PHOTOSYNTHESIS

VOLUME II · *Part 1*

PHOTOSYNTHESIS
AND RELATED PROCESSES

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VOLUME II

Part 1

PHOTOSYNTHESIS

and Related Processes

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VOLUME II · *Part I*

Spectroscopy and Fluorescence of Photosynthetic Pigments; Kinetics of Photosynthesis

1951

INTERSCIENCE PUBLISHERS, INC., NEW YORK

Interscience Publishers Ltd., London

INTERSCIENCE PUBLISHERS, Inc., 250 Fifth Avenue, New York 1, N. Y.

For Great Britain and Northern Ireland:
INTERSCIENCE PUBLISHERS Ltd., 2a Southampton Row, London W. C. I.

PRINTED IN THE UNITED STATES OF AMERICA
BY MACK PRINTING COMPANY, EASTON, PA.

PREFACE

to Volume II, Part 1

The manuscript of Volume II of this monograph was ready in draft form when the first volume was delivered for publication in 1944. After the interruption caused by war, I felt reluctant to publish the second volume without thorough revision in the light of new research data and of my own better understanding of some of the phenomena discussed. I began, in 1947, a revision of the manuscript; while I was revising the material, research in the field of photosynthesis picked up after the wartime slack, and the writing became something of an Achilles *vs.* turtle race. When, finally, the text achieved a temporary completeness, the count of the galleys revealed that it had become too long to be published under one cover. It was therefore divided into two half-volumes. The division cut through the part dealing with the kinetics of photosynthesis. Because of this, it seemed inadvisable to provide this half-volume with a separate subject index; an index for the whole work will be found at the end of the second part.* The latter will complete the treatment of the kinetics of photosynthesis (temperature effects, flashing light experiments, induction phenomena, and the function of the pigments, especially the energy transfer between them). The last three chapters will constitute an addition to Volume I, and will deal particularly with the new work on photochemistry of pigments (in solution and in chloroplasts), and with studies of the chemistry of carbon dioxide reduction by means of radioactive carbon.

The hopeful advance in these two fields has changed the appearance of the whole field of photosynthesis. The analysis of kinetic data, to which many pages in this half-volume are devoted, now seems somewhat like an attempt to reach a treasure chamber by drilling through steel walls while keys have been found to unlock the door. However, photosynthesis is not only a biochemical process in which all we want to learn is the chemical composition of the intermediates and the nature of the enzymes involved. It is also a most interesting physicochemical phenomenon; its kinetics will be worth continued study even after organic chemists

* As in Volume I, an index of the most important investigations described is provided at the end of this half-volume.

and biochemists have disentangled its chemical processes as thoroughly as they did those of respiration—which is still a long way to go. As a matter of fact, the kinetic aspects of respiration themselves are not adequately known, and will have to undergo hard study sooner or later.

Mr. Earl E. Jacobs not only kindly checked the derivation of kinetic equations in Chapters 26, 27, and 28, but has contributed much original thought and work to their development and interpretation; it is a pleasure to thank him for his unstinted assistance.

Much of the work on this volume was carried out while I was a member of the Solar Energy Research Project at the Massachusetts Institute of Technology. My thanks are due to the Project Committee and its chairman, Professor Hoyt C. Hottel, for generous assistance. I am equally indebted to the Photosynthesis Research Project, Department of Botany, University of Illinois, and Professor Robert Emerson, whose understanding and help have made the termination of the work possible.

Mrs. Carolyn Baer, Mrs. Marjorie Goodrich, and Mr. T. R. Punnett have given me valuable aid in the reading of the proofs and the checking of the bibliography.

EUGENE I. RABINOWITCH

Urbana
June 1951

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ABSORPTION SPECTRA OF PIGMENTS *IN VITRO*

A. Absorption Spectra of Chlorophyll and Its Derivatives*

1. Absorption Spectra of Chlorophylls *a* and *b*

The spectra of chlorophylls *a* and *b* have been studied in detail because of their theoretical interest, as well as because of their usefulness for the spectrophotometric determination of these pigments. Until recently, the results of different authors did not agree very well, either in the exact positions of the band maxima, or in the values of the extinction coefficients. Lately, improved chromatographic purification methods have enabled Zscheile and co-workers (*cf.* Zscheile 1934, 1935; Zscheile and Comar 1941; Comar and Zscheile 1941; Harris and Zscheile 1943) and Mackinney (1938, 1940, 1941) to obtain preparations of chlorophylls *a* and *b* that could meet high standards of spectroscopic purity and reproducibility.

Zscheile, Comar and Mackinney (1942) studied samples of chlorophyll prepared by the first two investigators at Purdue and by the third one at Berkeley, measuring the extinction coefficients by means of two different photoelectric spectrophotometers.

For Zscheile and Comar's "wet" preparation of chlorophyll *a* (*cf.* page 604), the two instruments gave practically identical extinction curves. (In the region between 430 and 660 mμ, all deviations were within 2%.) This shows how successfully large photometric errors (which are common in visual and photographic determinations of absorption curves) can be eliminated by the use of photoelectric devices.

The spectra of the solutions of chlorophylls *a* and *b* prepared by Mackinney in the dry state were, on the whole, similar to that of Zscheile's moist preparation; but differences up to 10% in chlorophyll *a* and 15% in chlorophyll *b* did occur between the extinction curves determined in the two laboratories, as well as between these two curves and that of Zscheile's preparation. The deviations varied irregularly with wave length, indicating the probable presence, in Mackinney's preparation, of an admixture of colored components. One may regret that no measurements were made below 430 mμ, since earlier experiments have shown a particularly strong variability of the absorption curve in this spectral region (*cf.* page 607).

The differences between the absorption curves of Zscheile and Mackinney appear minor when compared with the discrepancies that existed between the curves published by earlier investigators (*cf.* Table 21.I). These discrepancies must have been due to the use of less reliable photometric devices, and to the inferior purity of the earlier chloro-

* Bibliography, page 668.

phyll preparations—the latter clearly indicated by the relatively high absorption in the green (*cf.* last column in Table 21.IB).

A spectroscopically important impurity likely to be present in many chlorophyll preparations is the magnesium-free *pheophytin*, formed from chlorophyll whenever the latter comes in contact with acids. Elimination of magnesium from chlorophyll may take place even in living plants, *e. g.*, under the influence of acid fumes (*cf.* Stern 1935, 1938; and Tiegs 1938); it can easily occur during extraction, when the pigments are exposed to the action of acids contained in the cell sap. (Harris and Zscheile added magnesium carbonate to the extracting solvent to neutralize these acids.) This "primary" pheophytin is removed during chromatographic separation (according to Zscheile 1941, a pheophytin layer in the chromatogram was responsible for his earlier belief that leaf extracts contain a "chlorophyll *c*," *cf.* Vol. I, p. 402); but some pheophytin can again be formed afterward, *e. g.*, under the influence of atmospheric carbon dioxide.

As shown in figures 21.18 and 21.19, the pheophorbides (and this applies to pheophytins as well) have rather strong absorption bands in the green. Zscheile and Comar (1941) and Harris and Zscheile (1943) found that the ratios of the extinction coefficients in the maxima of the red chlorophyll bands (660 mμ for component *a* in ethyl ether and 642.5 mμ for component *b* in the same solvent) and of the green bands of pheophytin (505 and 520 mμ, respectively) reach 52 in solutions of the purest preparations of chlorophyll *a*, and 19 in similar preparations of chlorophyll *b*, but may drop to as low as 20 and 4.5, respectively, after these preparations have been allowed to stand for as little as a single day in the dry state. Zscheile and Comar (1941) recommended therefore that drying be avoided altogether in the preparation of spectroscopically pure chlorophyll solutions. More recently, Zscheile, Comar and Mackinney (1942) succeeded in preparing dry chlorophyll *a* which could be stored and still showed, upon dissolution, the high ratio of extinctions in the red and in the green indicative of high purity; but no standard procedure for obtaining such stable preparations could be given.

Zscheile, Comar and Harris (1944) found that the spectra of ethereal solutions of pure preparations of chlorophyll *a* show signs of deterioration after about one week storage at 0–5° C. in darkness. Crude ether extracts from leaves, on the other hand, proved to be comparatively stable—some gave no evidence of spectroscopic change even after 14 weeks storage (at −20° C.). Fresh corn leaves could be stored at −20° C., for a whole month without deterioration of chlorophyll.

Another problem of chlorophyll purification is the elimination of traces of chlorophyll *a* from chlorophyll *b*. According to Zscheile, supposedly "pure" chlorophyll *b*, used by many earlier observers, did contain up to

10% of chlorophyll *a*. Its presence can easily be recognized by increased light absorption at 614 mμ. According to Biermacher (1939), the fluorescence spectrum of chlorophyll *b* is even more senstive to contamination with chlorophyll *a* than the absorption spectrum (*cf.* chapter 23, page 744). He recommended extraction with hexane (which dissolves chlorophyll *a* much more easily than chlorophyll *b*) as a means of final purification of the *b*-component. Extraction is repeated until the fluorescence spectrum of the residue no longer shows the chlorophyll *b* band.

Meyer (1939) asserted that the band at 535 mμ, which is noticeable in most if not all extinction curves of pure chlorophyll *a* (*cf.* fig. 21.1B, and Table 21.1A), is not found in the spectra of *fresh* leaf extracts, and concluded that a mixture of the purified chlorophylls *a* and *b* is not identical with what he designated as "native" chlorophyll (*a* + *b*). This conclusion was criticized by Mackinney (1940, 1941), who found, to the contrary, that by mixing the two pure chlorophyll components one can reproduce the spectrum of a fresh leaf extract in all its details (except, of course, for the blue-violet region, where the absorption of the extracts is partly due to the carotenoids).

Figures 21.1A and B show the extinction curves of the two pure chlorophyll components in ethyl ether, according to Zscheile and Comar (1941). (The second figure is an enlarged detail of the first one.)

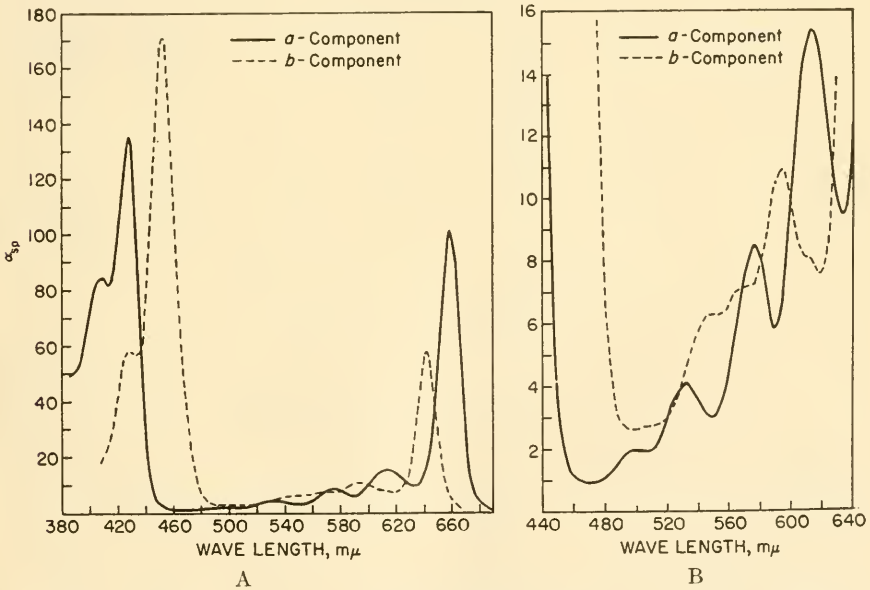


Fig. 21.1 Extinction curves of chlorophylls *a* and *b* in ethyl ether (after Zscheile and Comar 1941). Ordinates are specific extinction coefficients: $\log (I_0/I) = \alpha_{sp}cd$, where *c* is in g./l. and *d* in cm. To obtain molar extinction coefficients, multiply the data for chlorophyll *a* by 893 and those for chlorophyll *b* by 907. (These factors are uncertain to the extent of ∓1% because of the unknown degree of hydration of chlorophyll, *cf.* Vol. I, chapter 16.)

The absorption curves given by Mackinney (1938, 1940, 1941) and by Winterstein and Stein (1933), although less detailed, agree satisfactorily with figure 21.1; but those of Sprecher von Bernegg, Heierle and Almasy (1935) and of Hagenbach, Auerbacher and Wiedemann (1936) show considerable deviations, illustrated by Tables 21.I.

TABLE 21.I. CHLOROPHYLL SOLUTIONS IN ETHYL ETHER

A. ABSORPTION MAXIMA. Main bands in italics.

Band no.	Rudolph (1933)	Sprecher von Bernegg *et al.* (1935)	Hagenbach *et al.* (1936)	Egle[a] (1939)	Mackinney (1940)	Zscheile and Comar (1941)	Harris and Zscheile (1943)
			Chlorophyll *a*				
1	*660*	*657.3*	*655.6*	*662.2*	*660*	*660.0*	*660.0*
2	613	612.5	608.5	613.6	—	612.5	614.0
3	577	575.4	574.6	574.1	—	572.5	—
4	—	534.8	531.8	530.6	—	527.5	—
5	—	—	510.8	—	—	—	—
6	—	503.8	494.2	490.5	—	497.5	—
7	—	—	464.1	—	—	—	—
8	—	*425.8*	*430.7*	*431.1*	*430*	*427.5*	*429.0*
9	—	407.0	—	—	—	410.0	410.0
			Chlorophyll *b*				
1	*643*	*642.2*	*637.6*	*644.6*	*642.5*	*642.5*	*642.5*
2	597	594.7	589.1	595.6	—	592.5	594.0
3	—	—	566.7	567.3	—	567.5	—
4	558	—	559.4	544.6	—	—	—
5	—	—	552.6	—	—	—	—
6	—	537	540.4	—	—	547.5	—
7	—	—	499.9	—	—	502.5	—
8	—	*451.8*	*449.9*	*457.3*	*453*	*452.5*	*453.0*
9	—	425.8	—	426.5	—	430.0	428.5

B. MOLAR EXTINCTION COEFFICIENTS. $\alpha = \log (I_0/I)/cd$, where c is in mole/l. and d is in cm. Most probable values in italics.

Chlorophyll *a* in ethyl ether

Observers	$\alpha \times 10^{-4}$ Red peak (660 mμ)	Blue peak (430 mμ)	Green min. (472 mμ)	Ratios of coefficients blue peak / red peak	red peak / green min.
Sprecher von Bernegg *et al.* (1935)	7.4	8.5	0.27	1.15	27
Hagenbach *et al.* (1936)	7.23	14.3	0.25	1.98	29
Mackinney (1940)	7.65	9.75	0.11	1.28	70
Zscheile and Comar (1941)	*9.10*	*12.0*	*0.08*	*1.32*	*114*
Zscheile, Comar and Mackinney (1942)[b]	9.00	11.7	0.08	1.35	108
Same[c]	8.34	10.4	0.09	1.39	85
Same[d]	8.78	11.5	0.09	1.31	84
Harris and Zscheile (1943)	—	—	—	1.33	—

Table continued

TABLE 21.I—*Continued*

Chlorophyll *b* in ethyl ether					
Observers	(643 mμ)	(453 mμ)	(510 mμ)		
Sprecher von Bernegg *et al.* (1935)	4.7	9.4	0.28	2.0	17
Hagenbach *et al.* (1936)	7.10	20.9	0.34	2.91	21
Mackinney (1940)	5.00	13.6	0.26	2.72	19
Zscheile and Comar (1941)	*5.15*	*15.5*	*0.24*	*3.01*	*21*
Zscheile, Comar and Mackinney (1942)[c]	4.80	12.9	0.24	2.65	20
Same[d]	4.98	13.9	0.24	2.79	21
Harris and Zscheile (1943)	—	—	—	2.98	—

[a] Band "axes"—*i. e.*, arithmetic means of the wave lengths of the limits of blackening on a photographic plate (all other data in this table were obtained by photoelectric photometry).

[b] Zscheile and Comar's moist preparation measured by Mackinney.

[c] Mackinney's dry preparation measured by Zscheile and Comar.

[d] Mackinney's dry preparation measured by Mackinney.

The reproducibility of the red band encourages its use for the spectrophotometric assay of the two chlorophylls. This method was developed by Ghosh and Sen-Gupta (1931), Zscheile (1934, 1935), Sprecher von Bernegg, Heierle and Almasy (1935), Haskin (1942), Comar and Zscheile (1942), Comar (1942) and Comar, Benne and Buteyn (1943). Measurements at two different wave lengths are required to calculate the concentrations of the two components. The use of the absorption maxima at 642.5 and 660 mμ permits the most sensitive determination, but requires precise work, since the extinction values in the sharp absorption peaks are very sensitive to variations in the width of the spectrometer slit or to slight errors in the adjustment of the monochromator. Cross-checks at other wave lengths are therefore desirable. All errors could be eliminated by the use of monochromatic light; but the spectrum of the mercury arc—the usual source of monochromatic light in the laboratory—does not contain suitable lines in the red and orange regions.

As pointed out once before, particularly wide discrepancies can be noted between the different extinction curves in the *blue-violet region* (*cf.* Table 21.IB). It was noted by Albers (1941) that the subsidiary violet band (situated, in ethereal solution, at 410 mμ in chlorophyll *a* and 430 mμ in chlorophyll *b*) sometimes appears as a slight hump on the main band, and sometimes as a separate peak, almost as prominent as the main one. The observed variations in the maximum height of the violet peak may be caused by the more or less complete separation of this doublet structure.

Differences of this type cannot be attributed to the presence of carotenoids, or other impurities. A renewed photometric study of this spectral region is desirable. If the deviations will not disappear upon further purification of the material and improvement of the photoelectric methods, one may have to consider, as a possible explanation, the existence of *tautomers*.

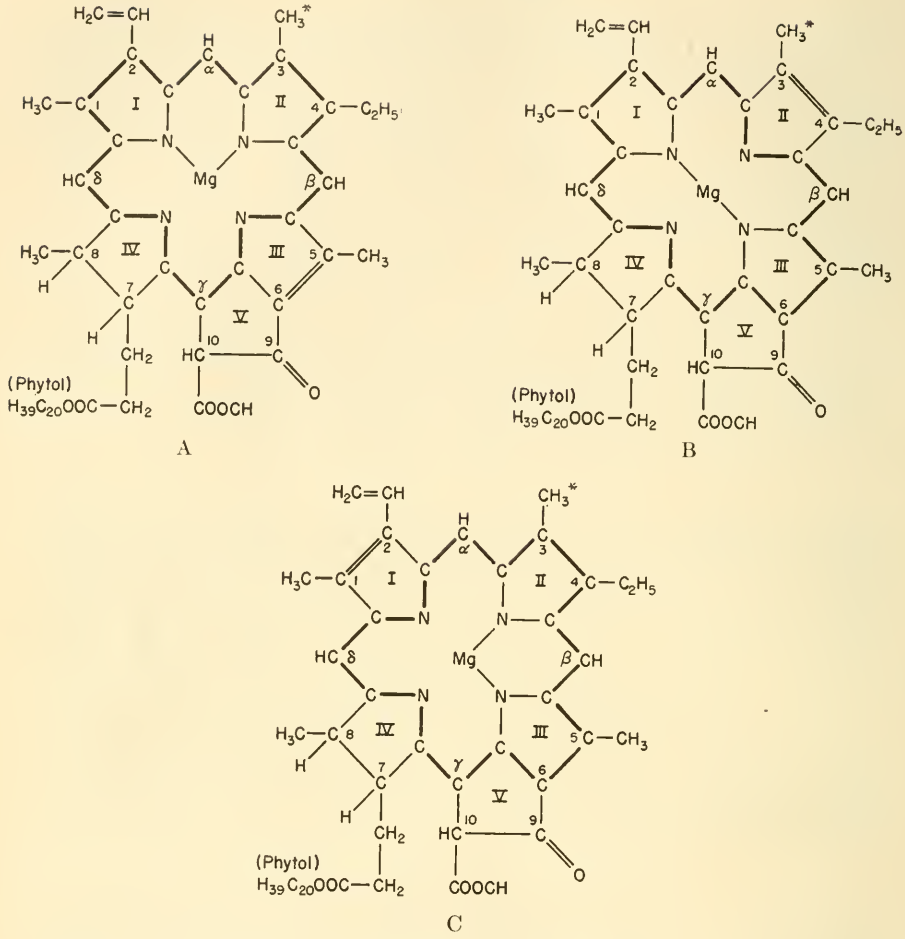


Fig. 21.2. Chlorophyll *a* structure according to Hans Fischer. A, B and C are three isomeric or tautomeric (or mesomeric) structures, distinguished by the routing of the all-round conjugated ring system (heavy line) and the positions of the "semi-isolated" double bond and of the Mg—N bonds (all of which depend on this routing). The asterisk designates the position of a carbonyl group in chlorophyll *b*. A, semi-isolated double bond in nucleus III; Mg bound to nuclei I and II. B, semi-isolated double bond in nucleus II; Mg bound to nuclei I and III. C, semi-isolated double bond in nucleus I; Mg bound to nuclei II and III.

We recall that the three structures of chlorophyll, A, B and C, described in chapter 16, Vol. I, (*cf.* fig. 21.2), were characterized there as probably tautomeric rather than mesomeric. We also recall that Strain and Manning found (*cf.* page 403) in the chromatograms of leaf extracts, two new

chlorophylls, which they called *a'* and *b'* and interpreted as tautomers of chlorophylls *a* and *b*. The forms A, B and C have different double bond arrangements in the nonhydrogenated pyrrole nuclei, but the same hydrogenated nucleus IV. Since the red absorption band is somehow associated with the hydrogenation of this nucleus (*cf.* page 621), modifications A, B and C may have identical red bands. They could, however, differ in the positions or shapes of the blue-violet bands, associated with the conjugated porphin system as a whole.

Erdman and Corwin (1946) noted the spectroscopic similarity of etioporphyrin and *N*-methyl etioporphyrin, and deduced from this that the two "central" H-atoms in the porphin system must be fixed at definite nitrogen atoms for $\gg 10^{-8}$ sec. This supports the hypothesis that structures such as those represented by the three formulas in figure 21.2 are tautomeric rather than mesomeric.

We have used so far only data obtained with *ethyl ether* as solvent, since they alone offered the possibility of comparison between the results of several observers. Determinations of extinction curves of chlorophyll in solvents other than ether are listed in Table 21.II.

TABLE 21.II

CHLOROPHYLL EXTINCTION MEASUREMENTS IN VARIOUS SOLVENTS

Solvent	Reference
Methanol	Rabinowitch and Weiss (1937) (ethyl chlorophyllide); Harris and Zscheile (1943); McBrady and Livingston (1948, 1949)
Ethanol	Meyer (1939); Sprecher von Bernegg, Heierle and Almasy (1935)
Butanol (*n*- and *iso*-)	Harris and Zscheile (1943)[a]
Octanol	Harris and Zscheile (1943)[a]
1-Propyl ether	Harris and Zscheile (1943)[a]
Dioxane	Harris and Zscheile (1943)[a]
Benzene	Winterstein and Stein (1933); Hausser (*cf.* Fischer and Stern, 1940; Harris and Zscheile (1943)
Cyclohexane	Harris and Zscheile (1943)
Acetone	Mackinney (1940); Harris and Zscheile (1943)
Carbon tetrachloride	Harris and Zscheile (1943)[a]
Methyl oleate	Harris and Zscheile (1943)[a]
Olive oil	Harris and Zscheile (1943)[a]

[a] Several of the curves of Harris and Zscheile (1943) are reproduced in figure 21.26.

The absorption spectra of the two chlorophylls in the *ultraviolet* are shown in figure 21.3. The absorption remains considerable all the way down to 200 mμ; the most prominent band is a double band of chlorophyll *b* at 310 and 335 mμ. In the spectrum of chlorophyll *a*, distinct maxima

appear at 325 and 375 mμ. Below the region shown in figure 21.3, both components have an absorption maximum at 250 mμ ($\alpha_{sp.} \simeq 30$).

An absorption band at 330 mμ was first noticed in the spectrum of alcoholic extracts from nettle leaves by Lewkowitsch (1928).

The absorption spectra of chlorophyll, ethyl chlorophyllide and phytol in the *infrared* were described by van Gulik (1914) and Stair and Coblentz

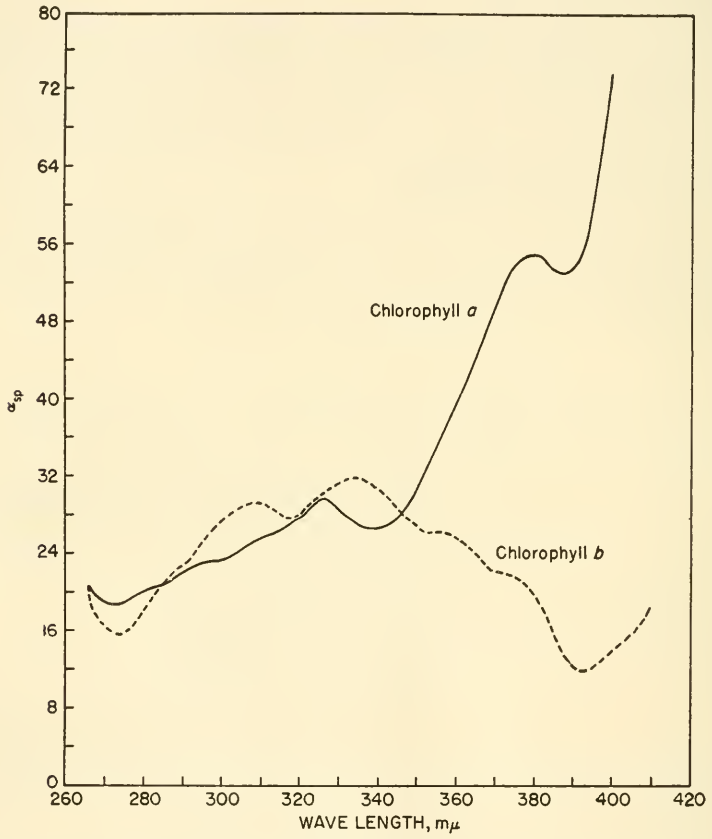


Fig. 21.3. Ultraviolet spectrum of chlorophylls *a* and *b* in ethyl ether (after Harris and Zscheile 1943). Specific extinction coefficients; *c* in g./l.

(1933). Chlorophyll is transparent between 0.7 and 3 μ (this may be useful in preventing the overheating of leaves in direct sunlight). It has several absorption bands at 3–4, and 5.8 μ; most of them are found also in the spectrum of phytol, and are absent from that of ethyl chlorophyllide (*cf.* fig. 21.4 and Table 21.IIA); they thus belong to the phytyl chain rather

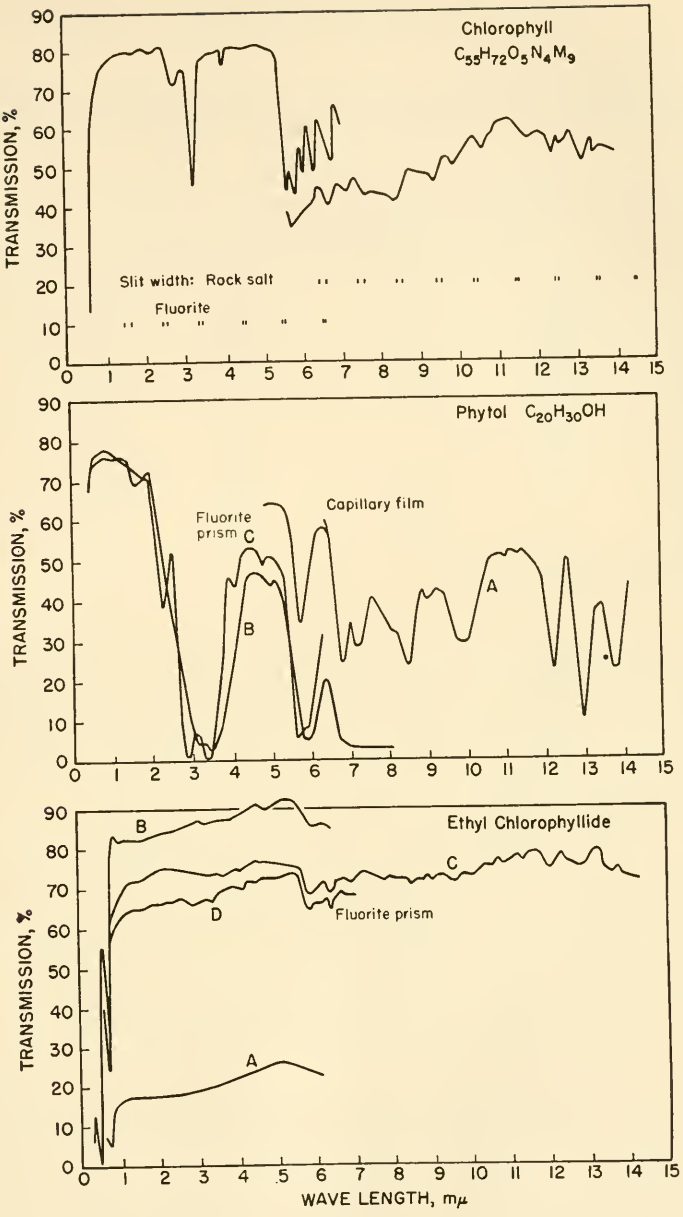


Fig. 21.4. Infrared spectra: top, chlorophyll (a + b) layer prepared by evaporation of ethanol solution on rock salt cleavage plate; center, liquid phytol in cell 0.3 mm. thick with rock salt windows (curves B and C) and in capillary layer between two rock salt cleavage plates (curve A); bottom, thin evaporated films of ethyl chlorophyllides (a + b) on fluorite (curves A and B) and on rock salt (curves C and D) (after Stair and Coblentz 1933).

than to the chlorophyllin ring system. The frequencies 1045, 1265, 1450, 1545 and 1610 cm.$^{-1}$, on the other hand, seem to belong to chlorophyllin.

TABLE 21.IIA

INFRARED ABSORPTION FREQUENCIES OF CHLOROPHYLL (a + b), PHYTOL AND ETHYL CHLOROPHYLLIDE (a + b)[a]

Chl., a + b, cm.$^{-1}$	Phytol, cm.$^{-1}$	Et. chl., a + b, cm.$^{-1}$	Chl., a + b, cm.$^{-1}$	Phytol, cm.$^{-1}$	Et. chl., a + b, cm.$^{-1}$
—	5260	—	1370	*1375*	—
4255	4200	—	1265	—	—
3365	3380	3445	—	*1220*	—
2915	*2915*	2940	1160	1165	—
2360	*2360*	—	—	1100	—
—	2035	—	1045	—	1040
1730	*1745*	—	995	*1005*	—
1675	1675	*1695*	920	—	—
1610	—	—	835	*815*	820
1545	—	*1550*	795	*775*	780
1450	*1445*	*1450*	750	*720*	735

[a] Strongest bands in italics.

In considering Table 21.I and figures 21.1 A and B, one notices the alternation of the absorption peaks of the chlorophylls a and b. This relationship was interpreted by Willstätter and Stoll (as well as by Hagenbach, Auerbacher and Wiedemann) as an indication that the two sensitizers support each other in ensuring complete utilization of all wave lengths of the visible spectrum. However, the slight differences in position of the two main bands in the red, and the alternation of low maxima in the yellow and orange, can have but slight influence on the efficiency of light absorption by living plants, as a detailed comparison (at present unavailable) of the absorption spectra of green algae with those of the chlorophyll b-deficient brown algae will certainly confirm. There is, however, one spectral region in which the presence of chlorophyll b markedly improves the absorption. This is in the blue, between 450 and 530 mμ (*cf.* chapter 22, page 720). This fact may explain why plants that grow in the shade often contain more chlorophyll b than sun plants (*cf.* page 403). In brown algae, which carry no chlorophyll b, a similar improvement of absorption in the blue is achieved by the presence of fucoxanthol (*cf.* page 725).

A much more efficient absorption throughout the visible spectrum could result from the addition, to the yellow-green chlorophyll a, of a purple pigment, with an absorption maximum in or near the middle of the visible spectrum. Nature has provided this type of pigmentation in red algae. These often inhabit the dim regions deep under the sea, and efficient absorption of all radiations that reach them may be a question of life or death for them. For sun-exposed plants, on the other hand, com-

plete absorption of all visible light appears to be unnecessary, or even harmful. As stated before (compare Volume I, chapter 19), chlorophyll probably was adopted as sensitizer for photosynthesis not because of a particularly favorable absorption spectrum, but because of its peculiar photosensitizing properties. Once having found a suitable sensitizer, nature then made adjustments to improve the supply of light energy to species living in unfavorable habitats—by increasing the quantity of chlorophyll *b* in shade plants, and by providing brown algae with fucoxanthol, and red algae with phycobilins. The presence of the latter makes the red algae capable of growth even under a thick layer of blue-green sea water.

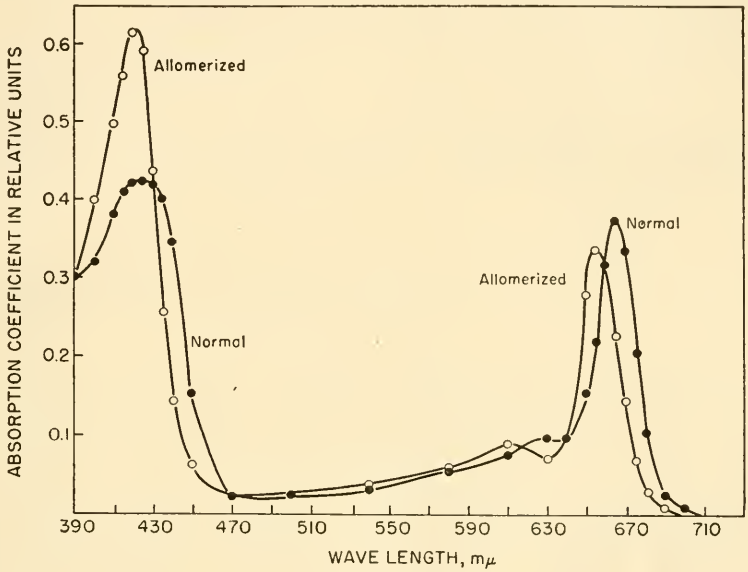


Fig. 21.4A. Absorption spectrum of allomerized chlorophyll *a* in methanol (after Livingston 1948).

A word must be said about the absorption spectrum of *allomerized* chlorophyll. When chlorophyll in alcoholic solution is permitted to stand in air, it is "allomerized," *i. e.*, according to Conant and Fischer, oxidized at the C(10) atom (*cf.* Vol. I, page 460). This reaction is catalyzed, according to Livingston and co-workers, by salts such as $LaCl_3$ and $BaCl_2$. Spectroscopic evidence indicates that a similar, or identical, oxidation occurs also under the influence of iodine or bromine even in the absence of air: Fischer's chemical observations indicate that quinone has the same effect. According to Livingston (1948, 1949), allomerization of chlorophyll is characterized by a spectral change, illustrated by figure 21.4A. The two

curves in this figure were obtained by chromatographic separation of a partially allomerized solution; a curve identical with curve *b* is obtained by leaving a methanolic solution of chlorophyll *a* stand in air at 80°C. for two days, or by adding to it 2–3 equivalents of iodine, or by adding traces (10^{-5} ml.) of $LaCl_3$ or $CaCl_3$. Standing in air, or addition of traces of iodine, has no effect on the spectrum of chlorophyll *a* in ether or carbon tetrachloride, except for slow general bleaching. If some methanol is added to the nonpolar solvent, allomerization proceeds more slowly, but ends with being as complete as in pure methanol; transfer of allomerized product into pure ether or carbon tetrachloride does not reverse the change.

In cresol, allomerization also seems to occur, but is complicated by other changes, probably due to the acid nature of this solvent.

Livingston and co-workers also have measured the absorption spectrum of the yellow and brown unstable intermediates in the reactions of chlorophyll (in methanol) with $FeCl_3$ (Vol. I, page 464) and with alkali (see "phase test," Vol. I, page 459). The results will be described in chapter 37, in the section dealing with new observations on the chemistry of chlorophyll.

2. Absorption Spectra of Chlorophylls *c* and *d*, Bacteriochlorophyll and Protochlorophyll

The absorption spectrum of "*chlorophyll c*," also called *chlorofucin* (*cf.* Vol. I, page 406), is characterized, according to the earlier authors (*e. g.*, Tswett), by a band in the region of 630 mμ. Strain and Manning (1942) determined the extinction curve of this compound, first by subtraction of the extinction curve of pure chlorophyll *a* from that of the chlorophyll extract from brown algae, and later by direct spectrophotometry of isolated chlorophyll *c*. Figures 21.5A and B show that the results of the two methods are in approximate agreement. Two "chlorofucin" bands are situated in the orange and red—with peaks at 575.5 and 627 mμ, respectively, in methanol, and at 581 and 631 mμ, respectively, in 80% acetone. Figure 21.5C also shows a band in the blue (at 446 mμ) almost ten times stronger. The ratio between the intensities of the bands in blue and red is thus much larger than in chlorophyll *b* (where it is about 3), not to speak of chlorophyll *a* (where the two bands are approximately equal in intensity, *cf.* Tables 21.IB and 21.VIII), but the general pattern of the spectrum is similar.

Wassink and Kersten (1946) and Tanada (1951) gave similar absorption curves for a "chlorophyll *c*" fraction from chromatographic fractionation of the pigments of diatoms. The three absorption peaks appear, in methanol, at about 450, 590 and 635 mμ, with the first band about ten times more intense than the other two (*cf.* p. 623).

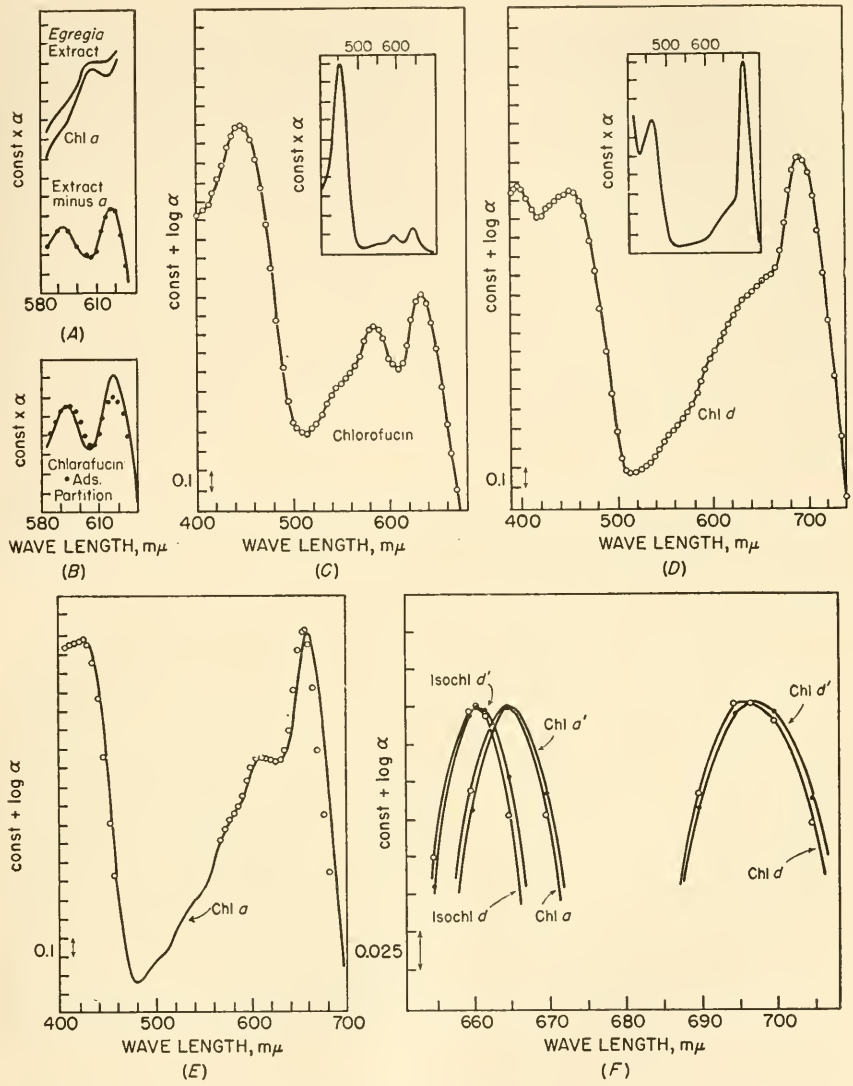


Fig. 21.5. Spectrum of chlorophyll *c* (chlorofucin) in methanol. Absorption coefficients, α, in relative units (after Strain and Manning 1942). (*A*) Chlorofucin from *Egregia*, a brown alga (spectrum calculated by difference). (*B*) Same spectrum measured with chlorofucin prepared by adsorption (dots) or partition (solid line). (*C*) Chlorofucin from *Nitzschia*, a diatom (measured spectrum). (*D*, *E*, *F*) Absorption spectra of chlorophylls *a*, *d*, *d'*, and of isochlorophylls *d* and *d'*; absorption coefficients, α, in relative units (after Manning and Strain 1943). Open circles in *E*, absorption coefficients of isochlorophyll *d*.

The absorption spectrum of the "*chlorophyll d*" of red algae also was determined by Manning and Strain (1943). Since this investigation was only briefly mentioned in Volume I, a few words may be said here about this newly discovered pigment. Its presence is revealed by a bulge on the red side of the chlorophyll *a* band, observed in the spectra of methanol extracts from red algae. In pure chlorophyll *a* solution in methanol, the ratio of extinction coefficients at 665 and 700 mμ is 90; in extracts from twenty red algae, this ratio was between 15 and 65. *Short* extraction leads to products with even lower ratios; *e. g.*, two minutes extraction of *Gigartina agardhii* gave a product with a ratio of only 10:1. Apparently, chlorophyll *d* is much more easily extracted by methanol than chlorophyll *a*. Chromatographic purification can be used for the preparation of pure chlorophyll *d*. Figure 21.5D shows the spectrum of this pigment in methanol. In ethyl ether, the band maxima of chlorophyll *d* lie at 686 and 445 mμ, and below 395 mμ.

In spectrum as well as in solubility and other chemical properties, chlorophyll *d* resembles chlorophyll *a* more than chlorophyll *b*. Careful search for chlorophylls *b* and *c* in the chromatograms of pigments from red algae gave negative results (the upper limit for the content of chlorophyll *b* is 0.3% of that of chlorophyll *a*).

The *isomerization* of chlorophyll *d* was mentioned in chapter 16 (Vol. I). It occurs in the methanolic solution upon standing in the dark, in the presence or absence of air, and leads to three new pigments, which seem to be interconvertible. They were designated chlorophyll *d'*, isochlorophyll *d* and isochlorophyll *d'*. The spectra of the components *d* and *d'*, respectively, are very similar, and the same is true of those of the isomers iso-*d* and iso-*d'*. The latter two spectra are almost identical with those of the chlorophylls *a* and *a'* (*cf.* fig. 21.5E); but the maximum of chlorophyll iso-*d* and iso-*d'* lies about 5 mμ further toward the blue (*cf.* fig. 21.5 F). The red band of chlorophyll *d* (and *d'*) lies about 37 mμ further toward the infrared than that of chlorophylls *a* and *a'* (*cf.* fig. 21.5F).

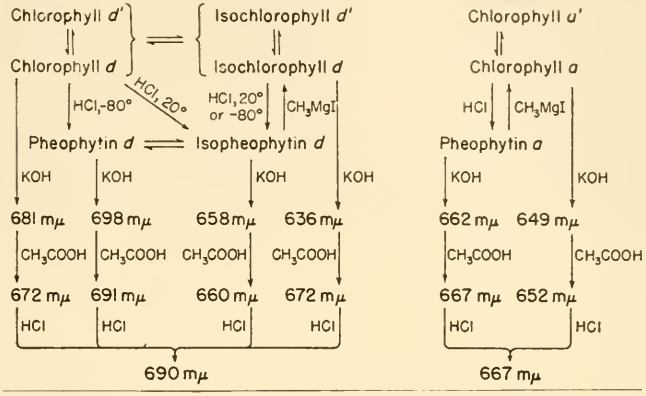
Isochlorophylls *d* and *d'* are not found in fresh extracts from algae. The conversion *d* → iso-*d* appears to be slower than the conversions *d* → *d'* and iso-*d* → iso-*d'*.

Four interconvertible pheophytins with different spectra were produced by the action of acids on the four *d*-pigments; but successive treatment with alkali and acid led to spectroscopically identical products for all four *d*-pigments. These products are distinct from the compounds obtained by a similar treatment of either of the two *a*-pigments. Table 21.III illustrates the relationships between the six pigments *a*, *a'*, *d*, *d'*, iso-*d* and iso-*d'* and their transformation products.

The main absorption band of the *bacteriochlorophyll* of purple bacteria

TABLE 21. III

ISOMERIZATION AND OTHER REACTIONS OF CHLOROPHYLLS *d* AND *a* [a]



[a] After Manning and Strain (1943).

Fig. 21.6. Relative absorption curve of green pigment from bacterium *Spirillum rubrum* in methanol (after French 1937). 0.2 ml. moist cells extracted in the dark at 0° with 5.2 ml. absolute methanol. Extract kept in dark and measured at room temperature with very weak light. A similar curve was given by Vermeulen, Wassink and Reman (1937) for alcoholic extract from *Chromatium* (major peak at 790 mμ, minor peaks at 705, 600, 510, 470 and 440 mμ).

lies in the near infrared and has not yet been measured precisely; its general shape is shown by figure 21.6. According to this figure, the absorption spectrum of bacteriochlorophyll contains *three* main bands—one in the infrared (770 mμ in methanol, shown also in fig. 21.21), one in the orange (605 mμ) and one in the violet (~400 mμ). One may assume (*cf.* below, page 622) that the two latter bands are analogous to the red and the blue-

violet bands of chlorophyll *a*, respectively (the whole system having been shifted by about 60 mμ toward shorter waves), while the band in the near infrared has no analogue in the spectrum of ordinary chlorophyll.

The status of the "orange" bacteriochlorophyll band needs additional clarification. Our interpretation is based on figure 21.6. A strong band on the short-wave side of the main red band is also recognizable in the spectrum of bacteriopheophytin as observed by French (fig. 21.21), but is situated much further toward the green, at 530 mμ; in addition, there is a weaker band at 680 mμ and indications of a still weaker one at about 630 mμ. Dutch observers state (see page 702, chapter 22) that "alcoholic

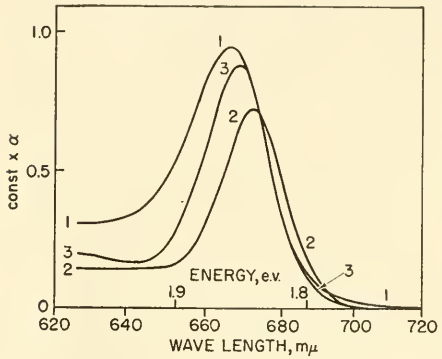


Fig. 21.7. Red absorption bands of alcoholic extracts of (1) green alga, *Chlorella*, (2) green sulfur bacteria, and (3) a blue-green alga, *Oscillatoria* (after Katz and Wassink 1939).

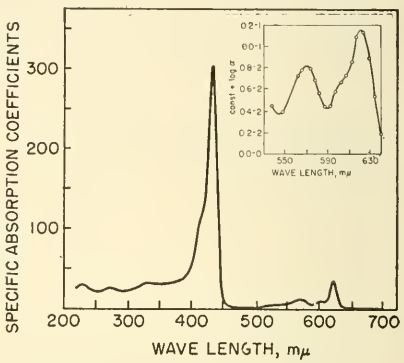


Fig. 21.8. Absorption spectrum of protochlorophyll after Rudolph (1933) in the insert, and after Koski and Smith (1948) in the main figure.

extracts from purple bacteria show only one absorption maximum at 774 mμ," but it is not certain how wide a region this statement is supposed to cover. The extract absorption curves in figures 21.30A and B extend only down to 730–740 mμ.

One question to be clarified is the possible contribution to the absorption curves of bacteriochlorophylls of derivatives analogous to chlorophylls *b*, *c* and *d*. The existence of pigments of this kind was suggested by Seybold and Egle (1939) (*cf.* Vol. I, page 407), who gave some provisional figures for the positions of their absorption bands.

The absorption spectrum of *bacterioviridin*—the pigment of green bacteria (*cf.* Vol. I, page 445)— was observed by Metzner (1922) and, more recently, by Katz and Wassink (1939). Apparently, it is very similar to that of chlorophyll *a*. Figure 21.7 shows the red adsorption band in

alcoholic extracts from *Chlorella* (green alga), *Chlorobium limicola* (green bacteria) and *Oscillatoria* (blue alga). The difference between the curves 1 and 3 can be attributed to the absence of chlorophyll *b* in blue algae; while the larger difference between the curves 3 and 2 indicates a chemical distinction between chlorophyll *a* and bacterioviridin. The absorption peak of the latter pigment in ethanol lies at 668 mμ, while that of the former one is situated at about 662 mμ.

The absorption spectrum of *protochlorophyll* (from squash seeds) was described by Noack and Kiessling (1929, 1930, 1931) as well as by Rudolph (1933), Seybold (1937) and Koski and Smith (1948). The long-wave bands are listed in Table 21.IV; the whole spectrum is shown in figure 21.8.

TABLE 21.IV

ABSORPTION BANDS OF PROTOCHLOROPHYLL

In chloroform–pyridine		In ether				
Noack and Kiessling (1929, 1930)		Rudolph (1933)	Seybold (1937)			
			Component *a*		Component *b*	
λ, mμ	Order of intensities	λ, mμ	λ, mμ	Order of intensities	λ, mμ	Order of intensities
641–621	1	621	650–620	1	645–632	3
		(602)	620–603	3	632–605	1
582–567	2	571	592–572	2	620–605	4
			572–555	5	587–570	2
540–521	3	536	545–530	4	545–520	5
			<480	—	<500	

3. Relation between Absorption Spectrum and Molecular Structure of Porphin Derivatives

To understand the role of chlorophyll in photosynthesis, it would be important to have a detailed knowledge of the nature of the lowest excited state of the chlorophyll molecule, since sensitization must be due to the interaction of chlorophyll in this excited state with the primary sensitization substrate or substrates (*e. g.*, with the CO_2-acceptor complex $\{CO_2\}$, or with the oxidant $\{H_2O\}$; *cf.* Vol. I, chapter 7). If this interaction is in the nature of a reversible oxidation-reduction (which is probable) analysis of the nature of the excited state may permit conclusions as to the type of oxidations (or reductions) most likely to be involved. Theoretical analysis of porphin spectra was initiated too late for use in the following discussion, which is based on empirical relationships only. The papers by Kuhn (1949), Simpson (1949), and Platt and co-workers (1950) will be summarized in the last chapter.

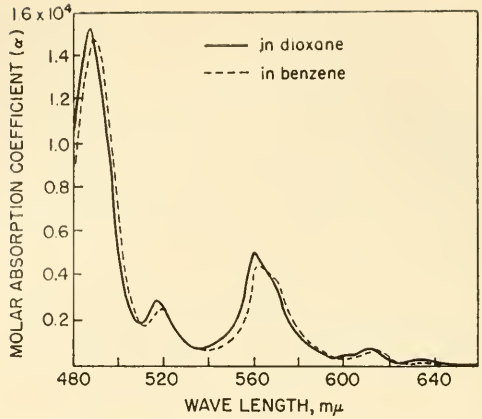


Fig. 21.9. Absorption spectrum of porphin (after Stern, Wenderlein and Molvig 1936).

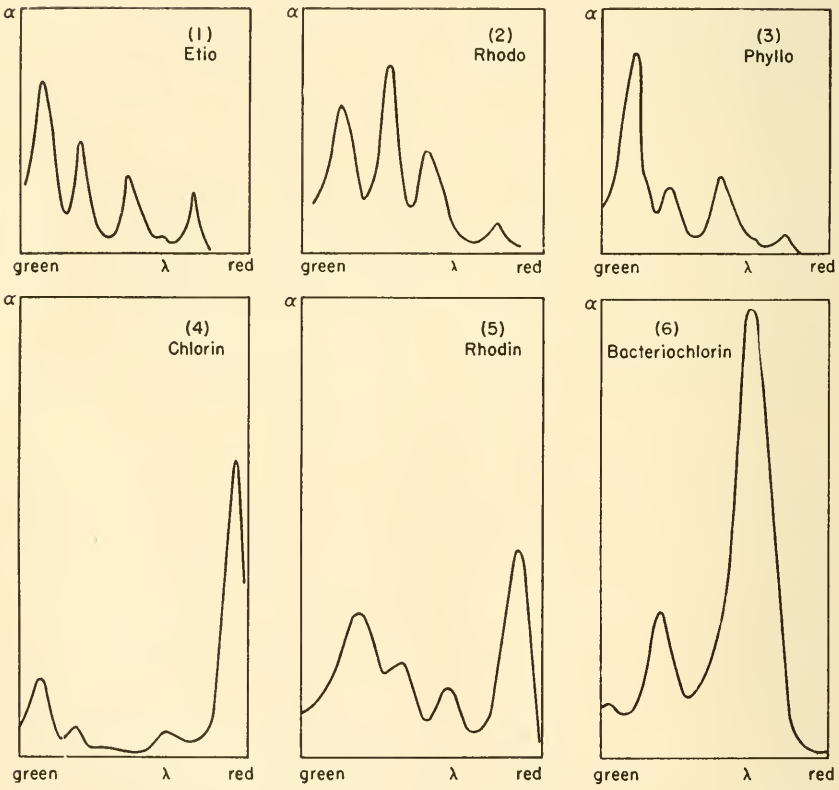


Fig. 21.10. Typical spectra of porphin derivatives above 500 mμ (not showing the strongest band, at 420 mμ) (after Stern 1938). (1, 2, 3) porphyrin spectra; (4, 5) chlorin spectra; (6) bacteriochlorin spectrum.

The conjugated double bond system of porphin (which is the basic structure of all chlorophyll pigments as well as of the porphyrins) is a chromophore, capable of producing strong absorption bands in the visible and near ultraviolet. This is illustrated by the absorption spectrum of the parent substance of the group, porphin (*cf.* fig. 21.9). This spectrum has a typical pattern of four bands between 480 and 700 mμ, generally increasing in intensity toward the violet, but with the third band from the red weaker than the second one. Stern and Wenderlein (1936) called this pattern the "phyllo type" (fig. 21.10, 3); it is exhibited by many porphyrins. Other

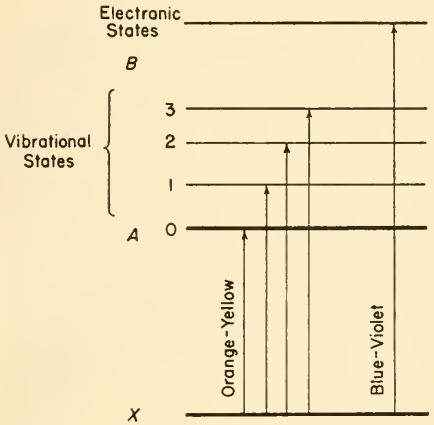


Fig. 21.11. Interpretation of porphin spectrum in terms of transitions from the ground state, X, to two excited electronic states A (vibrational states A_0, A_1, A_2, A_3...) and B.

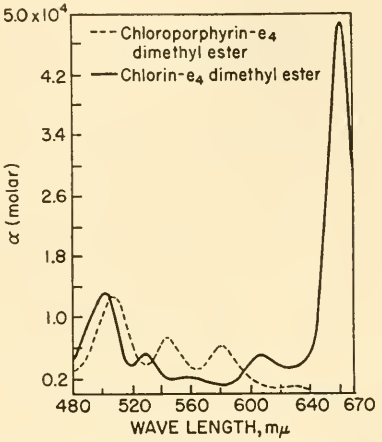


Fig. 21.12. Spectroscopic effect of transition from the porphin to the dihydroporphin (chlorin) system (in dioxane) (after Stern and Wenderlein 1935).

porphyrins have similar four-band spectra with a somewhat different distribution of intensities; these are called by Stern the "etio type" and the "rhodo type" (fig. 21.10, 1 and 2). Compounds of these three types transmit freely in the red; their color is red or purplish, hence the name "porphyrin."

In addition to the series of bands shown in figure 21.9 and 21.10, all porphin derivatives have the so-called "Soret" band in the blue-violet (similar to the blue-violet band of chlorophyll). The distances between the four bands in the green, yellow and red are such as to make plausible their interpretation as vibrational bands, corresponding to a common electronic transition; while the blue-violet band stands apart and probably corresponds to a different electronic excitation (*cf.* Table 21.V and fig. 21.11).

The porphin spectrum undergoes a far-reaching change (*cf.* spectra 4 and 5 in fig. 21.10) upon the hydrogenation of one pyrrole nucleus, *i.e.*,

upon the transition from the prophin system to the *dihydroporphin* (*chlorin* or *rhodin*) system. ("Rhodins" are chlorins derived from chlorophyll *b*; *cf.* page 447, Vol. I.) Chlorins are *green* (as their name implies) and this

TABLE 21.V

PORPHIN BANDS

λ (mμ)	613	560.5	517.5	487	430
ν (cm.$^{-1}$)	16300	17820	19300	20530	23260
$\Delta\nu$ (cm.$^{-1}$)	1520	1480	1230	(2730)	

indicates strong absorption in the red, as well as in the blue and in the violet, and transparency in the middle of the visible spectrum. Figure 21.12 shows the spectrum of a compound of the etio type, chloroporphyrin-e_4 dimethyl ester, together with that of its hydrogenation product, chlorin-e_4 dimethyl ester. While the bands in green and yellow appear weakened and displaced toward shorter waves in consequence of hydrogenation, a new and very intense band arises in the red (at about 660 mμ), which completely overshadows all the other bands of the orange-yellow system (its intensity is about equal to that of the blue-violet band, not shown in fig. 21.12). The hydrogenation of a second pyrrole nucleus, which is characteristic of bacteriochlorophyll and other derivatives of *tetrahydroporphin* (*cf.* Vol. I, page 447), causes a renewed transformation of the spectrum (fig. 21.10, 6). As stated on page 617 (*cf.* fig. 21.6), the strongest band of bacteriochlorophyll is situated in the near infrared. According to Stern and Pruckner (1939) the "bacterio" type bands in the visible region (red, yellow and green) are generally weaker (with the notable exception of one comparatively strong green band), and situated at shorter waves than the corresponding bands of the chlorin type. It thus seems that the effect of the second hydrogenation is similar to that of the first one, *i. e.*, the majority of the previously existing bands (at least, of those in the region above 450 mμ) are weakened and shifted toward the violet, while a new band of dominant intensity arises at the long-wave end of the spectrum We made use of this interpretation on page 618, when we suggested that the orange (rather than the infrared) band of bacteriochlorophyll be considered as analogous to the red band of ordinary chlorophyll (see also page 631).

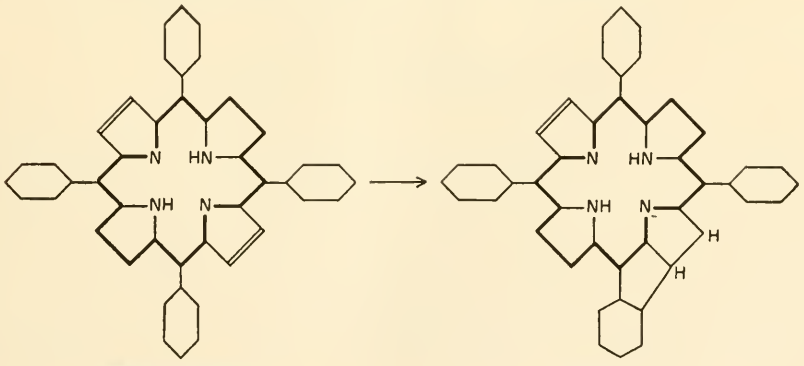
The rule that the dominant red or infrared band in the absorption spectrum of porphin derivatives is associated with the presence of one or two hydrogenated nuclei may be very significant from the point of view of the mechanism of the photosensitizing action of chlorophyll and bacteriochlorophyll, and it is therefore important to mention some apparent exceptions to this rule. One such exception was mentioned on page 619—

protochlorophyll, a green compound which, according to H. Fischer (*cf.* Vol. I, page 445), nevertheless is a prophyrin rather than a chlorin or phorbin. The spectrum of protochlorophyll (*cf.* fig. 21.8 and Table 21.IV) does not show the predominance of the red band over the bands in yellow and green to the same extent as do the typical spectra of dehydro- or tetrahydroporphin derivatives; but it resembles these spectra somewhat more than it does the typical porphyrin spectra in figure 21.10. A re-examination of the structure of the protochlorophyll molecule is therefore desirable. It is noteworthy that "protopheophytin," obtained from protochlorophyll by the action of acids, was found to have a typical porphyrin spectrum.

A similar case is presented by chlorophyll *c*. As stated on p. 614, this pigment has an arrangement of bands similar to those of the chlorophylls *a* and *b*, but the red band is very weak compared to the Soret band; in fact, the spectra of chlorophyll *c* (insert in fig. 21.5C) and protochlorophyll (fig. 21.8) are extremely similar. It is therefore significant that Granick concluded, from chemical evidence, that chlorophyll *c*, too, is a porphin rather than a chlorin derivative (*cf.* chapter 37).

Another interesting problem of the same character was raised by an investigation of Aronoff and Calvin (1943). They prepared (by condensation of benzaldehyde with pyrrole) several compounds that they interpreted as isomeric hexaphenylporphins. Some of these compounds had porphyrin spectra of the "etio type" (fig. 21.10, 1), but others had spectra with a predominant sharp band in the red (fig. 21.13, curve B). The authors thought at first that these green isomers may contain one pyrrole nucleus turned around, placing its N atom on an outside corner.

Rabinowitch (1944) suggested they could perhaps be interpreted as *chlorins*: Chlorins isomeric with hexaphenylporphin could be formed, *e. g.*, by attachment of one phenyl group to a pyrrole nucleus, and shifting of the two liberated hydrogen atoms to another pyrrole nucleus, thus:



Tetraphenylporphin Tetraphenylchlorin

Subsequently, Calvin, Ball and Aronoff (1943) found indications that the two "isomers" whose spectra are shown in figure 21.13 actually belong to two different reduction levels of the porphin system. Thus, in this case at least, the dominant red band was confirmed as an indicator of partial hydrogenation.

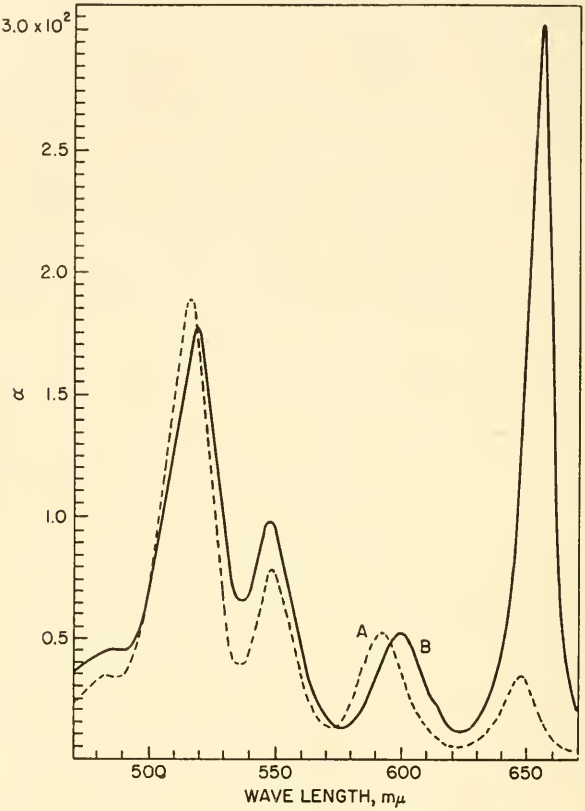


Fig. 21.13. Molar absorption spectra of two tetraphenylporphin "isomers" (after Aronoff and Calvin 1943).

No such explanation can as yet be given to another observation of Aronoff and Calvin—that addition of *hydrochloric acid* to tetraphenylporphin solutions with spectra of the etio type causes a reversible transition to chlorin type (fig. 21.14). Aronoff and Calvin attributed this to *salt formation*: porphin + 2HCl → (porphin H_2)$^{++}$(Cl^-)$_2$. The addition of 2 *hydrogen ions* thus appears to have the same effect on the porphin spectrum as does the addition of two *hydrogen atoms*. If this is true, the problem of the spectroscopic difference between prophins and chlorins becomes cognate

to the problem of acid–base color changes (concerning the latter, see Epstein, Karush and Rabinowitch 1941, and Lewis and Bigeleisen 1943).

According to Pruckner (1942), *imidoporphyrins* (which differ from porphin derivatives by the substitution of an NH group for a bridge carbon)

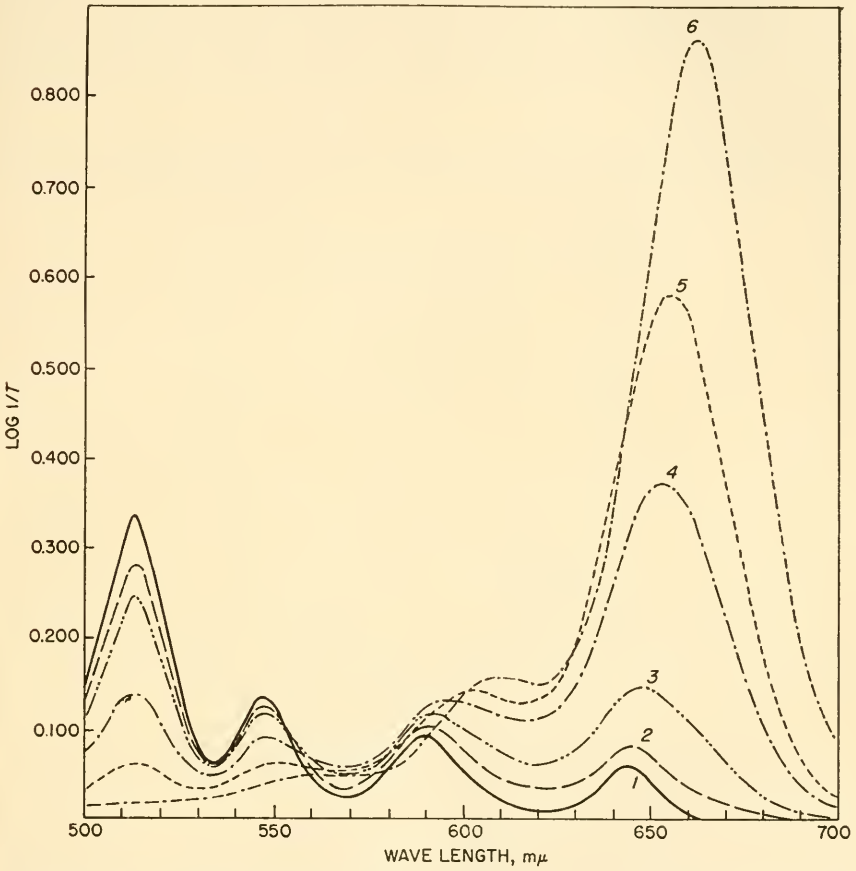


Fig. 21.14. Effect of increased acidity on spectrum of a tetraphenylporphin (after Aronoff and Calvin 1943). 10 ml. alcohol, containing 5 ml. 3×10^{-3} *M* solution of free base and variable amounts of hydrochloric acid. (*1*) no acid, (*2*) 0.0204 *N* HCl, 0.5 ml., (*3*) same, 1 ml., (*4*) same, 2 ml., (*5*) same, 3.50 ml., (*6*) 6.3 *N* HCl, 5 ml.

also have a strong absorption band in the red. She suggested that the appearance of this band is generally associated with increased molecular symmetry; but it is not quite clear why hydrogenation of one or two pyrrole nuclei, or substitution of NH groups for C atoms, should lead to higher symmetry.

The "chlorin type" spectrum remains almost unaffected by all transformations leading from the parent substance chlorin to chlorophyll *a*. This is in accordance with general experience as far as the introduction of methyl and ethyl groups is concerned. It is noteworthy, however, that the introduction of an unsaturated (vinyl) substituent in nucleus I also causes only a slight shift of the bands, as illustrated by figure 21.15. In other words, a difference of two hydrogen atoms in a *side chain* is almost without influence on the spectrum, while a similar difference *in the nucleus* has a strong effect.

The spectroscopic effect of *carboxyl* groups in chlorophyll also is small. The alcohols esterifying these carboxyls have a certain influence on the

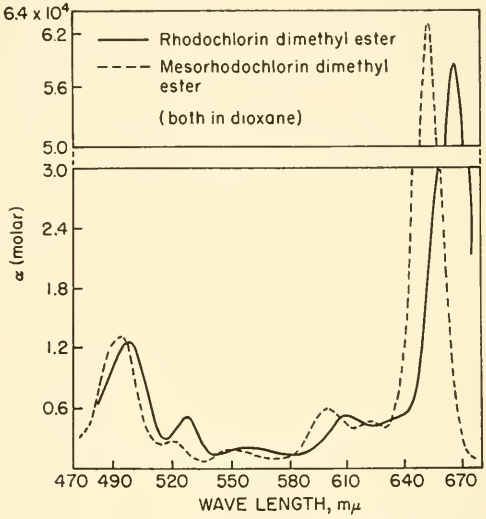


Fig. 21.15. Effect of vinyl group on chlorin spectrum in dioxane (after Stern and Molvig 1937).

intensity of the absorption bands (but none on their *position*): The shorter the alcohol, the sharper the absorption peak. As an example, figure 21.16 shows the extinction curve of *phytyl* pheophorbide (pheophytin), together with that of *methyl* pheophorbide.

It is further noteworthy that closure of the *carbocyclic ring, i. e.*, the transition from chlorin to phorbin, also hardly affects the spectrum at all, as shown by figure 21.17. The *carbonyl* group in nucleus II, whose presence distinguishes chlorophyll *b* and its derivatives from the corresponding compounds of the *a* series, has a much stronger effect on the spectrum: The two chlorophylls have distinctly different colors—one blue-green and the other yellow-green. Figure 21.1 shows that this difference is caused by

different positions of the blue-violet bands: That of chlorophyll *a* is confined to the violet and ultraviolet regions, allowing free transmission of blue light, whereas the absorption peak of the *b* compound is situated in the blue, so that this compound transmits only green light. The arrangement of the weaker bands in the middle of the visible region is also affected by the carbonyl in position 5, to such an extent that Stern classified the spectra of chlorophyll *b* and its derivatives as a separate "rhodin" type (fig. 21.10, 5) distinct from the "chlorin" type (fig. 21.10, 4).

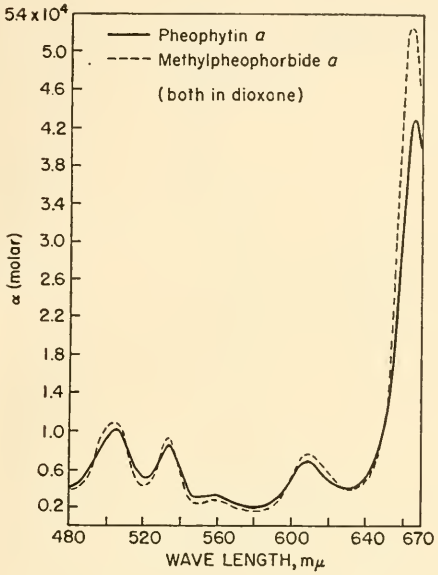


Fig. 21.16. Effect of esterification on chlorin spectrum (after Stern and Molvig 1937).

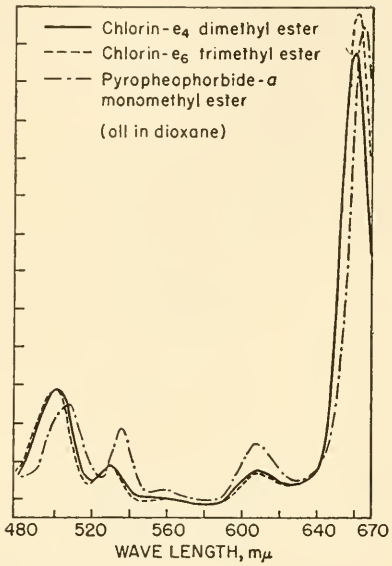


Fig. 21.17. Effect of closure of carbocyclic ring (transition chlorin → phorbin) on chlorin spectrum in dioxane (after Stern and Wenderlein 1935).

The other carbonyl group of chlorophyll, the one in the carbocyclic ring (in position 9), has no pronounced influence on the spectrum of chlorin derivatives. This is striking because, in the case of porphyrins, a carbonyl group in a similar position affects the spectrum to a considerable degree. Stern (*cf.* Fischer and Stern 1940, page 343) suggested an explanation of this difference, based on Fischer's earlier assignment of the two extra hydrogen atoms to nucleus III. By this assignment, the C=O double bond in position 9 was removed from conjugation with other double bonds in the molecule (*cf.* Volume I, page 441), and this could explain why its effect

on the spectrum is less pronounced than that of the conjugated C=O bond in position 3. As stated on page 441, Volume I, Fischer later concluded from chemical degradation experiments that the two extra hydrogen atoms are located in nucleus IV. A certain difference between the conjugation in nuclei II and III exists, however, also in the latter structure (formula A, page 608)—namely, the double bond 3–4 in nucleus II is part of the all-round "aromatic" ring system, while the double band 5–6 in nucleus III is

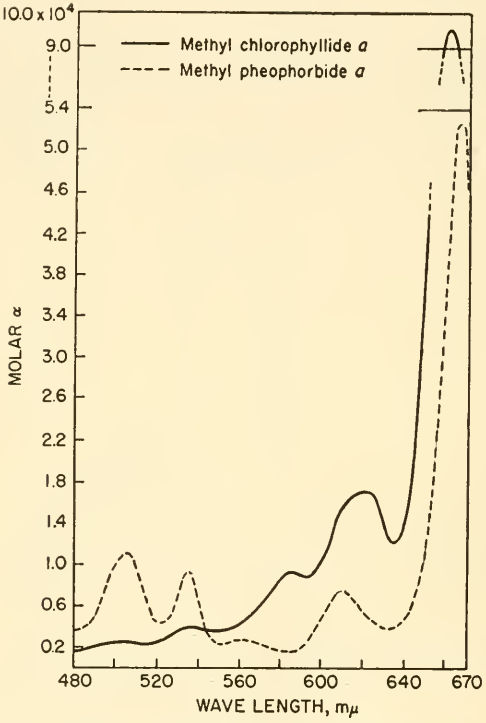


Fig. 21.18. Effect of magnesium on a porphin spectrum (in dioxane) (after Stern and Wenderlein 1936).

merely in "one-sided" conjugation with this system. In structure B, on the other hand, bond 5–6 is part of the all-round conjugated system, and bond 3–4 is in one-sided conjugation. Finally, in structure C, both bonds, 3–4 and 5–6, participate in all-round conjugation. Thus, a difference between the chromophoric effects of carboxyls in nuclei II and III appears plausible in structures A and B, but not in structure C. We note further that, according to Stern and Pruckner (1939), a carbonyl group in nucleus I also has no strong effect on the spectrum. This indicates that, with re-

spect to conjugation, the role of nucleus I is similar to that of nucleus III—and this points to structure B as the true structure of chlorophyll, in preference to structure A, advocated by Fischer (page 443). (As mentioned on page 444, Vol. I, structure B also has the advantage of providing a direct link to bacteriochlorophyll.)

If this interpretation of the spectroscopic data is correct, it means that the introduction of a carbonyl group has a stronger effect on the spec-

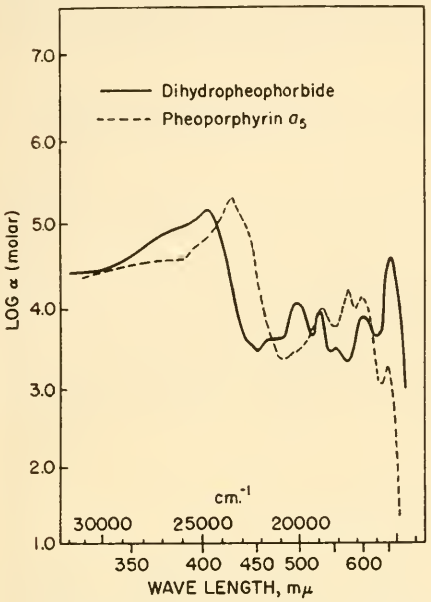


Fig. 21.19A. Effect of porphin → chlorin transition on the visible and ultraviolet spectrum (after Hagenbach, Auerbacher and Wiedemann 1936).

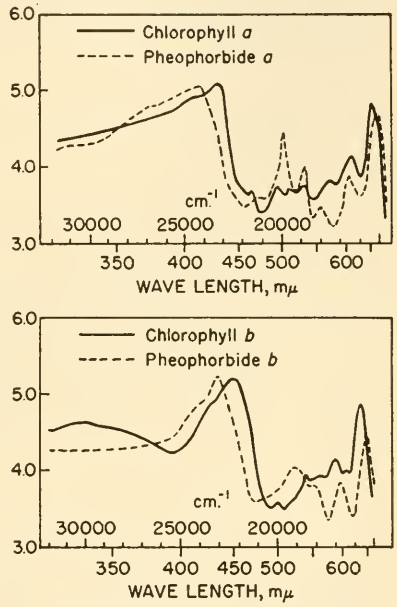


Fig. 21.19B. Extinction curves of chlorophyll and pheophorbides in the visible and near ultraviolet (after Hagenbach, Auerbacher and Wiedemann 1936).

trum if this group comes into conjugation with a C=C bond, which is merely in one-sided conjugation with the "aromatic" system, than if it is attached directly to the latter system.

Introduction of *magnesium* into the molecule (transition from phorbin to phyllin) has the effect of enhancing further the main red absorption band, and of weakening the bands in the green, as illustrated by figure 21.18. The result is the beautiful pure green color of chlorophyll—so different from the dull olive-green of pheophytin.

While the porphyrins, chlorins, phorbins and rhodins differ in their absorption spectra in the green, yellow, orange and red, their spectra in the

violet and ultraviolet all show the same pattern. As pointed out by Stern (1939), neither the transition from porphin to chlorin nor the introduction of magnesium has much effect on the intensity of the blue-violet absorption band. This is shown by figures 21.19A and B. The blue-violet band is shifted by hydrogenation toward shorter waves, but suffers no appreciable change of intensity.

Comparison of figure 21.19A and B shows that, while both magnesium and the extra hydrogen atoms enhance the main red absorption band, these two substituents have antagonistic effects on all the rest of the spectrum, below 600 mμ.

4. Some Theoretical Remarks on the Spectrum of Chlorophyll

(a) The Term System

In section 3 (*cf.* fig. 21.11) we interpreted the four absorption bands of porphin in yellow and green as vibrational bands belonging to the same band system (electronic transition $X \rightarrow A$). A similar interpretation has been suggested by Prins (1934) for the bands of chlorophyll in the red, orange, yellow and green; it is made plausible by the magnitude of the $\Delta\nu$ values given in Table 21.VI. (*cf.* the infrared absorption frequencies in Table 21.IIA). The blue-violet and the two ultraviolet bands are best interpreted as separate electronic transitions.

Table 21.VI

Chlorophyll *a* Bands

λ (mμ)	660	612.5	572.5	527.5	497.5	427.5	375	325
ν (cm.$^{-1}$)	15100	16300	17500	18900	20100	23400	26700	30800
$\Delta\nu$ (cm.$^{-1}$)		1200	1200	1400	1200	(3300)	(3300)	(4100)

According to this interpretation, the term scheme of figure 21.11 could apply also to chlorophyll. However, instead of a (more or less) uniform change in probability in the series of transitions $X \rightarrow A_0$, $X \rightarrow A_1$, $X \rightarrow A_2$ (as revealed by a gradual increase in intensity of the corresponding bands in porphin and its derivatives), one would have to postulate in the case of chlorophyll a predominant probability of the transition $X \rightarrow A_0$ (to account for the outstanding intensity of the first absorption band at 660 mμ). Furthermore, the relationship between the spectra of porphins and dihydroporphins, illustrated by figure 21.12, suggests a shift of the bands in the hydrogenated compounds toward the *shorter*, rather than toward the longer, waves. Therefore, if the red band of the dihydroporphins is $X \rightarrow A_0$, the first absorption band of the porphins must be interpreted as

$X \rightarrow A_1$, and the band $X \rightarrow A_0$ must be considered missing because of low intensity. However, this interpretation is made untenable by the observation—to be discussed in chapter 23—that both in porphins and in dihydroporphins the main *fluorescence bands* are close to the first absorption band in the red—whether this band is the weakest of all absorption bands, as in porphin, or the strongest one, as in chlorophyll. This shows that in both cases, the first observed absorption bands lead to the vibration-free upper level A_0. If the first absorption band of porphin were $X \rightarrow A_1$ (as tentatively suggested above), we would expect to find the main fluorescence

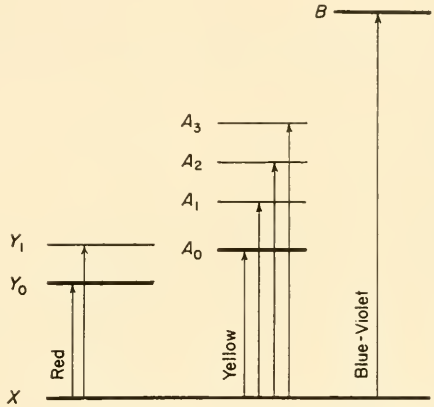


Fig. 21.20. Hypothetical term system of the chlorins.
Band $X \rightarrow A_0$ is submerged by band $X \rightarrow Y_1$.

band some distance toward the red from it—in the approximate position of the "invisible" $X \rightarrow A_0$ absorption band (Rabinowitch 1944).

Since this is not the case, an alternative hypothesis must be considered. It is represented in figure 21.20, and suggests that the hydrogenation of one pyrrole nucleus creates a *new low electronic excitation state* Y, situated a little lower than the level A_0, "inherited" from the nonhydrogenated system.* Figure 21.12 makes one suspect that the second chlorin band (612.5 mμ in chlorophyll), which is stronger than the corresponding band in the nonhydrogenated compound, may also belong to the system $X \rightarrow Y$ (as a second band of this system, $X \rightarrow Y_1, \ldots$), and that it masks the weak band $X \rightarrow A_0$. A similar interpretation can be suggested for the infrared band of bacteriochlorophyll and other tetrahydroporphin derivatives: We can attribute the strongest infrared band of bacteriochlorophyll (fig.

* Another possibility—suggested by the spectra of protochlorophyll and chlorophyll *c*—is that the band $X \rightarrow Y_0$ exists also before hydrogenation, but is strongly enhanced by the latter.

21.6) to a new electronic transition, $X \rightarrow Z$, added (in consequence of the hydrogenation of a second pyrrole nucleus) to the three transitions $X \rightarrow Y$, $X \rightarrow A$ and $X \rightarrow B$ present in the spectrum of chlorophyll. Simultaneously with the creation of a new excited electronic level Z, the "old" levels Y, A and B are shifted upward, thus accounting for the "violet shift" of all bacteriochlorophyll bands "inherited" from chlorophyll. The infrared band $X \rightarrow Z$ dominates the spectrum of bacteriochlorophyll to an even greater degree than the red band, $X \rightarrow Y$, dominates the visible spectrum of ordinary chlorophyll.

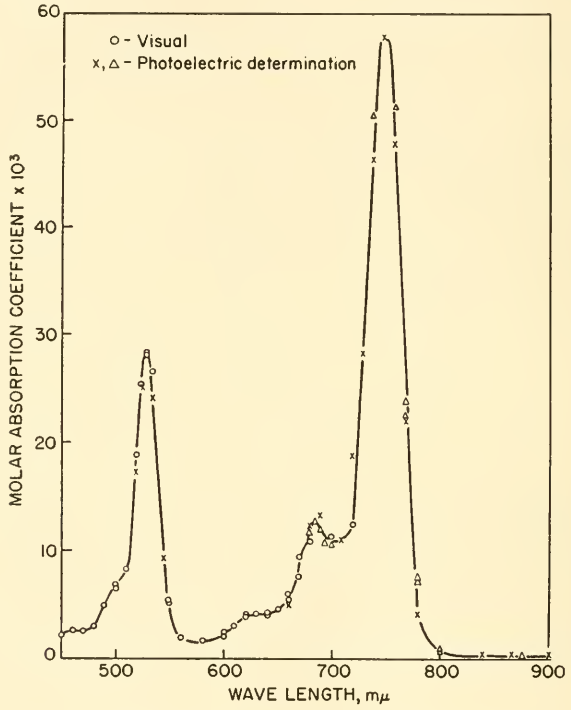


Fig. 21.21. Absorption spectrum of bacteriopheophytin from *Spirillum rubrum* (after French 1940). Specific absorption coefficients, c, in mg./l., d in cm.

Since the absolute extinction coefficients of bacteriochlorophyll are as yet unknown (fig. 21.6 gives only the optical densities), it is not certain whether the predominance of the 770 mμ band is caused by its great absolute height, or by a relatively low intensity of the other bands. A specific extinction curve of *bacteriopheophytin* was given by French in a later paper (1940), and is reproduced in figure 21.21. It shows that the molar absorption coefficient of bacteriopheophytin (in methanol) reaches 2.7×10^4

in the maximum of the orange band, while the infrared peak is almost exactly twice as high. According to figure 21.16, the maximum absorption coefficient of ordinary pheophytin *a* in the red is about 4.2×10^4 (in dioxane, where the peaks are usually sharper than in methanol). It thus seems that the dominant position of the infrared band is due both to its own outstanding intensity *and* to the comparative weakness of all other bands.

The addition of a new low electronic level in consequence of each hydrogenation step of the porphin system offers an interesting problem for theoretical discussion. Offhand, one would expect increased saturation to *decrease* rather than to increase the number of excited electronic states.

If the red bands of dihydroporphin (and the infrared bands of tetrahydroporphin) are brought about (or enhanced) by the presence of electrons associated with the additional carbon-hydrogen bonds, it seems plausible that light could specifically activate the "extra" hydrogen atoms. This would make excited chlorophyll (or bacteriochlorophyll) an effective hydrogen donor—a property which may be of decisive importance for the photochemical function of this pigment. In Volume I (chapter 19, pages 552–554) the primary photochemical oxidation of chlorophyll was discussed as a possible mechanism of sensitization in photosynthesis. This hypothesis would gain considerably in plausibility if it could be proved that absorption of light actually activates chlorophyll as a hydrogen donor. The effect of light on the chlorophyll–ferric iron equilibrium (*cf.* Vol. I, page 488) is the only observation at present that lends experimental support to the concept of chlorophyll as a light-activated reductant.

Stoll (1936) thought that the excitation of chlorophyll by light activates especially its "odd" hydrogen atom in position 10.

Studies by Krasnovsky (*cf.* chapter 35) indicate the capacity of chlorophyll to act also as a light-activated *oxidant.*

(*b*) *Life-Time of the Excited States of Chlorophyll*

The natural life-time of the state Y can be calculated from the integral area of the red absorption band $X_0 \rightarrow Y_0$.

Strictly speaking, one should take into account also the probabilities of transitions from Y_0 to the vibrating states $X_{1,2}\ldots$, which could be derived from the relative intensities of the successive bands in the fluorescence spectrum (*cf.* fig. 23.2); but we are concerned here with orders of magnitude only.

Prins (1934), who made this integration, obtained for the number of "absorption electrons" (*i. e.*, the number of harmonic oscillators with the charge e that could account for the observed intensity of absorption according to classical electromagnetic theory):

(21.1) $f = \begin{cases} 0.24 \text{ per molecule chlorophyll } a \text{ (in ethanol)} \\ 0.22 \text{ per molecule chlorophyll } b \text{ (in ethanol)} \end{cases}$

In the quantum theory, f is the measure of the *transition probability* between the states X_0 and Y_0; and the reciprocal of the transition probability is the *mean life-time*, τ, of the excited state (as far as the latter is limited only by the fluorescent transition $Y_0 \rightarrow X_0$). The relation between f and τ is:

(21.2) $$\tau = \frac{3}{8} \times \frac{mc^2}{e^2\pi^2} \times \frac{1}{f} = \frac{1.96 \times 10^{-8}}{f}$$

where m, c, e and π have the usual meaning. The theoretical mean life-time of chlorophyll molecules in the lowest excited state (reached by absorption of red light) is therefore:

(21.3) $\tau = \begin{cases} 8.2 \times 10^{-8} \text{ sec. for chlorophyll } a \text{ (in ethanol)} \\ 8.9 \times 10^{-8} \text{ sec. for chlorophyll } b \text{ (in ethanol)} \end{cases}$

The higher intensity of the blue-violet absorption band (particularly in chlorophyll *b*) indicates that the natural life-time of the excited state B, is somewhat shorter than that of state Y—probably 5×10^{-8} sec. or less.

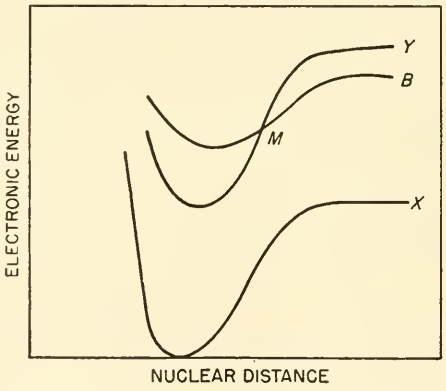


Fig. 21.22. Crossing of potential curves.

The *actual* life-times of chlorophyll in states B, A and Y are considerably shorter than the "natural" life-times. This is indicated by the complete absence of fluorescence originating in the levels B and A and the relative weakness of fluorescence originating in level Y.

From the complete absence of blue-violet fluorescence in chlorophyll solutions (*cf.* page 748) it follows that the energy of state B must be dissipated within 5×10^{-12} sec. or less. (With the natural life-time of $5 \times$

10^{-8} sec., energy dissipation within 5×10^{-12} sec. would reduce the yield of fluorescence to $<0.01\%$ and thus make it practically unobservable.)

Similar considerations apply to state A. The ease with which states A and B are transformed into state Y (as shown by the excitation of red fluorescence with yellow or blue light, *cf.* page 748) indicates that the potential energies of states A, Y and B (plotted against some appropriate "configuration co-ordinate") give curves of the type shown in figure 21.22. At point M, the electronic excitation energy of state B is easily transformed into the (smaller) electronic excitation energy of state Y, plus a large amount of vibrational energy.

The yield of red fluorescence of chlorophyll in solution is of the order of 10% (*cf.* chapter 23). This shows that the actual life-time of state Y in solution is of the order of one tenth of the above calculated "natural" life-time, *i. e.*, about 5×10^{-9} sec. The various "quenching" processes that may contribute to this shortening of the life-time of excited molecules will be discussed in chapter 23 (page 755).

B. Influence of Medium on Absorption Spectrum of Chlorophyll and Bacteriochlorophyll*

We have spoken so far of the absorption spectra of chlorophyll and its derivatives in solution as though they were determined only by the chemical structure of these compounds. However, the absorption spectra also are affected by changes in the nature of the solvent, and even more strongly by adsorption on solids, or by the formation of colloidal aggregates. These spectroscopic changes are caused by interaction between the light-absorbing molecules and their neighbors. Kundt had noticed as early as 1878 that the absorption bands of many dyestuffs are shifted toward longer waves with increasing refractivity of the solvent. This relation appears plausible in the light of London's theory, which establishes a connection between the capacity of molecules to refract light and the intensity of intermolecular forces—both properties being determined by *polarizability* of the molecules.

Parallelism between molecular attraction and polarizability presumes the absence of other, chemical or physical forces between the interacting molecules. Such forces may arise if the molecules bear electric charges, dipole moments, or possess incompletely saturated valencies; therefore, we can expect Kundt's rule to apply primarily only to neutral, nonpolar, saturated molecules in nonpolar, saturated solvents. The rule may also apply to series of polar or nonsaturated molecules in which the additional interactions due to dipoles or residual valencies are approximately constant (*e. g.*, to a homologous series of alcohols).

* Bibliography, page 669.

London's theory of molecular attraction predicts that solvents with a high refractive index (*i. e.*, strong polarizability) should exercise strong attraction on solute molecules, thereby causing considerable deformation of their electronic systems and considerable shifts of their energy levels. The *direction* of the resulting displacement of the absorption bands depends on the comparative polarizability of the solute molecules in the ground state and in the excited state. Figure 21.23 shows that the excitation

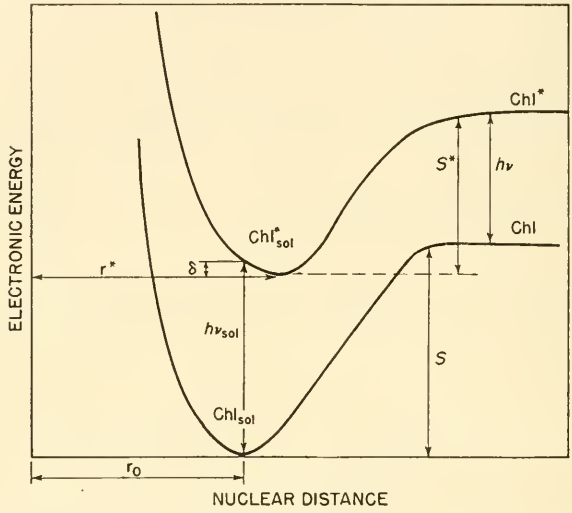


Fig. 21.23. Influence of solvation energy on energy of excitation (if $S^* > S, h\nu > h\nu_{sol}$). $S + h\nu = S^* + h\nu_{sol} - \delta$. $\delta + \Delta S = h\nu_{sol} - h\nu$.

energy of a bound (*e. g.*, solvated) molecule, $h\nu_{sol.}$, is related to that of the free molecule, $h\nu$, by the equation:

(21.4) $$h\nu_{sol.} = h\nu + S - S^* = h\nu + \Delta S$$

where ΔS is the difference between solvation energies of the normal and the excited molecule. If $\Delta S < 0$, *i. e.*, if the excited molecule is attracted by the solvent more strongly than the normal one, $h\nu_{sol.}$ is smaller than $h\nu$ and the absorption band is shifted toward the red (as postulated by Kundt). That ΔS should be negative is plausible, since excited molecules usually have a looser electronic structure and are therefore more easily polarizable than the normal ones.

Figure 21.23 shows that equation (21.4) is only correct if the equilibrium distances r and r_0 are identical. This will not generally be the case (r^* is likely to be somewhat larger than r_0). The exact equation for the "red shift" according to figure 21.23 is:

(21.4a) $$h\nu_{sol.} - h\nu = \Delta S + \delta$$

the red shift thus being decreased by the amount δ.

Probably no other compound has been so often studied from the point of view of Kundt's rule as chlorophyll. The origin of this interest was the fact, first noticed by Hagenbach in 1870, that the maximum of the red band of chlorophyll in living plants is displaced by about 20 mμ toward the red end of the spectrum, compared to its position in solution. Gerland (1871) found that a similar displacement occurs in the case of the absorption bands of chlorophyll in the yellow and green. It was early suggested that this position of the bands indicates a peculiar state of chlorophyll in the living cell, and numerous attempts have been made to reproduce this state *in vitro*. We will see, however, in the following review of experimental data, that the "red shift" is not a specific effect, and could be caused by various types of aggregation or complexing.

We will first deal with chlorophyll solutions in different organic solvents, and then with colloidal solutions, complexes and adsorbates, in which chlorophyll is associated with proteins, lipides or other carriers.

1. Solvent Effect in the Spectra of Chlorophyll and Bacteriochlorophyll

Chlorophyll was one of the dyestuffs whose study caused Kundt (1878) to postulate a relation between the refractive index of the solvent and the position of the absorption bands, which became known as "Kundt's rule." Since then, numerous observations have been made of the spectrum of chlorophyll in different solvents, *e. g.*, by Baas-Becking and Koning (1934), Hubert (1935), Wakkie (1935), Katz and Wassink (1939), Biermacher (1939), Egle (1939) and Harris and Zscheile (1943). The agreement between the different authors is not very satisfactory—for example, Hubert found 662.5 mμ for the position of the absorption peak of chlorophyll *a* in methanol, and 664 mμ for its position in ether, while Katz and Wassink obtained, for the same solvents, 664 and 661 mμ, Harris and Zscheile 664 and 660 mμ, and Bass-Becking and Koning 656 and 666 mμ, respectively.

Many discrepancies probably have been caused by the use of poor spectrophotometric equipment; Mackinney (1938) stressed, for example, the errors inherent in the identification of the band *maximum* with the so-called band "axis" (*cf.* above, page 607, and chapter 23, page 744). Other, and perhaps more important, differences may have been caused by the preparations used—often leaf extracts containing chlorophylls *a* and *b* in unknown proportions. It is difficult enough to obtain spectroscopic reproducibility even with purified preparations of a single chlorophyll component! Small solvent impurities, too, may strongly change the spectrum (*cf.* p. 647).

TABLE 21.VII

POSITIONS OF THE ABSORPTION PEAKS OF CHLOROPHYLLS *a* AND *b* IN DIFFERENT SOLVENTS

Values shown in italics are those used in fig. 21. 24

Solvent	n_D	Baas-Becking and Koning (1934)	Hubert (1935)	Katz and Wassink (1939)	Harris and Zscheile (1943)				Egle (1939)[a]		Biermacher (1939)[b]	
					(*a*)		(*b*)					
		(*a* + *b*)	(*a* + *b*)	(*a* + *b*)	Red	Blue	Red	Blue	(*a*)	(*b*)	(*a*)	(*b*)
Methanol	1.329	656	662.5	*664.0*	*664.0*	434.0	651.0	471.0	666.9	655.5	663.5	653.5
Ethyl ether	1.353	666	664.0	661.2	660.0	429.0	642.5	453.0	664.3	645.6	663	642
Pentane	1.357	—	—	*660.5*	—	—	—	—	662.6	645.3	658.5	640
Acetone	1.359	664	666.5	661.2	661.5	431.5	643.5	456.0	662.9	645.8	663.5	644.5
Ethanol	1.362	667	668.0	*664.0*	—	—	—	—	665.5	651.2	663.5	648
Isopropyl ether	1.37	—	—	—	661.0	430.0	—	—	—	—	—	—
Ethyl acetate	1.372	—	—	662.0	—	—	—	—	—	—	—	—
Hexane	1.375	—	—	*660.0*	—	—	—	—	663.2	645.5	658.5	640
2-Methyl-1-propanol	1.39	—	—	—	*664.5*	433.0	—	—	666.6	652.4	—	—
1-Butanol	1.402	—	—	—	665.0	432.5	—	—	667.3	652.4	—	—
Amyl acetate	1.402	—	—	663.1	—	—	—	—	—	—	—	—
1-Amylol	1.410	—	—	*665.6*	—	—	—	—	—	—	—	—
1,4-Dioxane	1.42	—	—	—	661.0	433.0	—	—	—	—	663.5	644.5
2-Ethyl-1-hexanol	1.43	—	—	—	*666.5*	429.5	—	—	—	—	—	—
Cyclohexane	1.431	—	—	*660.5*	660.0	434.0	—	—	—	—	—	—
Ethylene chloride	1.444	—	—	666.1	—	—	—	—	—	—	—	—
Chloroform	1.446	668	—	665.0	—	—	—	—	666.2	648.8	668	647.5
Piperidine	1.454	—	—	642.0 (!)	—	—	—	—	—	—	—	—

Solvent	n_D	Baas-Becking and Koning (1934)	Hubert (1935)	Katz and Wassink (1939)	Harris and Zscheile (1943) (a)		Harris and Zscheile (1943) (b)		Egle (1939)[a]		Biermacher (1939)[b]	
		(a + b)	(a + b)	(a + b)	Red	Blue	Red	Blue	(a)	(b)	(a)	(b)
Methyloleate	1.46	—	—	—	662.0	431.5	—	—	—	—	—	—
Cyclohexanol	1.466	—	—	665.8	—	—	—	—	—	—	667	649
Carbon tetrachloride	1.466	—	664.0	*664.7*	663.5	432.5	644.0	457.0	—	—	664	645
Toluene	1.498	—	—	*664.1*	—	—	—	—	661.1	648.8	—	—
Benzene	1.501	673	672.0	*665.3*	664.0	432.5	645.0	459.0	668.1	650.9	664	645.5
Xylene	1.507	—	—	*665.6*	—	—	—	—	—	—	668	645
Pyridine	1.509	—	—	668.7	—	—[c]	—	—	671.6[c]	656.1	669.5[c]	655
Benzyl chloride	1.542	—	—	665.0	—	—	—	—	—	—	—	—
Nitrobenzene	1.553	—	—	665.2	—	—	—	—	—	—	668.5	651
Aniline	1.586	667	—	666.0	—	—	—	—	—	—	673	657
α-Bromonaphthalene	1.659	—	671	670.2	—	—	—	—	—	—	—	—
Carbon disulfide	1.630	674	672.5	*672.0*	—	—	—	—	671.8	654.9	674	650
Methylene iodide	1.756	676	674	—	—	—	—	—	—	—	677	655
Lecithin	—	662	—	—	—	—	—	—	—	—	—	—
Olive oil	1.48	—	—	—	669.5	433.0	—	—	—	—	—	—
Paraffin oil	—	668	—	—	—	—	—	—	—	—	668.5	645
Nujol	—	672	—	—	—	—	—	—	—	—	—	—
Cedar oil	—	674	—	—	—	—	—	—	—	—	—	—

[a] Band axes in dilute solution (2 mg./100 ml.).

[b] Band axes.

[c] 442 and 669 ± 1mμ (photoelectric measurement) after Krasnovsky, Brin and Vojnovskaja (1949).

Tables 21.VII and 21.VIII contain a summary of the experimental results. Considering the former table as a whole, one finds only a very rough confirmation of Kundt's rule—it consists mainly in the fact that $\lambda_{max.}$ values greater than 670 mμ are found only with solvents whose refractive index is greater than 1.5. Between $n_D = 1.33$ and 1.5, the variations of $\lambda_{max.}$ appear small and irregular.

However, if one sorts out solvents of the same chemical type, a more regular shift at least of the red absorption maximum becomes apparent. Figure 21.24 summarizes, as an example, the data of Katz and Wassink and Harris and Zscheile for nonpolar solvents (curve A) and for alcohols (curve B).

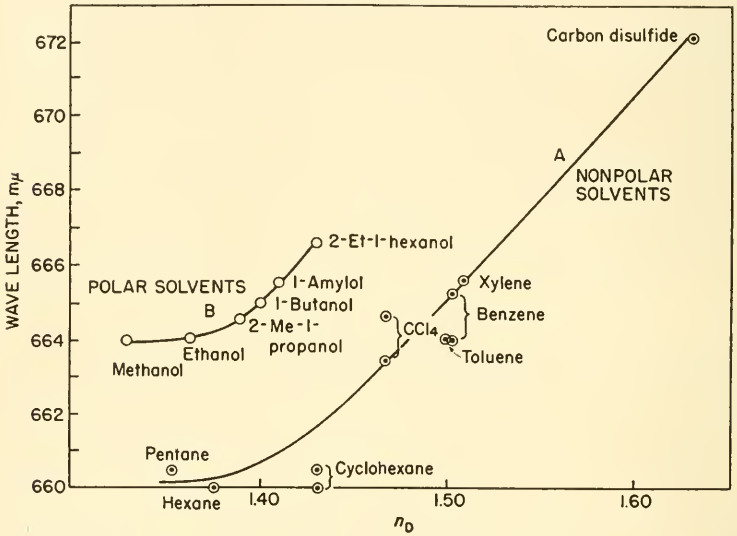


Fig. 21.24. Absorption maxima of chlorophyll *a* in solvents of different refractivity.

The shift of the blue band remains irregular, even in selected series of solvents. The highest value of $\lambda_{max.}$ of the blue band was found in the solvent with the lowest refractive index, methanol. (This fact may perhaps be attributed to a specific sensitivity of the blue-violet band to tautomerization—a hypothesis discussed on page 607; tautomerization equilibra are known to be strongly affected by solvent changes.)

Livingston and co-workers (1949) noted that, in the series of chlorophyll *a* solutions in alcohols (from methanol to octanol), the relation between the two peaks in the blue-violet region changes systematically. In methanol, they are equally high and separated only by a dip; in octanol, the short-

wave peak appears as a lower hump separated from the main peak by a trough.

Wakkie (1935), too, divided solvents into classes and gave four separate $\lambda_{max.} = f(n)$ curves—one for nonpolar solvents, one for weakly polar solvents (ethers, ketones), one for alcohols and one for colloidal solutions in water and glycerol. However, the last curve is not directly comparable with the other three, since, as we shall see later, the position of the

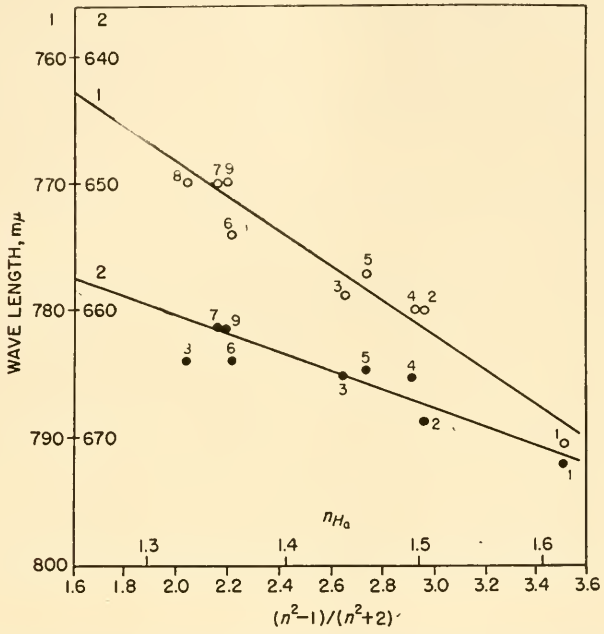


Fig. 21.25. Wave lengths of absorption maxima of green pigment extracts in different organic solvents, in relation to their refractive indices. (1) Carbon disulfide, (2) pyridine, (3) chloroform, (4) benzene, (5) carbon tetrachloride, (6) ethanol, (7) ether, (8) methanol, (9) acetone (after Katz and Wassink 1939). Top scale: extracts from purple sulfur bacteria (strain D) (O). Bottom scale: extracts from *Chlorella* (●).

absorption bands of *colloidal* chlorophyll solutions depends not only on the solvent, but also on the degree of dispersion of the sol.

Wakkie's curves (as well as our two curves in fig. 21.24) are displaced toward longer waves with increasing dipole moment of the solvent—a relation that can be explained by the superposition of attraction forces between solvent and solute caused by *permanent polarization* upon forces due to *polarizability*. This dipole effect is stronger for chlorophyll *b* than

for chlorophyll *a*—the former being the more po'ar of the two compounds. For example, according to Harris and Zscheile, the red band of chlorophyll *a* lies at 660 mμ in ether and 664 mμ in methanol ($\Delta\lambda = 4$ mμ), while the corresponding values for chlorophyll *b* are 642.5 and 651 mμ, respectively ($\Delta\lambda = 8.5$ mμ). Hydrogen bonding, too, may have to be taken into consideration.

If one compares the effects of varying refractivity of the solvents on the spectra of different homologous solutes, one may expect the solute with the stronger polarizability to exhibit the strongest shift. Polarizability increases with the intensity and wave length of the first absorption band. Thus, the spectra of dyestuffs should be more sensitive to solvent changes than the spectra of noncolored substances, and the sensitivity of dyes of different color should increase with the shift of the main absorption band toward longer waves. This is confirmed by the finding of Pruckner (1940) that the solvent effect increases strongly from porphins through dihydroporphins (chlorins and phorbins) to tetrahydroporphins (bacteriochlorophyll). The first absorption band of chlorophyll is situated further toward the red and is more intense than the first absorption band of the porphyrins; and the same is true of the first absorption band of bacteriochlorophyll compared to that of chlorophyll. The two (or four) additional hydrogen atoms contained in these compounds contribute electrons that are easily excitable by light (thus giving rise to long-wave absorption bands) and easily displaceable in electric fields (thus producing strong polarizability).

Figure 21.25 shows the shifts of the band maxima of bacteriochlorophyll and ordinary chlorophyll in the same solvents. This figure indicates that on the wave length scale the solvent effect is about twice as strong for the first band of bacteriochlorophyll as for the first band of ordinary chlorophyll.

Katz and Wassink (1939) extrapolated the curves in figure 21.25 to vacuum (refractive index 1) and predicted that the absorption peak of free chlorophyll molecules—if it can ever be determined—will be found at 648 $\pm$ 5 mμ, and that of free bacteriochlorophyll molecules at about 740 mμ.

In piperidine solution, the absorption peak of chlorophyll lies at 642 mμ, *i. e.*, beyond the extrapolated limit for the free molecule. This demonstrates the existence of exceptions to Kundt's rule, probably caused by specific chemical interactions between solvent and solute. Other (less striking) exceptions from Kundt's rule have been discussed by Mackinney (1938, 1940) and Egle (1939).

Theoretically, it would be more appropriate to plot, in figures 21.24 and 21.25, wave numbers (or frequencies) since these are proportional to energies and therefore bear direct relationship to the terms in equation (21.4). In a narrow spectral range, such as is used here for a given band of a single pigment, linear extrapolation on a wave number scale would give results not significantly different from those obtained by linear extrapola-

tion on a wave length scale. In the comparison of the shifts of bands in different parts of the spectrum, on the other hand, the use of wave lengths may be quite misleading; for example, a shift by 10 mμ at 440 mμ is equivalent, on the energy scale, to a shift by 22.5 mμ at 660 mμ.

The effect of solvents on absorption maxima of chlorophyll other than the main red peak has not been studied systematically, but it is known that in general all of them experience shifts toward longer waves with increasing refractivity of the solvent (see *e. g.*, Egle 1939). Exact measurements of this shift may prove useful in the interpretation of the spectrum, since absorption bands that lead to the same electronic upper state can be expected, according to page 636, to show the same shift. Krasnovsky *et al.* (1949) found bands II and III to be shifted, in pyridine, by 30–35 mμ (to 643 and 622 mμ), while other bands were shifted by 8–15 mμ only.

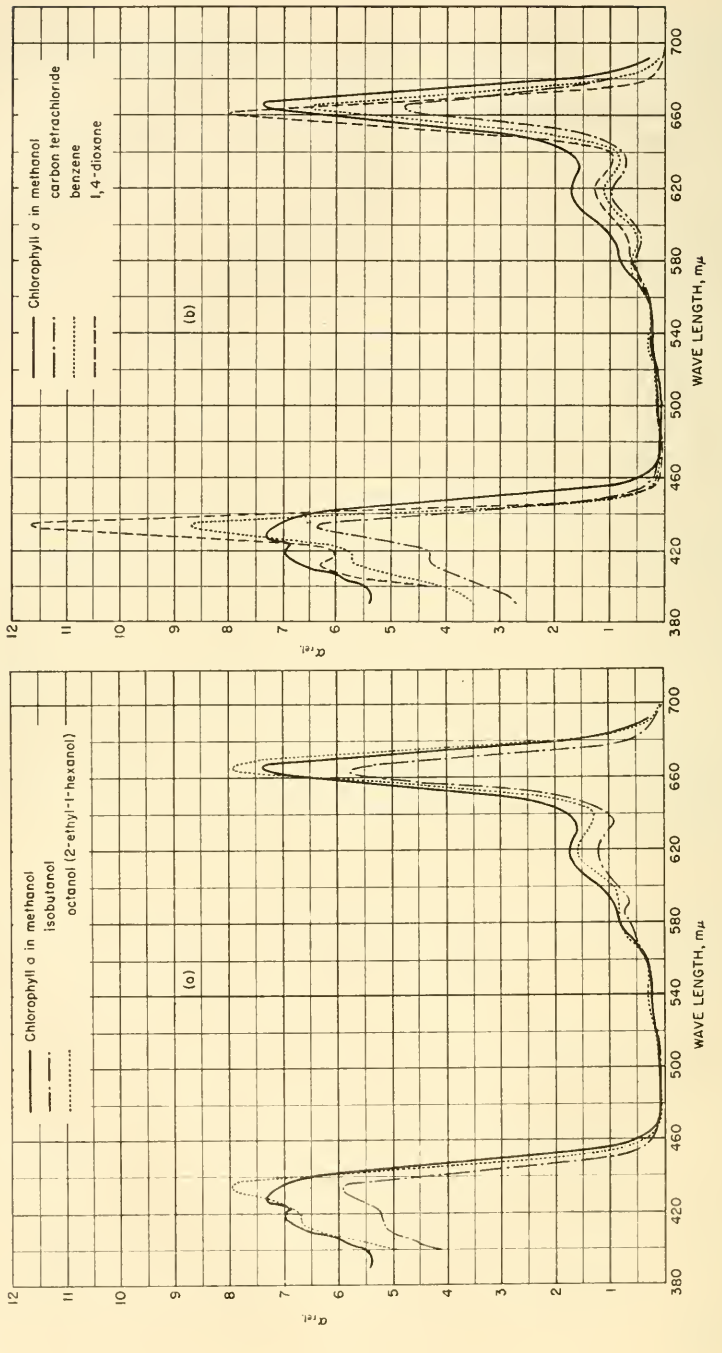
The solvent effect is not restricted to *band shifts*, but also involves changes in the width and shape of the bands and (perhaps as a consequence of these changes) alterations in the absolute and relative intensities of the band maxima. In this case, too, bands leading to the same excited electronic level must show a similarity of behavior (*cf.* Pruckner 1940). In the case of chlorophyll, the ratio between the intensities of the blue-violet and the red peak is quite different in different solvents. While Table 21.IB showed a value of 1.3 for ether solutions of chlorophyll *a*, the ratio drops to 1 in methanol (Mackinney 1940, Albers 1941, Harris and Zscheile 1943) and rises to approximately 1.5 in dioxane (Harris and Zscheile 1943) and perhaps also in benzene (according to Hausser's measurements, *cf.* Fischer and Stern 1940; not confirmed by Table 21.VIII).

Figure 21.26 shows the absorption curves of chlorophylls *a* and *b* in a variety of solvents, according to Harris and Zscheile.

TABLE 21.VIII

RELATIVE INTENSITY OF CHLOROPHYLL ABSORPTION PEAKS IN DIFFERENT SOLVENTS (AFTER HARRIS AND ZSCHEILE 1943)

Solvent	Blue max./red max.	
	Chl. *a*	Chl. *b*
Methanol	1.00	2.85
2-Ethyl-1-hexanol	1.00	—
2-Methyl-1-propanol	1.02	—
1-Butanol	1.03	—
Methyl oleate	1.25	—
Acetone	1.26	2.95
Olive oil	1.29	—
Isopropyl ether	1.29	—
Carbon tetrachloride	1.32	2.54
Benzene	1.33	2.45
Ethyl ether	1.33	2.98
Cyclohexane	1.36	—
1-*n*-Dioxane	1.46	—


(a)
Chlorophyll a in methanol
isobutanol
octanol (2-ethyl-1-hexanol)
(b)
Chlorophyll a in methanol
carbon tetrachloride
benzene
1,4-dioxane
WAVE LENGTH, mμ
$\alpha_{rel.}$

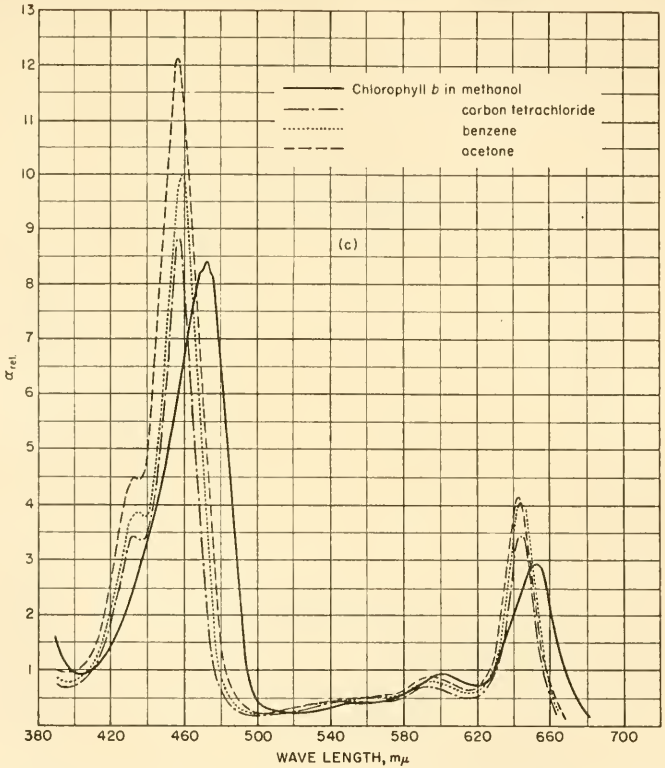


Fig. 21.26. Relative extinction coefficients of chlorophyll in different solvents (after Harris and Zscheile 1943): (a) Chlorophyll *a* in different alcohols. (b) Chlorophyll *a* in solvents of different type. (c) Chlorophyll *b* in different solvents.

Characteristic changes apparently occur in the "doublet structure" of the blue-violet band. In chlorophyll *a* in alcohols, the two components of this band are well separated and almost equal in prominence. (This separation of the band into two almost equal components may be the cause for the fact that the blue-violet peak is in this case not much higher than the red peak.) In dioxane, on the other hand, the violet component is a "satellite" only half as high as the main blue peak (the latter being in this case almost 50% higher than the peak of the red band). It was mentioned

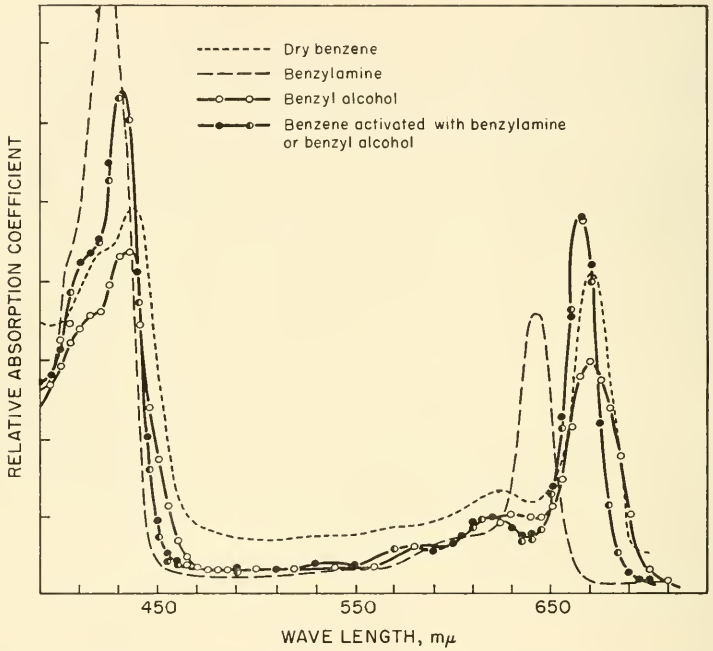


Fig. 21.26A. Absorption spectra of chlorophyll *a* in pure hydrocarbon and hydrocarbon containing an alcohol or amine (after Livingston *et al.* 1949).

on page 607 that these differences may perhaps be indicative of tautomeric equilibria; but this is merely a conjecture.

It would be interesting to evaluate the total areas under the different curves to find whether the *transition probabilities* are changed by the solvent, or whether the latter merely affects the *shapes* of the bands, without changing their *total areas*.

An as yet little investigated subject is the absorption spectrum of dyestuffs in general, and of chlorophyll in particular, in *mixed* solvents. Observations of this type could give information about the occurrence, and

energy, of complex formation of chlorophyll with different organic molecules. Livingston, Watson and McArdle (1949), in a study devoted primarily to the strong effect of small admixtures of polar solvents on the fluorescence of chlorophyll solutions in hydrocarbons, noted that these admixtures also changed the absorption spectrum. As an example, figure 21.26 B shows the effect of traces of water on the absorption spectrum of chlorophyll *b* in benzene. In the dry solution, both main peaks are lower and a shoulder appears at about 670 mμ on the long-wave side of the red

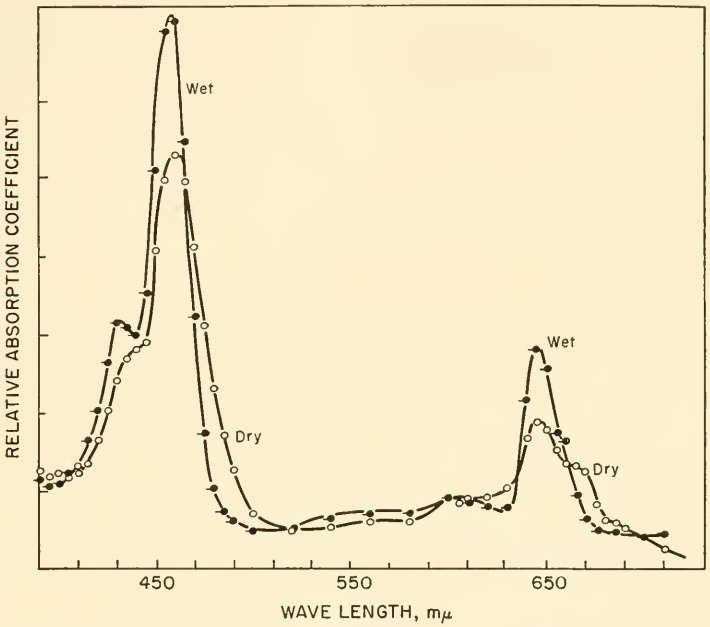


Fig. 21.26B. Absorption spectra of chlorophyll *b* in dry and wet benzene.

peak. Figure 21.26 A shows the effects of two other polar solvents, benzylamine and benzyl alcohol, on the absorption spectrum of chlorophyll *a* in dry benzene. In this case, the main absorption peaks are *higher* in dry nonpolar solvent, and no "shoulder" appears on the long-wave end of the spectrum. A remarkable fact shown by this figure is that the spectrum of the activated solution (the term "activated" refers to fluorescence, which is absent in pure benzene), is the same whether activation is due to amine or to alcohol, although the absorption spectra of chlorophyll *a* in pure benzylamine and in pure benzyl alcohol are quite different. This could mean that polar molecules associate preferentially with a certain tautomeric form of chlorophyll, and in this way stabilize it; the presence of a small

number of such molecules thus converts the spectrum of "normal" chlorophyll into one of "tautomerized" chlorophyll. In pure polar solvent, on the other hand, polar molecules surround the chlorophyll molecule from all sides and thus cause a different and more radical change in its absorption spectrum.

The absorption spectrum of the "activated" solution is affected by increasing temperature, indicating a shift toward dissociation of the equilibrium

$$\text{Chl} + \text{M}^{+-} \rightleftharpoons \text{tChl} \cdot \text{M}^{+-}$$

(M^{+-} = polar molecule, tChl = tautomeric chlorophyll). The interpretation of these interesting results will be discussed in chapter 23 (page 769) after presentation of the corresponding fluorescence data.

Evstigneev, Gavrilova and Krasnovsky (1949[2]) noted that polar molecules have no effect on absorption spectrum and fluorescence of magnesium-free compounds (pheophytin and phthalocyanine) and therefore ascribed this effect to the binding of these molecules by the residual valencies of magnesium.

A few words can be added here on the effect of *dissolved gases* on the absorption spectrum of chlorophyll solutions. Padoa and Vita (1932) described changes in the absorption spectra of chlorophylls *a* and *b* (in benzene solutions) in contact with nitrogen, oxygen, carbon monoxide and carbon dioxide. A strong effect was observed in the case of carbon monoxide—a result taken as an indication of the existence of a chlorophyll–carbon monoxide complex, similar to carboxyhemoglobin. However, the spectra reproduced in the paper of Padoa and Vita are so different from the true spectrum of chlorophyll, that they must have been obtained with some decomposition products rather than with the intact pigment. Katz and Wassink (1939) found practically identical extinction curves for colloidal aqueous bacteriochlorophyll extracts in atmospheres of oxygen, hydrogen sulfide, nitrogen, hydrogen and air.

Evstigneev, Gavrilova and Krasnovsky (1949[1]) asserted that the presence of oxygen does have a certain effect on the spectrum of chlorophyll (*a* + *b*, or pure *b*) in *toluene*. Upon evacuation, the absorption coefficient decreased in both maxima, which were shifted slightly toward the red. In chlorophyll *b*, a new maximum of absorption appeared, when air was removed, at 670 mμ. These changes were reversible; but irreversible changes were noted in the ultraviolet part of the spectrum. Similar changes were observed in carbon tetrachloride and heptane, but not in pyridine, ethanol, acetone or benzene. Addition of one drop of alcohol, pyridine or acetone to 10 cc. toluene destroyed the effect of evacuation. Later (1949[2]) the same investigators found that the effects they had ascribed to the admission of air were actually caused by the admission of water vapor.

These results obviously bear a relation to Livingston's conclusions that chlorophyll is present, in nonpolar solvents, in a state different from that to

which it is converted by the presence of even traces of an alcohol or water. Evstigneev *et al.* (1949[1]) suggested that in the absence of polar molecules chlorophyll is dimerized, and that the dimer is dissociated by oxygen molecules, dimerization being due to unsaturated magnesium valencies, which can also be saturated by oxygen. Their subsequent results (1949[2]) made this interpretation of oxygen action unnecessary.

In solvents of intermediate type, or in mixed (or simply not extremely purified) solvents, both types of interaction may occur simultaneously.

Changes in the absorption spectra of chlorophyll can be caused, according to the observations of Livingston and co-workers (1948, 1949), also by small quantities of admixtures other than polar solvents. Examples are iodine, bromine, ferric and ceric salts (Rabinowitch and Weiss, 1937), and, in the case of chlorophyll *b* (but not chlorophyll *a*!), phenylhydrazine. Some effects of this type might be similar to those of polar solvents, *i.e.*, they may be caused by reversible formation of molecular complexes. Mostly, however, they are due to irreversible chemical changes such as allomerization (or, more generally, oxidation), or reduction (which is likely in the case of chlorophyll *b* and phenylhydrazine); these phenomena do not belong under the heading of "effects of the medium on the absorption spectrum of chlorophyll," but rather under that of "chemical reactions of chlorophyll, revealed by spectroscopic measurements" (*cf.* pages 450–467 in Vol. I, and chapter 37 in this volume).

2. Absorption Spectrum of Colloidal and Adsorbed Chlorophyll

Colloidal aqueous solutions of chlorophyll are obtained by mixing a molecular solution of the pigment (in alcohol or acetone) with water. The spectrum of the resulting solution depends on the conditions of mixing; this is the reason that earlier investigators could not agree on the position of the band maximum of colloidal chlorophyll. Herlitzka (1912), Willstätter and Stoll (1918) and Baas-Becking and Koning (1934) reported that this maximum coincides with that of chlorophyll in leaves, *i. e.*, lies close to 680 mμ. Ivanovski (1907, 1913) and Hubert (1935), on the other hand, found the maximum at 668 mμ, *i. e.*, in the same region as in many true solutions. Hubert noticed, however, that the position of the maximum was affected by the degree of dispersion of the colloidal system: Addition of magnesium chloride, which caused a growth of the particles (and finally led to flocculation) shifted it by as much as 8 mμ—from 668 to 676 mμ. Later, Wakkie (1935) and K. P. Meyer (1939) found that the essential factor is not the *size* of the colloidal particles, but their internal *density, i. e.*, the concentration of chlorophyll molecules in them.

A colloidal solution prepared by Meyer by rapid addition of 3 volumes of water to 1 volume of a chlorophyll solution in ethanol was clear, transparent and nonfluorescent. Its particles were 0.5 to 3 μ in diameter. The band maximum was at 670 mμ, and the shape of the extinction curve was similar to that of the original solution in ethanol. On the other hand, a colloidal solution prepared by adding quickly 0.6 volume of water to 1 volume of ethanol and then diluting by 6.4 volumes of water, was turbid and opalescent. Its particles had a diameter of 1–3 μ, *i. e.*, were not substantially larger than those of the first, transparent colloid, but they contained more pigment. The absorption maximum of this colloid was situated further toward the red, at 673 mμ, and the whole shape of the extinction curve was more like that of the leaves, as shown by figure 21.27 (Meyer described the spectrum of this colloidal preparation as "identical" with that of the leaves, but figure 21.27 does not justify this statement). By counting the particles of the colloid, Meyer found that the concentra-

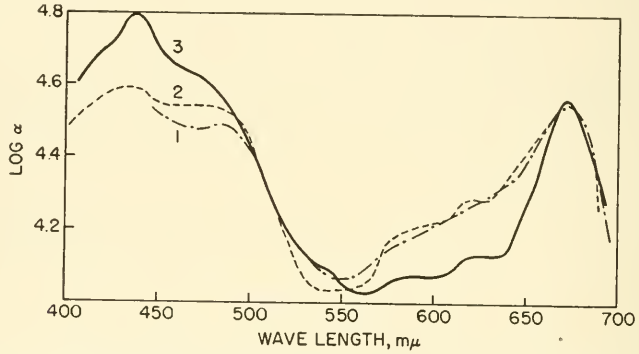


Fig. 21.27. Transmission curves of leaves (1 and 2) and of colloidal chlorophyll solutions (3) (after K. P. Meyer, 1939).

tion of chlorophyll in the particles of the turbid solution was of the order of 0.13 mole/l., *i. e.*, similar to that in the chlorophyll grana in the leaves (*cf.* Vol. I, chapter 15, page 411). Even these "densely packed" colloid particles are still far from "solid," but contain up to 90% solvent.

Because of the intensity of the absorption bands, the extinction curves of dyestuffs usually are measured with concentrations of the order of 10^{-6} to 10^{-4} mole/l. No deviations from Beer's law (*i. e.*, no changes in the extinction curve with concentration) were observed in chlorophyll solutions in this range of concentrations. With very thin glass cells ($\sim$0.1 mm. deep), dyestuff solutions containing 10^{-3} mole/l. can be investigated, but even this is a hundred times more dilute than the 0.1 mole/l. reached in Meyer's colloidal particles and also present in the chlorophyll grana in

leaves. Wakkie (1935) emulsified a chlorophyll solution in ether in a saturated water–ether mixture, bubbled air through it and watched the changes of the absorption spectrum as the ether evaporated and the chlorophyll in the drops grew more and more concentrated. At a certain concentration (not estimated in the paper) the absorption band began to shift to the red, from 666 to 676 mμ. This indicates that a shift similar to that caused by the accumulation of chlorophyll in colloidal particles can be produced also by an increase of its concentration in true molecular solution. When the ether was completely evaporated, the remaining dry chlorophyll had an absorption maximum at 679 mμ. This agrees approximately with the measurements of Hubert (1935), who gave 680.5 mμ for the absorption maximum of solid chlorophyll (thin film of dried chlorophyll on glass).

The possibility of energy exchange between excited and normal chlorophyll molecules and the spectroscopic effects of this exchange—which must increase with increasing concentration of the pigment—will be discussed in chapter 32, in connection with the concept of the "photosynthetic unit" and similar hypotheses.

A similarity between the absorption spectra of solid chlorophyll in suspension and of chlorophyll in the living cell was first claimed by Ivanovski (1907, 1913).

It thus appears that a shift of the red chlorophyll band toward the longer wave lengths (approaching its position in the leaf spectrum) can be achieved not only by interaction with a solvent of high polarity or polarizability, but also by interaction with other chlorophyll molecules. This offers several alternatives for the interpretation of the state of chlorophyll *in vivo*. In the case of *bacteriochlorophyll*, Katz and Wassink (1939) noted that the absorption band of evaporated pigment was shifted by not more than 2.5 mμ from its position in solution, while in live bacteria (and in colloidal extracts from bacteria) the same band is shifted toward longer waves by as much as 80–100 mμ. In this case, the position of the band in the spectrum of the living cells definitely indicates interaction with other cell components and not merely close mutual proximity of the pigment molecules.

A "red shift" of the absorption bands of chlorophyll probably can be obtained also by *adsorption* on appropriate carriers: According to Seybold and Egle (1940), the red absorption band of chlorophyll adsorbates on *starch* is situated at 662 mμ, *i. e.*, in the same region as in solution. Figure 21.27A, taken from Seybold and Weissweiler (1942), shows the absorption peaks of chlorophylls *a* and *b*, adsorbed on sugar, in the following positions: Chlorophyll *a*, 670 and 450 mμ, and chlorophyll *b*, 662 and 488 mμ—values that correspond to shifts by 10 and 20 mμ for the *a*-component and 19 and 35 mμ for the *b*-component (compared to band positions in ether). The

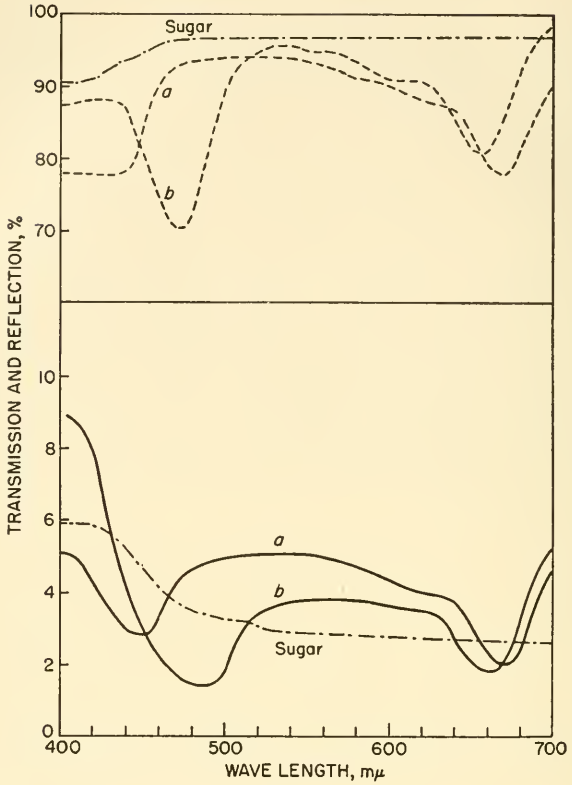


Fig. 21.27A. Transmission and reflection curves of chlorophylls *a* and *b* adsorbed on sugar (after Seybold and Weissweiler 1942).

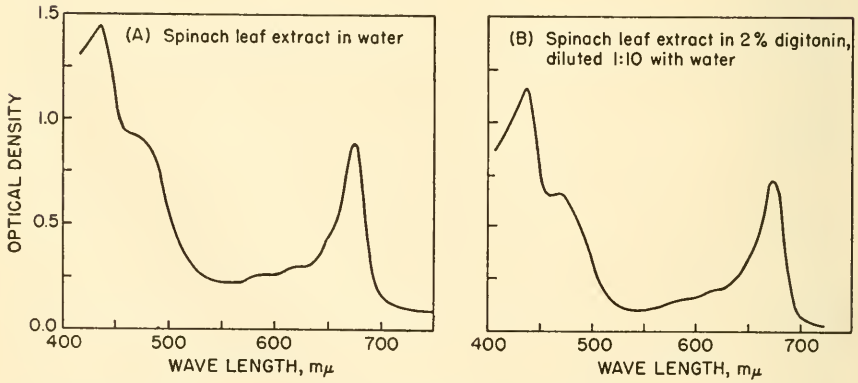


Fig. 21.28. Absorption curves of spinach leaf extracts (after Smith 1941).

figures for the blue-violet bands are so high as to call for a recheck (see chapter 22, page 705, for the position of the blue-violet bands in the living cells). Eisler and Portheim (1922) and Noack (1927) stated that the absorption spectra of chlorophyll adsorbates on *proteins* are "similar to those of the living cells," but gave no figures.

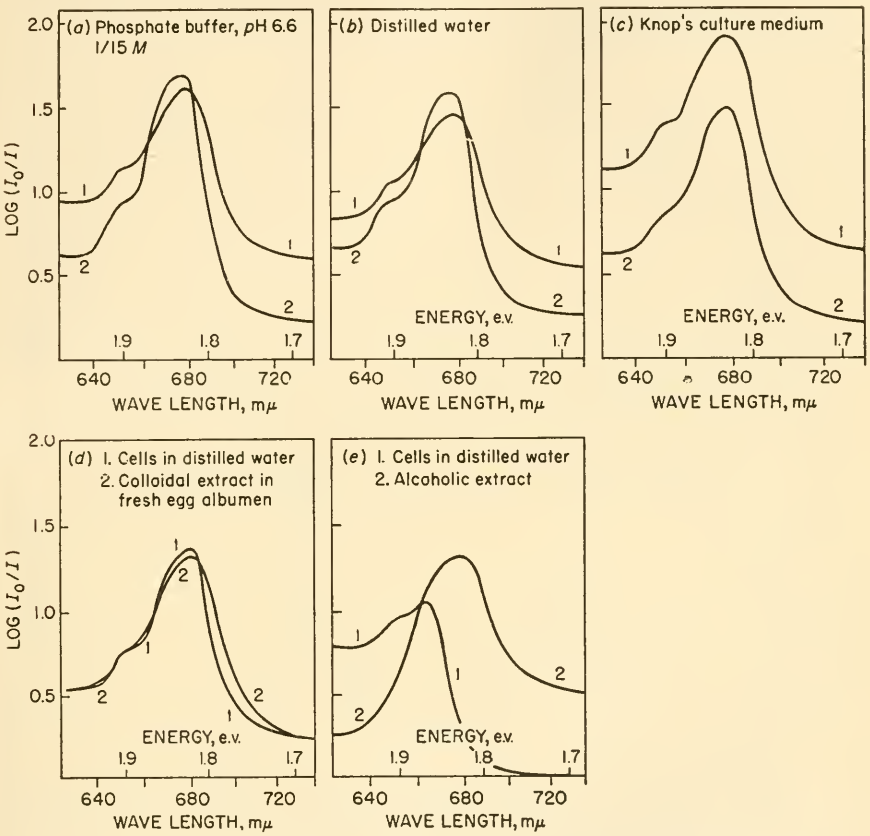


Fig. 21.29. Comparative absorption spectra of cell suspensions and pigment extracts of *Chlorella* in different media (after Katz and Wassink 1939). Curve 1, cells; curve 2, extracts. See page 656.

Absorption curves are available for "natural" chlorophyll–protein colloids extracted from leaves, algae and bacteria. Smith (1938, 1941) gave an absorption curve of the crude extract from spinach leaves, which contains broken chloroplasts or grana, and another curve for the same extract clarified by digitonin (*cf.* fig. 21.28, curves A and B). The maximum of curve A is at 678 mμ, that of curve B at 675 mμ.

The relatively strong absorption of the crude extract in the violet may be due to the presence of yellow pigments; its stronger absorption in the far red (>700 mμ) was attributed by Smith to scattering. However, increased absorption in the far red has also been observed by Noddack and

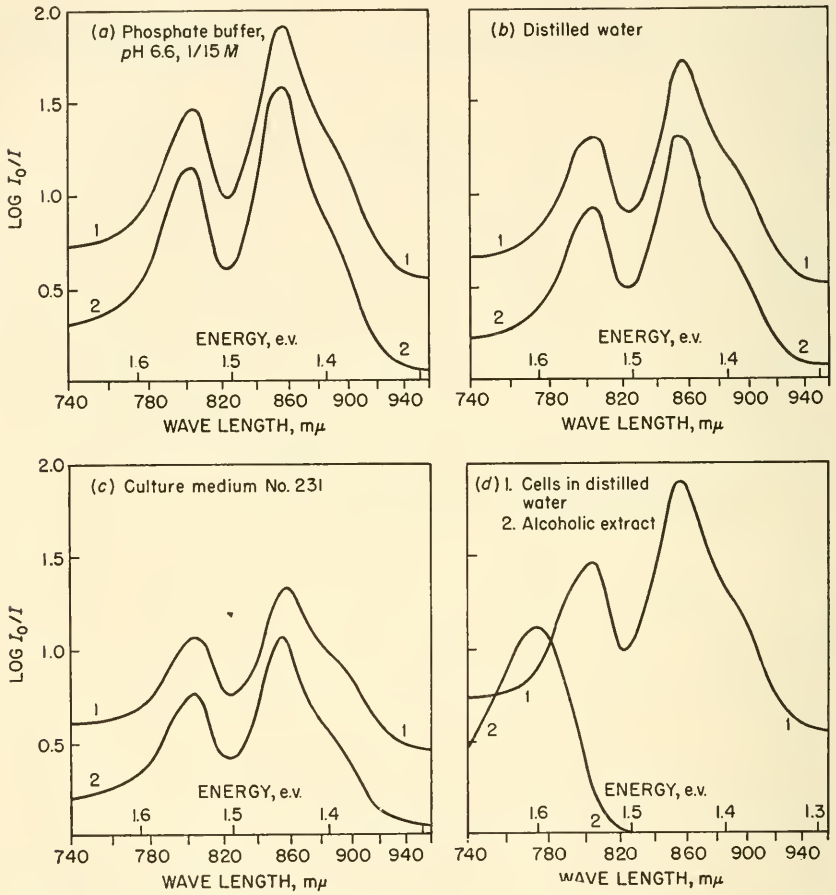


Fig. 21.30A. Comparative absorption spectra of cell suspensions and pigment extracts of strain D purple sulfur bacteria in different media (after Katz and Wassink 1939). Curve 1, cells; curve 2, extract. See page 656.

Eichhoff in *Chlorella* suspensions (see fig. 22.21), although these investigators used an integrating method, which was supposed to give true absorption values, free of scattering effects.

Rabideau, French and Holt (1946) gave absorption curves (obtained with an Ulbricht sphere) and transmission curves (obtained with a Beckman spectrophotometer) for chloroplast dispersions prepared by means

of supersonic waves. These curves, which can be found in figure 22.15, indicate that enhanced extinction in the far red is characteristic of transmission much more than of true absorption—thus supporting Smith's explanation and contradicting the results of Noddack and Eichhoff.

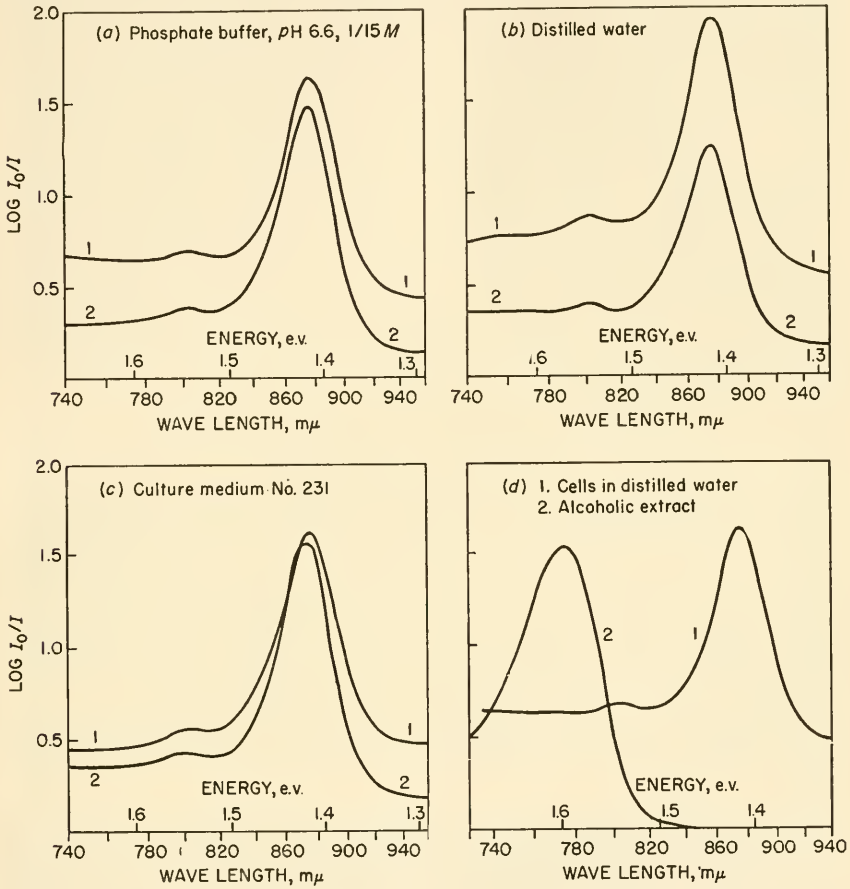


Fig. 21.30B. Comparative absorption spectra of cell suspensions and pigment extracts of *Rhodospirillum rubrum* in different media (after Katz and Wassink 1939). Curve 1, cells; curve 2, extract. Concerning the difference between the cell (and the colloidal extract spectra), in A and B, see page 703.

According to Smith, the molar extinction coefficient of chlorophyll in the maximum of the red band is approximately the same in the aqueous extract containing digitonin and in ether or acetone solution. In other words, the shift in the position of this band from 660 to 675 mμ occurs without a change in its intensity.

Absorption curves of colloidal chlorophyll–protein extract were given also by Katz and Wassink (1939). Figure 21.29 shows the red band in the spectra of *Chlorella* suspensions, and in colloidal extracts from the same cells (*a*) in phosphate buffer *p*H 6.6, (*b*) in distilled water, (*c*) in Knop's culture medium and (*d*) in fresh egg albumen. The position of the maxima are but slightly different in all these curves (approximately 680 mμ); the shapes of the curves are, however, affected by the nature of the medium, particularly in extracts *a* and *b*. The solution in egg albumen has an extinction curve practically identical with that of the living cells. Fig. 21.29*e* shows, as a contrast, the strong shift occurring upon extraction of the pigment with alcohol. Similar curves were given by Katz and Wassink (1939) and French (1940) for colloidal extracts from purple bacteria (*cf.* figs. 21.30).

A transmission curve of a water extract of the blue-green alga *Chroococcus* can be found in figure 22.48B. In these extracts, the phycocyanin–protein forms a true colloidal solution, while the other pigments probably are in the same state of dispersion as in extracts from green algae and leaves.

C. Absorption Spectra of the Carotenoids*

1. Experimental Results

The extinction curves of carotenoids in organic solvents have been investigated by numerous authors, among whom we may mention Willstätter and Stoll (1913), Pummerer and Rebmann (1928), McNicholas (1931), Smakula (1934), Gillam (1935), Sprecher von Bernegg, Heierle and Almasy (1935), Miller (1935, 1937), Strain and co-workers (1938, 1942, 1943, 1944), French (1941), Beadle, Zscheile and co-workers (1942, 1944, 1945) and Zechmeister and co-workers (*cf.* review by Zechmeister 1944).

Recently, a number of absorption curves were determined by Karrer and co-workers, and were reproduced in a monograph by Karrer and Jucker (1948).

The absorption spectra of carotene and "leaf xanthophyll" in the *infrared* were observed by Stair and Coblentz (1933). They show a series of absorption bands characteristic of long unsaturated carbon chains; many of them coincide closely with the absorption bands of phytol (*cf.* Table 21.IIA).

The spectra of all carotenoids in the *visible* are characterized by two or three intense bands near the violet end of the spectrum. Depending on how far these bands extend into the blue and green, the color of the pigments may be yellow, orange or even red. The position of the absorption bands depends, often even more strongly than in the case of chlorophyll,

* Bibliography, page 670.

on the state of the pigment and the surrounding medium. Table 21.IX shows that the direction of the band shift in different solvents is the same as for chlorophyll—*i. e.*, they are displaced toward the longer waves with increasing polarity and polarizability of the solvent. From ether to carbon disulfide, the "red shift" amounts to 43 mμ for carotene β and 35 mμ for luteol—as compared to only 10 mμ for chlorophyll. However, the effect of transition from a nonpolar to a polar solvent of approximately the same polarizability—*e. g.*, from ether to ethanol—seems to be smaller for the carotenoids than for the more polar chlorophylls (*cf.* Table 22.IX).

A still stronger displacement sometimes occurs in aqueous colloidal solutions. According to Karrer and Strauss (1938), the maximum of band I of carotene is shifted, from 480 mμ in hexane and ether, to 510 mμ, or even 535 mμ, in hydrosols. This, too, is a much wider shift than was observed in chlorophyll colloids. (Because of this shift, some carotene sols are red, while their molecular solutions are yellow.)

In addition to a shift of the band maxima, changes of medium may also cause a broadening of the carotenoid bands. This effect appears to be particularly strong in the case of some algal carotenoids. For example, the curves given for fucoxanthol spectrum by Strain, Manning and Hardin (1944) show a considerable flattening of the absorption peaks and extension of the absorption band toward the longer waves in ethanol as compared to petroleum ether. The spectrum of peridinol, a pigment of the dinoflagellates, shows a similarly strong solvent effect.

The spreading of the absorption bands of the carotenoids into the green, rather than a shift of their peaks, probably explains the color of brown algae and diatoms. The striking difference between their color and that of green plants appears inexplicable if one considers only the solution spectra of fucoxanthol (*cf.* fig. 21.35A) or peridinol, since these are almost identical with the spectra of the carotenols of green plants (*e. g.*, luteol and zeaxanthol).

Recently, Karrer and co-workers (1943, 1948) published an absorption curve of fucoxanthol in hexane (fig. 21.36) which shows a comparatively slow decline of absorption toward the longer waves. The absorption remains marked up to 550 mμ, while that of most other carotenoids drops to zero at 500 mμ. The reason for the difference between this curve (which together with that of chlorophyll *could* explain the brown color of fucoxanthinol-bearing algae) and that given by Wald for the same solvent, remains unexplained.

The curves given by Wassink and Kersten (1946) for the absorption spectrum of the "yellow" and the "orange" fraction of the carotenoids from the diatom *Nitzschia dissipata* also show an extension of the absorption in the second fraction (in methanol) to about 550 mμ, the peaks being situated

TABLE 21.IX

ABSORPTION MAXIMA OF CAROTENOIDS IN SOLUTION

Pigment	Occurrence	Solvent	Bands I	Bands II	Bands III	Reference
Carotene β	All plants	Ethyl ether	478	449	—	Rudolph (1933)
		n-Hexane	480	450	—	Smakula (1934)
		n-Hexane	478	452	—	Wald (unpublished)
		n-Hexane	477	450	—	Beadle and Zscheile (1942)
		Cyclohexane	482	451	—	Pummerer and Rebmann (1928)
		Ethanol	478–492	448–459	—	Willstätter and Stoll (1913)
		Chloroform	497	466	—	von Euler, *et al.* (1932)
		Carbon disulfide	521	486	—	Kuhn and Brockmann (1932–1933)
		Water (coll.)	510–535	475–488	—	Karrer and Strauss (1938)
Neocarotene β	Green leaves	*n*-Hexane	467	442.5		Beadle and Zscheile (1942)
Luteol	Green leaves and algae	Ethyl ether	470	440	—	Rudolph (1933)
		Ethyl ether	472	447	420	Strain (1938)
		Ethanol	473	451		" "
		Ethanol	480	450	421	Kuhn, Winterstein and Kaufmann (1930)
		Ethanol	477.5	446.5	424	Zscheile *et al.* (1942)
		1,4-Dioxane	482	453	423	Strain (1938)
		Chloroform	486	455	424	Kuhn, Winterstein and Kaufmann (1930)
		Chloroform	485	457	430	Strain (1938)
		Carbon disulfide	505	473	441	Kuhn, Winterstein and Kaufmann (1930)
		Carbon disulfide	506	474	440	Strain (1938)

Pigment	Occurrence	Solvent	Bands I	Bands II	Bands III	Reference
Violaxanthol	Green leaves, brown algae	Ethanol	472	442	417	Strain, Manning and Hardin (1944)
Zeaxanthol	Green leaves	Ethanol	479	452	—	Zscheile *et al.* (1942); Manning, Hardin and Strain (1944)
Fucoxanthol	Brown algae	Chloroform	484	459	—	von Euler, *et al.* (1932)
		n-Hexane	477	451	—	Wald (unpublished)
		Ethanol	492	457	—	von Euler *et al.* (1932)
		Ethanol	—	453	—	Strain, Manning and Hardin (1944)
		Petroleum ether	478	451	423	" " " " "
		Methanol	476	450	426	Wassink and Kersten (1946)
Neofucoxanthol A	Diatoms	Ethanol	—	447	—	Strain, Manning and Hardin (1944)
Neofucoxanthol B	Diatoms	Ethanol	—	446	—	" " " " "
Diadinoxanthol	Diatoms, flagellates	Ethanol	478	448	—	" " " " "
Diatonanthol	Diatoms	Ethanol	481	453	—	" " " " "
Peridinol	Dinoflagellates	Ethanol	475	—	—	" " " " "
Neoperidinol	Dinoflagellates	Ethanol	464	—	—	" " " " "
Dinoxanthol	Dinoflagellates	Ethanol	471	441.5	—	" " " " "
Neodinoxanthol	Dinoflagellates	Ethanol	466	438	—	" " " " "
Neodiadinoxanthol	Dinoflagellates	Ethanol	471	442	—	" " " " "
Rhodoviolascin	Purple bacteria	Ethanol	526	491	(465)	Karrer and Solmssen (1935)
		Carbon disulfide	573.5	534	496	" " " "
Rhodopol	Purple bacteria	Ethanol	505	474	(445)	" " " "
		Carbon disulfide	547	508	478	" " " "
Flavorhodin	Purple bacteria	Ethanol	472	443	—	" " " "
		Carbon disulfide	502	472	—	" " " "

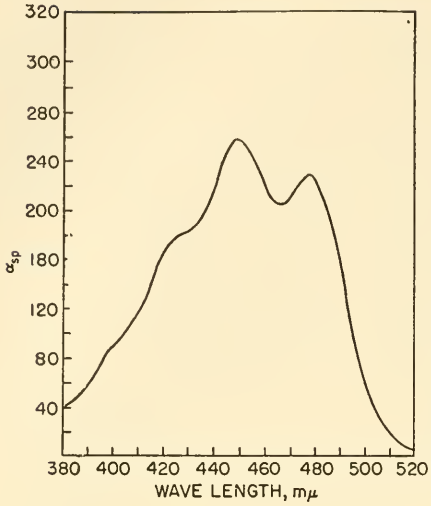


Fig. 21.31. Absorption spectrum of β-carotene in hexane (after Zscheile, White, Beadle and Roach 1942).

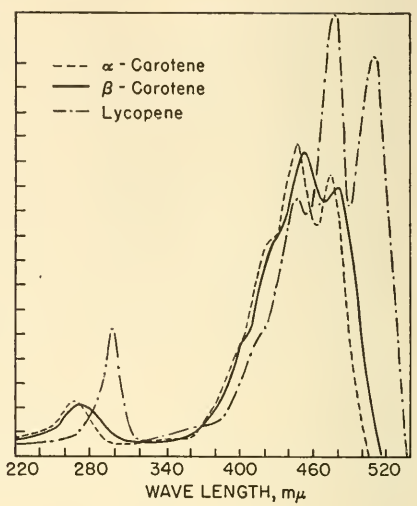


Fig. 21.32. Absorption spectra of α-carotene, β-carotene, and lycopene in 80% ethanol + 20% diethyl ether (after Miller 1937).

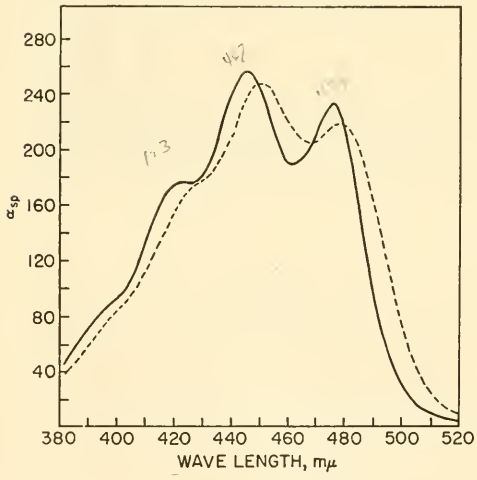


Fig. 21.33. Specific absorption spectra of luteol (solid line) and zeaxanthol (broken line) in ethanol (after Zscheile, White, Beadle and Roach 1942): *c* is in grams per liter; *d* is in centimeters.

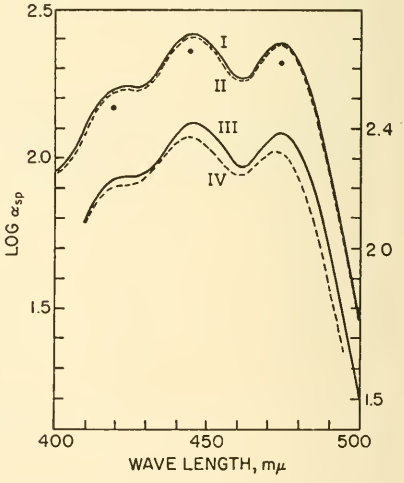


Fig. 21.34. Specific absorption coefficients of luteol in ethanol (after Strain 1938). I, dried luteol. II, luteol with 2.65% petroleum ether. Dots, luteol in ethanol as reported by Smakula (1934). III, pure luteol. IV, same after heating 3 hrs. at 77°. Values of log α for I and II at left, for III and IV at right.

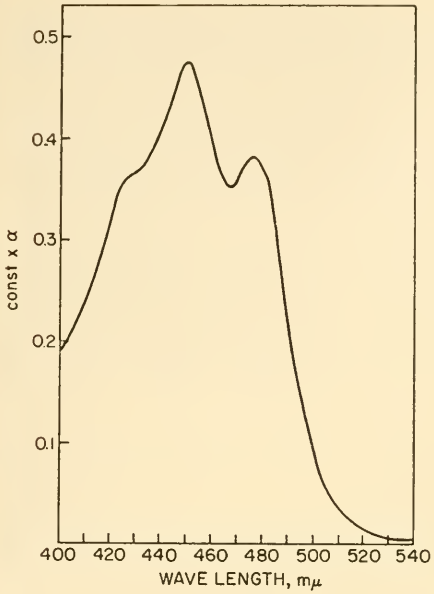


Fig. 21.35A. Absorption spectrum of fucoxanthol in hexane (after Wald 1942). (For a different curve for the same solution see fig. 21.36.)

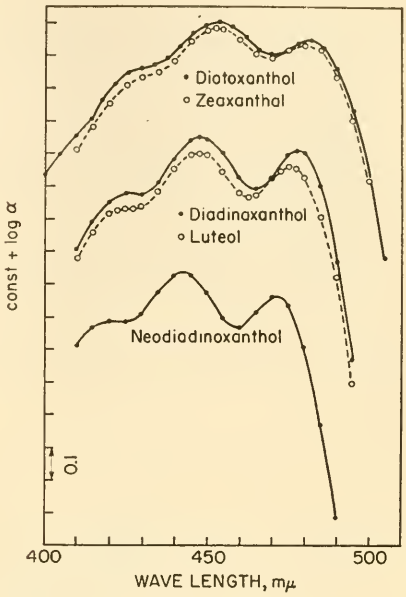


Fig. 21.35B. Absorption spectra of diatoxanthol, diadinoxanthol, neodiadinoxanthol, zeaxanthol and luteol (after Strain, Manning and Hardin 1944).

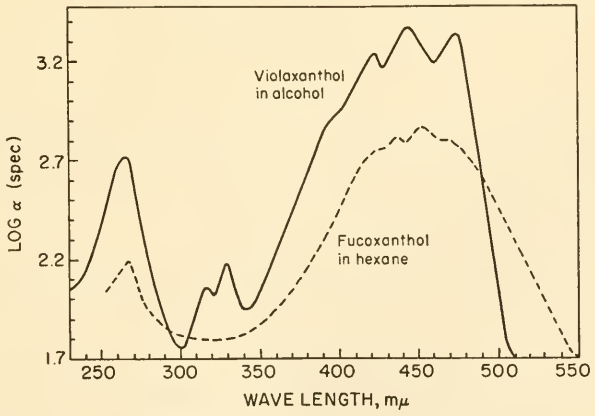


Fig. 21.36. Specific absorption coefficients of fucoxanthol in hexane and violaxanthol in ethanol (after Karrer and Würgler 1943). Concentration in g./100 cc.

at 426, 450 and 476 mμ (Table 21.IX). The curves given by them for live diatoms, colloidal extracts and pigment solutions in organic solvents indicate a considerably increased absorption, *in vivo*, in the region 500–560 mμ.

Table 21.IX shows that the absorption bands of many *bacterial* carotenoids are situated further toward the red than those of green plants and algae—a relation that reminds one of that between chlorophyll and bacteriochlorophyll.

Figures 21.31 and 32 show the extinction curves of three carotene isomers and figures 21.33 and 21.34 those of luteol—the most common carotenol of green plants (*cf.* Vol. I, page 415). Figures 21.35A and B represent the spectra of several carotenols of brown algae, diatoms and dinoflagellates.

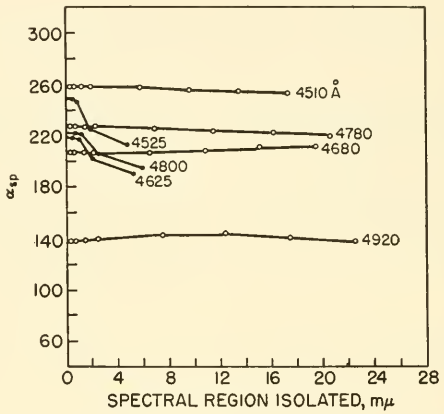


Fig. 21.37. Effect of width of spectral region isolated on the specific absorption coefficient of β-carotene at selected wave lengths (after Zscheile, White, Beadle and Roach 1942). The changes in α observed by Miller with single monochromator (●) were not found in the work with a double monochromator (O).

The absorption bands of the carotenoids are much broader than those of chlorophyll; they are therefore less sensitive to changes in the width of the slit, as illustrated by figure 21.37.

2. Theoretical Considerations

Carotenoids are *polyene dyestuffs, i. e.*, their color is due to the chromophoric properties of a straight chain of conjugated double bonds. Synthetic polyenes, and the cyanine dyestuffs used in photographic sensitization, are other examples of the same type. With increasing length of the

conjugated chain, the absorption bands are shifted regularly toward longer wave length, and their intensity becomes greater.

These simple relations between color and molecular structure make polyene dyes particularly suitable objects for theoretical studies. This problem was treated by Pauling (1939), who used the method of atomic orbitals and the concept of resonance, and by Mulliken (1939, 1941), who used the method of molecular orbitals. According to Pauling, the fundamental resonance possibilities of polyene molecules are provided by the shifting of the double bonds, which results in the transfer of an electron from one end of the molecule to the other. If we consider, *e. g.*, a straight conjugated chain with an even number of carbon atoms (*A*), the shift of all double bonds to the left will produce structure *B* and a shift to the right, structure *C*:

(*A*) $CH_3CH{=}CHCH\ldots\ldots CHCH{=}CHCH_3$

(*B*) $^{+}CH_3{=}CHCH{=}CH\ldots\ldots CH{=}CHCHCH_3$

(*C*) $CH_3CHCH{=}CH\ldots\ldots CH{=}CHCH{=}CH_3{}^{+}$

Each of the states *B* and *C* has a large dipole moment; but, since the two moments have opposite directions, and *B* and *C* have equal probabilities, the molecule will show no dipole moment at all, both in the ground state of the molecule and in the lowest excited states formed by resonance between the same three structures. However, the transition from the normal state to an excited state of this type has a "transition moment" whose order of magnitude is that of the dipole moment of the individual structures *B* and *C*. This is a very large moment, and it increases with length of the chain. In wave mechanics, the probability of a spectroscopic transition between two states (*i. e.*, the intensity of the corresponding absorption line or band) is determined by the magnitude of the "transition moment." This explains why polyene molecules have strong absorption bands, and why their intensity increases with the greater chain length.

The second approach to the same problem is that of the theory of "molecular orbitals." It considers the actual state of the molecule without decomposing it into imaginary resonating components. It tries to assign the electrons not to definite atoms or bonds, but to definite ψ-functions (orbitals) of the molecule as a whole. In a long chain of conjugated double bonds, some of these orbitals include the nuclei of all atoms in the chain, and electrons assigned to them can be considered as moving freely through the whole chain (this being the counterpart to the "shifting of double bonds" in the resonance theory). A conjugated double bond chain has, in this theory, a certain similarity to a metallic wire.

An investigation of a molecule by this theory consists in the determination of the qualitative characteristics of available orbitals and the evaluation of the relative energies of the states obtained by different assignments of the electrons to the orbitals.

Let us consider (Mulliken 1939) a straight chain of n carbon atoms (n = even number) and $n/2$ double bonds (the presence of symmetrical end groups on both ends of this conjugated chain—which is common in carotenoids—does not alter the problem). It contains n "unsaturation orbitals," sweeping over the whole conjugated chain, of which $n/2$ are "bonding" (*i. e.*, electrons assigned to them stabilize the molecule), and $n/2$ "antibonding." Each orbital can, as usual, hold two electrons, so that the n available "unsaturation electrons" are just enough to fill the $n/2$ bonding orbitals, thus giving a singlet normal state. The transfer of any one of these electrons into any one of the $n/2$ antibonding orbitals leads to an excited state; there are therefore $n^2/4$ groups of excited states. Each group consists (because of the interaction of orbitals with the electron spin) of one triplet and one singlet state; however, because of the prohibition of

singlet–triplet transitions in light atoms, only the $n^2/4$ singlet states are of importance for absorption. The longer the chain, the more numerous are the excited states. It can be shown that the "center of gravity" of these states in the energy diagram remains more or less unchanged, while the lowest excited states shift closer and closer to the ground state as the chain grows longer. This is shown schematically in figure 21.38. At the left we have the energy diagram of an A_2-molecule, at the opposite end, that of an infinite chain of A nuclei; the long-wave absorption limits are represented by the arrows; they become shorter and shorter, *i. e.*, the absorption shifts further and further to the red, with increasing chain length, until it extends into the infrared.

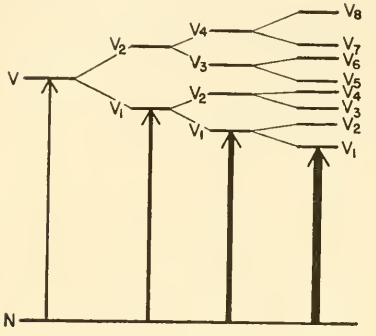


Fig. 21.38. Shift of first absorption band with increasing length of conjugated chain (thickness of arrow indicates intensity).

Mulliken has shown that, if the chain is a straight as possible (*i. e.*, if all carbon atoms in the chain are in *trans* positions), the transition to the lowest excited state (arrow in fig. 21.38) is more probable than all the other transitions together. This means that the intensity of the absorption band with the lowest frequency must increase steadily with increasing chain length. We have thus obtained theoretical interpretations, both of the gradual shift of the absorption band to longer waves, and of the increase in its intensity with the growing length of the chain.

The two or three separate maxima observed in carotenoid spectra may mean as many distinct electronic transitions; but more probably they correspond to coexcitation of one or several vibrational quanta. The distance between maxima (*ca.* 1500 cm.$^{-1}$) is of the order of magnitude of vibrational quanta in organic molecules.

D. Absorption Spectra of the Phycobilins*

The absorption spectra of the phycobilins have been observed in living algae, in aqueous colloidal extracts of chromoproteids and in organic solutions of chromophores. The results are somewhat confused because both phycocyanin and phycoerythrin apparently occur in several modifications of slightly different color. (These modifications might be due either to minor variations in the structure of the chromophores, or to the association of the same chromophore with different proteins.)

The first extensive data on the absorption spectra of the phycochromoproteids were given by Schütt (1888). Among the more recent papers on this subject are those of Lemberg (1928, 1930), Svedberg and Lewis (1928), Svedberg and Katsurai (1929), Dhéré and Fontaine (1931), Svedberg and Eriksson (1932), Roche (1933), Katz and Wassink (1939) and French and co-workers (1948,1951).

* Bibliography, page 671.

The only available data on the absorption spectra of the isolated (*i. e.*, protein-free) chromophores are those of Lemberg (1930). He gave the following wave lengths for the maxima of the absorption bands:

Compound	Medium	Wave length
Cyanobilin (from *Porphyra tenera*)	HCl (conc.)	598 mμ
	Acid $CHCl_3$	606 mμ
Erythrobilin (from *Porphyra tenera*)	HCl (conc.)	498 mμ

Combination of the pigments with protein shifts the bands toward the red; but the amount of this shift is not adequately described by comparison of the above-quoted figures with the positions of the absorption maxima of aqueous extracts from algae, because the data on free pigments refer to strongly acid solutions, while those on chromoproteids relate to neutral or only weakly acid solutions. The main absorption maximum of the phycocyanin–protein complex lies at 615 mμ in the *p*H range between 3.5 and 7, but is displaced, in concentrated hydrochloric acid, by as much as 41 mμ toward the red (to 656 mμ). Comparing this strongly acid solution of the chromoproteid with an equally strongly acid solution of the chromophore, we find a "red shift" by as much as 58 mμ; comparison with a neutral chromoproteid solution would indicate a shift of only 17 mμ.

Similar figures were given more recently by Wassink (1948) for cyanobilin from blue-green *Oscillatoria* ($\lambda_{max.}$ = 620 mμ for the chromoproteid, 610 mμ for the solution of the cyanobilin in chloroform, and 600 mμ for its solution in HCl).

Extinction curves of aqueous chromoproteid colloids were given by Svedberg and Lewis (1928), Svedberg and Katsurai (1929), Svedberg and Eriksson (1932), and French and co-workers (1948,1951).

Figure 21.39 shows the extinction curves of the phycoerythrins from five different algae. Three maxima (566, 540 and 498 mμ) are always present, but with variable relative intensities, pointing to the existence of three different forms of the pigment (perhaps the same chromophore linked to different proteins). Van Norman *et al.* (1948) found only two absorption peaks (550 and 495 mμ) in aqueous extract from *Iridaea*. It also has several bands in the ultraviolet.

Similar observations were made with phycocyanin. In phycocyanin from a *Rhodophycea* (*e. g.*, *Ceramium rubrum* and *Porphyra tenera*), Svedberg and Katsurai (1929) found two bands in the visible, at about 615 and 550mμ and ultraviolet bands at 355, 271 and 240 mμ. In the phycocyanin from a *Cyanophycea* (*e. g.*, *Aphanizomenon flos aquae*) they found only one visible band, at 615 mμ, and ultraviolet bands at 368 and 277 mμ.

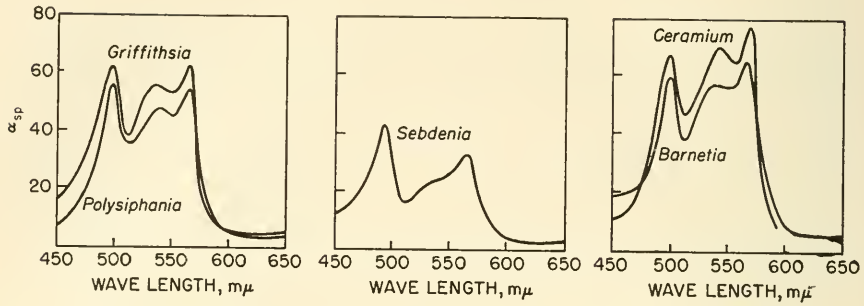


Fig. 21.39. Specific absorption spectra of phycoerythrins from different algae (after Svedberg and Eriksson 1932).

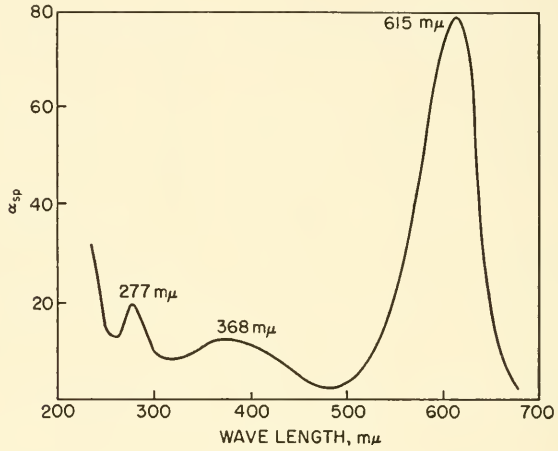


Fig. 21.40. Specific absorption spectrum of phycocyanin from *Aphanizomenon flos aquae* (after Svedberg and Katsurai 1929).

Figure 21.40 shows the extinction curve of the phycocyanin from *Aphanizomenon flos aquae*, according to Svedberg and Katsurai (1929).

Using Lemberg's estimate of 2% pigment in the chromoproteid (with a molecular weight of 636 for the chromophore, this corresponds to one mole pigment in 3.2×10^4 g. of the complex) we can convert the specific extinction coefficients, given by Lemberg (1928, 1930) and Svedberg and Katsurai (1929) into molar extinction coefficients. The resulting values (*cf.* Table 21.X) are exceptionally high—up to 3×10^5, as against only 4×10^4 in the maximum of the red band of chlorophyll and 1.5×10^5 in the maximum of the main absorption band of the carotenoids. This makes the correctness of Lemberg's analytical data somewhat doubtful. Lemberg

(1930) himself noted that the specific extinction of the phycobilins is ten times stronger than that of hemoglobin—while his analysis indicated the presence of only one half mole pigment per Svedberg unit of protein in phycobilins, as against one mole pigment per unit of protein in hemoglobin. If Lemberg's analysis is in error, and the content of phycobilins in the chromoproteids is as high or even higher than that of hemin in hemoglobin, the molar extinction coefficients of the phycobilins, given in Table 21.X will have to be proportionally reduced.

TABLE 21.X

ESTIMATED MOLAR EXTINCTION COEFFICIENTS OF PHYCOBILINS (IN THE BAND MAXIMA)

Pigment	State	λ, mμ	$\alpha_{mol.}$	Observer[a]
Phycoerythrin.....	Pigment in HCl	495	1.8×10^5	L
	Chromoproteid from *Ceramium*	565	2.6×10^5	S,K
	Chromoproteid from *Ceramium*	565	2.5×10^5	L
	Chromoproteid from *Porphyra*	565	2.5×10^5	L
Phycocyanin.....	Pigment in HCl	598	*ca.* 10^5	L
	Chromoproteid from *Ceramium*	615	1.3×10^5	S,K
	Chromoproteid from *Ceramium*	615	2.0×10^5	L
	Chromoproteid from *Porphyra*	615	3.1×10^5	L
	Chromoproteid from *Aphanizomenon*	615	2.6×10^5	S,K

[a] L = Lemberg (1928, 1930). S,K = Svedberg and Katsurai (1929).

A comparison of the intensity of the phycocyanin band at 615 mμ with that of the chlorophyll band at 680 mμ in the absorption spectrum of live *Oscillatoria* cells (*cf.* fig. 22.18) leads to similar doubts concerning Lemberg's analytical data. According to Lemberg (1928), the content of the chromoproteids in algae (determined with another species, *Ceramium rubrum*) is of the order of 0.5%, with only 2% chromophore in the complex. This corresponds to as little as 0.01% phycobilin in the dry matter of the algae —while the concentration of chlorophyll in red algae usually is of the order of 0.1% (*cf.* Table 15.II, Vol. I). The predominance of the phycocyanine band over the chlorophyll band in figure 22.18 therefore leads to the improbable conclusion that the molar extinction coefficient of the phycobilins is at least ten, and perhaps one hundred, times higher than that of chlorophyll—unless we prefer to assume that Lemberg's analytical figures are too low.

In addition to the fact that the estimate of the chromophore content in the complex (~2%) is probably too low, the content of the chromoproteid in the algae (~0.5%) may also have been underestimated (*cf.* chapter 15, page 418).

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Absorption Spectra of Pigments *in Vitro*

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Chapter 22

LIGHT ABSORPTION BY PIGMENTS IN THE LIVING CELL

The determination of light absorption in solutions or other homogeneous media is a routine measurement, and the results permit a simple interpretation (based on Beer's law) in terms of molecular absorption coefficients (also called "extinction coefficients"—since attempts to discriminate between these two terms have not been successful in practice). The experimental determination of the absorptive power of plants is less simple, and often the exact meaning of the results is problematical. The *measurement* of light energy absorbed by leaves, algal thalli or cell suspensions is complicated by scattering, which is significant not only in multicellular tissues, but even in suspensions of single cells (because the dimensions of the cells, $\sim 10^{-3}$ cm., are larger than the wave length of visible light, $\sim 5 \times 10^{-5}$ cm.). The *interpretation* of the results in terms of the absorption constants of the pigments is complicated, not only by the *light scattering* on phase boundaries, but also by *inhomogeneous distribution* of pigments in cells and tissues, and by the *shifting and deformation of the absorption bands* caused by adsorption and complexing. Let us assume, for example, that we have measured the energy, I, of a beam of light falling on a vegetable object—leaf, thallus or cell suspension—and the energy, I', emerging from this object, taking care to integrate the latter over all directions so as to include both the light transmitted forward (T) and the light reflected backward (R), and thus to avoid the "gross" errors that may be caused by scattering. If now we try to apply to the results Beer's law:

$$I' \ (= T + R) = I \times 10^{-\alpha c d} \tag{22.1}$$

with the intention of calculating an absorption coefficient, α, we find, first of all, that *scattering* has made the length of the *path* of the light in the absorbing medium—d in eq. 22.1—indefinite (even its average—Mestre's "detour factor"—is not constant, but depends on wave length, *cf.* Kok 1948). In the second place, we note that the *local accumulation* of pigments in the chloroplasts has made the *concentration* of absorbing molecules in the path of the individual light beams—c in equation (22.1)—variable: Some light beams pass between the chloroplasts and encounter no pigment molecules at all (a phenomenon to which we will refer later as the "sieve effect").

In the last place, if, overcoming these two difficulties, we succeed in obtaining a reliable value of α, it is an average absorption coefficient of a mixture of several pigments, whose individual absorption spectra in solution we may know, but whose bands are variously shifted and deformed, in the living cell, by adsorption and complexing. The task of apportioning the total absorption at a given wave length to the component pigments (which requires the knowledge of their individual absorption coefficients and of their distribution in the cell) often proves impossible of achievement, except by gross simplifications.

We shall deal first, in part A, with the determination of the amount of light energy absorbed by plants, and then, in part B, with the spectroscopic properties of individual pigments *in vivo* and their contribution to the total absorption.

A. Light Absorption by Plants*

1. General Remarks

In working with solutions in plane-parallel glass cells, the determination of the absorbed light energy (A) requires two measurements: Either one measures the incident light flux (I) and the transmitted light flux (T), or, more commonly, one compares T with the flux T_0 transmitted by a blank cell containing pure solvent. A is calculated by one of the following:

(22.2a) $$A = I - T \quad \text{or}$$

(22.2b) $$A = T_0 - T$$

Both are *first approximations.* Equation (22.2a) neglects *all* reflections; a *second approximation* can in this case be obtained by subtracting from I the light flux reflected from the front wall of the absorption cell:

(22.3) $$A = I(1 - r) - T$$

where r is the reflection coefficient of the cell material. However, reflection from the front wall is only part of the total reflection in the cell; to make our equation exact, we should write, in place of (22.3):

(22.4) $$A = I - T - R$$

meaning by R the total reflected flux.

Equation (22.2b) is a better first approximation than (22.2a), because it neglects only the difference between the reflections from the solution cell and the blank cell. The fluxes reflected from the front walls of both cells are identical, but those reflected from the back walls are different (because

* Bibliography, page 736.

of the weakening that light suffers in passing through the absorbing medium). A *second approximation*, which can be substituted for (22.2b), is:

$$A = T_0 - T + I(1 - r)[r(1 - 10^{-2\alpha d})] \tag{22.5}$$

where α is the absorption coefficient of the solution and d the thickness of the cell. In this equation, consideration has been given to one reflection from the front wall (factor $1 - r$) and one reflection from the back wall (factor in brackets). However, these reflections are only the beginning of an infinite series (as with two mirrors on opposite walls). The exact equation for A in terms of the properties of the blank cell is:

$$A = (T_0 - T) + (R_0 - R) \tag{22.6}$$

where R_0 and R are the total light fluxes reflected by the blank cell and the solution cell, respectively.

If α and r are known, exact values of T_0, T, R_0 and R can be obtained by the summation of infinite power series.

If the light "trapped" between the walls leaves the cell after an *odd* number of passages, it is added to the transmitted flux; if it escapes after an even number of passages, it is added to the reflected flux. Consequently, the series for T contains only even powers of r and odd powers of $10^{-\alpha d}$, and the series for R only odd powers of r and even powers of $10^{-\alpha d}$. (The ratio of the sums for T and T_0 is given in equation 22.12.)

If α and r are unknown, R (and R_0) must be determined experimentally.

Since repeated reflection lengthens the average path of the light in the absorption cell, it increases absorption. In the case of homogeneous solutions in plane-parallel glass cells, this increase represents only a minor correction (to be estimated on page 711); we mention it merely to illustrate the complications in the measurement of light absorption that arise from the presence of phase boundaries. In nonhomogeneous systems, the complications are similar in principle, but much more important quantitatively. Reflections are not only more numerous, but also stronger (because of the varying angles with which the light strikes the interfaces); and they are supplemented by refractions and total inner reflections, which all affect the length of the path of the light beam and the direction in which it leaves the medium.

Leaves and thalli are heterogeneous systems, with numerous phase boundaries between air channels, cell walls, cytoplasm, vacuoles, plastids and starch grains; and the passage of light through plants or plant organs is, therefore, a very complicated phenomenon. It has been repeatedly discussed—by Willstätter and Stoll (1918), Briggs (1929), Mestre (1935), Seybold and co-workers (1932[1,2], 1933[1,2], 1934, 1943), Schanderl and Kaempfert (1933), Meyer (1939) and Loomis (1941, 1949), among others—but these discussions have not gone far beyond the qualitative stage.

A suitable statistical theory (*cf.* page 713) may permit the *calculation* of A from measurements of light transmission in one direction, made with two or more different optical densities of the scattering material (*e. g.*, with a series of several leaves, or with several cell suspensions of different concentration or layer thickness). However, it is better not to rely on such theoretical equations, but, particularly in working with leaves or thalli, actually to *measure* the light fluxes transmitted and reflected in all directions. Having determined experimentally both T and R, one can use the exact equation (22.4) for the evaluation of A. The time to use theoretical equations for combined absorption and scattering comes when one is not satisfied with the knowledge of the amount of absorbed *energy*, but wants also to know the absorption *coefficients*, *e. g.*, as indicators of the molecular state of the pigment in the living cell.

Attempts have been made to use "blanks," for example, white parts of variegated leaves (*cf.* Linsbaur, 1901, Brown and Escombe 1905, Weigert 1911, Meyer 1939, and Seybold 1932[1,2], 1933[1], 1934), or algal thalli from which the pigments had been extracted (*cf.* Reinke 1886), or tissues bleached by long exposure to light (*cf.* Wurmser 1926), and to imitate in this way the method usually applied to transparent media. In the latter case, the blanks provide an automatic correction for reflection (*cf.* page 673); in the case of plants, they were intended to provide a correction also for scattering. However, the approximation (22.2b), which is generally satisfactory in work with transparent media, may give entirely erroneous results when applied to optically inhomogeneous systems. This was pointed out by Willstätter and Stoll (1918) and Warburg (1925) when they criticized the absorption calculations of Weigert (1911). The error is caused by the large difference between the fluxes R and R_0 (*cf.* equation 22.6) reflected by the green and the colorless leaf. A green leaf may transmit about 10% and reflect another 10% of incident white light, while a similar, pigment-free leaf may transmit 50% and reflect the other 50%. If the absorption of the green leaf is calculated from these figures by means of equation (22.2b), the result is $A = 40\%$, which is only one half the correct value (80%)!

Therefore, if one wants to determine absorption, A, by comparison of a green leaf with a pigment-free leaf, one has to use the complete equation (22.6), *i. e.*, to measure the *four* quantities T_0, R_0, T and R, while measurement of only *three* quantities, I, T and R, is sufficient to make the same determination with a single leaf, according to equation (22.4). Furthermore, in plant work, one is never certain whether the "blank" is entirely free of pigments: According to Seybold and co-workers (1933[1], 1942), so-called "white" leaves of *Acer negundo* absorb 10–20% of incident white light; this absorption may be caused by nonplastid pigments, or by a small quantity of residual chlorophyll or carotenoids.

The transmitted light flux (T) and the reflected light flux (R) can both contain a collimated component, T_s or R_s (light transmitted in the direction of the incident beam, or reflected according to the laws of specular reflection), and a *diffuse* component, T_d or R_d, so that equation (22.4) can be written more explicitly as follows:

$$A = I - (T_s + T_d) - (R_s + R_d) \tag{22.7}$$

Measurements of T and R must include both the collimated and the diffuse components.

If the leaf is sufficiently thick, and not glossy, it acts as an *ideal scatterer*, *i. e.*, the intensity of light scattered in a given direction is proportional to

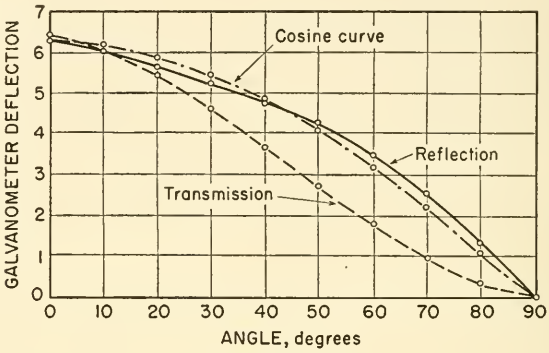

Fig. 22.1. Angular distribution of light transmitted and reflected by a leaf of *Coleus blumei* (after Loomis, Carr and Randall 1941). Reflection is diffuse and obeys the cosine law; transmission is only partly diffuse and therefore deviates from the cosine law.

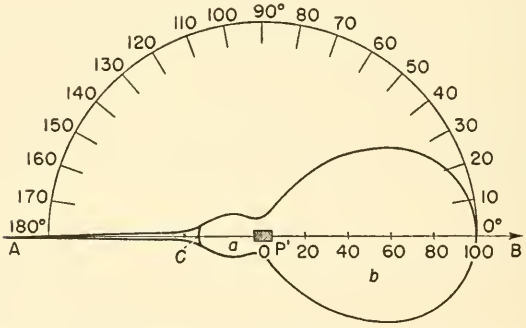

Fig. 22.2. Scattering of light by a dense suspension of *Chlorella* (in vessel O) (after Noddack and Eichhoff 1939). Direction of incidence A → B; area *a* represents backward scattering, or reflection (R); area *b* forward scattering or transmission (T).

the cosine of its angle with the direction of the incident light. Figure 22.1 shows the angular distribution of the light scattered by a comparatively thin leaf of *Coleus*. This leaf transmits some collimated light (as evidenced by the deviation of the angular distribution of T from the cosine law), but its reflection is entirely diffuse. Thicker leaves, with similarly dull surfaces, obey the cosine law with respect to both reflection and transmission,

whereas thick leaves with glossy surfaces may show a completely diffuse transmission, but a marked specular reflection.

Figure 22.2 shows the forward and back scattering of light by a small vessel containing a suspension of *Chlorella* cells, as observed by Noddack and Eichhoff (1939). The sharp peak (C) at 180° is caused by specular reflection from the glass wall.

In practical work, one can often drop the distinction between T and R and measure the total scattered flux $S = (T + R)$ by means of some integrating device:

$$A = I - S \tag{22.8}$$

An example is the study by Rabideau, French and Holt (1946) of the absorption spectra of leaves and pigment extracts.

2. Average Transmittance and Reflectance of Leaves and Thalli in White Light. Intensity Adaptation and Movements of Chloroplasts

The first measurements of the *proportion of white light transmitted by leaves* were carried out by Sachs in 1861. Later this magnitude was measured by Detlefson (1888), Linsbauer (1901), Brown and Escombe (1905), Purevich (Purjewitsch) (1914), Schanderl and Kaempfert (1933), Seybold (1932[1,2], 1933[1,2], 1943), Loomis, Carr and Randall (1941,1947,1949). The *transmittance of algae* was investigated by Reinke (1886), Wurmser (1921) and Seybold and co-workers (1934, 1942).

The first measurements of the *reflectance of leaves* were made by Coblentz in 1912, and were followed by those of Pokrovski (1925), Shull (1929), Seybold and co-workers (1932[2],1933[1,2],1942,1943) and Loomis, Carr and Randall (1941,1949). The only data on the *reflectance of algae* are those of Seybold and co-workers (1934, 1942, 1943).

Brown and Escombe (1905) and Purevich (1914) found comparatively high values—of the order of 20%—for the transmission of (infrared-free) white light by average leaves. Seybold (1932) suggested that these results were falsified by the inclusion, in the measured transmitted flux, of the thermal radiation of the leaves. In agreement with Pokrovski (1925), Seybold found that an average fully green leaf transmits not more than 10% of infrared-free white light. Leaves are almost transparent in the far red and near infrared (*cf.* figs. 22.30 and 31). Therefore, transmission values obtained by means of thermopiles (or other infrared-sensitive instruments) are deceivingly large if the light used for the measurements contains a large proportion of infrared radiations. According to Loomis, and co-workers (1941,1949), an average leaf transmits 30% of total sunlight, including the infrared. With artificial light sources of lower tempera-

ture, the over-all transmission may be much greater. Selenium barrier layer photocells ("photronic cells"), because of their low sensitivity in the infrared and in the far red, indicate, in direct sunlight, a transmission of only 5 to 10%, depending on the thickness of the leaf (*cf.* Seybold 1932[1], and Egle 1937).

The light absorption by a leaf depends on its thickness and the concentration of the pigments. As mentioned above, fully green leaves transmit and reflect only 10 or 15% of incident visible light, and absorb as much as 85 or 90%; on the other hand, green onion skins, one cell thick, transmit 85%, reflect 10% and absorb less than 5% (Seybold 1932[2]). In chapter 15

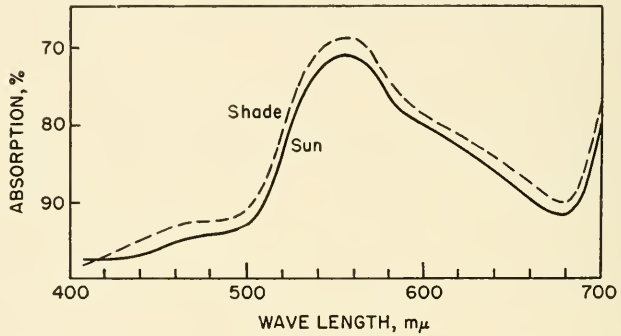

Fig. 22.3. Absorption spectra of a shade leaf and a sun leaf of *Fagus sylvatica* (the shade leaf contains 50% more chlorophyll and 80% more carotenoids) (after Seybold and Weissweiler 1943).

(Vol. I) we described the adaptation of plants to the intensity and composition of the incident light. We found there that typical "shade plants" contain two or three times more pigment than typical "sun plants." The probable purpose of this "intensity adaptation" is to ensure an adequate supply of energy to plants that grow in the shade, or to algae that live deep under the sea, and, inversely, to prevent light injury to plants or algae exposed to direct sunlight.

There is no doubt that the presence of fucoxanthol or of the phycobilins in brown, red and blue algae has considerable influence on the amount of light adsorbed; this will be brought out in detail in part C. The effects of variations in the concentration of chlorophyll are much smaller. Even "light-green" plants absorb so large a proportion of incident light that a doubling of their chlorophyll content can increase the absorption only comparatively little. Three examples can be found in our illustrations: Figure 22.3 shows the very slight enhancing effect that a 50% excess of chlorophyll and 80% excess of carotenoids have on the absorption of light by a

shade leaf of *Fagus*, as compared to a sun leaf of the same species. The two lowest curves in figure 22.14 indicate a sightly larger difference between the spectral transmission curves of a dark-green and a light-green *Hibiscus* leaf. Finally, figure 22.10 illustrates the effect of *extreme* variations in chlorophyll content, such as occur in *aurea* leaves. Here, a few per cent of the normal chlorophyll content (*cf.* Table 15.I, Vol. I) suffice to produce from two thirds to nine tenths of normal absorption in the region between 520 and 700 mμ.

Without varying the concentration of the pigments, many plants have it in their power to adjust the light absorption by the *displacement or reorientation of the chloroplasts*. These tactic reactions, discovered by Böhm in 1856, were investigated by Stahl (1880, 1909), Senn (1908, 1909, 1917, 1919), Liese (1922) and Voerkel (1933), among others. It was found that each chloroplast moves independently, *i. e.*, it is not carried by streaming of the protoplasm. In *moderate light*, the chloroplasts gather on the illuminated front walls and orient themselves so as to present their large cross-

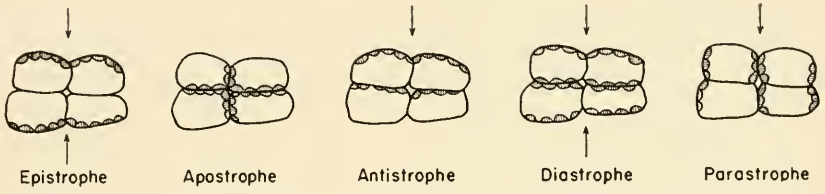

Fig. 22.4. Schematic representation of different chloroplast orientations (after Benecke and Jost 1924). Arrows show direction of light incidence.

sections to the light ("antistrophe," "epistrophe" and "diastrophe" in figure 22.4; the first one is produced by one-sided, and the other two by two-sided, illumination). In *strong direct light*, on the other hand, the chloroplasts turn their axes parallel to the light beams and line the side walls of the cells ("parastrophe" in fig. 22.4). During the night, they often assume characteristic "night positions"—congregate around the nuclei, or disperse throughout the cytoplasm, or line the internal walls ("apostrophe" in fig. 22.4). The largest variety of chloroplast movements has been observed by Senn in green and brown algae, and in diatoms. In leaves, the chloroplasts in the parenchyma cells assume positions similar to those shown in figure 22.4, but the chloroplasts in the palisade tissue usually remain arrayed along the side walls, leaving the end walls free. Instead of moving bodily these chloroplasts merely change their shape: In strong diffuse light, they spread flat against the walls, whereas in weak light they protrude into the cytoplasm, without losing contact with the walls. Illu-

mination with strong parallel light may, however, produce antistrophe in these cells as well.

Obviously, the effect of parastrophe is to decrease, and that of epistrophe, antistrophe or diastrophe to increase the absorption of light. How

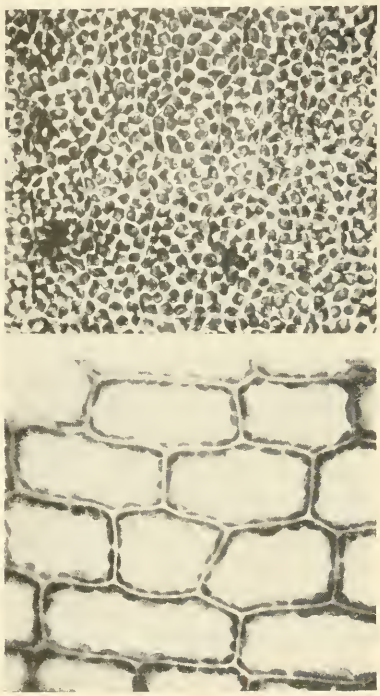

Fig. 22.5. Chloroplasts in *Funaria* (after Voerkel 1933): above, in light (epistrophe); below, in darkness (apostrophe).

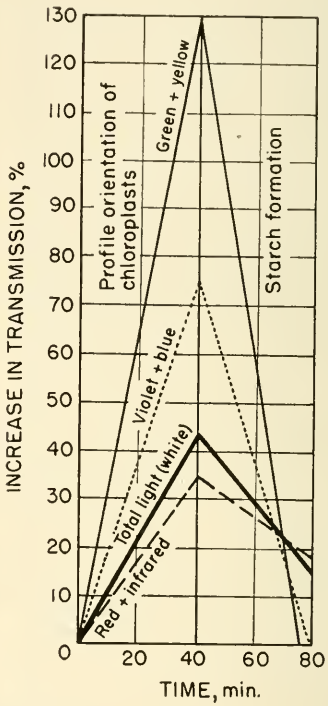

Fig. 22.6. Changes in light transmission through leaves of *Tradescantia viridis*, caused by chloroplast orientation and starch formation (after Schanderl and Kaempfert 1933).

successfully this can be achieved is illustrated by figure 22.5, which shows how *Funaria* cells change their appearance upon transition from epistrophe to apostrophe.

Differences in the transmittance of leaves in light of different intensity, caused by the regrouping of chloroplasts, have first been actually observed by Detlefson (1888) and Stahl (1880, 1909). A quantitative investigation was made by Schanderl and Kaempfert (1933); typical results are shown

in Table 22.I. This table indicates that in blue-violet light increase in transmittance may be by as much as one-third (from 19 to 25%).

TABLE 22.I

TRANSMITTANCE OF A LEAF OF *Adiantum cuneatum*
(AFTER SCHANDERL AND KAEMPFERT 1933)

Light exposure	White light (including infrared)	Red and infrared	Yellow and green	Violet and blue
In diffuse room light, %	31	34	16	19
After 4 hr. exposure to sun, %	37	42	18	25

With some plants (*e. g.*, *Tradescantia viridis*) Schanderl and Kaempfert found a *reversal* of the effect after the first half hour of illumination (*cf.* fig. 22.6); they attributed it to increased scattering, caused by the formation of starch grains.

Schanderl and Kaempfert did not prove that increased transparency of sun-exposed leaves was due entirely to reorientation of the chloroplasts, and not, *e. g.*, to a partial bleaching of the pigments. However, the results of Willstätter and Stoll (1918), which showed no change in chlorophyll concentration after strong illumination (*cf.* Vol. I, chapter 19, page 549), argue against the second explanation.

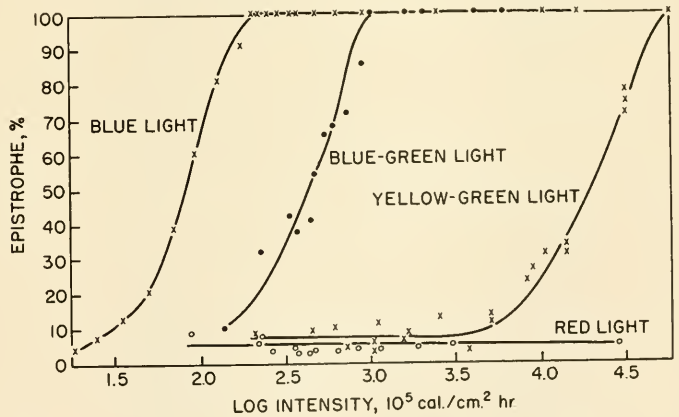

Fig. 22.7. Orientation of chloroplasts in *Funaria* in light of different color (after Voerkel 1934).

The phototaxis of the chloroplasts is caused mainly by blue-violet light and is entirely absent in red light (fig. 22.7). It must thus be sensitized by the carotenoids rather than by chlorophyll. This is not a proof

(*a*) (*b*)

Fig. 22.8. Leaves of *Naegelia zebrina* (after Mecke and Baldwin 1937). Normal leaves, left; water-filled leaves, right; photographed on (*a*) panchromatic plate and on (*b*) infrared-sensitive plate (max. 850 mμ). Increase of transmittance by decreased scattering best recognizable on the infrared photograph.

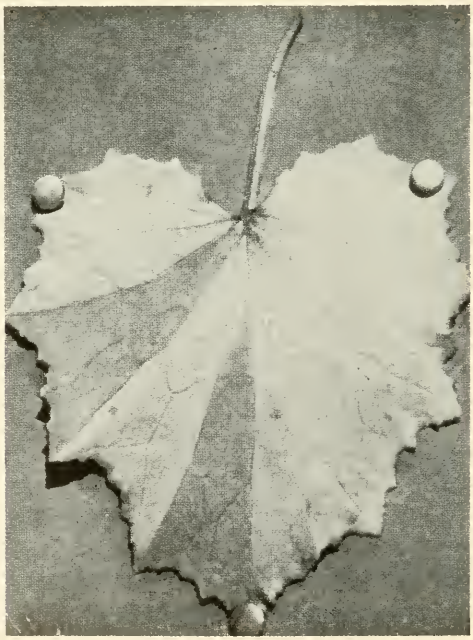

Fig. 22.9. Leaf of *Tussilago farfara* on black background (after Schanderl and Kaempfert 1933). Dark sections imbibed with water, therefore more transparent than the air-filled section.

that the whole phenomenon is unrelated to photosynthesis, since in natural light fields, red light does not occur without the presence of some blue-violet light as well.

Detlefson (1888) and Purevich (1914) found that leaves become less transparent in the presence of carbon dioxide, but Ursprung (1918) was unable to confirm this result. This effect, if at all real, could be attributed to increased formation of starch grains.

Scattering of light in leaves can be decreased by injection of water into the air channels (*e. g.*, by evacuation under water; *cf.* figs. 22.8 and 22.9). According to Seybold (1933[2]), water-filled leaves of land plants (as well as those of aquatic plants of the type of *Elodea*) transmit about twice as much light as air-filled leaves. For example, a submerged leaf of *Potomageton alpinus* was found to transmit as much as 22% of white light (Seybold 1932); similar figures were obtained by Seybold (1934) for the transmission by algae (*Chlorophyceae*, *Phaeophyceae* and *Rhodophyceae*). Increased transparency of water-filled leaves or thalli is due to reduced diffuse reflection (5% instead of >10%), rather than to weaker absorption. As a rough approximation, it can be assumed that average land leaves transmit 10% of (infrared-free) white light (400–700 mμ), reflect 10% and absorb 80%; while average aquatic plants or algal thalli reflect 5%, transmit 15% and absorb 80%.

Loomis (1947) found, for 28 species of leaves, transmissions between 2 and 9%, and reflections of the same order of magnitude. Yellow tobacco leaves transmitted ~ 30% of visible light, and light yellow leaves, 36% of visible and 53% of total sunlight. Normal leaves of *Nicotiana* and *Quercus* transmitted 5–7% of visible, and 25–30% of total sunlight.

According to Seybold, a single chloroplast transmits from 30 to 60% of visible light (depending on spectral distribution), absorbs 30 to 60% and reflects about 10%. As mentioned in chapter 19 (Vol. I) an average leaf contains the equivalent of from five to ten complete layers of chloroplasts; these can easily account for the above-estimated absorption of 80% of the incident white light. For a detailed discussion of the light absorption in successive layer of chlorophyll molecules or plastides, see Seybold and Weissweiler (1943).

In regularly patterned systems of colored and colorless materials, the absorption sometimes depends upon the angle of incidence. When this is the case the absorption of diffuse light may be different from that of collimated light. Seybold (1933[2]) made a search for such an effect in leaves, but without success. The reason probably is that leaves are such strong scatterers that collimated light is converted into practically completely diffuse light, long before it emerges from the leaf.

3. Absorption by Nonplastid Pigments

When the total absorption of light by a leaf, thallus or cell suspension has been determined, the question arises as to what part of this absorption is due to the chloroplast pigments. It has been assumed by many authors —from Reinke (1886) to Noddack and Eichhoff (1939)—that a certain part of the absorption of white light in plants is due to the "colorless" parts of the tissue, the cytoplasm, cell sap, nuclei, starch and cellulose. Seybold arbitrarily ascribed one eighth of the total absorption to these components, and seven eights to the chloroplast pigments. An absorption curve of a white *Pelargonium* leaf, given by Seybold and Weissweiler (1942), shows considerable absorption near the blue-violet end of the visible spectrum.

Of course, no really colorless substance can absorb visible light. But the plant cells contain coloring materials associated with the cell walls or with the cell sap rather than with the plastids, such as flavones, tannins, etc. Some of these substances are only weakly colored—usually yellow; others, although intensely colored, are present only in small quantities, as compared to the plastid pigments. In some species, however, flavones and anthocyanines are so abundant as to give the leaves a striking red color (leaves of the *purpurea* variety, and many young leaves in the spring). The color of these leaves advertises the fact that much of the light energy they absorb goes to nonplastid pigments.

Figure 22.10 shows, as an example, the spectral transmission and reflection curves of three varieties of *Corylus avellana*, normal, *aurea* and *purpurea*. Curves 1 and 2 illustrate the effect on light absorption of extreme variations in the concentration of chlorophyll (*cf.* page 678), while curve 3 shows the considerable increase in absorption, particularly in the green, caused by the presence of anthocyanines in *purpurea* leaves. (Light absorption in *aurea* and *purpurea* leaves will again be discussed in chapters 28 and 30, in relation to the yield of photosynthesis.)

As far as *green* leaves and algae are concerned, the participation of nonplastid pigments in light absorption remains a moot question. It probably varies widely from species to species. For example, according to Thimann (unpublished), leaves of *Phaseolus vulgaris* contain a large quantity of yellow, water-soluble pigments. The same is true of the needles of the conifers (Burns 1942).

It was mentioned above (page 675) that "white" leaves may absorb 10 or 20% of incident white light (Seybold 1933[1], 1942, *cf.* fig. 22.12). This absorption, too, is probably due to nonplastid pigments.

Most investigators who measured the yield of photosynthesis in relation to the amount of absorbed light silently assumed that the absorption

by the nonplastid components was negligible; but this assumption is not, or is not always, justified, particularly in work with blue-violet light. It is therefore advisable to ascertain, before undertaking quantitative work on photosynthesis, whether the plants chosen are free from, or at least poor in, nonplastid pigments.

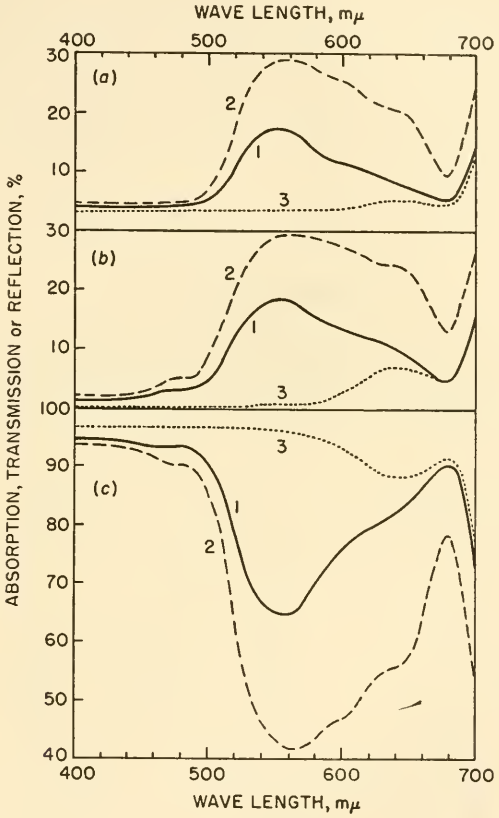

Fig. 22.10. Spectra of normal (curves 1), *aurea* (curves 2) and *purpurea* (curves 3) leaves of *Corylus avellana* (after Seybold and Weissweiler 1942). (*a*) Transmission curves, (*b*) reflection curves, (*c*) absorption curves. New absorption curves of yellow, red and white air-filled and air-free leaves can be found in the paper by Seybold and Weissweiler (1943[1]).

Noddack and Eichhoff (1939) attempted to determine the absorption of light by the "colorless" components of *Chlorella*, and concluded that it is negligible. This conclusion may be correct, but the method employed—comparison with the absorption by

cells bleached with bromine—does not appear reliable, since bleaching by bromine may not be restricted to plastid pigments.

It was suggested that the proportion of the total light absorption by leaves due to plastid pigments can be determined by extracting the latter and comparing the absorption of the extract with that of the original leaf. Timiriazev (1885) and Lazarev (1924, 1927) based this suggestion on the assumption that the absorption of white light by extracted pigments is about equal to the absorption by the same pigments in the leaf. However, light scattering, "sieve effect" and band shifts can make the two magnitudes quite different. True, some of these factors enhance the absorption, while others weaken it, but it would be an unusual coincidence if the net result were exactly nil. Experimentally, Noddack and Eichhoff (1939) found that the increased absorption by live *Chlorella* cells in the far red almost exactly compensates for the decreased absorption in the region 520–680 mμ (*cf.* fig. 22.21); but Seybold and Weissweiler (1942) could not confirm this result, and found, to the contrary, that throughout the visible spectrum the absorption by live cells is more complete than that by the extracts.

B. Spectral Properties of Plants*

1. Empirical Plant Spectra

It is clear from the preceding discussion that what is usually called a "leaf spectrum," or even a "spectrum of a cell suspension," is *not* the true spectrum of the pigments contained in these materials (meaning by "true spectrum" the plot of the absorption coefficient of the pigment mixture

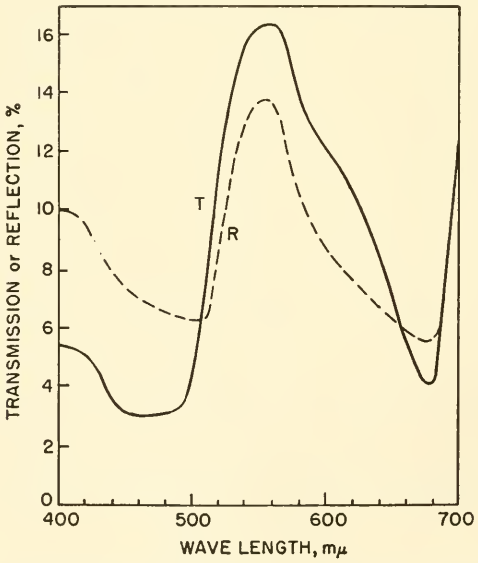

Fig. 22.11. Transmission and reflection spectrum of a leaf of *Parietaria officinalis* (after Seybold and Weissweiler 1942).

* Bibliography, page 737.

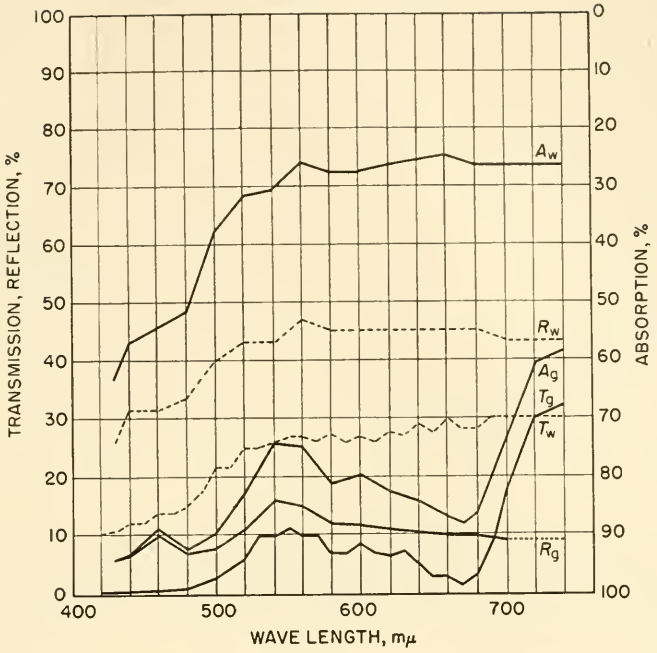

Fig. 22.12. Transmission (T_g), reflection (R_g) and absorption (A_g) of light by a green leaf of *Pelargonium zonale*, and the corresponding constants for a white leaf (index w) (after Seybold 1933). The figure indicates considerable absorption by "white" leaves, particularly in the blue-violet region. For other absorption curves of "white" leaves, see Seybold and Weissweiler (1943[1]).

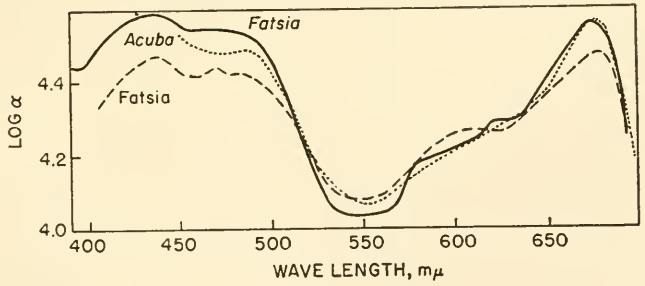

Fig. 22.13. Transmission spectrum (log T_0/T) of leaves of *Fatsia* and *Acuba* (after Meyer 1939). Absolute values of ordinates adjusted to give best agreement with spectrum of extracted pigments.

against wave length). One may measure I, T and R (or $S = T + R$), plot T/I, R/I, or A/I $(= [I - (T + R)]/I = [I - S]/I)$ against wave length, λ, and call the resulting curves "transmission spectra," "reflection

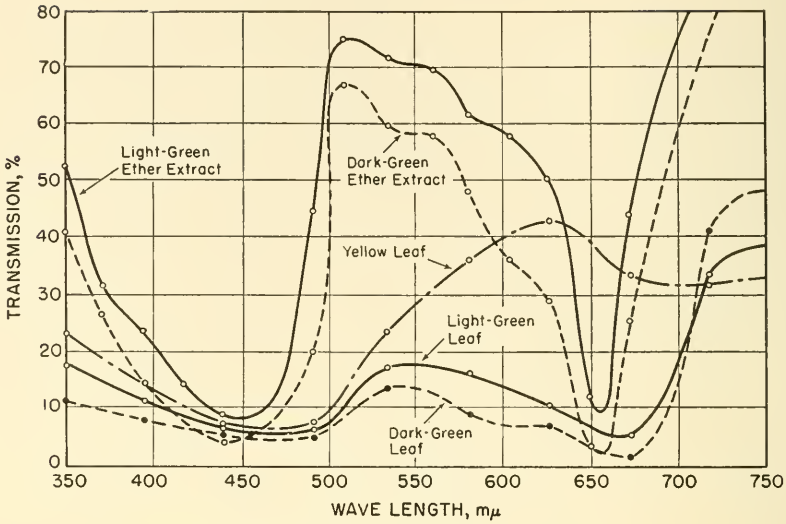

Fig. 22.14. Transmission (T/I) of dark-green, light-green and yellow leaves of *Hibiscus rosa-sinensis*, and of equivalent quantities of pigments in ether (after Loomis, Carr and Randall 1941). With allowance for reflection, the leaves absorbed more light than the extracted pigments in the green part of the spectrum, and less in the blue and red.

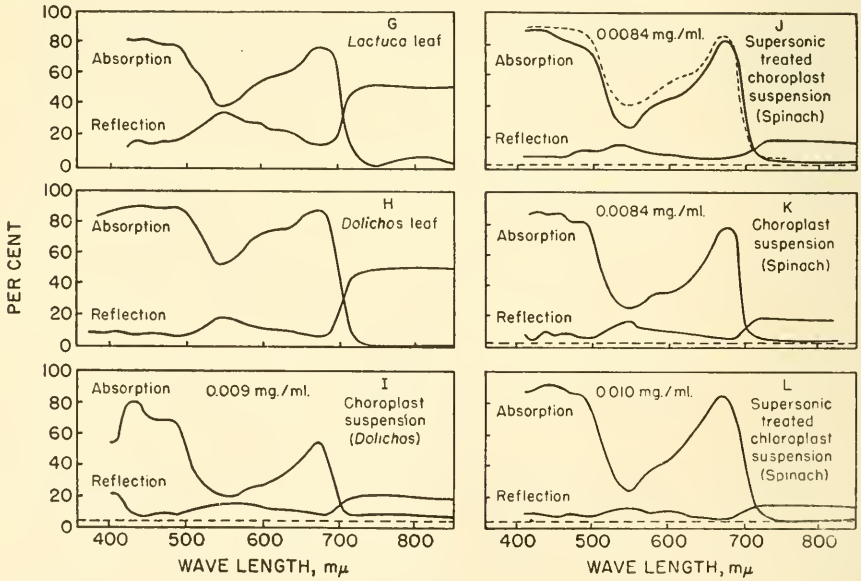

Fig. 22.15. Absorption and reflection spectra of leaves (G, H), of chloroplast suspensions (I, K) and of the supernatant fraction after centrifugation of disintegrated chloroplasts (J, L) (as measured with the Ulbricht sphere) (after Rabideau, French and Holt 1946). The chlorophyll concentrations of the suspensions are given on figures.

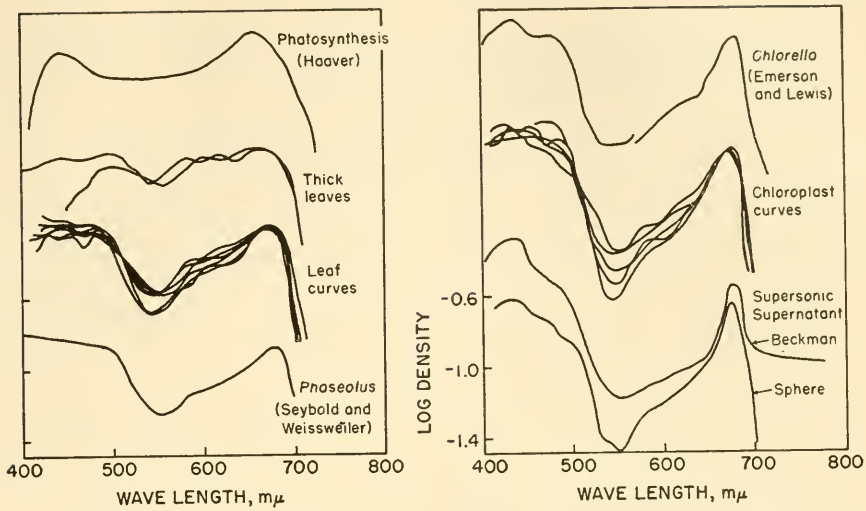

Fig. 22.16. Leaf and chloroplast absorption spectra (on a log density basis and adjusted to the same height at 670 mμ) (after Rabideau, French and Holt 1946). The Beckman curve of the chloroplastin solution (chloroplasts disintegrated by supersonics) is an $I - T$ plot (*i. e.*, it represents absorption plus scattering); the Ulbricht sphere curve is an $I - T - R$ plot (pure absorption). An absorption curve of *Chlorella* (after Emerson and Lewis) and a photosynthesis action curve (after Hoover *et al.*) are given for comparison.

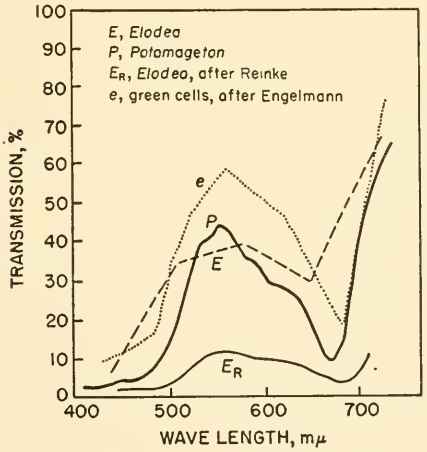

Fig. 22.17. Transmission spectra of water plants (after Seybold 1934).

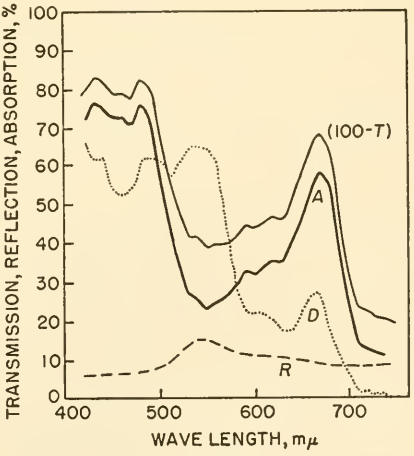

Fig. 22.18. Transmission T, absorption A and reflection R by green alga *Monostroma*. Absorption curve D of red alga *Delesseria* given for comparison (*cf.* fig. 22.20) (after Seybold 1934).*

* For an absorption curve of *Ulva lactuca* and its extracted pigments, see Seybold and Weissweiler (1943[1]).

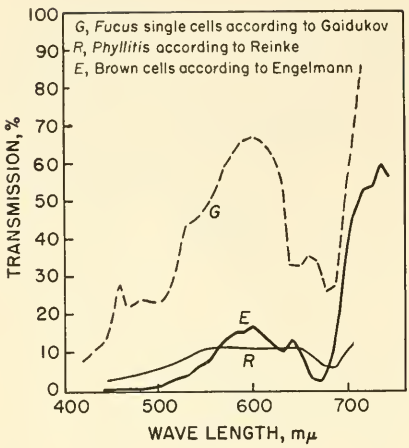

Fig. 22.19. The transmission spectra of brown algae (after Seybold 1934). Maximum of transmission is at 600 mμ instead of ~550 mμ as in green plants and algae (figs. 22.17–18) because of the presence of fucoxanthol. For absorption curves of *Fucus* and *Laminaria*, see Seybold and Weissweiler (1943[1]).

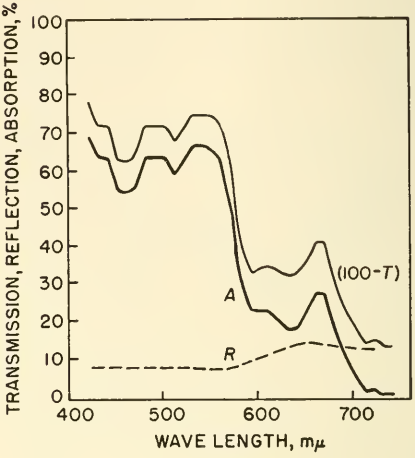

Fig. 22.20. Transmission T, reflection R and absorption A of the red alga *Delesseria* (according to Seybold 1934). More recent curves for *Delesseria* and *Porphyra* are given in Seybold (1943); for *Iridaea* and *Gigartina*, see Van Norman, French and Macdowall (1948).

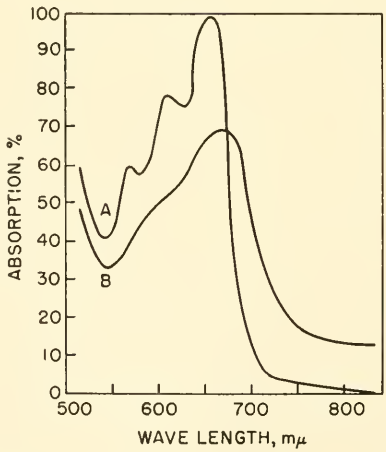

Fig. 22.21. Absorption spectra, $(I - S)/I = (I - T - R)/I$, of *Chlorella* suspension (after Noddack and Eichhoff 1939). About 1.1×10^7 cells and 1.6×10^{-5} g. chlorophyll *a* and *b* in 1 ml. A, 35 ml. pigment extract in a vessel 3.90 cm. thick; B, cell suspension, same volume, same vessel.

spectra" or "absorption spectra," respectively; or one may use colorless specimens as "blanks," determine T_0 and R_0 (or $S_0 = T_0 + R_0$), plot T/T_0, R/R_0 or S/S_0 against λ and designate *these* curves as "transmission," "reflection" or "absorption" spectra, respectively—but, although each of these plots is legitimate as representation of a certain property of the specimen investigated, they are all different. (For example, fig. 22.11, p. 686, shows the transmission spectrum and the reflection spectrum of the same leaf.) The true absorption spectrum on the other hand is an intrinsic property of a molecular species (or, in the case of a mixture, the average of intrinsic properties of several molecular species).

We will discuss the quantitative analysis of the empirical "leaf spectra"

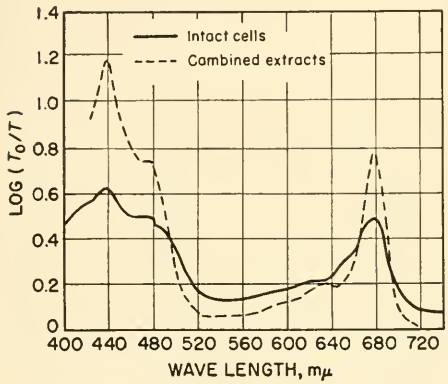

Fig. 22.22. Transmission spectrum of (T_0/T) *Chlorella* suspension (after Emerson and Lewis 1943).

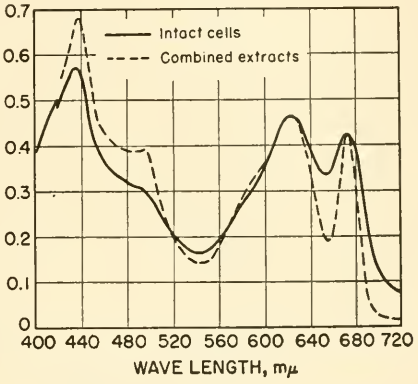

Fig. 22.23. Transmission spectrum (T_0/T) of suspension of *Chroococcus* (blue alga) (Emerson and Lewis 1943).

or "cell spectra" below, in section 4. First, we will present a selection of experimental results, and discuss their qualitative aspects.

Spectral data on *leaves of land plants* have been collected by Ivanovski (1907, 1913), Willstätter and Stoll (1918), Ursprung (1918), Wurmser (1921), Lubimenko (1927), Seybold and co-workers (1932[1,2], 1933, 1934, 1936, 1942[1,2], 1943), Meyer (1939), Loomis *et al.* (1941, 1949), Iljina (1946) and French *et al.* (1946), among others. The curves on pages 686 to 689 are from some of the more recent investigations. Figure 22.17 shows the transmission spectrum of the *water-filled leaves* of the aquatic plants *Elodea* and *Potomageton*, according to Seybold (1933). Transmission spectra of *algal thalli* were measured by Reinke (1886), Engelmann (1884, 1887), Gaidukov (1904), Wurmser (1926) and Seybold and co-workers (1934, 1942[1,2], 1943). Figures 22.18–22.20 show curves given by

the last-named observer. Transmission spectra of *suspensions of unicellular algae* were studied by Noddack and Eichhoff (1939), Katz and Wassink (1939), Emerson and Lewis (1941), Wassink and Kersten (1946), Van Norman, French and Macdowall (1948) and Tanada (1951). Several such curves are reproduced in figures 22.21–22.24.

Albers and Knorr (1937) measured the absorption spectra of single chloroplasts, in the narrow region 664–709 mμ (*cf.* fig. 22.35). Vermeulen, Wassink and Reman (1937), Katz and Wassink (1939), Wassink, Katz and

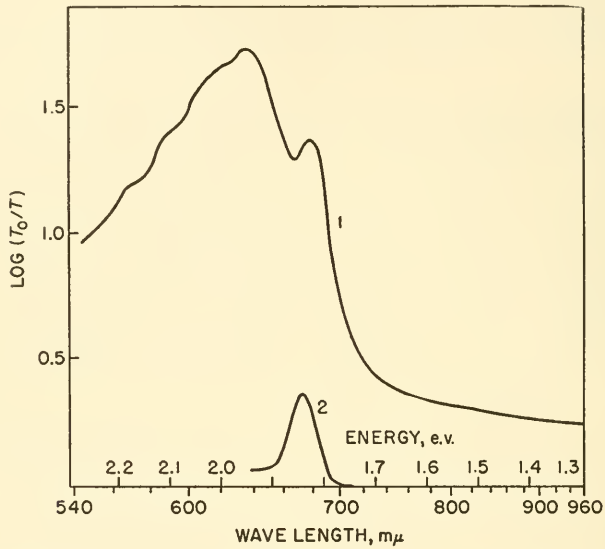

Fig. 22.24. Transmission spectrum (T_0/T) of *Oscillatoria* (blue alga) (after Katz and Wassink 1939). Curve 1, cell suspension; curve 2, chlorophyll extract.

Dorrestein (1939) and French (1937, 1940) observed the transmission spectra of purple bacteria (figs. 22.25 to 22.28).

Egle (1937) and Loomis, Carr and Randall (1941) investigated the transmission and reflection of leaves in the *infrared.* Figures 22.29 and 22.30 show that, from 800 to 1300 mμ, $T + R$ accounts for 85 or 90% of the incident light in the (comparatively thin) potato leaf, and for 75 or 85% in the (0.6 mm. thick) leaf of *Ficus elastica.* This region includes most of the infrared radiation of the sun that reaches sea level. (About 75% of the latter belong to the region 700 to 1500 mμ.) Absorption bands at and above 1.5 μ, shown in figures 22.29 and 22.30, are due to water and carbon

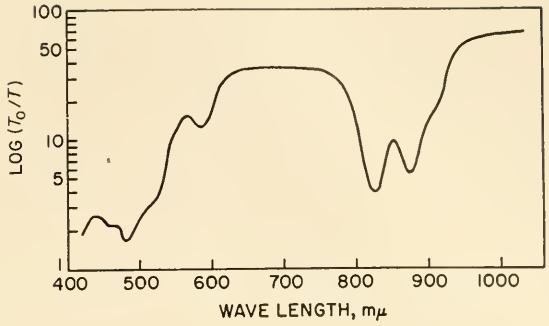

Fig. 22.25. Transmission spectrum (T_0/T) of a *Chromatium* suspension (after Vermeulen, Wassink and Reman 1937).

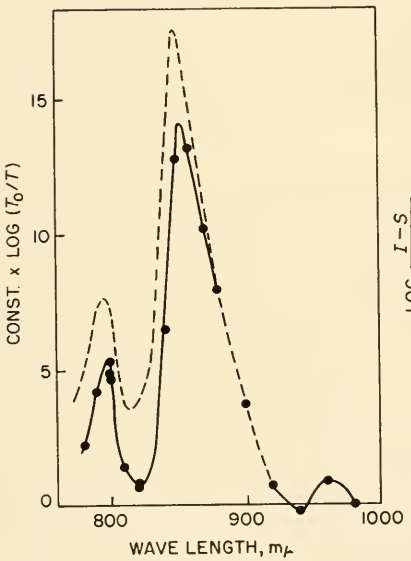

Fig. 22.26. Absorption curve of extracted pigment (broken line) compared with transmission curve of pigment in live cells of *Streptococcus varians* (strain C11) (after French 1940). Absorption bands of extracted pigment shifted to let maxima coincide with those of the live cells.

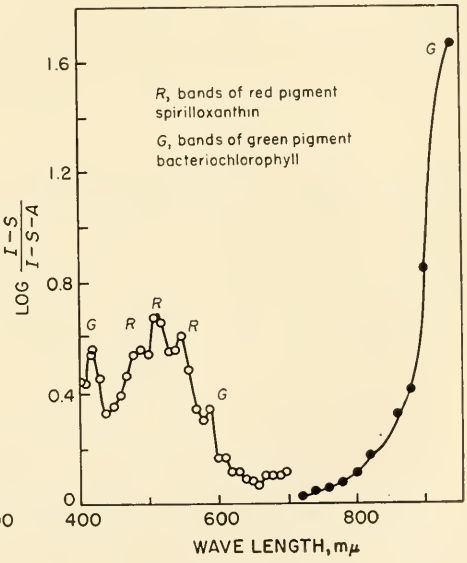

Fig. 22.27. Relative absorption curve of the pigments of *Spirillum rubrum* in living bacteria (measured photoelectrically using a scattering control of bleached bacteria) (after French 1937).

dioxide. Altogether, green plants absorb comparatively little light above 700 mμ—in the region where absorption would probably be useless for photosynthesis, but could cause strong heating.

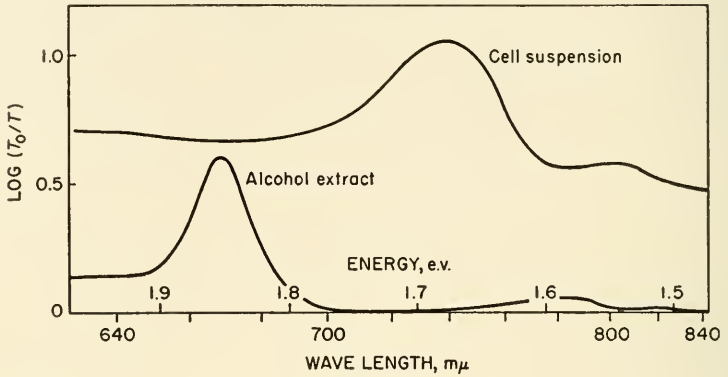

Fig. 22.28. Absorption spectra of pigments of green sulfur bacteria in the red and infrared region (after Katz and Wassink 1939).

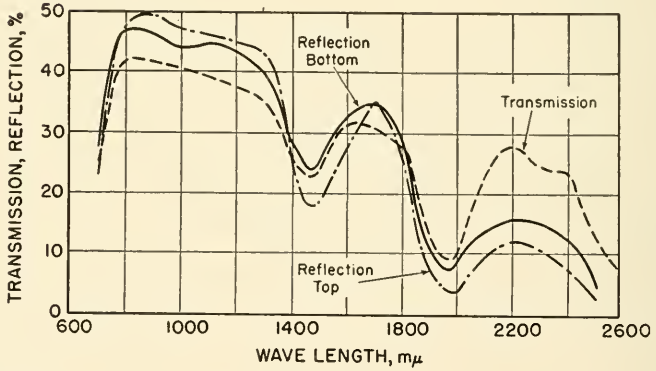

Fig. 22.29. Transmission and reflection of near infrared by potato leaf (*Solanum tuberosum*) (after Loomis, Carr and Randall 1941). Note absorption bands due to water, > 1300 mμ, but very low absorption (reflection + transmission = 90–100%) in the 800 to 1300 mμ region, which contains most of the near infrared rays of sunlight.

Figure 22.31 illustrates the "whiteness" of leaves and conifer needles in the near infrared, by a comparison of the reflection of infrared light by leaves with that by a sheet of white paper. According to Mecke and Baldwin (1937) this lack of absorption (rather than infrared fluorescence) causes the striking brightness of vegetation on infrared landscape photo-

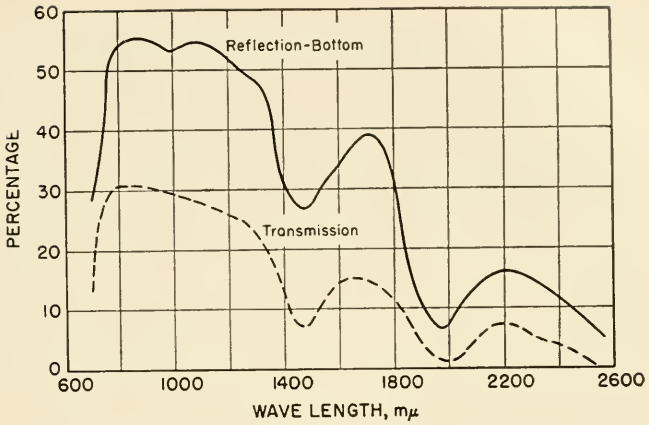

Fig. 22.30. Transmission and reflection of near infrared by a rubber leaf (*Ficus elastica*) (after Loomis, Carr and Randall 1941). This thick (0.8 mm.) leaf has normal reflection, but shows rather general absorption in the transmission spectrum.

Fig. 22.31. Leaves photographed in infrared light on white background (after Loomis, Carr and Randall 1941).

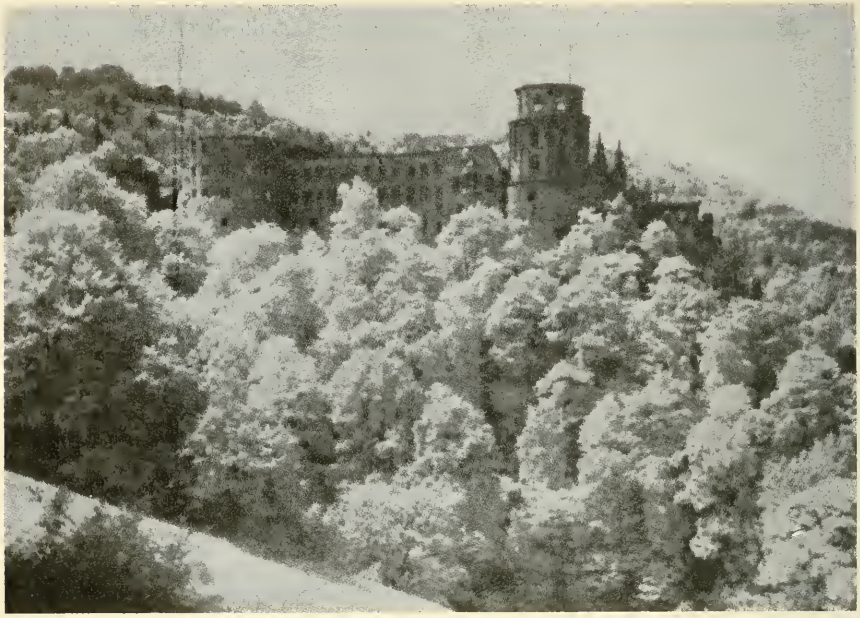

Fig. 22.31A. Summer landscape photographed in infrared light (after Mecke and Baldwin 1937).

graphs (fig. 22.31A). Figure 22.32 shows the *reflection* spectra of three leaves of the same species, but of different ages, as given by Shull (1929).

TABLE 22.II

REFLECTION TRANSMISSION AND ABSORPTION OF LIGHT BY LEAVES OF *Fraxinus excelsior* (AFTER POKROVSKI 1925)

Wave length, mμ	480	500	550	600	620	650
Reflected, %	2.5	4.1	11.5	7.7	6.7	5.4
Transmitted, %	6.4	8.6	17.5	12.4	11.1	9.8
Absorbed, %	91.1	87.3	71.0	79.2	82.2	84.8

The figures of Pokrovski (1925), given in Table 22.II, illustrate the fate of light of different wave lengths falling on a leaf of *Fraxinus excelsior*.

Figure 22.33 shows the reflection spectra of autumnal leaves of different colors (averages for 20–80 different species), as given by Loomis, Carr and Randall (1941). Spohn (1934) observed that the position of the absorption maximum of chlorophyll is shifted in autumnal leaves, from 670 to about 660 mμ, probably indicating the liberation of chlorophyll from the pigment–protein–lipide complex present in photosynthetically active cells.

Two characteristics strike the eye in all the above-reproduced curves; the *shift* of band maxima toward longer waves (as compared with their position in solution spectra), and the *diffuse appearance* of all bands, leading to considerable absorption (or apparent absorption) in those regions (green and extreme red) where pigment solutions are almost completely transparent. Of these two characteristics, the first one can be safely attributed to changes in the intrinsic absorption curves of the pigments in the cell (*cf.* page 698). The shapes of the bands, on the other hand, are affected largely, but probably not exclusively, by scattering, "sieve effect," and other phenomena of geometrical optics.

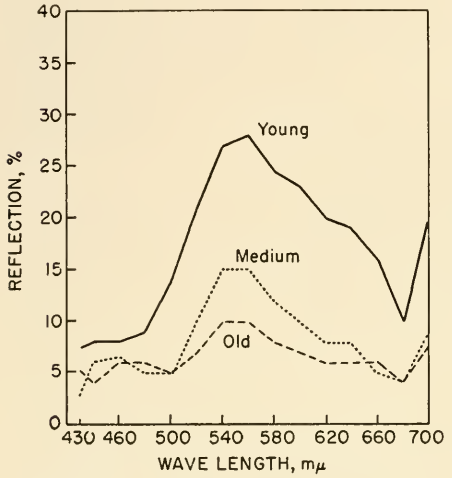

Fig. 22.32. Reflection (R/I) of leaves of *Tilia americana* (after Shull 1929).

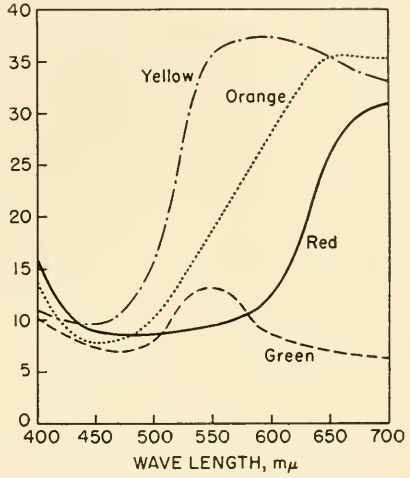

Fig. 22.33. Average reflection from green and fall-colored leaves (after Loomis, Carr and Randall 1941). 20–80 species averaged for each curve.

2. Band Maxima of Chlorophyll and Bacteriochlorophyll in the Spectra of Living Plants

(*a*) *Red Band of Chlorophyll a*

Chlorophyll is responsible for practically all absorption of green plants above 550 mμ (*cf.* page 719), and the main red absorption peak of chlorophyll *a* is recognizable in all plant spectra. Hagenbach in 1870, noticed that this peak is displaced by about 20 mμ toward the infrared compared with its position in ether, alcohol or similar solvents; and Gerland, in 1871, found that a similar shift also affects other, less prominent, chlorophyll bands in the yellow and orange part of the spectrum.

Timiriazev (1872), in an early discussion of the effect of scattering on the spectrum of leaves, suggested that scattering may not only broaden the absorption bands, but also shift their maxima. However, the attribution of the "red shift" to scattering is not permissible, as shown, *e. g.*, by the return of the red band to the position it occupies in solution, after soaking the leaves with ether, or immersing them in boiling water (fig. 22.34) (Willstätter and Stoll 1918, Seybold and Egle 1940, Seybold and Weissweiler 1942). These treatments do not dissolve the pigments, and do not make the tissues more homogeneous; they merely destroy the association

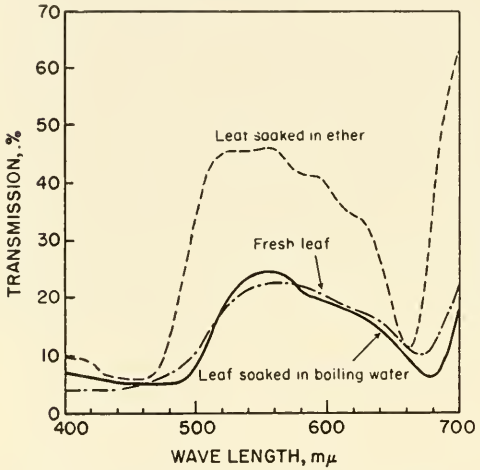

Fig. 22.34. Effect of boiling and immersion in ether on transmission spectrum of a *Parietaria* leaf (after Seybold and Weissweiler 1942).

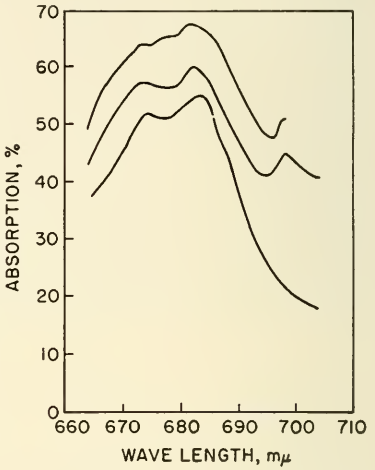

Fig. 22.35. Absorption spectra of single *Protococcus* chloroplasts (after Albers and Knorr 1937).

of chlorophyll with proteins and lipides. Drying, on the other hand, which changes the scattering of light by leaves to a much larger extent than immersion in hot water, does *not* affect the position of the red band maximum (Seybold and Egle 1940).

The conclusion that the position of the red absorption peak in the living cell is not affected by scattering, is confirmed by determinations of the absorption spectra of single *Euglena* cells (Baas-Becking and Ross 1925), and of single chloroplasts (Albers and Knorr 1937). Figure 22.35 shows that the absorption maximum of single *Protococcus* chloroplasts lies close to 680 mμ, *i. e.*, in the same region as in whole plants.

Scattering effects must be weaker than in leaf spectra not only in the spectra of single cells, but also in those of cell suspensions. Several such spectra were reproduced above (*cf.* figs. 22.21 and 22.22 for *Chlorella*,

TABLE 22.III

ABSORPTION MAXIMA IN THE SPECTRA OF LIVING CELLS

Plants	Observer	Type of spectrum[a]	Position of: Main red maximum, λ, mμ	Position of: Other bands (approx.)
LAND PLANTS				
Fatsia (fig. 22.13)	Meyer (1939)	T	677	486, 468, 440
Hibiscus (fig. 22.14)	Loomis (1941)	T	665	470
Pelargonium (fig. 22.12)	Seybold (1932)	A	670	580, 480, 440
Tilia (Fig. 22.32)	Shull (1929)	R	678	
Eight spp. (figs. 22.15, 16)	Ralideau, French and Holt (1946)	A, R	670–680	500–480; 460–430; 660–690; 600–620; 588–580
Fifty different spp.	Lubimenko (1927)	T	663–690	*Cf.* below
Various spp.	Seybold (1942)	T R	678–684 680–684	
AQUATIC PLANTS				
Potomageton (fig. 22.17)	Seybold (1934[2])	T	667	440, 420
ALGAE				
Chlorella (fig. 21.29)	Katz, Wassink (1939)	A	680	
Chlorella (fig. 22.21)	Noddack, Eichhoff (1939)	A	668	
Chlorella (fig. 22.22)	Emerson, Lewis (1941)	T	672	488, 473, 432
Chlorella	Wassink, Kersten (1946)	T	675,625(?)	475, 430
Chlorella	Seybold (1942)	A	680	
Laminaria (fig. 22.19)	Seybold (1933)	T	669	
Nitzschia	Dutton, Manning (1941)	T	680	
Nitzchia	Wassink, Kersten (1946)	T	675,630(?)	580, 470, 440
Delesseria (Fig. 22.20)	Seybold (1933)	T	668,605	540–490[c]
Iridaea	Van Norman *et al.* (1948)	T	675,620[b]	550[c], 480
Chroococcus (fig. 22.23)	Emerson, Lewis (1941)	T	683,625[b]	436
CHLOROPLASTS				
Protococcus, Spirogyra, Zygnema (fig. 22.35)	Albers, Knorr (1937)	T	681–684	698, 687, 676, 679, 673, 675, 667, 669

[a] T = transmission spectrum, R = reflection spectrum, A = absorption spectrum (transmission + reflection). [b] Phycocyanin. [c] Phycoerythrin.

22.23 for *Chroococcus*, and 22.24 for *Oscillatoria*). The "red shift" is recognizable in all of them, particularly in the last three figures, in which the spectra of extracted pigments are compared with those of live cells. True. Noddack's figure (fig. 22.21) indicates only a comparatively small shift —from 660 to 668 mμ—in live *Chlorella* cells, but Katz and Wassink (1939) and Seybold and Weissweiler (1942) found, for the wave length of the red band in *Chlorella*, the value generally given for leaves—about 680 mμ. Finally, it was stated on page 653 (*cf.* figs. 22.15, 22.16 and 21.29) that the red absorption peak is situated at about 675 mμ also in aqueous chloroplastin extracts. The position of the red absorption peak in live cells is thus determined, beyond doubt, by the *state of the pigment* (which is preserved, to a certain extent, in colloidal extracts), and *not* by geometrical–optical conditions.

It has often been suggested that the position of the red absorption maximum of chlorophyll (and the number and position of secondary maxima) varies in different species. Table 22.III gives a summary of experimental results. It shows the red peak at 675 mμ $\pm$ 15 mμ, with the average position corresponding to a "red shift" of 15 mμ, or 370 cm.$^{-1}$ (compared to the position of the corresponding absorption band in ethereal solution of chlorophyll *a*). The extreme limits of variation of $\lambda_{max.}$ in Table 22.III are 665 and 690 mμ—quite a wide range. However, because of the diffuse character of the spectra, the position of the maximum often cannot be determined precisely. It is therefore not certain whether any of the tabulated variations in λ_{max} are real. (It will be noticed that four values are given for *Chlorella*, 680, 668, 672 and again 680 mμ!)

Lubimenko (1927), who made photographs of the absorption spectra of a large number of leaves, insisted that they do exhibit real differences—not only in the positions of the main peak, but also in the number end positions in the secondary absorption maxima in the yellow, green and blue.

According to Lubimenko, the spectra of some species ("group 1") contain *eight* bands in the visible region. An example is nettle (*Urtica dioica*), with the following bands: I, 680–660; II, 650–645; III, 630–606; IV, 600–570; V, 550–540; VI, 512–480; VII, 450–430; and VIII, below 420 mμ. The bands III, IV and V become visible only when two leaves are used. Leaves of "group 2," which includes the largest number of species, show *seven* bands (band VII of the above list is missing). *Elodea canadensis* and *Hedera helix* belong to this group. "Group 3" (*e. g.*, *Prunus laurocerasus*) shows only *six* bands (bands I and II are fused). Finally, plants of "group 4" (*e. g.*, the alga *Ulva lactuca*) have only *four* bands: I, II, V, VI and VIII. The absence of band III in these spectra is particularly remarkable. Table 22.IV shows the limits of variation in the positions of the above-listed bands, as given by Lubimenko, and also gives a tentative identification of these bands with the bands of chlorophylls *a* and *b* in solution. According to Lubimenko, in passing from species to species, different bands are displaced by different amounts, or even in different directions.

TABLE 22.IV

ABSORPTION BANDS IN LEAVES (AFTER LUBIMENKO 1927)

Band	I (*a*)	II (*b*)	III (*a*)	IV (*a,b*)		V (*a,b*)	VI (*a,b*)	VII (*b,a*)		VIII
Chl (*a* + *b*) in ether[a], λ.....	669–655	648–638	619–605	599–570	571–559	547–523	506–489	467–446	439–424	415→
Leaves, λ from	700–680	660–650	630–620	600–570	—	555–542	510–490	450–430	—	418→
to.....	680–655	648–638	620–610	595–575	—	545–535	505–480	—	—	430→

[a] *cf.* Table 21.I.

Photometric curves prove that many of Lubimenko's visually recorded "secondary bands" are only shoulders on the slopes of the main bands or slight ripples on almost uniformly high absorption plateaus (*cf.*, for example, figs. 22.13 and 22.15). Apparent shifts in the positions of these "maxima," or even the disappearance of some of them, could easily be caused by changes in the ratios of chlorophylls *a* and *b*, as well as by variations in scattering. Lubimenko saw in these differences an evidence of variability of chemical composition of "natural chlorophyll" (a name given by him to a hypothetical complex formed by proteins with all the plastid pigments).

Albers and Knorr (1937) found that the number and position of the absorption maxima in single chloroplasts of *Protococcus*, *Spirogyra* and *Zygnema* vary not only from species to species, but also from specimen to specimen. In addition to the maxima at 681–683 mμ and 672–675 mμ, which may be attributed to chlorophylls *a* and *b*, respectively, some chloroplasts showed secondary maxima at 667–669 mμ, 678–679 mμ and—rather unexpectedly—also in the far red, at 698 mμ (*cf.* fig. 22.35).

Albers and Knorr considered these results as indicating variations in the chemical nature of chlorophyll (*e. g.*, oxidations or reductions) that they thought might be associated with the participation of chlorophyll in photosynthesis (*cf.* Vol. I, chapter 19).

(*b*) *Red Band of Chlorophyll b*

The main red absorption band of *chlorophyll b* is noticeable, according to Lubimenko (1927), in some spectra of leaves (groups 1 and 2, *cf.* page 700) but not in others (groups 3 and 4). There is no doubt that this band, too, is shifted toward the red from its position in solution (642.5 mμ in ether), but the extent of the shift is difficult to measure, because the band appears merely as a hump on the short-wave side of the main chlorophyll *a* band. Lubimenko's table (Table 22.IV) gives, for the band axis,

values from 643 to 655 mμ. This seems to indicate a somewhat lesser shift than is found with the *a*-component; Table 21.VI gives the impression that the same may be true also for the spectra of the two chlorophylls in different solvents *in vitro*.

The clearest indication that chlorophyll *b* retains its identity in live cells is provided by the *fluorescence spectrum*. According to Tables 24.I and 24.II, the axis of the main fluorescence band of chlorophyll *b in vivo* lies at about 656 mμ, *i. e.*, only about 5 mμ further toward the red than in ether solution (while the axis of the corresponding *a* band is displaced at least twice as much). This, too, indicates that the absorption band of chlorophyll *b* in the living cell probably lies at 647–648 mμ.

(*c*) *Red and Infrared Bands of Bacteriochlorophyll*

It was stated on page 642 that the bands of *bacteriochlorophyll* are more susceptible to shifts under the influence of the solvent than are those of chlorophyll. We are therefore not astonished to find that the two bands of bacteriochlorophyll, which are situated in methanol solution at about 605 and 770 mμ, respectively (*cf.* page 617, fig. 21.6), are replaced in live bacteria by bands situated much further in the infrared—at approximately 800 and 850–870 mμ in figs. 21.30, 22.26 and 22.36, and at 825 and 875 mμ in fig. 22.25. Figures 21.30A and 22.26 (*cf.* also Table 22.V) indicate a third absorption band close to or beyond 900 mμ, while figure 22.27 shows only one, very strong infrared band beyond 900 mμ.

Wassink, Katz and Dorrestein (1939) found that the absorption spectra of purple bacteria vary from strain to strain, as illustrated by Table 22.V and figure 22.36.

Alcoholic extracts from all organisms listed in Table 22.V showed only one absorption maximum in the red (at 774 mμ, *cf.* fig. 21.30B) in place of the *two* maxima of most *Athiorhodaceae* (a weak one at 800 and a sharp one at 875 mμ), and *three* of some *Athiorhodaceae* and all *Thiorhodaceae* (at approximately 800, 850 and 895 mμ). Analysis of the band shapes made it probable that the spectra of *all* purple bacteria contain three infrared bands, even if one of them may sometime be concealed by the other two. Similar variations in the spectra of different species and strains of purple bacteria were observed by French (1940).

The correlation of the "cell bands" with the "solution bands" of bacteriochlorophyll (fig. 21.6) is not clear. Shall the cell band at 800 mμ be considered as the displaced solution band at 605 mμ, and the cell band at 850–870 mμ as the displaced solution band at 770 mμ? This would mean a shift by as much as 4000 cm.$^{-1}$ for the "orange" and 1500 cm.$^{-1}$ for the "red" band. The first shift is so large that one is inclined to doubt the

correlation. An alternative is to consider *both* cell bands, that at 800 as well as that at 850–870 mμ (and perhaps that at 900 mμ, too) as related to the one solution band at 770 mμ. This means "red shifts" varying between 500 and 2000 cm.$^{-1}$, and implies that bacteriochlorophyll is present, in purple bacteria, in at least three different pigment-bearing complexes.

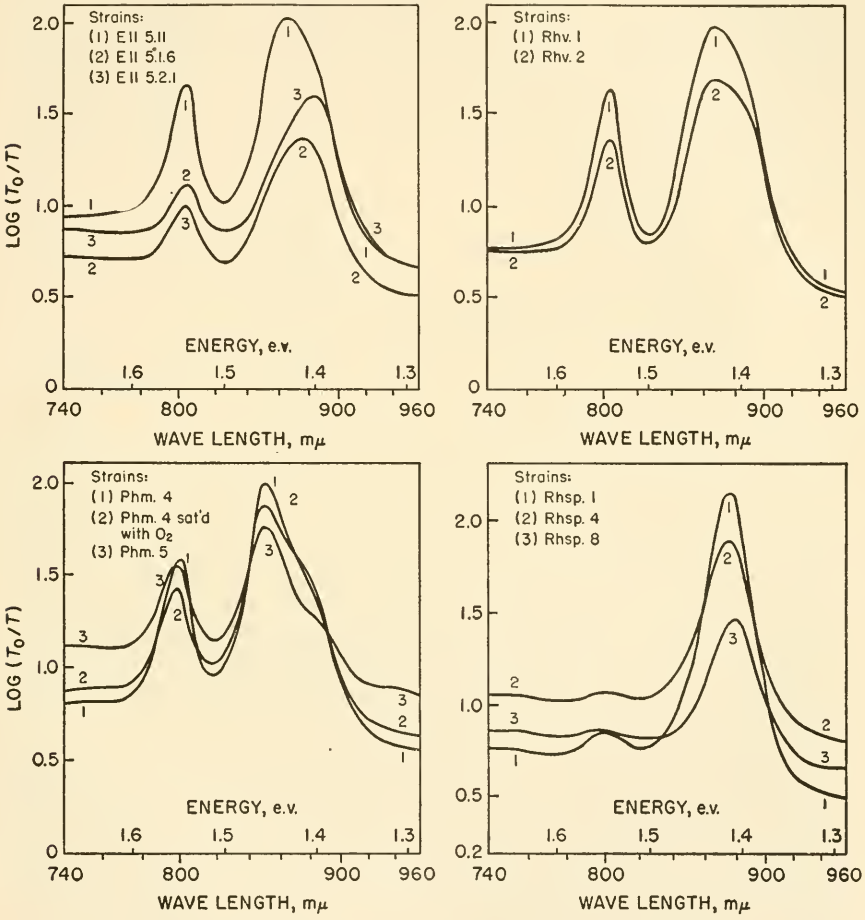

Fig. 22.36. Infrared absorption spectra of suspensions of different strains of *Athiorhodaceae* (after Wassink, Katz and Dorrestein 1939).

The wide variations in the relative intensities of the three absorption peaks (illustrated by the several curves in figure 22.36, and even more strikingly by the curves in figures 21.30A, 21.30B, 22.26 and 22.7) can then be attributed to differences in the relative amounts of the several

TABLE 22.V

ABSORPTION MAXIMA OF PURPLE BACTERIA (AFTER WASSINK, KATZ AND DORRESTEIN 1939)

Species and strain	$\lambda_{max.}$, mμ		
	I	II	III
Thiorhodaceae			
Strain D (Roelofson)	895	854	803.5
Strains 1, 4, 7,12 (van Niel)	895	850.5 856	796
Strains 9, b, 19 (van Niel-Muller)	895	852.5	796
Strains 101, 201	Weak	865	803.5
Strain, 301, 401	895	858	
Athiorhodaceae			
Rhodovibrio (2 strains)	865	—	802.5
	864	—	803
Rhodobacillus palustris (3 strains)	881 873 862.5	—	802
Phaeomonas varians (*Streptococcus varians*) (3 strains)	885 885 892.5	850.5	799 799 798.5
Rhodospirillum rubrum (2 strains)	875 878	—	800

complexes in the individual species and strains. The complexes may be formed by combination of the same pigment (bacteriochlorophyll) with different proteins, each complex being perhaps specifically adapted to the utilization of one reductant, such as hydrogen, sulfide, thiosulfate or an organic hydrogen donor (this hypothesis was suggested by Wassink, Katz and Dorrestein). Other possibilities include several isomeric or tautomeric forms of bacteriochlorophyll, or small differences in chemical composition, or in the reduction level, of the pigment.

If this interpretation of the three cell bands is correct, the question arises as to the reasons for the absence in live cells of a counterpart to the 605 mμ solution band of bacteriochlorophyll. No answer can be given to this question—except that the matter requires renewed, and more systematic, investigation. It was mentioned in chapter 21 (page 618) that the role of the 605 mμ band is not quite clear even in solution spectra.

According to Katz and Wassink (1939), live *green sulfur bacteria* have two absorption maxima (at 740 and 810 mμ, respectively) instead of the single band found in bacterioviridin extracts (at 668 mμ, *cf.* fig. 22.28). According to the statement on page 642 the width of the "red shift" is in agreement with the hypothesis that bacterioviridin is a derivative of tetrahydroporphin (as assumed on page 445 in Vol. I).

(*d*) *Blue-Violet Bands of Chlorophyll*

The position, in live cells, of the second main band of chlorophyll or bacteriochlorophyll—that situated in the blue-violet part of the spectrum—has received much less study than that of the red band, the main reason being that the presence of carotenoids and other yellow pigments tends to make absorption in this region very heavy and diffuse. Table 22.VI contains some values read from figures reproduced earlier in this chapter. This table indicates a "red shift" by 5 or 10 mμ. On the frequency scale, this shift (250–500 cm.$^{-1}$) is about equal to that of the main red band.

TABLE 22.VI. BLUE-VIOLET ABSORPTION MAXIMA IN LIVING PLANTS

Organism	$\lambda_{max.}$, mμ
Fatsia (*cf.* fig. 22.13)	438
Chlorella (*cf.* fig. 22.22)	432
Chroococcus (*cf.* fig. 22.23)	436
Chlorophyll *a* in ether	427.5

(*e*) *Protochlorophyll*

French (1951) estimated, from the action spectrum of chlorophyll formation, that the red absorption band of protochlorophyll lies, *in vivo*, at 650 mμ. Because of its weakness, it has not yet been directly observed.

3. Absorption Bands of Accessory Pigments in Live Cells

Lubimenko (1927) could not find, in the spectra of green leaves, absorption maxima identifiable with the absorption bands of the carotenoids (which could easily be observed, at 510–480 mμ, in the spectra of yellow leaves or yellow parts of variegated leaves). He pointed out that the first carotenoid maximum may be masked by band VI of chlorophyll (*cf.* Table 22.IV) and that the second one, at 470–450 mμ, should be visible between the chlorophyll bands VI and VII. Lubimenko concluded that the yellow pigments do not exist "as such" in the green plastids. This conclusion, obtained by a purely qualitative examination of the spectra, is untenable. The spectra of leaf extracts, in which the chlorophylls and the carotenoids certainly exist as separate entities, also do not always show the individual maxima of all these pigments. In figure 22.47, for example, the absorption curve of the extract from barley leaves shows only three maxima between 400 and 500 mμ—at 410, 428 and 450 mμ—instead of the six maxima of the separated green and yellow pigments. The two main carotenoid

maxima (at 422 and 467 mμ) and one chlorophyll maximum (at 500 mμ) are lost by the superposition of the absorption curves.

Upon closer examination, carotenoid maxima *can* be identified at least in some plant spectra. Peaks, which probably belong to the carotenoids, are noticeable, for example, at 468 and 486 mμ in the spectrum of the leaves of *Fatsia* (Fig. 22.13, *cf.* also Table 22.III), and at 473 and 488 mμ in that of *Chlorella* (Fig. 22.22). If one attributes both these peaks to luteol, whose absorption bands in ethereal solution are situated at 442 and 472 mμ (*cf.* Table 21.I), it follows that the carotenoid bands in live cells are shifted toward the red by as much as 25–30 mμ. ($\Delta\nu$ = 1250 cm.$^{-1}$, or three times the shift of the red and blue-violet absorption bands of chlorophyll.) Emerson and Lewis (1942) postulated, in their interpretation of the *Chroococcus* spectrum (*cf.* page 723), a shift of the carotenoid bands *in vivo* by 14 mμ from their position in ethanolic solution; according to Table 21.I, this corresponds to a shift by 24 mμ relative to ethereal solution, in good agreement with the preceding estimate (25–30 mμ).

Menke (1940) extracted chlorophyll from chloroplast preparations (made from spinach leaves; *cf.* Vol. I, page 369); and found that a brick-red residue was left. A suspension of this residue in water showed absorption bands at 490 and 540 mμ—much further toward the red than the carotenoid bands have been observed in live green plants (470 and 490 mμ were the positions quoted above for *Fatsia* leaves and *Chlorella* cells). Moistening with ether led to a change of color, and a shift of the absorption bands to their usual positions in carotenoid solutions (442 and 472 mμ).

The absorption spectrum of the brown alga *Laminaria* was found by Menke to exhibit bands— probably due to fucoxanthol—at even longer waves, namely, 499 and 545 mμ. Heating to 70° C. led to a shift of these bands to below 510 mμ, and to a color change from brown to green.

The transmission curves of diatoms (*Nitzschia dissipata*) published by Wassink and Kersten (1946), as well as the absorption curves of brown algae given by Seybold (1934, 1943), clearly show an increased absorption (in comparison to the green algae, such as *Ulva* or *Chlorella*) in the region 500–580 mμ, but give no indication as to the position of the absorption peak (or peaks) of the carotenoid responsible for this absorption. From the comparison of the transmission curves of live diatoms (and of aqueous colloidal cell extracts, whose brownish color is similar to that of cell suspensions) with the transmission curves of the (green) pigment extract in an organic solvent (methanol and petroleum ether), Wassink and Kersten estimated that the fucoxanthol bands are shifted *in vivo* by about 20 mμ (corresponding to about 700 cm.$^{-1}$), toward the longer waves; but this estimate is not at all reliable because of the absence of pronounced maxima. These curves, and Karrer's absorption curves of fucoxanthol in solution, make it appear uncertain whether the increased absorption of diatoms and brown algae in the green (500–560 mμ) is due mainly (or exclusively) to a

strong red *shift* of the fucoxanthol band as a whole, or to a *broadening* of this band toward the longer waves.

These observations give some information about the strength with which the carotenoids are bound to the protein–pigment–lipide complex of the chloroplasts. According to equation (21.4) and figure 21.23, the "red shift" can be caused by association of the light-absorbing molecule with other molecules (by adsorption, solution or complexing). The shift is approximately equal (on the energy scale) to the difference between the binding energies of the normal and the excited pigment molecule. In the case of green leaves and algae, we found the chlorophyll bands to be shifted *in vivo* by about 370 cm.$^{-1}$, while the luteol bands were shifted by as much as 1250 cm.$^{-1}$ (according to Meyer, and Emerson and Lewis), perhaps even by 2220 and in some cases 2530 cm.$^{-1}$ (according to Menke). The fucoxanthol bands in brown *Laminaria* are, according to Menke, shifted still more widely—by 2585 and 2815 cm.$^{-1}$.

All these shifts are relative to the band position in ether solution. A better idea of the binding energies could be obtained by comparison with extrapolated positions of the bands of isolated pigment molecules. Such an extrapolation was made for chlorophyll and bacteriochlorophyll on page 642, but it is not yet possible for the carotenoids.

The strong red shifts of the carotenoid bands—particularly those of fucoxanthol—indicate clearly that these pigments form part, in chloroplasts, of some complex, and that the binding becomes particularly strong when the carotenoid molecules are electronically excited. This fact may be relevant for the transfer of electronic excitation energy from the carotenoids (particularly fucoxanthol) to chlorophyll, a phenomenon that is revealed by the occurrence of fucoxanthol—sensitized fluorescence of chlorophyll *in vivo* (*cf.* chapter 24, page 814)—and that probably explains also the participation of carotenoids in the sensitization of photosynthesis (*cf.* chapter 30).

The absorption peaks of the carotenoids appear especially clear on French's (1937) spectral transmission curves of *purple bacteria* (*cf.* fig. 22.27), because in this case the carotenoid bands fall between the two absorption bands of bacteriochlorophyll, at about 600 and 400 mμ.

Differences in the carotenoid bands of brown and red varieties of *Streptococcus arians* were described by French in a later paper (1941).

The absorption bands of the *phycobilins* are clearly discernible in the spectra of blue and red algae, *e. g.*, in figure 22.20 at about 550 mμ (phycoerythrin) and in figure 22.23 in the neighborhood of 625 mμ (phycocyanin). According to Emerson and Lewis (1942), the phycocyanin maximum is shifted by about 6 mμ toward shorter waves in the aqueous cell extract (figs. 22.23 and 22.48), while the absorption peaks of other pigments retain the positions they had in living cells. This indicates that in the extract,

TABLE 22.VII

RATIOS OF OPTICAL DENSITIES OF CHLOROPLAST PIGMENTS IN THE RED MAXIMUM, GREEN MINIMUM AND VIOLET MAXIMUM

Material	System	Authority	*Cf.* fig.	Ratios: green/red	Ratios: violet/red	Quantity plotted[a]
Solutions..	1. Chlorophyll *a* in ether	Zscheile, Comar (1941)	21.1	0.01	1.35	$\log (T_0/T)$
	2. Chlorophyll $(a + b)$ + carotenoids in ether	Strain (1938)	22.47	0.05	1.75	$\log (T_0/T)$
	2a. Diatom pigments in methanol and petroleum ether	Wassink, Kersten (1946)	—	0.12	3.0	$\log (T_0/T)$
Colloids...	3. Chlorophyll $(a + b)$, colloidal	K. P. Meyer (1939)	21.27	0.29	1.62	$\log (T_0/T)$
	4. Aqueous protein–pigment suspension (from spinach)	Smith (1941)	21.28(A)	0.25	1.66	$\log (T_0/T)$
	5. Aqueous protein–pigment solution in digitonin	Smith (1941)	21.28(B)	0.13	1.60	$\log (T_0/T)$
	5a. Diatom pigments in aqueous suspension	Wassink, Kersten (1946)	—	0.63	2.0	$\log (T_0/T)$
Algae.....	6. *Chlorella* suspension	Noddack, Eichhoff (1939)	22.21	0.33	—	$\log (I/S)$
	7. *Chlorella* suspension	Emerson, Lewis (1941)	22.22, 22.43	0.28	1.40	$\log (T_0/T)$
	8. *Monostroma* (thallus)	Seybold (1934)	22.18	0.29	1.55	$\log (I/S)$
	8a. *Chroococcus* suspension	Emerson, Lewis (1942)	22.48	(0.4)[b]	1.36	$\log (T_0/T)$
	8b. *Nitzschia dissipata* suspension	Wassink, Kersten (1946)	—	0.50	1.5	$\log (T_0/T)$
	8c. *Navicula minima* suspension	Tanada (1951)	—	0.50	1.34	$\log (T_0/T)$
	8d. Same, in glycerol	Tanada (1951)	—	0.37	1.36	$\log (T_0/T)$
Leaves....	9. *Fatsia* (leaves)	K. P. Meyer (1939)	22.13	0.41	1.01	$\log (T_0/T)$
	10. *Pelargonium* (leaves)	Seybold (1933)	22.12	0.63	1.19	$\log I/S$
	11. *Hibiscus* (light green leaves)	Loomis *et al.* (1941)	22.14	0.57	0.89	$\log (T_0/T)$

[a] I = incident flux, T = transmitted flux, S = scattered flux in all directions (includes T) (*cf.* equation 22.8).
[b] Phycocyanin absorption.

the cyanobilin-proteid becomes dissociated from the pigment–protein–lipide complex present in the live cell. It was stated in chapter 15 (Vol. I, page 399) that, of all plastid pigments, only the phycobilin chromoproteids pass into true colloidal solution upon extraction with water.

To sum up, it is certain that all the pigments found in extracts from plants retain their spectroscopic identity in live cells, despite their probable close association in a common complex. The association causes, however, considerable band shifts, and probably also changes the shape of the bands, particularly those of accessory pigments, such as fucoxanthol.

4. True Absorption Spectrum of the Pigment Mixture in the Living Cell

In the two preceding sections, we tried to derive as much information as possible from the positions of the absorption maxima in the empirical plant spectra described and reproduced in section 1. It was mentioned repeatedly that the difference between the "plant spectra" and the spectra of extracted pigments is not limited to band shifts, but includes also changes in the height, width and shape of the individual bands. However, as stated on page 697, the latter changes are, to a large extent, the product of scattering and other geometrical–optical phenomena. In the present section, we will deal first with a more detailed description of the appearance of the absorption bands in living plant cells, and then with the possibility of deriving from the empirical plant spectra the true absorption curves of the pigment mixtures contained in them.

General diffuseness was stated on page 697 to be the most striking characteristic of plant spectra as compared to the absorption spectra of the pigment extracts. The ratios of the "optical densities," log (I/S) or log (T_0/T) in the absorption "peaks" and "valleys" can serve to illustrate this statement.

Table 22.VII shows that the ratio of the optical densities in the "green minimum" and the "red maximum," which, according to Table 21.IB is less than 0.01 in pure chlorophyll *a*, and about 0.05 in an ether extract from barley leaves (which contains all the chloroplast pigments), is as high as 0.3 in live algae and may reach 0.6 in green leaves.

The ratio (violet peak : red peak) also is changed: it declines from 1.75 in ethereal extract, to 1.6 in colloidal aqueous extracts, 1.4 to 1.5 in live algae, and 0.9 to 1.2 in green leaves.

It will be noted that the ratios derived from true absorption spectra, log (I/S), are not very different from those derived from the transmission

spectra, log (T_0/T), obtained by comparison of colored with colorless tissues (or, in the case of cell suspensions, by comparison with pure water).

Similar data for a variety of green, yellow and red leaves can be found in Seybold and Weissweiler's paper (1943).

The question arises: can the leveling off of the absorption peaks and the filling in of the absorption valleys be attributed *entirely* to geometrical–optical effects (scattering and "sieve effect") or do they indicate a genuine deformation of the absorption curves of the pigments (which could be caused by close packing of pigment molecules in the chloroplasts, as well as by their association with proteins and lipides)?

The fact that the leveling off is much stronger in the spectra of leaves than in those of algae indicates that geometrical–optical effects account for a considerable part of the phenomenon. Tanada's (1951) observations with glycerol (table 22.VII) support this interpretation. Clearly, scattering and "sieve effect" must lead to an apparent decrease in the selectivity of absorption, as far as plots of log (I/T) or of log (T_0/T) are concerned, since, in these two representations, losses of the weakly absorbed (green and extreme red) light by scattering obviously simulate absorption. One could attempt to explain in this way the results under 4, 5, 7, and 8a–8d in Table 22.VII, which were obtained by the use of blank cells with pure water. On the other hand, the similarity of results of the experiments listed under 6 and 8, in which the *true absorption* of *Chlorella* cell suspensions was determined, with the results listed under 7, in which the *transmission* of a similar suspension was measured, cannot be explained in this way.

In the case of leaf spectra too, it is not immediately obvious why diffuse scattering should lead to transmission curves, log (T_0/T) (obtained by comparison of green with white specimens), characterized by high optical density in the regions of weak pigment absorption (green and far red).

If one would assume, for example (*cf.* Meyer 1939), that the weakening of the transmitted beam by passage through a green leaf can be represented by the equation:

$$T = I \times 10^{-(K\alpha_\lambda + \sigma_\lambda)} \tag{22.9}$$

where K is a proportionality constant, equivalent to the product (concentration × thickness of the absorbing layer) in Beer's law, and σ a "scattering coefficient"; and assuming also that the corresponding equation for the white leaf is:

$$T_0 = I \times 10^{-\sigma_\lambda} \tag{22.9A}$$

then the "transmission curve" would be given by the equation:

$$\log T_0/T = -K\alpha_\lambda \tag{22.10}$$

In other words, the "transmission curve" would follow faithfully—except for a proportionality factor K—the true absorption curve of the

pigments in the cell. Plotted on a semilogarithmic scale (log log $[T_0/T]$ as function of λ), the curves with and without scattering would run parallel. If this were the case, the ratios in table 22.VII derived from the transmission curves, log (T_0/T), would be unaffected by scattering. Closer examination shows why scattering can produce a distortion of the absorption curves in the sense observed. This can be shown with the help of the simple example discussed on page 673—that of repeated reflections in a plane-parallel absorption cell. The equation usually applied for the calculation of the absorption coefficient, α, is:

$$\alpha = (1/d) \log (T_0/T) \tag{22.11}$$

where d is the depth of the absorption cell, and T and T_0 the fluxes transmitted through the solution and the pure solvent, respectively. This equation was shown on page 674 to be a first approximation, neglecting the difference in the reflectances of the two cells. As mentioned on page 674, correct expressions for T and T_0 can be obtained by summation of infinite series. This summation leads to the following relationship:

$$T = T_0\, 10^{-\alpha d} \left(\frac{1 - r^2}{1 - r^2\, 10^{-2\alpha d}} \right) \tag{22.12}$$

and thus to:

$$\alpha = \frac{1}{d} \left[\log \frac{T_0}{T} - \log \left(\frac{1 - r^2\, 10^{-2\alpha d}}{1 - r^2} \right) \right] \tag{22.13}$$

Since the term in parentheses is >1, equation (22.13) shows that the value of α, calculated in the usual way from (22.11), is *too large* and that *the relative error increases with decreasing absorption.* With $r = 0.1$ and $T_0/T = 1.01$, *i. e.*, an absorption of only 1%, the error in α is 2%. Such an error can be neglected in most absorption measurements. What matters to us, however, is the fact that the percentage error depends on the value of α and *therefore changes with wave length.* In other words, it causes a *distortion of the transmission curve.*

The character of this distortion—increase of apparent α values for *all* wave lengths, but particularly for those in which the true absorption coefficient is small—remains the same in scattering media, where the effect becomes much stronger than in plane-parallel cells filled with transparent materials.

The loss of selectivity by scattering can be even more pronounced in *absorption* spectra, log (I/S), than in *transmission spectra,* log (T_0/T). Radiations that would be only weakly absorbed by straight passage through a leaf (*e. g.*, green or extreme red light) become more strongly absorbed

when their optical path in the leaf is increased by scattering. On the other hand, a certain proportion of radiations that would be almost completely

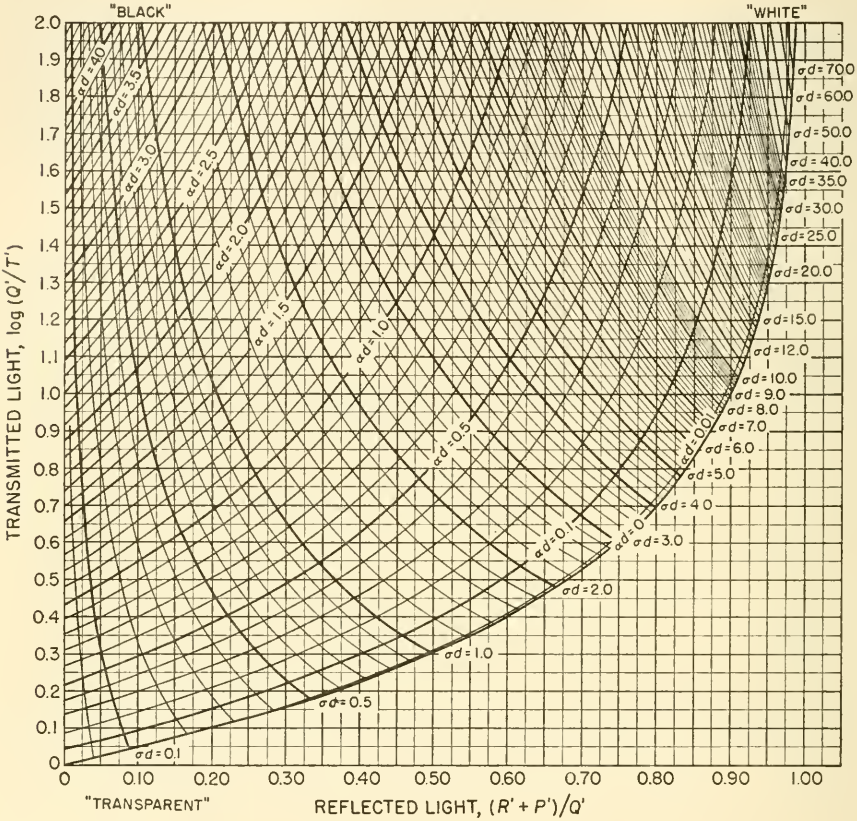

Fig. 22.37. Nomograph for determination of absorption and scattering coefficients in turbid media for diffuse reflection and transmission measurements (after Duntley).

$$(22.14) \quad \frac{R' + P'}{Q'} = \frac{\sigma d \sinh Kd}{(\alpha d + \sigma d) \sinh Kd + Kd \cosh Kd}$$

$$(22.15) \quad \log \frac{1}{T'} + \log Q' = \log \frac{(\alpha d + \sigma d) \sinh Kd + Kd \cosh Kd}{Kd}$$

$$(22.16) \quad Kd/K = \sqrt{\alpha d \, (\alpha d + 2\sigma d)}$$

absorbed if forced to pass straight through the leaf (*e. g.*, blue-violet or red light) will be enabled to escape from it by diffuse reflection, which shortens the optical path in the leaf. Therefore, in a plot of log (I/S) versus λ

in a scattering medium, we will find that not only are the valleys less deep, but also the peaks are less high than in a similar plot in a nonscattering system.

This qualitative explanation of the effect of scattering on selective transmission and absorption can be replaced by an exact analysis if the system satisfies certain conditions—namely, random distribution of scattering centers and—in the simplest forms of the theory—equal probability of scattering in all directions.

Equations for combined absorption and scattering in systems of this type have been derived by several authors; we will mention here, as examples, the investigations by Wurmser (1941), Duntley (1942, 1943) and Saunderson (1942). Figure 22.37 represents a nomograph constructed by Duntley (1942). In this diagram, the abscissae are the expressions: $(R' + P')/Q'$, and the ordinates the expressions: $\log (1/T) + \log Q'$, whose relation to the absorption coefficient (per unit path), α, and the scattering coefficient (per unit path), σ, is shown in the inserted formulae (d being the depth of the layer measured). The constants P' and Q' are characteristic of the asymmetry of the scattering. If scattering is isotropic, and the incident, transmitted and reflected light are perfectly diffuse, $P' = 0$ and $Q' = 1$, and the abscissae in the figure are simply the measured reflectances, while the ordinates are the measured optical densities (logarithms of inverse transmittance). The nomograph remains approximately correct also if the incident light is collimated, if only the reflected and transmitted light are completely diffuse (*cf.* page 676). Under these conditions, the absorption coefficient, α, and the scattering coefficient, σ, can both be read from the graph, if reflectance and transmittance have been determined.

Another method of determination of the two coefficients was described by Saunderson (1942). It requires two reflection measurements—one with a thin layer and one with a layer of "infinite thickness" (*i. e.*, having practically negligible transmission).

Wurmser (1941) suggested that transmission measurements with two layers of different optical density can be used to determine the coefficients of absorption and scattering, and gave a sample nomograph for two specific depths of the cell (0.035 and 0.1 cm.), which is reproduced in figure 22.38. However, he did not point out that his two-constant formula is correct only if both layers scatter sufficiently to ensure complete diffuseness of the transmitted light.

Procedures of the above-described type can be applied with a fair degree of reliance to *cell suspensions*, and it is desirable that future investigations of spectra of such systems make use of them. The application of Duntley's nomograph to *leaves* or *thalli* also is possible, but one has to re-

member that the statistical theory deals with *random* distributions of large numbers of small colored particles, and therefore does not take into account the "sieve effect," which may be caused by regular alignment of the comparatively large chloroplasts. The result of this effect is the admixture

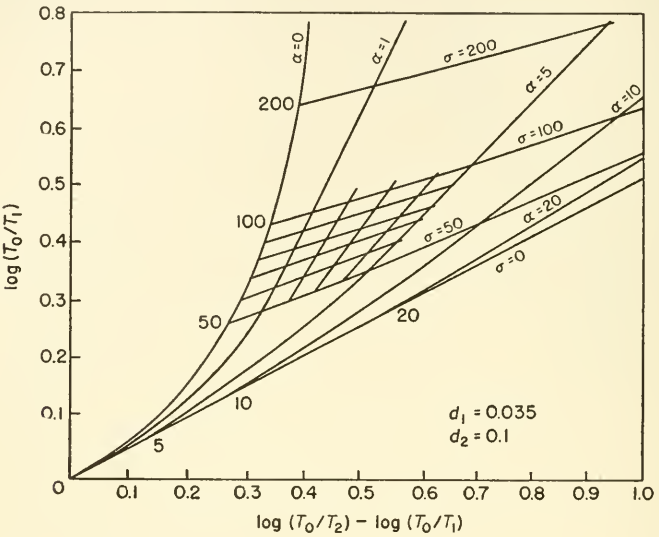

Fig. 22.38. Nomograph for calculation of absorption and scattering coefficients in turbid media from two transmission measurements (after Wurmser 1941).

$$(22.17)\quad T_0/T = \cosh d\sqrt{\alpha(\alpha+\sigma)} + \frac{2\alpha+\sigma}{2\alpha(\alpha+\sigma)}\sinh d\sqrt{\alpha(\alpha+\sigma)}$$

of white light to the transmitted flux, *i. e.*, a decrease in absoption at all wave lengths. In the determination of the apparent absorption coefficient, this decrease must have the largest effect in the absorption peaks, and a lesser effect in the regions of low absorption. It thus helps to make absorption less selective.

The sieve effect is negligible in *Chlorella* suspensions, since the cells in such a suspension—unlike the chloroplasts in a leaf—are distributed at random. The suspensions used in the experiments of Noddack and Eichhoff contained about 1×10^7 cells/ml. in a plane-parallel vessel 3.9 cm. thick. The average diameter of a *Chlorella* cell is about 5 μ, and its average cross section is 10^{-7} cm.^{2}, so that 4×10^7 cells would cover a surface of 1 cm.^{2} with eight complete layers. Statistics predict that the probability of a beam's traversing such a suspension without striking a single cell is $e^{-8} = 0.0003$ and thus negligible.

According to page 683, the number of chloroplasts in a fully green leaf is sufficient to form five or ten continuous layers; the statistical probabil-

ity that a beam of light passes between all these chloroplasts, distributed at random, is between 2×10^{-3} and 5×10^{-5}. Consequently, if the distribution of the chloroplasts were random, the sieve effect would be negligible. However, the effect can become important if the alignment of chloroplasts attains a high degree of regularity. The results of Schanderl and Kaempfert (1933) (Table 22.I) point toward a measure of success that nature has achieved in this regulation (*cf.* fig. 22.5). In very young or thin tissues, the sieve effect is important even without an alignment of the chloroplasts. Meyer (1939) mentions that he was unable to measure the absorption spectra of the seedlings of *Tradescantia*, and of oat, because "chlorophyll in these seedlings was so granulated that they appeared in transmitted light not green but checkered, consisting of white and dark spots."

One way of viewing the sieve effect is to consider the mutual "shading" of molecules in each colored particle, which prevents them from exercising their full absorbing capacity; this interference obviously cannot become effective unless the light is markedly weakened by the passage through a single particle. This condition is satisfied in the chloroplasts, since in the peaks of the absorption bands of chlorophyll a single chloroplast absorbs more than 50% of incident light (*cf.* page 683).

Until quantitative theories have found actual systematic application to cell suspensions, if not to leaves and thalli, the question—what, if any, changes in the true shape of the absorption bands can be deduced from the spectra of live plants—will beg detailed answer. Several qualitative indices that such changes do occur can be noted even now, but none of them is entirely reliable. These indications are: enhanced absorption in the far red and near infrared (to which we referred on page 654), decreased absorption in the maximum of the red band, and the comparatively weak absorption in the blue-violet region.

Increased absorption in the far red is exhibited not only by leaves (*cf.*, for example, fig. 22.15) but also by *Chlorella* and *Chroococcus* suspensions (figs. 22.21, 22.22 and 22.23) and by aqueous protein–pigment suspensions (fig. 21.28A). In the experiments of Smith (1941), this excess absorption was observed to disappear upon the addition of a detergent, digitonin (compare figure 21.28A with B); he therefore attributed it to scattering. A difference that may be explained in the same way was noted by Rabideau, French and Holt (1946) between the transmission and the absorption spectra of chloroplastin dispersed by ultrasonic waves (*cf.* fig. 22.15). However, as mentioned before, a strongly enhanced absorption in the far red by live *Chlorella* cells is recognizable also in Noddack and Eichhoff's figure (fig. 22.21), which (unless the integrating apparatus failed to function as intended) could not be affected by scattering in the way assumed by Smith. It is true that, as explained on page 711, scattering, by

changing the average length of the light path in the medium, could also change the absorption spectrum ($\log I/S$ as function of wave length, which is what fig. 22.21 represents). However, it appears impossible that the light path in the *Chlorella* suspension could be so lengthened by scattering as to replace the practically complete transparency of the pigment extract at 800 mμ, by an absorption of over 10%. If Noddack and Eichhoff's results can be relied upon (which is not certain) we are led to consider the spread of the chlorophyll absorption in living cells into the far red and infrared, a genuine change in the absorption curve of the green pigment. It may be noted (*cf.* fig. 22.48B) that the absorption curve of *Chroococcus* (a *Cyanophycea*), calculated by superposition of the absorption curves of all the extracted pigments, although it is very close to the transmission curve of a living cell suspension (probably, because of the absence of chloroplasts and consequent reduction of scattering), nevertheless also shows enhanced absorption in the far red.

Increased absorption in the green, which is so striking a feature of leaf spectra, may or may not indicate a genuine change of the chlorophyll spectrum. The above-mentioned *Chroococcus* curve shows no similar effect. In interpreting the absorption in this region, one has to consider, in addition to scattering, also the possible presence in living cells of protochlorophyll, pheophytin or other relatives of chlorophyll that have absorption bands in the middle of the visible spectrum. The possible extensive spread toward the longer waves of the absorption bands of the carotenoids also has to be taken into account.

Decreased absorption by live cells in the maximum of the red band, shown in figures 22.21 and 22.22, cannot be explained by a sieve effect (which is negligible in cell suspensions); diffuse reflection, too, probably is insufficient to account for this effect. A certain flattening of the red band may therefore also be characteristic of the true absorption curve of chlorophyll in the living cell.

Table 22.VII shows that (as first noticed by Wurmser in 1921) *the ratio of the apparent absorptions in peaks of the blue-violet band and of the red band is low in algae* (about 1.5) and particularly in leaves (about 1.0), as compared with pigment extracts (1.75). The blue-violet rays are scattered more strongly than red ones; but this could not explain a *decrease* in their absorption. (Such an effect would only be understandable if the diffuse reflection of blue-violet light by leaves were *stronger* than that of red light; but no such effect is revealed by figures 22.12 or 22.32.) The comparatively weak absorption of blue-violet light by plants is made even less understandable by the fact that yellow, water-soluble pigments present in leaves must enhance the absorption in this region.

However, Seybold and Weissweiler (1943) opposed Noddack and Eich-

hoff's assertion (fig. 22.21) that live *Chlorella* cells absorb less light than the pigment extract in the region 550–680 mμ; they found the absorption of the cells to be the same as that of extracts in the peaks of the bands, and greater everywhere else.

The weaker absorption by living cells, compared with the pigment extract, in the blue and violet region was noticed by Emerson and Lewis (1942) also in the spectrum of the blue-green alga *Chroococcus* (*cf.* fig. 22.48B), which is almost free of scattering effects.

C. Distribution of Absorbed Energy among Pigments*

The allotment of absorbed light energy to the several pigments is very important for the interpretation of the quantum yield of photosynthesis and, in particular, for the understanding of the role of the accessory pigments—carotenoids and phycobilins.

1. Effects of Spatial Distribution of Pigments in the Cell

The first step in the apportionment of absorbed energy is separation of the absorption by the "photosynthetic" pigments—chlorophylls, carotenoids and phycobilins—from that by pigments such as the flavones and anthocyanines, which probably bear no relation to photosynthesis at all. This question was discussed before (*cf.* page 685); figure 22.10 was given as illustration of the extreme case of leaves of the "purpurea" variety, in which a very considerable part of incident light, particularly in the green, is absorbed by the water-soluble red pigments.

The presence of pigments of this type complicates matters not only by adding new components to the composite absorption spectrum, but also by raising the problem of "color filters": Generally the apportionment of the absorbed light energy to different pigments, in the region of common absorption, requires the knowledge not only of the true absorption curves of the pigments in the state in which they are present in the living cells, but also of their microscopic and submicroscopic distribution. In the case of flavones and anthocyanines, it is definitely known that their distribution is different from that of chlorophyll—they are concentrated, not in the chloroplasts, but in the cell walls and vacuoles, and thus form "color filters," before or between the chloroplasts (*cf.* the calculations of Noddack and Eichhoff 1939). This makes it particularly advisable to use, for quantitative study of photosynthesis, plants containing as little nonplastid pigments as possible.

Even if the flavones and anthocyanines are absent, and the object studied contains no separate carotenoid-bearing bodies, it is by no means certain

*Bibliography, page 738.

that the submicroscopic structure of the plastids (discussed in Vol. I, chapter 14, and illustrated by fig. 14.6) does not place some pigments in a different position with respect to light absorption than others. We may hope that studies with the electron microscope and investigations of the optical properties of the plastids (birefringence, dichroism etc.) will reveal more about the arrangement of molecules in these bodies. Pending these developments, all estimates of the relative contributions of different pigments to the absorption of light by plants must be based on the assumption of an identical proportional composition of the pigment mixture in every point of the cell or tissue.

2. Apportionment of Absorption in Uniform Mixture

If it can be assumed that the pigment mixture in the cell or tissue under investigation has uniform composition—meaning that, wherever the mixture is present, it has the same relative composition (but not that the same absolute concentration of mixture is present everywhere)—the contribution of the ith pigment to the total absorption by the mixture at a given wave length, A_i, is proportional to the product $c_i\alpha_i$, where c_i is the concentration and α_i the absorption coefficient of this component.

$$A_i = A(c_i\alpha_i/\Sigma_i c_i\alpha_i) \tag{22.18}$$

Whatever the length and shape of the path of the light beam in the medium, equation (22.18) applies to absorption in every infinitely small element of this path; therefore, it applies also to the integral light absorption, independently of scattering or other geometrical–optical phenomena.

The relative concentrations, c_i, can be determined, *e. g.*, by extraction and photometric estimation; the correct value of the total absorption, A (*i. e.*, the value corrected for reflection and scattering), can be determined, for each wave length, by the methods discussed in part A. The application of equation (22.18) therefore hinges primarily on the knowledge of the true absorption coefficients of all pigments *in the state in which they are present in the cell*, and here the difficulty comes in.

In part B, we referred to statistical theories whose application may permit the determination of the *average* absorption coefficients of the pigment mixture, from measurements of transmission *and* reflection (or two reflection measurements, or two transmission measurements with different optical densities). In figures 22.37 and 22.38 we gave examples of nomographs that could be used for this purpose, provided both the transmitted and reflected light fluxes are perfectly diffuse; and we suggested that these (or other similar theories) be used in the future in the optical study of cell suspensions, leaves and thalli.

Even after the *average* absorption curve of the pigment mixture has been determined, this will not give us the desired knowledge of the absorption curves of the *individual pigments*; but it will be a step in the right direction: Some sections of this average absorption curve will be due to a single pigment or a small group of related pigments (*e. g.*, the part above 550 mμ in green plants, to chlorophylls *a* and *b*, and in brown algae, to chlorophylls *a* and *c*). The changes in shapes of the absorption bands, found in these regions, may be considered, by analogy, as valid also for the bands of the same pigments in the regions of composite absorption. (However, the different polarizabilities of a molecule in different electronic states, and the possibility of resonance effects between molecules with overlapping absorption bands call for caution in the use of such analogies.)

By constructing true absorption curves of cells or plastids with varying contents of the individual pigments, one can hope to assemble material whose analysis will permit derivation of the absorption curves of the individual components. (Here, too, caution will be needed because resonance phenomena may destroy simple additivity of absorption coefficients.)

In the light of these considerations, all attempts undertaken so far to apportion the light energy absorbed by plants, among individual pigments, are but first crude approximations. In some of these studies, the analysis was made entirely on the basis of comparison of the spectra of extracts with those of the solutions of separated pigments. In others, a certain improvement was achieved by assuming that all bands were shifted *in vivo* by the same amount, without change of shape. In a third group of investigations, the analysis was further improved by assuming individual values for the shifts of the bands of different pigments; but still no attempt was made to take into consideration the possible changes in shapes of the bands.

3. Absorption by Chlorophylls *a*, *b*, *c* and *d*

All absorption of light by chlorophyllous plants or plant organs at wave lengths longer than 550 mμ can be attributed to the chlorophylls, except in red and blue algae, where phycobilins may absorb light up to 650 or 700 mμ (*cf.* figs. 21.39 and 21.40), and purple bacteria, which contain carotenoids with absorption maxima at 550–570 mμ (*cf.* fig. 22.27 and Table 21.VIII).

No serious attempts have been made to apportion the absorption between chlorophylls *a* and *b*. A crude idea of this distribution in an extract from a green alga is given by Montfort's figure (fig. 22.39). (It will be noted that the abscissae in this and other figures of Montfort and Seybold are absolute values of absorption in each separate solution, not percentages of the total absorption by the mixture, and thus do not add to

100%.) This figure is too crude to reveal an increased participation of chlorophyll *b* in the region of its band maximum in the red; but shows

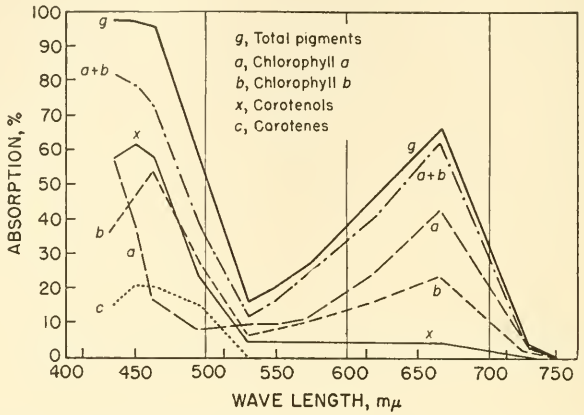

Fig. 22.39. Absorption of pigment extracts from *Ulva lactuca* (green alga) (after Montfort 1940).

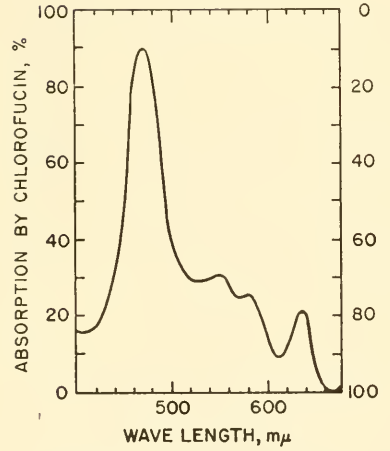

Fig. 22.40. Light absorption by chlorofucin (chlorophyll *c*), in per cent of total absorption, by a methanol extract from diatoms (after Strain and Manning 1942).

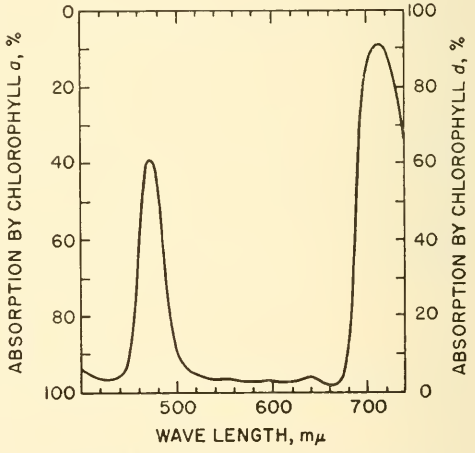

Fig. 22.41. Proportions of light absorbed by chlorophylls *a* and *d* in a methanol extract of *Erythrophyllum delesserioides* (after Manning and Strain 1943). Absorption by red and yellow pigments is not considered in the calculation.

clearly such an effect in the blue, between 450 and 530 mμ, where the absorption by the *b*-component is up to five times stronger than that by chlorophyll *a*.

Much more precise measurements were made by Strain and Manning (1942) with the chlorophyll $a + c$ mixture extracted from diatoms, and by Manning and Strain (1943) with the chlorophyll $a + d$ mixture from red algae. Figures 22.40 and 22.41 show that chlorophyll *c* (chlorofucin) contributes about 90% of total chlorophyll absorption at 570 mμ; while chlorophyll *d* accounts for 60% of the total chlorophyll absorption at 470 and 90% at 710 mμ.

These figures are for methanol extracts. The relative roles of the chlorophyll components in the absorption of light *in vivo* are uncertain. In the case of chlorophyll *b*, not even the position of its red absorption peak *in vivo* is known with any certainty (*cf.* page 701). It was suggested (*cf.* page 612) that alternation of the absorption maxima of chlorophylls *a* and *b* in the red, orange and yellow, best shown by figures 21.1A and B, may be nature's means to ensure most effective light absorption throughout this region; but all these bands are so broadened in leaf absorption spectra that it is doubtful whether the absorption by the natural mixture $a + b$ is, at any wave length above 550 mμ, markedly different from what would prevail if either of the two components were alone present in equivalent concentrations. The situation is different below 550 mμ. The region of prevalent absorption by the *b*-component, which we noted in extract at 450–530 mμ (fig. 22.39), in all probability exists also in live green cells. Because of the "red shift" it probably extends *in vivo* from about 460 to about 540 mμ. In this region, the presence of chlorophyll *b* may be of considerable importance from the point of view of enhanced light absorption by green leaves and algae.

4. Absorption by Carotenoids in Green Plants

The distribution of light between the chlorophylls and the yellow carotenoids of green plants in the region below 550 mμ has been much discussed. The first estimate was made by Warburg and Negelein (1923). In their calculations of the quantum yield of photosynthesis (*cf.* chapter 29), they decided, from extract spectra, that the carotenoids of *Chlorella* account for 30% of the total absorbed light at 436 mμ.

Figure 22.42 shows the results of the first more detailed estimate by Seybold (1936), based on spectroscopic measurements with an extract from leaves of *Phaseolus vulgaris.* Figure 22.39 represented similar data for the multicellular green alga *Ulva lactuca;* this graph shows the absorption by carotene and the carotenols separately (as well as that by the chlorophylls $a + b$, *a* and *b*). The two figures show the absorption by the carotenoids becoming noticeable below 530 mμ (in solvents of low polarizability) and below about 570 mμ in carbon disulfide. The largest percentage

participation of the carotenoids in total absorption is indicated between 450 and 520 mμ in methanol, and up to 550 mμ in carbon disulfide.

A considerably more precise analysis was made by Emerson and Lewis (1941, 1942, 1943). They measured the transmission of a *Chlorella* sus-

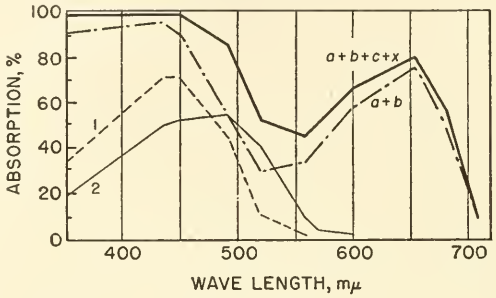

Fig. 22.42. Absorption by all pigments ($a + b + c + x$) from 100 cm.^{2} of leaves of *Phaseolus vulgaris* in methanol, compared with absorption by an extract of the chlorophylls $a + b$ in methanol, and by extracts of the carotenoids $c + x$ in methanol (1) and carbon disulfide (2) (Seybold 1936).

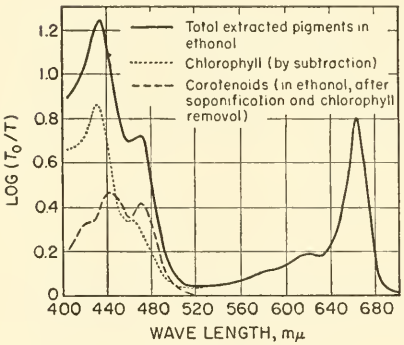

Fig. 22.43. Absorption spectra of ethanol solutions of pigments extracted quantitatively from *Chlorella* cells (after Emerson and Lewis 1943). At wave lengths over 520 mμ, the spectrum of chlorophyll fraction coincides with that of the total extract.

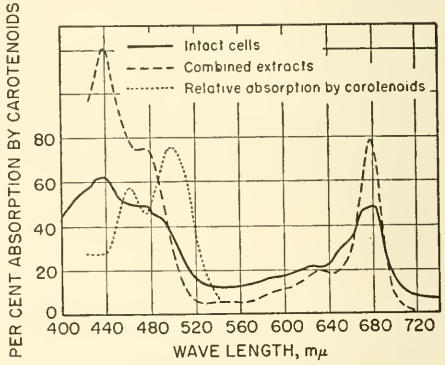

Fig. 22.44. Comparison of absorption spectra of extracted pigments (shifted as described in text) and of intact *Chlorella* cells (after Emerson and Lewis 1943). Curve for intact cells shows absorption due to cell suspension 1.4 cm. thick, containing 0.96 mm.^{3} cells/ml.

pension and the spectra of the total pigment extract and of the individual pigments. The results are represented in figures 22.43 and 22.44. The first one refers to conditions in the extract, and shows that, in ethanol, the absorption by the *Chlorella* carotenoids becomes marked below 520 mμ

and accounts for about 50% of total absorption between 500 and 450 mμ; below 450 mμ, the absorption by the chlorophylls again becomes predominant.

Figure 22.44 is an attempt to interpret the conditions in living cells. It shows the transmission spectrum, log (T_0/T), of the cell suspension—it would be better if it were the absorption spectrum, log I/S. Also shown is a composite absorption spectrum of the pigments obtained by shifting the chlorophyll bands, derived from the preceding figure, above 550 mμ, toward the red by 10 mμ, and below 580 mμ, by 6 mμ (it would be simpler to make the plot on the frequency scale and use a uniform shift!); the carotenoid bands, derived from the preceding figure, were shifted by 14 mμ. (The values used for chlorophyll may be a little low; *cf.* page 706.)

Two breaks in intact cell curve are at points where cell suspension was stirred and filters in monochromator were changed. The dotted curve shows fraction of total absorbed light absorbed by carotenoids, based on the curve for extracts after introduction of wave length shifts.

The difference between the composite absorption curve for total pigments and the—much more diffuse— transmission curve of the cell suspensions can be due (as discussed in part B) partly to scattering and partly to intrinsic changes in band shapes. A theoretical treatment, as described on pages 711–714, could eliminate the scattering effect and reveal more clearly the intrinsic alterations of the pigment spectra; but no attempt was made to take scattering into account, and the proportion of light absorbed by the carotenoids was calculated simply by comparison of the absorption curve of the combined extracts in figure 22.44 with those of the individual extracts in figure 22.43 (after appropriate "red shifts" of the latter). The results, indicated by the dotted line, show 40% absorption by the carotenoids at 520 mμ, a maximum of 75% at 500 mμ, a secondary maximum of about 55% at 460 mμ and a decline to about 25% below 440 mμ. According to these results, carotenoids contribute significantly to the total absorption of plastid pigments in *Chlorella* from 530 mμ downward.

5. Absorption by Carotenoids in Brown Algae

Brown algae (including diatoms) contain no chlorophyll *b*, and should thus, according to page 720, transmit more freely than green plants in the region 460–540 mμ. Instead, as their color shows, they absorb considerable amounts of green light (510–580 mμ)—much more than do the green cells. This must be ascribed to the presence of a specific carotenoid, fucoxanthol. If figure 21.35A is correct, and the absorption spectrum of fucoxanthol in solution does not extend toward the red any further than

that of luteol—*i. e.*, not much beyond 510 mμ—the brown color can be attributed to fucoxanthol only by assuming a very wide shift of absorption bands (or their strong broadening toward longer waves). According to page 706, Menke had in fact found indications that the absorption peaks of fucoxanthol in live *Laminaria* cells are situated as far toward the red as at 499 and 545 mμ (instead of 457 and 492 mμ in ethanol solution, according to Table 21.IX). Attempts to analyze the absorption by brown algae, described below, have not taken into account the possibility of such a wide shift—not to speak of any changes in the width of the bands.

On page 657 we referred to a more recent measurement of the extinction curve of fucoxanthol in hexane by Karrer and Würgler, which showed a considerably broadened band, extending to or even beyond 530 mμ (fig. 21.36).

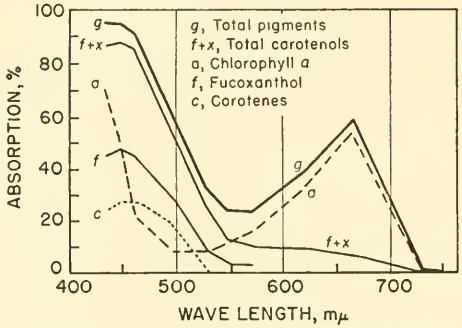

Fig. 22.45A. Absorption by extracted pigments from *Fucus vesiculosus* (brown alga, extreme sun form) (after Montfort 1940).

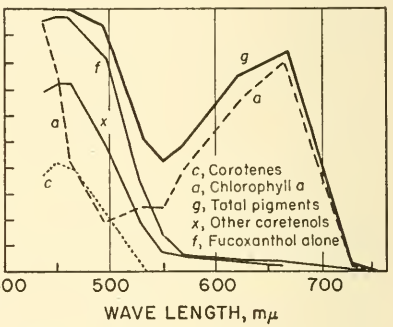

Fig. 22.45B. Absorption of extracted pigments from *Laminaria digitata* (brown alga, 12 m. depth) (after Montfort 1940).

Figures 22.45A and B are analyses of the absorption of light by pigment extracts from brown algae, as published by Montfort (1940). They are reproduced here, despite their crudeness, as examples of variations in the relative importance of fucoxanthol as light-absorbing agent in the "heliophilic" surface forms of brown algae, and the "umbrophilic" forms of the same algae that live in considerable depth. In the latter, fucoxanthol accounts for most of the extract absorption between 450 and 540 mμ, while in the former its importance is very much smaller.

Seybold and Montfort discussed their results in terms of the increase in light absorption in the spectral regions 550–500 and 500–450 mμ caused by the presence of the carotenoids. Table 22.VIII shows that, in extracts from green leaves and green algae, the presence of the carotenoids increases

this absorption by 40 to 60% in the region 500–550, and by 30% in the region 450–500 mμ. In brown algae and diatoms, the absorption increase due to the carotenoids is much larger, from 160% to 400%. This is a consequence of the absence of chlorophyll *b* and the presence of fucoxanthol; these two pigments substitute for each other, despite their difference in color.

TABLE 22.VIII

RATIO OF ABSORPTION BY INDIVIDUAL PIGMENTS IN METHANOL OR BENZENE AND BY THE TOTAL PIGMENT IN METHANOL[a] (AFTER MONTFORT 1940)

	550–500 mμ			500–450 mμ			
Organisms	Chl.	X. (including F.)	β[b]	Chl.	X. (including F.)	C.	β[b]
Diatoms	0.34	0.40[c]	3	0.20	0.82	0.24	5
Phaeophyceae							
Laminaria digitata taken from 12 m. depth	0.38	0.69[c]	2.6	0.32	0.93	0.33	3.1
Fucus vesiculatus from the surface.	0.24	0.80 (0.33)[c]	4.2	0.22	0.91 (0.47)[c]	0.30	4.5
Chlorophyceae							
Ulva lactuca	0.73[d]	0.34	1.37	0.75[d] (0.19)[e]	0.56	0.25	1.33
Leaves							
Phaseolus vulgaris[f].	—	—	1.6	—	—	—	1.3

[a] The figures in the table do not add to unity because they do not represent the proportions in which the energy absorbed by the pigment mixture is divided between the individual pigments, but rather the ratios (absorption by the separated pigments): (absorption by the mixture). Chl. = chlorophyll (in methanol), X. = carotenols (in methanol), F. = fucoxanthol (in methanol), C. = carotene (in benzene).

[b] β = factor by which total absorption is increased by the presence of carotenoids.

[c] F. alone.

[d] Chl. *a* + *b*.

[e] Chl. *a* alone.

[f] According to page 684, the leaves of this species contain a considerable quantity of water soluble yellow pigments.

A considerably more detailed analysis of the absorption by diatom pigments was made by Dutton and Manning (1941); the results are reproduced in figure 22.46. They are based on measurements with acetonic pigment extracts from *Nitzschia closterium*. In constructing the figure, Dutton and Manning postulated a shift of *all* absorption bands, those of chlorophyll as well as those of the carotenoids, by 20 mμ. (They based this assumption on the observation that, in the spectrum of this diatom, the maximum of the red chlorophyll band was recognizable at 680 mμ; the maximum of the blue-violet band, although not distinct in the cell spectrum

itself, could be recognized in the spectrum of a bile salt extract and appeared to be shifted by about the same amount—15 mμ.) We know, however, that the blue-violet band of chlorophyll is shifted by approximately the same amount as the red band on the *frequency scale* (and not on the *wave length scale*), and the bands of the carotenoids—particularly those of

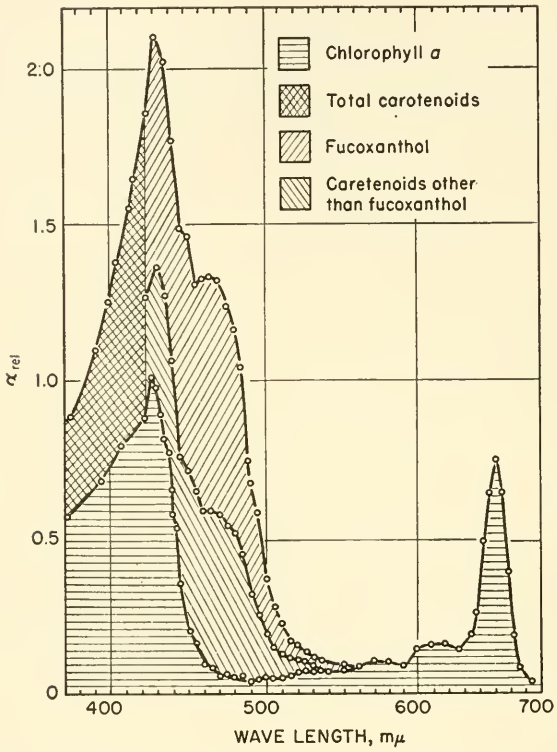

Fig. 22.46. Distribution of absorption in acetone extracts from *Nitzschia closterium* (after Dutton and Manning 1941). Lowest curve, chlorophyll *a*. Middle curve, chlorophyll *a* + carotenoids other than fucoxanthol. Top curve, all pigments.

fucoxanthol—probably are shifted much further than those of chlorophyll. Thus, figure 22.46 must contain considerable errors. The absorption by fucoxanthol above 500 mμ probably is much stronger than shown, with the consequent increase in general absorption in this region. (Figure 22.46 represents a green rather than a brown mixture!)

A still more detailed analysis of the spectrum of a diatom, *Navicula minima*, was undertaken by Tanada (1951). By extracting the pigments stepwise with aqueous methanol of different concentrations, he obtained evidence of the following "red shifts" in the blue-violet region (compared to methanolic solution): chlorophyll *a*, 8 mμ, chlorophyll *c*, 20 mμ; fucoxanthol, 40 mμ; other carotenoids, 20 mμ. A comparison of the cell spectrum with the sum of so adjusted pigment spectra can be found in fig. 30.9B.

How wary one must be in drawing conclusions based on a uniform shift of the bands of several pigments is illustrated by figure 22.47, taken from Strain (1938): It shows the absorption curves of barley leaf extracts in ethanol and in ether, and the corresponding curves for the separated yellow and green pigments, in the same solvent. In ether, up to 90% of the total absorption in the region 470–500 mμ is due to the yellow pigments, and less than 10% is absorbed by chlorophyll; in alcohol, on the other hand, the green

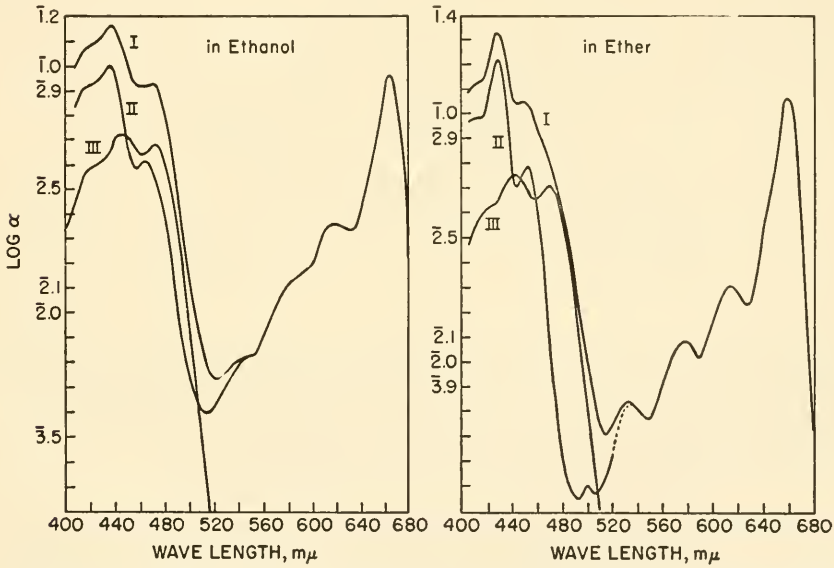

Fig. 22.47. Effect of solvent on absorption spectra of barley leaf pigments (after Strain 1938). α referred to 1 g. fresh leaves in 1 l. solution. I, all ether-soluble pigments; II, chlorophyll *a* and *b*; III, carotenoids.

pigments account for about one half of the total absorption in the same region. The difference is due to the fact that the transition from ether to ethanol causes a stronger shift of the bands of the more polar pigment—chlorophyll—than of those of the less polar carotenoids (11 and 5 mμ *versus* 1 and 4 mμ, respectively).

6. Absorption by Carotenoids and Phycobilins in Blue-Green Algae

Emerson and Lewis (1943) analyzed absorption by the pigments in the blue-green alga *Chroococcus,* in the manner described on page 722 for *Chlorella.* Figure 22.48A illustrates the absorption in alcoholic extract containing chlorophyll and the carotenoids, and in aqueous extract containing the phycocyanin–protein complex. The separation of the absorption regions is much neater than in green plants: above 650 mμ,

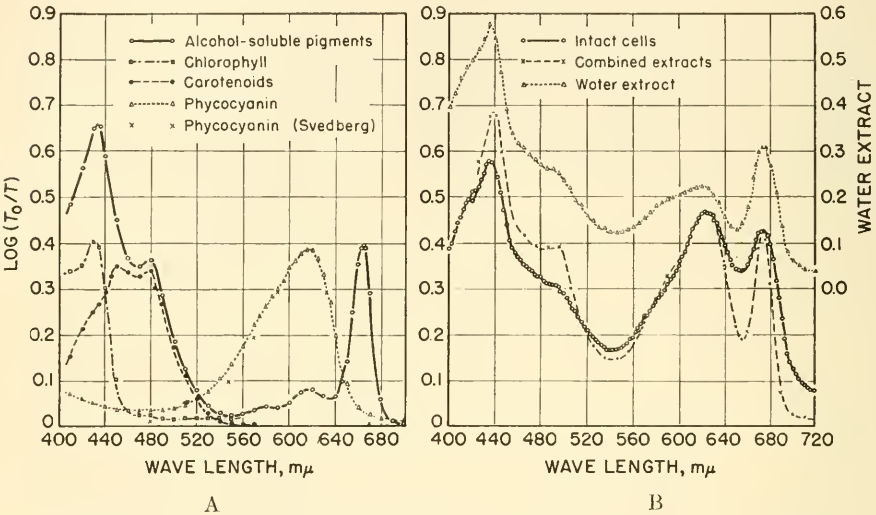

Fig. 22.48. Analysis of spectrum of Chroococcus (blue alga) (after Emerson and Lewis 1943). (A) Absorption spectra of extracted pigments in ethanol, and of phycocyanin in water. (B) Absorption spectra of intact cells (1.26 mm.^{3} cells/ml., layer 1.4 cm. thick), combined extracts (calculated from A by shifting bands as described in text) and of water extract (containing all pigments). Water extract curve has changed shape, indicating alteration of the phycocyanin–protein complex by extraction.

absorption is due mainly to chlorophyll; between 530 and 640 mμ, to phycocyanin; and between 460 and 510 mμ, to the carotenoids (because of the absence of chlorophyll *b*). Below 440 mμ, chlorophyll again becomes the main absorber.

Figure 22.48B is the attempt to reconstruct conditions in the cell. The chlorophyll and carotenoid bands were shifted, as in the treatment of *Chlorella,* the first ones by 10 mμ in the red, and 6 mμ in the blue and violet, and the second ones by 14 mμ. The phycocyanin band was shifted by 6 mμ compared to its position in aqueous extract. In the case of chlorophyll and the carotenoids, the extraction was assumed to be complete; in that of phycocyanin, the fact that the 620 mμ absorption peak in the ex-

tract was considerably lower than in the cell spectrum was taken as an indication of extraction losses (*cf.* Vol. I, page 418, about difficulties of quantitative extraction of phycobilins). Therefore, in the construction of the "combined extract" curve in figure 22.48B, the phycocyanin curve of figure 24.48A was not only shifted, but also increased in height by a factor sufficient to make the phycocyanin peak of this curve coincide with that of the cell curve.

Emerson and Lewis pointed out that the agreement between the "calculated" absorption curve and the empirical transmission curve (fig. 22.48) is much better than in a corresponding construction for *Chlorella*. They saw the explanation of this fact in the absence of chloroplasts in *Cyanophyceae*, and consequent reduction of scattering. The remaining differ-

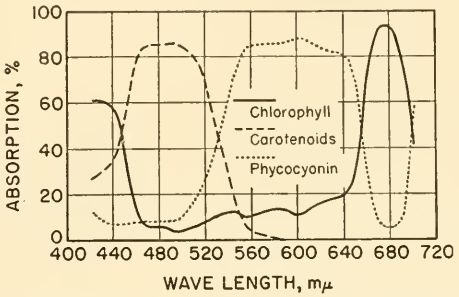

Fig. 22.49. Distribution of cell absorption in *Chroococcus* among pigments (derived from fig. 22.48A and broken curve in fig. 22.48B) (after Emerson and Lewis 1943).

ences—the enhanced absorption by living cells in the far red, and their weaker absorption in the blue and violet—may reflect significant changes in the true absorption spectrum of the pigment *in vivo* (*cf.* part B). The curve for the water extract of all *Chroococcus* pigments, also shown in figure 22.48B, may reflect changes due to separation of the phycobilin–protein from the "chromoplastin" complex as a whole.

Figure 22.49 shows the apportionment of the absorption by living *Cyanophyceae* to the three types of pigments. The neat separation of the absorption regions, noted above for the extracts from these cells, appears even clearer in this figure. It shows that blue algae (and perhaps red algae as well) offer particularly favorable conditions for the study of the role of the different pigments in the photosynthetic apparatus.

The *purple bacteria*, too, may present favorable conditions for distinguishing between the absorption by bacteriochlorophyll and by the carotenoids, because the absorption maxima of bacteriochlorophyll are situated in the infrared. Figures 22.25 and 22.27 showed the alternation of the bands of bacteriochlorophyll and of the red bacterial carotenoids in the region below 600 mμ; the strongest bands at about 500 mμ are due to the

carotenoids. Above 700 mμ, the absorption by purple bacteria is due entirely to bacteriochlorophyll, as shown in figure 22.26 by comparison of the cell spectrum with the spectrum of the extracted green pigment.

Appendix. Natural Light Fields*

The importance of the various pigments in the light economy of plants cannot be fully understood on the basis of curves discussed in the preceding section, because it also depends on the spectral composition of the light available to the species or individual plant under natural conditions.

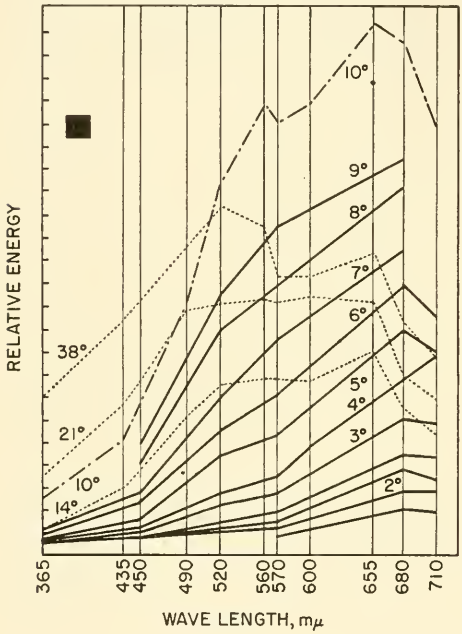

Fig. 22.50. Energy distribution curves for solar radiation at different heights of the sun over the horizon (after Seybold 1936). For 38°, 21° and 14° curves, black square = 2.50×10^{-3} cal./(cm.^{2} min.); for 10° to 2° curves, black square = 0.25×10^{-3} cal./(cm.^{2} min.).

Plants live in "light fields" whose normal character depends on the climatic zone and habitat, and which are also subject to variations with the season of the year, the time of day and the meteorological conditions. Three characteristics of natural light fields are important to plants: their total *intensity*, their spectral *composition* and their *periodicity*. There are strong indications that plants adapt their life processes in general, and their photosynthetic apparatus in particular, to all these three factors. The *intensity adaptation* reveals itself in the different characters of "*umbrophilic*" and "*umbrophobic*" (or "heliophilic") plants (shade and sun plants); *chromatic adaptation* is most strikingly shown by the occurrence of red algae

* Bibliography, page 738.

in the depths of the sea; and the *periodicity adaptation* shows itself in the distinction between long-day plants of the arctic zone and short-day plants of the temperate and tropical zones.

The spectral composition and intensities of light fields in different habitats of plants have been measured and discussed by several authors, for example, Wiesner (1907), Ursprung (1918), Seybold (1934, 1936) and Egle (1937). The factors that determine the total *intensity* of the available light (in the photosynthetically important region 400–700 mμ) are the height of the sun over the horizon, the clearness or haziness of the air, cloudiness and the position of the plant in direct sunlight or in the shade.

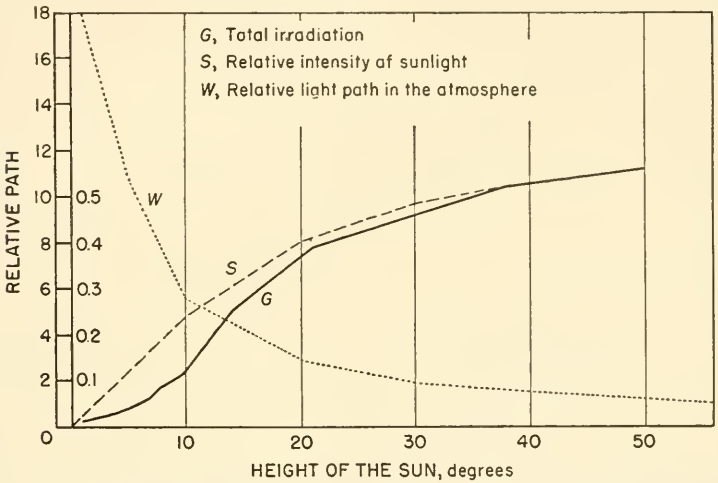

Fig. 22.51. Total irradiation and relative intensity of sunlight in relation to height of sun over the horizon (after Seybold 1936). Inner scale at left, cal./(cm.^{2} min.).

Reflection by the surrounding surfaces may also be of importance, particularly for the evergreens (reflection by snow!) and shore plants (reflection by water!) (*cf.* Egle 1937). Figure 22.50 shows the intensity distribution of the combined light of the sun and the sky for different heights of the sun over the horizon. The curves show the transition from the red light of the setting sun (maximum intensity at 680 mμ) to the yellow light of the sun in the zenith (maximum intensity at 520 mμ). Figure 22.51 shows the increase in the total energy of the light, from 0.1 cal./cm.^{2} min., at 10° elevation, to 0.6 cal./cm.^{2} min., at an elevation of 50°. (The figures are for a surface normal to the direction of the incident light; for a horizontal surface the variations are much wider.) When the sun is obscured by light clouds, the total light intensity decreases to 10 or 20% of its full value (*i. e.*, to 0.1–0.2 cal./cm.^{2} min. at midday). When the sky is entirely overcast.

the light intensity can drop to as low as 0.02 cal./cm.^{2} min., or even less, *i. e.*, to only 1% of full sunshine. At the same time, the spectral composition changes in the way characterized by figure 22.52, taken from a paper by Taylor and Kerr (1941); the light intensity becomes almost uniform throughout the visible spectrum, and the common designation of such days as "gray" proves to be correct.

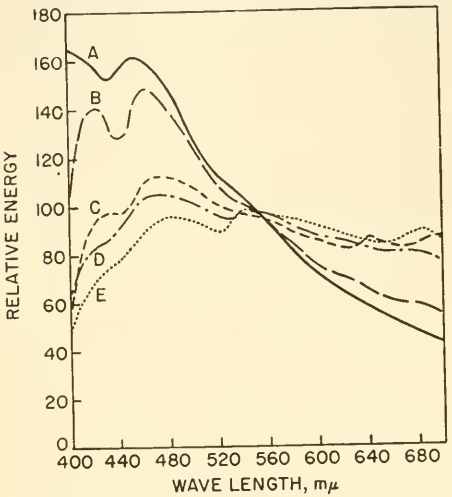

Fig. 22.52. Average energy distribution curves for daylight (after Taylor and Kerr 1941). (A) Zenith sky, color temperature 13,700° K.; (B) north sky on 45° plane, color temperature 10,000° K.; (C) totally overcast sky, color temperature 6,500° K.; (D) sun plus sky on horizontal plane, color temperature 6000° K.; (E) direct sunlight, color temperature 5335° K.

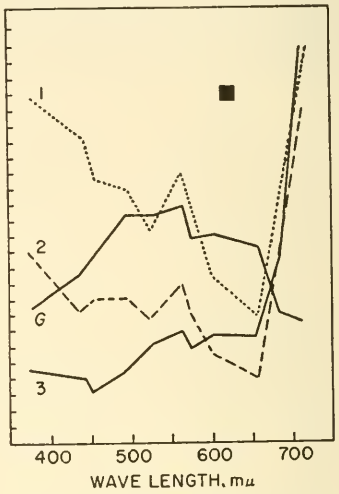

Fig. 22.53. Energy distribution in the shade (after Seybold 1936). (*G*) Edge of wood (black square = 2.5 × 10^{-3} cal. per (cm.^{2} min.); (1), (2), (3) three shade habitats of *Oxalis* (black square = 0.025 × 10^{-3} cal. per (cm.^{2} min.).

When the plant is in the shadow of a rock, house or mountain, and receives light mainly from the blue sky ("blue shade"), the spectral composition of its light field is entirely different from that to which it is exposed in direct sunlight, as shown by figure 22.52. The intensity of radiation from a clear blue sky is of the order of 20% of that of full sunlight (*i. e.*, about 0.1 cal./cm.^{2} min.) at sea level. It decreases with increasing altitude, as the scattering air layer above becomes thinner and the color of the sky a deeper blue.

The plants that live in the shadow of other plants, *e. g.*, the floor vegetation in the forest, receive their light filtered through the chlorophyll layers

of the overhanging foliage. They live in the "green shade." Figure 22.53, taken from Seybold (1936), shows the spectral compositions of the light field in the midst of a forest, compared with that at the edge. Characteristic is the minimum at 650 mμ, clearly corresponding to the absorption maximum of chlorophyll. A large part of radiations reaching the floor of a forest are either infrared or deep red, scarcely visible to the eye and useless for photosynthesis. The total intensity of the light field under the trees (400–700 mμ) is less than 10% of that of full sunlight above the forest and can drop to as low as 1% in a dense pine forest (Seybold).

An even stronger alteration in the intensity and spectral composition of the light field occurs when sun rays pass through thick layers of water.

Table 22.IX gives some data on the decrease in *total intensity* with depth. The main cause for this drop in light intensity is the absorption by water

Table 22.IX

Decrease in Light Intensity with Depth

	0	50	100	200 ft.
Atlantic Ocean Brightness[a]	1076	114	37	4.4

	0	1	2	5	10	20 m.
Titisee Lake Intensity[b]	100	57	32	21	13	10
Bodensee Lake Intensity[b]	100	54	30	15	9	6

[a] Beebe and Hollister (1930), Hulburt (1932).
[b] Seybold (1936).

itself. Some higher bands (overtones) of the vibrational spectrum of water lie in the visible spectrum (the fundamental frequencies are in the near infrared). They decrease in intensity from red to blue; in the violet and near ultraviolet, however, the absorption increases again, probably due to weak electronic bands. Figure 22.54 shows the extinction curve of water in the region 360–800 mμ, according to the measurements of Aschkinass (1895) and Sawyer (1931) (*cf.* Dorsey 1940). A water layer 10 m. thick reduces the light intensity at 640 mμ by the factor of 10; a layer 1 m. thick does the same at 760 mμ; 100 m. are required for a similar reduction at 440 mμ. Because of this absorption in red, yellow and violet, thick layers of pure water are bluish green in color.

The absorption by natural waters in the blue and in the violet is usually much larger than that by pure water, due partly to the presence of certain inorganic ions (*e. g.*, iron) and partly to that of organic matter (*e. g.*, the chlorophyll of the phytoplankton). This increased absorption at the short-wave end of the spectrum gives natural waters a pure green or even

a yellowish-green tinge, as compared with the bluish-green color of water as such. Figure 22.55 shows the spectral composition of light in different depths of the Mediterranean on a clear day according to Seybold (1934).

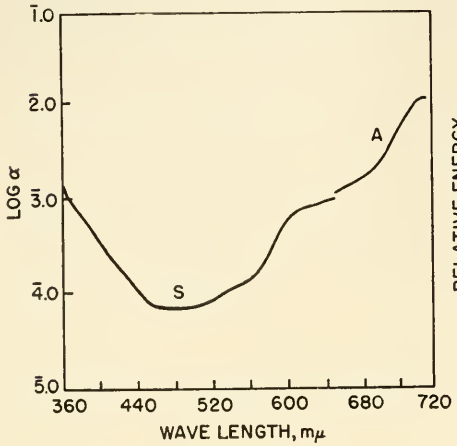

Fig. 22.54. Absorption curve of pure water (α = specific absorption coefficient). S, Sawyer (1931). A, Aschkinass (1895).

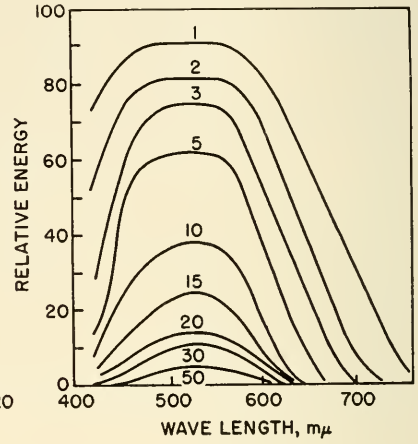

Fig. 22.55. Energy distribution under 1–50 m. water (after Seybold 1934).

TABLE 22.X

SPECTRAL COMPOSITION OF LIGHT UNDER WATER[a]
Italics indicate position of maximum of transmission.

A. TRANSMISSION BY A FIVE METER THICK LAYER[b]

Water	mμ 700	650	600	550	500	450	400	350
Pure water (blue)	7	27	44	83	90	*95*	79	62
Danish Sea (blue-green)	5	15	27	37	*41*	18	7	5
Pudget Sound (green)	3	10	18	*33*	30	28	17	8
Lake Mendota (green)	0	3	*8*	8	6	4	2	0

B. SPECTRAL COMPOSITION OF LIGHT IN DIFFERENT DEPTHS[c]

Depth	Per cent Red	Orange	Yellow	Green	Blue	Violet
0	28	16	15	13	18	16
1	18	20	21	12	15	14
3	9	17	27	19	16	12
5	5.5	17.5	36	22	12.5	6.5
7	0	13	46	27	10	4
9	0	6	52	29	10	3

[a] For more information, see *e. g.*, Schmidt (1908), Knudsen (1922), Hulburt (1928), Atkins (1932) and the book by Dorsey (1940). [b] Shelford (1929). [c] Trout Lake, Wisconsin, according to Manning, Juday and Wolf (1938).

Similar sets of curves, showing a less rapid decline in intensity at the red end of the spectrum, were given by Johnson and Kullenberg (1946) and Levring (1947) for sea water at the West Coast of Sweden. Levring attributed the absorption in violet and blue—which often converts the blue-green color of pure water into a yellow-green—to the presence of suspended particles. Some additional data can be found in Table 22.X.

Algae are found to a depth of 120 m. These deep water species live in a light field that contains almost exclusively green radiation (practically no red light is available below 10–20 m.). The total intensity of light available to them is only a few per cent of the light enjoyed by the species living close to the surface of the sea.

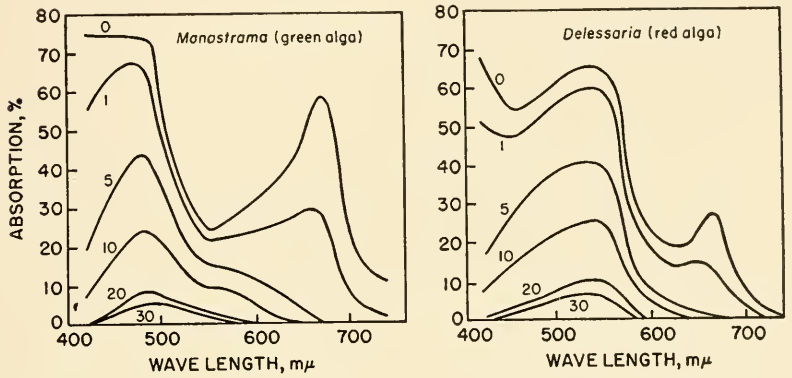

Fig. 22.56. Light absorption by algae in different depths (in meters) in per cent of light incident on the surface (after Seybold 1934).

We have discussed in chapter 15 (Vol. I) the ways in which plants adapt themselves to the chromatic composition of the light fields. There is no doubt that the brown algae, containing fucoxanthol, are capable of absorbing more light in the middle of the visible spectrum than the green plants, and that the presence of phycobilins in the *Rhodophyceae* and *Cyanophyceae* increases their absorbing capacity in this spectral region even more strongly. Table 22.VIII showed what these changes in the pigment system mean for the absorption capacity of the plants in different spectral regions. However, this table referred to illumination with light whose intensity is constant throughout the spectrum. The consequences of the same changes for the absorption of natural light were discussed by Seybold (1934), Montfort (1934) and Levring (1947), and we must refer to their papers for detailed results. We merely mention, as an example, that, according to Seybold, a *Rhodophycea* will absorb up to 95% of the light available in a 50 m.

depth, where a *Chlorophycea* will be able to absorb only 30–40% (*cf.* fig. 22.56).

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Light Absorption by Pigments in the Living Cells

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Chapter 23

FLUORESCENCE OF PIGMENTS *IN VITRO*

Fluorescence phenomena have two aspects. In the first place, the fluorescence *spectrum* offers a welcome addition to the absorption spectrum in the study of the term system and molecular structure of a chemical compound. In the second place, the *yield* and *duration* of fluorescence gives significant information as to the fate of the excitation energy and thus provide clues to the mechanism of photochemical reactions of the light-absorbing compound. In the case of chlorophyll, we are particularly interested in the second, photochemical aspect of the fluorescence phenomena.

Because of the division of this treatise into a chemical and a physical part, the photochemistry of chlorophyll already was dealt with in the first volume (chapters 18 and 19), while fluorescence could first be discussed in the present, second volume. Certain conclusions derived from fluorescence studies had to be anticipated in Volume I; some of them will have to be repeated and amplified here.

Some new facts and considerations have been added to this field since the appearance of Volume I; their fluorescence aspects are discussed in the present chapter, and their photochemical aspects in chapter 35.

A. Fluorescence of Chlorophyll *in Vitro**

1. Fluorescence Spectra of Chlorophyll and Its Derivatives in Solution

The fluorescence of chlorophyll was discovered by Brewster more than a hundred years ago (1834). It was first studied spectroscopically by Stokes in 1852, and a by-product of this study was the discovery that the leaf pigment consists of two green and two yellow components. Dhéré (1914) and Wilschke (1914) contributed the first photographs of the fluorescence spectrum. However, because the fluorescence bands of chlorophyll are situated in the far red and infrared, and the sensitivity of red- and infrared-sensitized plates varies strongly with wave length, the photographic method is not very suitable for the quantitative study of chlorophyll fluorescence.

* Bibliography, page 801.

Figure 23.1A shows the appearance of the fluorescence spectrum of chlorophyll on a panchromatic plate, and figure 23.1B, on an infrared-

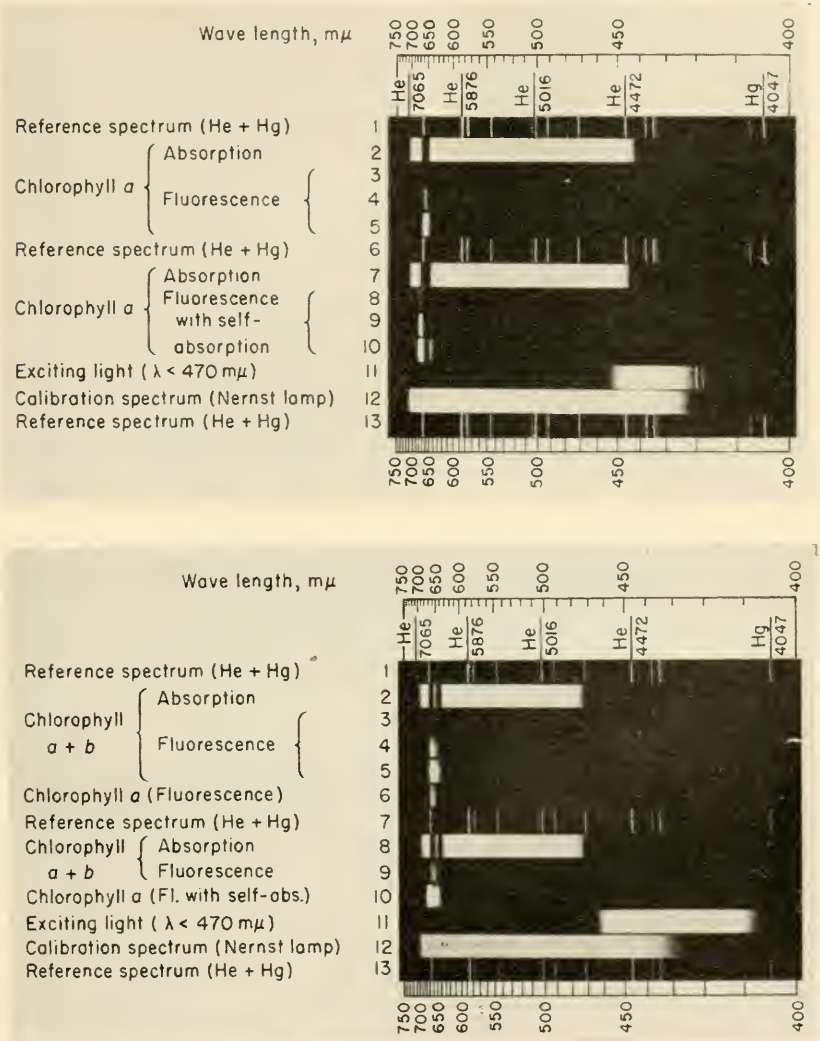


Fig. 23.1A. Fluorescence spectra of chlorophyll (*a* at top and *a* + *b* below) in ether on panchromatic plates (after Dhéré and Fontaine, 1931).

sensitive plate. Fig. 23.2 shows spectrophotometric curves of the fluorescence of chlorophylls *a* and *b* in ether, determined by Zscheile and Harris

(1943) by means of a photoelectric spectrophotometer. The maximum of the first emission band lies, in this figure, at 664.5 mμ for chlorophyll *a* and

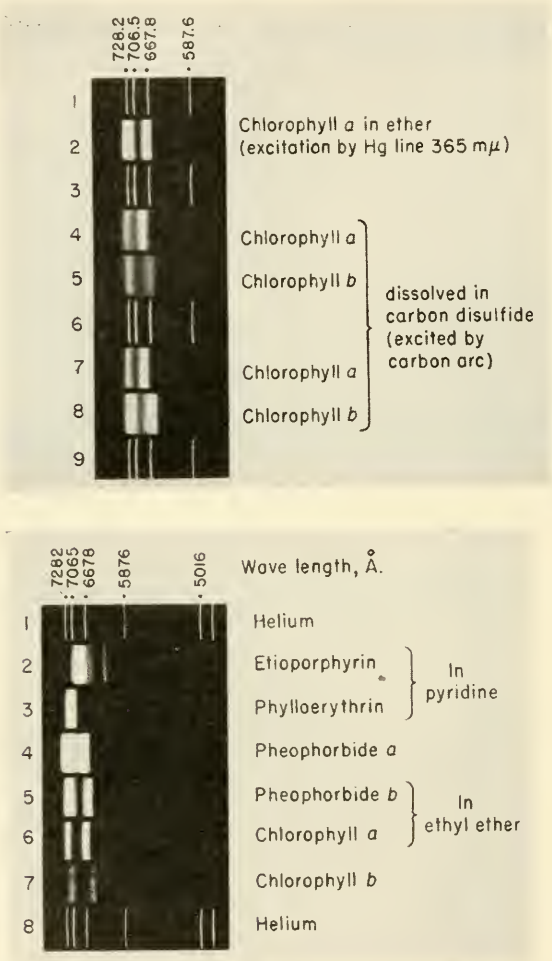


Fig. 23.1B. Fluorescence spectra of chlorophyll and some related compounds on infrared-sensitive plates (after Dhéré and Raffy 1935). Top: (2) Chlorophyll *a* in ether (excited by λ365 mμ); (4) and (7) chlorophyll *a* in CS_2 (excited by carbon arc); (5) and (8) chlorophyll *b* in CS_2 (excited by carbon arc). Bottom: (2) etioporphyrin in pyridine; (3) phylloerythrin in pyridine; (4) pheophorbide *a* in ether; (5) pheophorbide *b* in ether; (6) chlorophyll *a* in ether; (7) chlorophyll *b* in ether.

648.5 mμ for chlorophyll *b*—only very slightly on the long-wave side of the maxima of the corresponding absorption bands (*cf.* Table 23.IC). The

first fluorescence band is followed by a second one, of lower intensity, situated in the far red, which is visible in figure 23.IB as well as in figure 23.2; and by a third, weak one, situated in the near infrared, which is not recorded in these figures.

In an earlier investigation of Zscheile (1935), an additional band was observed in the fluorescence spectrum of chlorophyll *b* at 672.8 mμ; but Dhéré and Biermacher (1936) and Biermacher (1936) ascribed it to contamination with chlorophyll *a*, and this explanation was accepted by Zscheile and Harris (1943).

According to Biermacher (1936), the fluorescence spectrum provides the most sensitive test for the purity of chlorophyll *b*. A purification method based on this test was described in chapter 21.

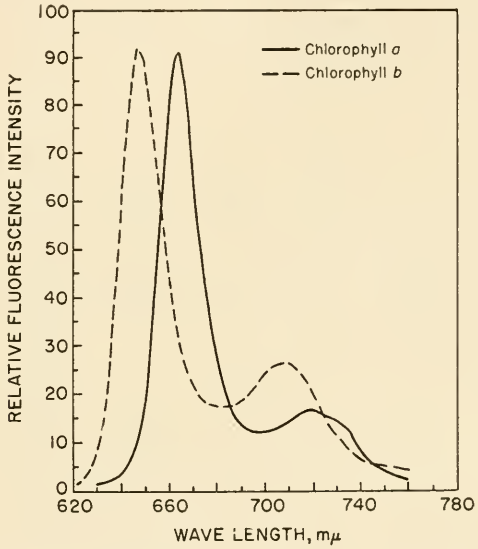


Fig. 23.2. Fluorescence spectra of chlorophylls *a* and *b* in ether. Photometric curves corrected for self-absorption (after Zscheile and Harris 1943).

Table 23.IA shows the positions of the main fluorescence bands of the two chlorophylls in ethyl ether, as found by several investigators, and Table 23.IB, the positions of the same bands in various solvents.

A spectrophotometric curve of the fluorescence of a benzine extract from *Brassica* (containing both chlorophyll components) can be found in a paper by Vermeulen, Wassink and Reman (1937).

In Table 23.I, some values represent band *maxima*, $\lambda_{max.}$, as determined by photoelectric photometry, and others band *axes*, $\bar{\lambda}$, *i. e.*, the arithmetic means of the extension limits of the bands on spectrum photographs. These limits depend strongly on the spectral sensitivity curves of the photographic plates used, and on the length of the exposure. Biermacher (1936) insisted, however, that, as long as only the peaks of the bands are photographed, *e. g.*, by using suitably short exposures, the axes coincide

TABLE 23.I

FLUORESCENCE BANDS OF THE CHLOROPHYLLS *a* AND *b*

A. BAND POSITION IN ETHYL ETHER

Component	Band	Maxima ($\lambda_{max.}$), mμ: Knorr, Albers (1933, 1935)[a]	Baas-Becking, Koning (1934)[a]	Zscheile (1935)[b]	Zscheile Harris (1943)[b]	Axis ($\bar{\lambda}$), mμ: Dhéré, Raffy (1935)[a]	Biermacher (1936)[b]
a	I	(633[c]), 672	675	668.5	664.5	665	663
	II	—	—	723	720	736	—
	III	—	—	—	—	801	—
b	I	(637[c]), 657	—	648.5	648.5	646	647
	II	—	—	705	708	713	—
	III	—	—	—	—	789	—

B. BAND POSITIONS IN DIFFERENT SOLVENTS

Solvent	n_D	Chlorophyll *a*: $\lambda_{max.}$, mμ: Baas-Becking, Koning (1934)	Knorr Albers (1933)	Zscheile, Harris (1943)	Axis ($\bar{\lambda}$), mμ: Biermacher (1936)	Chlorophyll *b*: $\lambda_{max.}$, mμ: Knorr, Albers (1933)	Axis, ($\bar{\lambda}$), mμ: Biermacher (1936)
Hydrocarbons							
Pentane	1.357	—	—	—	663	—	644.5
Hexane	1.375	—	—	—	663	—	644.5
Cyclohexane	1.431	—	—	665.5	—	—	—
Benzene	1.501	675	677	673	666.5	657	650
Paraffin (liq.)	—	678	—	—	668.5	—	645.5
Vaseline (white)	—	—	—	—	672.5	—	647.5
Ethers							
Ethyl ether	1.353	675	672	664.5	663.5	657	647
Isopropyl ether	1.37	—	—	670	—	—	—
Dioxane	1.42	—	—	668	666	—	699.5
Alcohols							
Methanol	1.320	666	675	674	667	657	—
Ethanol	1.362	675	—	—	666	—	654.5
2-Methyl-1-propanol	1.39	—	—	674	—	—	—
1-Butanol	1.402	—	—	673.5	—	—	—
2-Ethyl-1-hexanol	1.43	—	—	676.5	—	—	—
Cyclohexanol	1.466	—	—	—	669	—	653
Halogenides and Sulfides							
Carbon tetrachloride	1.466	—	—	671.5	668	—	648
Chloroform	1.446	680	—	—	670	—	650
Carbon disulfide	1.630	681	—	—	676	—	656
Methylene iodide	1.756	682	—	—	[d]	—	[d]
Tetralin	—	—	688.5[f]	—	—	—	—
Miscellaneous							
Acetone	1.359	668	672	670	665	657	653
Methyl oleate	1.46	—	—	670	—	—	—
Pyridine	1.509	—	—	—	674	—	658.5
Olive oil	1.48	—	—	672	—	—	—
Aniline	1.586	675	—	—	676	—	662
Lecithin	—	677[e]	—	—	—	—	—

Table continued

TABLE 23.I (*Continued*)

C. BAND SHIFTS IN DIFFERENT SOLVENTS[g]

Zscheile and Harris (1943)		Biermacher (1936)		
			Chl. *a*	Chl. *b*
Solvent	Chl. *a*, $\Delta\lambda_{max.}$, mμ	Solvent	$\Delta\bar{\lambda}$, mμ	
Methanol	10	Pentane	4.5	4.5
2-Ethylhexanol	10	Hexane	4.5	4.5
2-Methylpropanol	9.5	Pyridine	4.5	3.5
Benzene	9	Carbon tetrachloride	4	3
Isopropyl ether	9	Methanol	3.5	—
Acetone	8.5	Aniline	3	5
1-Butanol	8.5	Benzene	2.5	4.5
Methyl oleate	8	Dioxane	2.5	5
Carbon tetrachloride	8	Ethanol	2.5	6.5
Olive oil	7.5	Cyclohexanol	2	4
Dioxane	7	Chloroform	2	2.5
Cyclohexane	5.5	Carbon disulfide	2	6
Ethyl ether	4.5	Acetone	1.5	8.5
		Ethyl ether	0.5	5
		Paraffin (liq.)	0	0.5

[a] Photographic.
[b] Photoelectric photometer.
[c] Concerning subsidiary maxima, see page 748.
[d] Biermacher (1936) found no fluorescence at all in methylene iodide (as well as in nitrobenzene).
[e] This figure is quoted by Seybold and Egle (1940) from Stern.
[f] After Stewart, Knorr and Albers (1942).
[g] $\Delta\lambda = \lambda$(fluorescence) $- \lambda$(absorption).

with the true band maxima. He therefore denied that the difference between the $\bar{\lambda}$ values measured by him and the $\lambda_{max.}$ values found by earlier investigators could have been due to the decline in the sensitivity of his photographic plates in the far red; he suggested instead that this difference was caused by the failure of other observers to avoid "self-absorption," *i. e.*, reabsorption of fluorescent light before its escape from the chlorophyll solution. (Because of the position of the fluorescence band of chlorophyll close on the red side of the absorption peak, self-absorption must cause an apparent shift of the fluorescence band maximum toward longer waves.) The correctness of this interpretation was acknowledged by Zscheile and Harris (1943), who made a spectrophotometric redetermination of the fluorescence bands, varying the chlorophyll concentration systematically, and using a capillary vessel to reduce self-absorption. The extent of the self-absorption effect is illustrated by figure 23.3. It shows how large a part of the fluorescence band is overlapped by the (shaded) absorption band, and how, in consequence of this overlapping, the position of the maximum of the fluorescence band can be displaced, by self-absorption, by as much as 12 mμ. The position of the second fluorescence band (at 720 mμ) is found to be practically unaffected by reabsorption; this is natural, since this band leads to a vibrating state of the chlorophyll molecule and consequently does not occur in absorption—at least not with a marked intensity.

Table 23.IC compares the most reliable figures in Table 23.IB—those of Biermacher, and Zscheile and Harris—with the wave lengths of the absorption bands as listed in Table 21.IV. This comparison shows that the fluorescence maximum remains on the long-wave side of the absorption maximum in all solvents, but that the distance between the two maxima sometimes falls to as little as 1 mμ.

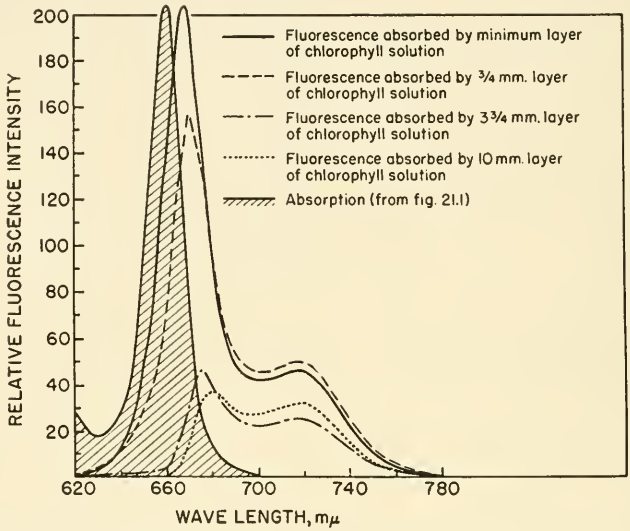


Fig. 23.3. Effect of self-absorption on fluorescence spectrum of chlorophyll *a* in ether (after Zscheile and Harris 1943).

It is difficult to say whether any of the variations in the shift, $\Delta\lambda$, indicated by Table 23.IC, are significant; in particular, whether the conspicuous difference in the order of solvents found for the two chlorophylls, *a* and *b*, is real.

Seybold and Egle (1940) suggested that $\Delta\lambda$ is abnormally large ($\simeq$15 mμ) in chlorophyll solutions in *lipides* such as lecithin. (This assumption was necessary for their "two-phase theory" of the state of chlorophyll *in vivo*; *cf.* Vol. I, page 393). This suggestion is not plausible in itself, and therefore cannot be accepted without confirmation by reliable measurements.

As to the reason for the "red shift" of the fluorescence bands compared with the absorption bands, the explanation must lie in the loss of vibrational quanta in the interval between excitation and re-emission, or after re-emission. Quite generally, the molecule has a somewhat different nuclear configuration in the excited and in the normal state. Therefore, according to the so-called Franck-Condon principle, electronic excitation is accompanied by the excitation of a certain amount of vibrations. A large part if not all these vibrational quanta are dissipated before re-emission of

light. After the emission, the molecule finds itself for a second time in a deformed state, and, for a second time in the fluorescence cycle, some energy is converted to vibrational energy. The magnitude of $\Delta\lambda$ indicates that the vibrational quanta concerned must be of the order of 100 cm.$^{-1}$, much smaller than the quanta (1000–1400 cm.$^{-1}$) postulated on page 630 to account for the sequence of the visible absorption bands of chlorophyll.

Another explanation of the red shift could be derived from the hypothesis (*cf.* page 631) that the main red absorption band, $X_0 \rightarrow Y_0$ (*cf.* fig. 21.20), conceals a weak band, $X_0 \rightarrow A_0$, which belongs to the yellow-orange band system. If this is true, and if the red fluorescence band is the pure $Y_0 \rightarrow X_0$ band, the somewhat different position of its maximum is understandable. This explanation is less likely because the displacement of the fluorescence band toward the red is a general phenomenon, while the overlapping of the bands $X_0 \rightarrow Y_0$ and $X_0 \rightarrow A_0$, if it exists at all, can be only an accidental occurrence.

The influence of the *solvent* on the position of the fluorescence band must be attributed to the same cause as its influence on the absorption spectrum, *i. e.*, to the difference in the solvation energy of the pigment in the ground state and in the excited state. Table 23.IC shows that within a homologous group of solvents an approximate parallelism exists between the position of the fluorescence band and the refractive index of the medium. This regularity already was noted and discussed in chapter 21, when we dealt with the absorption spectra of chlorophyll in different media.

A new light on the effect of solvents on the fluorescence of chlorophyll was thrown by the observations of Livingston, Watson and McArdle (1949), which will be described further below. These experiments indicate that the solvent effect is twofold: In the first place, the presence of at least a small amount of solvent molecules of a certain type (water, alcohols, amines) appears to be needed to bring out the fluorescence (presumably, by converting chlorophyll from a nonfluorescent into a fluorescent tautomeric form). After the fluorescence had been "activated" in this way, its spectrum and intensity are independent of the specific nature of the "activator," and determined only by the nature of the bulk solvent. In other words, whether the fluorescence of chlorophyll *a* in benzene is "activated" by methanol, or piperidine, or water, its spectrum and intensity are characteristic of benzene as medium. Of course, when larger quantities of the "activator" are added, the spectrum must sooner or later approach that characteristic of the chlorophyll solution in the pure activator; but these transitions have not yet been studied.

Like the two main chlorophylls, *a* and *b*, *chlorophyll c* (*chlorofucin*) also has a red fluorescence band; its axis lies at 631.5 mμ in ether (Dhéré and Fontaine 1931), and at 635 mμ in ethanol (Wilschke 1914).

Chlorophyll d, too, was described by Manning and Strain (1943) as exhibiting a deep red fluorescence. Its spectrum shows a first maximum at 693 mμ (in ethereal solution) and indications of a diffuse second maximum at 750 mμ.

No quantitative data appears to be available on the fluorescence spectrum of *allomerized* chlorophylls.

Protochlorophyll has a fluorescence band at 626.5 mμ, in ether (Dhéré 1930). The fluorescence bands of *pheophytin a* lie at 676 and 730.5 mμ in ether (Dhéré and Raffy 1935) and at 677.5, 717, 750.5 and 804 mμ in dioxane (Stern and Wenderlein 1936).

A photometric curve of the fluorescence spectrum of *bacteriochlorophyll* in solution was obtained by Vermeulen, Wassink and Reman (1937). It showed two bands, a weaker one at 695 mμ, and a stronger one at 810 mμ (fig. 23.4). The relationship of these two bands is not clear (*cf.* p. 751).

The fluorescence of these and many other porphin derivatives was reviewed by Dhéré (1937, 1939).

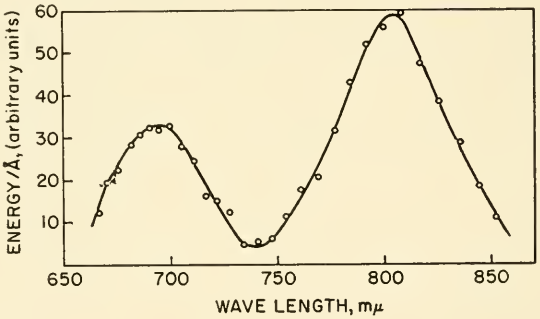


Fig. 23.4. Fluorescence spectrum of bacteriochlorophyll in solution (after Vermeulen, Wassink and Reman 1937).

No counterpart of the strong blue-violet absorption band appears in chlorophyll fluorescence, even if white, violet or ultraviolet light is used for excitation (*cf.* Dhéré and Raffy 1935, Prins 1934, and Vermeulen, Wassink and Reman 1937). The same is true of the weaker absorption bands in the middle of the visible spectrum. True, Prins (1934) said that the red band occurs in fluorescence without the yellow and orange bands only if fluorescence is excited by red light (660–680 mμ); and Knorr and Albers (1933, 1935) observed "subsidiary" fluorescence bands on the short-wave side of the main one—at 633 and 637 mμ in chlorophyll *a* and *b*, respectively (*cf.* Table 23.I); but Zscheile and co-workers (1935, 1943), who excited fluorescence with white light, and Vermeulen, Wassink and Reman (1937), who used ultraviolet and blue exciting light, obtained fluorescence curves without any indication of such additional bands (*cf.* fig. 23.2).

Zscheile and Harris (1943) found that the fluorescence spectrum of chlorophyll was exactly the same whether excited by the mercury lines

365, 404.7, 435.8 or 546 mμ, or by white light filtered through a red, orange yellow, violet, blue or green filter.

Whether chlorophyll is capable of emitting a weak, but long-lasting *infrared* fluorescence (originating in a metastable state, *cf.* page 753) is uncertain. Calvin and Dorough (1947) have described such a "phosphorescence," but Livingston and co-workers (1948) could not confirm their observations (*cf.* page 795).

Figure 23.1B shows, beside the fluorescence spectra of the two chlorophylls, also those of the two pheophorbides, and of two porphyrins. The pheophorbides (*i.e.*, chlorophyllides in which hydrogen has been substituted for magnesium) fluoresce not less strongly than the chlorophylls or chlorophyllides themselves; but certain other substitutions in the same position in the molecule (*e. g.*, copper instead of magnesium) cause complete disappearance of fluorescence.

Stern and Molvig (1935, 1936[1]), Stern and Deželić (1936) and Stern (1938) have investigated the fluorescence of numerous porphyrins and chlorins. They found that, similarly to chlorophyll, all of them fluoresce with red light, even when excited by violet or ultraviolet radiation. The main fluorescence band always lies close to the first absorption band in the red—whether this band is the weakest of the whole absorption spectrum (as in some porphyrins) or the strongest one (as in chlorins and phorbins). Stern (1938) found that tetrapyrrole compounds without the closed porphin ring system (*e. g.*, the bile pigments), as well as compounds in which the conjugation in the porphin ring is interrupted, do not obey this rule, and do not show *sharp* fluorescence bands at all. He therefore considered a sharp red fluorescence band as an important characteristic of the all-round conjugated porphin ring system.

According to the term systems given in figures 21.9 and 21.25, the appearance of the red band in fluorescence, to the exclusion of all the other bands, means that all excitation energy in excess of that corresponding to the lowest, nonvibrating, excited electronic state is dissipated before fluorescence can occur—probably first by internal distribution of this energy among vibrations within the pigment molecule, a process known as "internal conversion," and then by gradual transfer of vibrational quanta to the medium. The dissipation is interrupted at the lowest excited level, whether this is level *A* (in porphyrins), *Y* (in chlorins and phorbins) or *Z* (in bacteriochlorophyll), long enough to allow a significant proportion of the excitation energy to escape as fluorescence.

It was suggested by Franck and Herzfeld (1937) that the capacity of chlorophyll to convert rapidly quanta of larger size into smaller red quanta may be important for the function of this pigment in photosynthesis, because it prevents the occurrence of undesirable photochemical reactions that could be sensitized by the larger quanta. This surmise may or may not be correct, but since the same property is shared by all porphyrins and chlorins, it cannot explain the special suitability of chlorophyll as photocatalyst in photosynthesis.

TABLE 23.II

A. FLUORESCENCE BANDS OF PORPHIN IN DIOXANE

Band no.	−1	0	1	2	3
Interpretation	$A_1 \rightarrow X_1$	$A_0 \rightarrow X_0$	$A_0 \rightarrow X_1$	$A_0 \rightarrow X_2$	$A_0 \rightarrow X_3$
λ, mμ	591	616.5	644	669.5	684
ν, cm.$^{-1}$	16,900	16,200	15,500	14,950	14,600
$\Delta\nu$, cm.$^{-1}$		700	700	550	350

B. VIBRATIONAL QUANTA IN FLUORESCENCE SPECTRUM OF CHLOROPHYLLS *a* AND *b*

Value	Chlorophyll *a*			Chlorophyll *b*		
λ, mμ	665	736	801	646	713	789
ν, cm.$^{-1}$	15,000	13,580	12,480	15,500	14,020	12,670
$\Delta\nu$		1420	1100		1480	1350

The sequence of several bands observed in the fluorescence spectra of chlorophyll and its derivatives (as well as in those of the porphyrins) probably corresponds to transitions from the excited electronic state A_0 (or Y_0) to different vibrational levels of the ground state, X_0, X_1, X_2 ... As an example, we consider first the fluorescence spectrum of porphin, as observed by Stern and Molvig (1936). It consists of the five bands listed in Table 23.II. The band at 616.5 mμ is the strongest. Comparison with the absorption spectrum of the same compound (compare Table 21.V) shows that all five fluorescence bands probably are related to the one weak band in the absorption spectrum at 613 mμ. According to the term system in figure 21.9, this is the $0 \rightarrow 0$ band of the $A \rightarrow X$ system. The band designated as "−1" in Table 23.II must then be an "anti-Stokes band," originating in the next-to-lowest vibrational level of the excited electronic state A. According to Table 21.V, the level A_1 is situated 1520 cm.$^{-1}$ on the short-wave side of the $0 \rightarrow 0$ band; while the fluorescence band "−1" is displaced only 700 cm.$^{-1}$ in the same direction. This difference can be interpreted by assuming that the state in which the "−1" band originates belongs to a vibrational sequence not excited by light absorption. Or, one can postulate that the "−1" band does originate in the vibrational level A, but terminates in a vibrational level of the ground state situated about 800 cm.$^{-1}$ above the X_0 state.

Bands 1, 2 and 3 probably all originate in the nonvibrating excited state A_0, and terminate in the successive levels, X_1, X_2 and X_3, of the ground state. According to this interpretation, the vibrational quanta of the ground state of porphin, which are excited by fluorescence, are comparatively small and rapidly declining in size (700–800, 550 and 350 cm.^{1}). In chlorophyll, on the other hand, the two sets of vibrational quanta, derived from the absorption and the fluorescence spectrum, respectively, are of the same order of magnitude (1100–1500 cm.$^{-1}$). This is shown by a comparison of the $\Delta\nu$ values in Table 21.VI with those in the Table 23.IIB. (For

the sake of uniformity, all figures in Table 23.IIB are based on the data of Dhéré and Raffy in Table 23.IA.)

In *bacteriochlorophyll*, the distance between the two fluorescence bands (shown in fig. 24.4) is much larger than in chlorophyll—2400 cm.$^{-1}$; and the short-wave (red) band is weaker than the long-wave (infrared) band. This points to two different electronic transitions, rather than two vibrational bands in a common band system. The two fluorescence bands may even belong to two different molecular species. Only the stronger of them —that at 810 mμ—appears to be correlated with a known absorption band of bacteriochlorophyll, that at 770 mμ (*cf.* fig. 21.7). (This correlation implies that $\Delta\lambda$, the displacement of the fluorescence band relative to the absorption band, is of the order of 40 mμ, as against <15 mμ in ordinary chlorophyll.)

This may be the place to mention the luminescence that occurs when a chlorophyl solution in *tetralin* is heated to 125° C. This phenomenon was first described by Rothemund (1938) and investigated spectroscopically by Stewart, Knorr and Albers (1942). The maximum of the luminescence band was found at 677.5 mμ. After the tetralin solution was heated for five minutes, chlorophyll showed a change—its fluorescence band was shifted from its original position at 688.5 to 671.0 mμ and was reduced to one third its original intensity; the absorption spectrum also had undergone a transformation, especially in the blue-violet region. The origin of this luminescence is as yet unknown, and its interpretation as chemiluminescence, suggested by the investigators, although plausible, requires confirmation.

2. Yield of Fluorescence and Life-Time of the Excited States of Chlorophyll

The fluorescence yield can be defined either as the proportion of absorbed *energy* re-emitted in the form of radiation or, more significantly, as the proportion of re-emitted *photons*. The two figures coincide only in the case of resonance fluorescence; usually (particularly in condensed systems) the emitted light is of a lower frequency than the absorbed light ("Stokes' rule"), and the "energy yield," ϵ_f, is therefore smaller than the "quantum yield," φ.

The relation $\epsilon_f < \varphi$ holds true for the main fluorescence bands of all derivatives of porphin and chlorin, and the difference becomes particularly large if blue, violet or ultraviolet light is used for excitation. It was stated above (page 748) that only red light is emitted in the fluorescence of these compounds; this means that the absorbed ultraviolet, violet or blue energy quanta are transformed into much smaller red quanta, while up to 50% of the absorbed light energy is dissipated. Since the "theoretical" life-time of the excited state B (upper state of the blue-violet band system) is of the order of 5×10^{-8} sec. (*cf.* page 634), the absence of even 0.01% fluorescence in this system shows that the electronic energy of the state B is dissipated in less than 5×10^{-12} sec., *i. e.*, after less than one hundred molecular vibrations.

It appears that all porphyrins, chlorins and phorbins, when brought into an electronic state with an energy higher than that of their lowest excited state, rapidly lose this excess energy, and revert into state *A* (porphyrins), *Y* (chlorins and phorbins) or *Z* (bacteriochlorophyll and its derivatives). Differences in the yield of red fluorescence among various compounds of these three classes must then be due to variations in the longevity of the lowest excited states (*A*, *Y* or *Z*). In "nonfluorescent" compounds (*e. g.*, copper pheophorbide, with $\varphi < 0.01\%$) the energy of the lowest excited state must be dissipated within 10^{-11} sec.; while in "strongly fluorescent" compounds, such as chlorophyll itself, this state must persist for 10^{-9} or 10^{-8} sec. to allow at least several per cent of the excitation energy to be re-emitted as fluorescence.

Prins (1934) mentioned that the quantum yield of the fluorescence of chlorophyll *a* in ethanol is smaller if excited by blue than if excited by red light—an observation that seemed to indicate that the conversion of chlorophyll molecules in state *B* into those in state *Y* is less than 100% efficient, perhaps due to competition on the part of a photochemical reaction with the solvent (*cf.* page 756). However, this result needed confirmation; Dutton, Manning and Duggar (1943), who worked with acetonic solutions of chlorophylls *a* and *b*, found identical yields of fluorescence when using excitation by violet light (436 mμ) or yellow light (578 mμ). More recently, measurements of the relative yield of the fluorescence of chlorophyll, excited by the Hg lines 435.8 and 577–579 mμ, and by narrow bands centered at 645 and 681 mμ (isolated by means of Farrand's interference filters) were made by Livingston and co-workers (1949). Table 23.IIC shows their results.

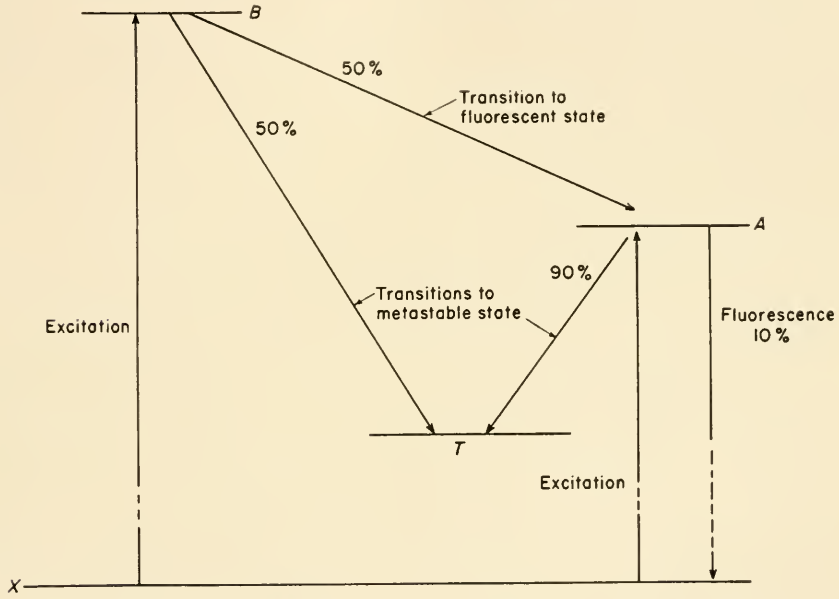
Table 23.IIC

Effect of Wavelength of Exciting Light on Relative Quantum Yield of Chlorophyll Fluorescence

Solvent	Chlorophyll *b*:	Chlorophyll *a*:	
	$\frac{\varphi(435.8\ m\mu)}{\varphi(642.5\ m\mu)}$	$\frac{\varphi(435.8\ m\mu)}{\varphi(580.0\ m\mu)}$	$\frac{\varphi(681.0\ m\mu)}{\varphi(645.0\ m\mu)}$
Ether	0.58	0.58	0.51
Methanol	0.62	0.63	..
Acetone	0.73	0.67	0.88

Table 23.IIC indicates that the yield of fluorescence of both chlorophylls is much higher when it is excited in the orange-red system, than when it is excited in the blue-violet band. Furthermore, the yield drops sharply in the far red—a result which recalls the drop in the quantum yield of photosynthesis observed in the same range by Emerson and Lewis

(*cf.* chapt. 30). Livingston mentioned that a similar decline of fluorescence in the long-wave region was noted by Solomin with other dyestuffs. Such a drop would be understandable if the long-wave wing of the red absorption band were covering another band, leading to a different excited electronic state. If, however, the long-wave wing is due to transitions originating in vibrational levels of the normal state and leading to the same excited state as that reached in the peak of the band (and this is what one would think offhand), then the lower quantum yield of fluorescence and sensitization is peculiar and awaits interpretation.



Scheme 23.I. The metastable state of chlorophyll (*T* may mean "triplet" or" tautomeric").

In contrast to the observations in the far red the confirmation by Livingston of Prins's observation—that the fluorescence yield of chlorophyll in solution is considerably smaller when fluorescence is excited by blue-violet than when it is excited by red light—has no parallel in the wavelength dependence of the quantum yield of photosynthesis in live cells (*cf.* chapter 30,), and (what seems to be even more remarkable) in the quantum yield of chlorophyll-sensitized autoxidations in the same medium (alcohol) (*cf.* chapter 18, page 513). This discrepancy confirms the supposition that photochemical sensitization by chlorophyll does not compete directly with fluorescence, but is brought about by transfor-

mation of chlorophyll into a long-lived active (tautomeric, isomeric or metastable electronic) state. This transformation may perhaps occur not only from state A, but also directly from state B (fig. 21.9 and scheme 23.I). Molecules excited to the higher state B, have the choice of going directly to T, or first to A and thence to T. Consequently, the total probability of conversion to a metastable state is higher for molecules excited by blue-violet, than for those excited by red light. In the numerical example indicated in scheme 23.I, this probability is 95% for molecules in state B and 90% for molecules in state A—leaving for fluorescence, 5% in the first case and 10% in the second.

More measurements of the yield of fluorescence of chlorophyll under different conditions are greatly needed for better understanding of the photochemistry of this compound. At present, our knowledge of the *absolute* yield of chlorophyll fluorescence in solution is limited to a single estimate by Prins (1934), who found it to be of the order of 10% (in a 10^{-4} M solution of chlorophyll a in ethanol). He did not take into account self-absorption (*cf.* fig. 23.3), and, not knowing the exact experimental arrangement, it is impossible to estimate its influence on the yield.

Perrin (1929) calculated 3×10^{-8} sec. for the life-time of chlorophyll fluorescence, from polarization measurements in four solvents of different viscosity.

If we accept the Prins estimate as substantially correct, it follows that, even in so-called "strongly fluorescent" chlorophyll solutions, 90% of the excited pigment molecules lose their excitation energy before they have an opportunity to fluoresce. The actual mean life-time of the excited state Y under these conditions must be of the order of $0.1 \times 8 \times 10^{-8} = 8 \times 10^{-9}$ sec. (*cf.* equation 22.3). Not all of the excitation energy needs to be lost during this period. The fate of the residual excitation energy is not definitely established, but in the next section we will discuss indications (mentioned in Volume I, page 483) that the chlorophyll molecules in state A, which fail to emit fluorescence, are converted into a *long-lived active form* (level T in scheme 23.I), which may represent a metastable triplet electronic state, or a tautomer, or an oxidized or reduced molecular species.

The fluorescence yield of *allomerized* chlorophyll a in methanol is, according to Livingston (1949) about *one-half*, and that of allomerized chlorophyll b about *twice*, that of the intact pigment. The change is the same when the "allomerization" of the pigments is caused by iodine, or accelerated by catalysts such as $LaCl_3$.

This confirms that all these reagents lead to the formation of the same product—which, according to Fischer (Volume I, page 461), is chlorophyll oxidized at the carbon atom C(10).

3. Factors Limiting the Yield of Fluorescence

What process (or processes) cause the abbreviation of the natural lifetime of the excited state A of dissolved chlorophyll molecules and prevent a 100% yield of fluorescence? If a single molecule in vacuum absorbs a light quantum, the probability of fluorescence is 100%, since fluorescence is the only way in which the molecule can get rid of excess energy. A temporary distribution of this energy between the internal degrees of freedom or even its transformation into chemical energy, by isomerization or dissociation, can *delay* the emission of fluorescence, but cannot prevent it. Sooner or later, the original composition and configuration of the molecule will be restored, excess energy will again be converted into the original form of electronic excitation and emission of fluorescence will take place.

In condensed phases, on the other hand, the yield of fluorescence is usually, if not always, less than unity. This weakening of fluorescence by the medium has several causes. The presence of a large number of foreign molecules can make both the above-mentioned "fluorescence-delaying" processes—the dissipation of the electronic excitation energy within the absorbing molecule ("internal conversion") and the transformation of excitation energy into chemical energy—partly or completely irreversible, and thus reduce the probability of fluorescence. The proximity of foreign molecules adds new possibilities of chemical utilization of light energy, since the excited molecule can now react with other molecules encountered during the excitation period. A *fourth* possibility of energy loss in condensed systems—in addition to (*1*) internal conversion, (*2*) chemical reaction within the excited molecule and (*3*) chemical reaction with molecules of the medium—is (*4*) bulk transfer of electronic energy from the excited molecule to a foreign molecule close by. Finally (*5*), if the fluorescent molecules are present in sufficient concentration, "self-quenching," *i. e.*, energy dissipation by interaction of excited with nonexcited pigment molecules, may become significant.

It is important to keep in mind that the interaction of a fluorescent molecule with a given medium can be complex. For example, the presence of the medium may make internal energy dissipation *irreversible*, but also *slower*. Furthermore, the general effect of the solvent surrounding the fluorescent molecule may be one of *quenching*, but the specific effect of association of certain groups in the medium with certain groups in the fluorescent molecule may be one of "protection", *i. e.*, *stimulation* of fluorescence, and so on.

We will now consider in more detail the five ways in which fluorescence can be affected by the mutual closeness of molecules in condensed systems.

(*a*) *Internal Conversion* (*Physical Dissipation of Excitation Energy*)

In complex molecules, *electronic excitation states* are likely to be converted, before fluorescence has had time to occur, into *strongly vibrating* states. The reason for this is that the nonvibrating (or weakly vibrating) excited electronic state may have not only the same energy, but also the same nuclear configuration as strongly vibrating, electronically nonexcited states. (This is the many-dimensional equivalent of the "crossing of potential curves" of diatomic molecules.) It was stated above that, in an isolated molecule, internal conversion is reversible, and therefore can only delay but not prevent fluorescence. In condensed systems, on the other hand, the vibrational quanta of the "converted" molecule can be lost, by collisions, to the molecules of the medium. The loss of one or a few vibrational quanta may be sufficient to make return into the original, electronically excited state impossible, and thus prevent fluorescence. The remaining vibrational quanta can then be lost, at leisure, one by one, to the surrounding molecules.

(*b*) *Isomerization or Dissociation* ("*Monomolecular*" *Chemical Quenching*)

The excitation energy can be used, within the absorbing molecule (either directly, or after "internal conversion" into vibrational energy), for a chemical change, *e. g.*, isomerization, or dissociation. Here again, the reversible character that the process has in vacuum may be lost in the presence of foreign molecules. The photochemically formed isomer or tautomer may, for example, lose one or several vibrational quanta by collisions, and thus become incapable of reconversion into the original, electronically excited form. Similarly, the recombination energy of the dissociation fragments may be lost to foreign molecules serving as "third bodies," so that the original molecule will be formed directly in the nonexcited ground state. (In this case, the dissociation remains *chemically* reversible, but recombination is not the exact reversal of photochemical dissociation, since it occurs without chemiluminescence.)

(*c*) *Reaction with Foreign Molecules* ("*Bimolecular*" *Chemical Quenching*)

The presence of foreign molecules opens the possibility of "bimolecular" chemical reactions of electronically excited molecules. The reaction partners may be the molecules of the solvent, or molecules of an accidental impurity (*e. g.*, dissolved oxygen) or specially provided "quenchers." In this case again, even if the photochemical reaction is reversed afterward, the reversal is likely to occur without chemiluminescence; in other words, the light energy used for the photochemical forward reaction will not be available for re-emission in the back reaction.

As to the specific nature of the quenching reactions, two types appear most likely: Oxidation-reductions and complex formation. The first type can be represented by equation (23.1A or B):

(23.1A) $\mathrm{Chl}^* + \mathrm{Q} \longrightarrow \mathrm{oChl} + r\mathrm{Q}$

(23.1B) $\mathrm{Chl}^* + \mathrm{Q} \longrightarrow \mathrm{rChl} + \mathrm{oQ}$

(where o stands for oxidized, r for reduced, Q for quencher and Chl* for excited chlorophyll). The second type is described by equation (23.2):

(23.2) $\mathrm{Chl}^* + \mathrm{Q} \longrightarrow \mathrm{Chl}^*\mathrm{Q} \longrightarrow \mathrm{ChlQ} \longrightarrow \mathrm{Chl} + \mathrm{Q}$

the quenching effect being due to accelerated internal conversion of excitation energy into vibrational energy in the complex Chl*Q.

If Q is the solvent, only reactions (23.1A and B) can be classified as "chemical quenching," while reaction (23.2) becomes identical with "physical" energy dissipation in the solvated pigment molecule.

Photochemical reactions with foreign molecules interfere with fluorescence only if they take place *directly, i. e.*, by encounters of electronically excited molecules with the quencher. If, on the other hand, reactions of this kind are preceded by monomolecular steps such as isomerization (or dissociation) of the excited molecule, their effect on fluorescence may become negligible (since the molecules that take part in the photochemical reaction are the ones lost for fluorescence anyhow; (*cf.* second scheme on p. 483, Vol. I). We will use this concept below; *cf.* page 788 in interpreting the nonquenching of chlorophyll fluorescence by certain compounds whose autoxidation is sensitized by this pigment.

Sometimes the isomeric molecule, formed after light absorption, has a certain chance of reverting into the original electronically excited state (*e. g.*, with the help of thermal energy). Similarly, photochemical dissociation products may have a certain chance of forming an electronically excited molecule by recombination. If this exact reversal of the primary photochemical process occurs after a period that is long compared to the duration of ordinary fluorescence (10^{-7} sec.), we observe the emission of "delayed fluorescence" or "phosphorescence." Chemical reactions of the metastable, isomeric photoproduct (or of the dissociation products) will cause quenching of this delayed emission. This is the mechanism of the strong quenching of phosphorescence of many dyestuffs by oxygen (*cf.* page 789).

(*d*) *Bulk Transfer of Electronic Energy*

Transfer of electronic excitation energy "in bulk" to another type of molecules can lead to the quenching of the fluorescence of the originally excited molecular species, either by substitution of a secondary (stronger

or weaker) "sensitized" fluorescence of the quencher, or (if the quencher is nonfluorescent) by complete conversion of the excitation energy into heat.

Three types (or rather three limiting cases) of electronic energy transfer mechanisms are known. The first, which is the only one possible when the distance between the excited molecule and the quencher is $>10^{-5}$ cm. (for visible light quanta), is trivial—the emission of a light quantum by the primarily excited molecule and its reabsorption by a molecule of the quencher, a process similar to the "self-absorption" of fluorescence (page 745). The second mechanism is energy transfer by kinetic collisions (so-called "collisions of the second kind"), or "encounters," to use a term more appropriate for molecules in solution. It is associated with the mutual disturbance of the electronic structures of the two molecules in contact, and requires approach to within the kinetic collision diameter (10^{-8} to 10^{-7} cm.). In this case, the energy exchange is not contingent on "resonance" between the electronic excitation states of the two partners, since a considerable fraction of electronic energy can be converted into vibrational or kinetic energy in the collision. A third and perhaps most interesting possibility is the "resonance transfer" of electronic excitation energy between two practically undisturbed molecules, which can occur when these molecules are within a distance smaller than the wave length of the exchanged quantum ($\simeq 10^{-5}$ cm. for visible light), and does not require an actual "contact" between them. The probability of this kind of transfer depends decisively on resonance between the energy-exchanging molecules (*i. e.*, on the mutual overlapping of the fluorescence band of the donor and the absorption band of the acceptor). The phenomenon was first discussed by Kallman and London in application to sensitized fluorescence *in gases*. Similar considerations were afterward applied to *solutions* by J. Perrin (1926, 1927), who used classical electrodynamics, and by F. Perrin (1929, 1932), who first attempted a quantum-mechanical treatment. F. Perrin used this energy transfer mechanism to interpret so-called "concentration depolarization" of fluorescence in solution (decrease in the degree of polarization with increasing concentration). Subsequently, several other phenomena in fluorescence and photochemistry have been ascribed to energy exchanges of this type, and improved theoretical treatments were evolved by Vavilov and co-workers (1942, 1943, 1944), Förster (1946, 1947, 1948) and Arnold and Oppenheimer (1950). Because of the importance of the resonance transfer concept for the photochemical mechanism of photosynthesis (in particular, for the possible participation of phycobilins and carotenoids in it), these papers will be discussed in greater detail in chapters 30 and 32. Here, we are concerned only with the possibility of quenching (or excitation) of chlorophyll fluorescence being

due, in some cases, to resonance transfer of excitation energy not requiring molecular contact. Examples will be found on p. 778 (quenching of dye fluorescence by other dyes), p. 790 (chlorophyll *a* fluorescence sensitized by *b*) and in chapters 24 and 32 (energy transfer between pigments *in vivo*). Self-quenching, too, may be caused by resonance (p. 797).

(*e*) *Self-Quenching*

Experience shows that quenching by molecules identical with the excited one often is particularly strong. This is revealed by rapid decrease in the yield of fluorescence of many substances with increasing concentration of the fluorescent pigment. This strong self-quenching probably is due to very close resonance between the fluorescent molecule and the quencher. However, resonance transfer of electronic energy does not in itself explain self-quenching, because, from the point of view of the yield of fluorescence, it should be irrelevant whether the excitation energy stays with the originally excited molecule or is transferred to another molecule of the same kind. Nevertheless, self-quenching can result from resonance, if some additional phenomena are taken into account. Effective energy dissipation can result either from kinetic encounters of excited and normal pigment molecules ("kinetic self-quenching"), or from their close average proximity ("static self-quenching"). In the first case, we can postulate transient formation of dimeric molecules during the encounter. Resonance between the two structures, $D^*D \rightleftharpoons DD^*$, creates an attraction force, leading to a more intimate contact of the two electronic systems than that established in an encounter of two nonresonating molecules. This can bring about accelerated conversion of electronic into vibrational energy; the molecules which met as $D^* + D$ will then separate as $D + D$.

Resonance transfer of excitation energy over distances wider than a collision diameter also can explain self-quenching, if one makes certain auxiliary hypotheses. Förster (1947, 1948) suggested, for example, that even dyestuff solutions which reveal no equilibrium dimerization (*i. e.*, show no effect of concentration on the absorption spectrum; *cf.* below), contain a small proportion of nonfluorescent, dimeric molecules. If the resonance exchange of excitation energy is so fast that this energy visits, during its life time, a considerable number (say > 100) pigment molecules, the presence of even a single dimer in this series of "hosts" may suffice to "trap" the excitation energy, and dissipate it into heat. (Of course, for this mechanism to be effective, the absorption band of the dimer and the fluorescence band of the monomer must overlap sufficiently to permit resonance exchange.)

Franck and Livingston (1949) suggested another possibility—that

energy traps are provided by monomers which are in the state of exceptionally strong thermal agitation. (This hypothesis explains also the decline in the intensity of fluorescence, usually observed with rising temperature.) Accelerated dissipation of electronic energy in "hot" molecules is plausible, since in order to convert electronic energy into vibrational energy, a configuration of the nuclei must be reached in which the electronic system has the same energy in the excited and in the normal state ("crossing point of two potential curves" in the diatomic model). This configuration usually can be achieved only by combination of electronic excitation with vibrations of appropriate kind; the excited molecule must wait until an accidental fluctuation of thermal agitation supplies the critical degree of freedom with the amount of vibrational energy required to make internal conversion possible. The higher the temperature, the shorter will be this waiting period, and the greater the probability of internal conversion occurring during the electronic excitation period, and competing successfully with fluorescence.

One may ask: how can resonance migration of excitation energy assist this mechanism of dissipation? Does it make any difference whether the excitation stays with one molecule and awaits there the thermal fluctuation that will permit it to be dissipated, or whether it visits a thousand molecules during the same total life-time, spending a correspondingly short time with each of them? We said elsewhere in this book that a man cannot change his life expectancy by sleeping every night in a different bed! Whether this analogy applies here or not depends on the relative duration of a thermal fluctuation and electronic excitation. If the fluctuation is short-lived, in comparison not only with the total duration of electronic excitation, but also with the time during which the excitation remains with a single host molecule, then migration can have no effect—the chance of being hit by lighting is the same whether one spends the thunderstorm under a single tree or shifts every minute from one to another (identical) tree. If, however, the state of abnormal thermal agitation lasts long compared to electronic excitation of a single molecule, then resonance exchange will increase the chances of the two meeting in one molecule. (If one house in a hundred in a town is quarantined for smallpox, then a visitor who comes to town will be much safer if he stays the whole time in the first house he has entered than if he visits a hundred houses, spending a correspondingly short time in each of them.)

In mathematical form, the probability of two independent events, one lasting T sec. (electronic excitation) and another t sec. (thermal fluctuation) overlapping each other in a single molecule, is changed, by subdividing T into n periods of T/n sec. each, by the factor:

$$(nt/T) + 1$$

which is significantly different from 1 if t is *not* $\ll T/n$, *i. e.*, if the duration of the thermal fluctuation is not much shorter than that of electronic excitation of a single molecule. The former can be postulated to last for a period of a few molecular vibrations, thus $t \simeq 10^{-12}$ sec. The total period of electronic excitation is $T \cong 10^{-8}$ sec. (for example, in alcoholic chlorophyll solution, the natural life-time of excitation is $\simeq 5 \times 10^{-8}$ sec.; the actual life-time must be ten times shorter, as indicated by a fluorescence yield of about 10%). Under these conditions, for t to be not much shorter than T/n, the number n must be higher than 10^4, *i.e.*, excitation energy must be exchanged more than ten thousand times before its dissipation (staying $< 10^{-12}$ sec. at each molecule visited). The role of thermally excited ("hot") monomeric dyestuff molecules in the concentration quenching of fluorescence thus is predicated on this minimum length of energy exchange chains, and on the possibility of internal conversion occurring during the extremely short sojourn of the electronic energy in the hot molecule. It may be suggested that conversion requires (at least) a period of a single molecular vibration ($\simeq 10^{-13}$ sec.). This would restrict quenching by hot molecules (in the case of chlorophyll) to exchange chains not shorter than 10^4 and not longer than 10^5 molecules.

In addition to the various *physical* mechanisms of self-quenching which were considered so far, two *chemical* mechanisms also are feasible, analogous to the two chemical mechanisms of quenching by foreign substances, discussed in section (*c*). They are: an oxidation-reduction reaction between the excited and a normal molecule (photodismutation):

(23.3) $$\mathrm{Chl^* + Chl \longrightarrow oChl + rChl}$$

and formation of nonfluorescent dimers by two normal molecules:

(23.4) $$\mathrm{Chl + Chl \rightleftharpoons Chl_2}$$

Dimerization of dyestuff molecules is favored by the fact that marked resonance attraction must occur not only between an excited and a normal molecule (as discussed above), but also between two molecules in the *non-excited* state. (This application of London's theory of intermolecular forces to pigment molecules was suggested by Rabinowitch and Epstein 1941.) The tendency of dyestuff molecules to dimerize (or polymerize) in solution may be attributed to such resonance phenomena. In many cases—perhaps the majority of those observed so far—self-quenching of fluorescence appears to be due to "permanent" dimerizations (or polymerizations) rather than to reaction (23.3) or dimerization after excitation. The decline in yield of fluorescence with increasing concentration is in this case a direct measure of the degree of association. Like the unstable dimers formed in light, the stable dimeric molecules formed in the dark can be

nonfluorescent either because of rapid internal conversion of excitation energy, or because of the occurrence of internal oxidation-reductions (disproportionations). It was already mentioned above that combination of dimerization with resonance exchange of energy can lead to strong quenching even when the number of dimeric molecules is very low.

Whether self-quenching is due to pre-existing dimers (or polymers) or to encounters between excited and nonexcited monomers (in which dimers are formed) can often be deduced from observations of the absorption spectra: In the first case the absorption spectrum of the dyestuff must change with concentration (as observed, *e. g.*, by Rabinowitch and Epstein with thionine and methylene blue); in the second case, Beer's law must be obeyed (*i. e.*, the absorption spectrum of the dye must be independent of concentration). Quenching by a chain of energy exchange reactions, with a dimer as occasional link in the chain, suggested by Förster, also does not require marked deviations from Beer's law, since the number of dimers present can be very small.

Which of the several possible quenching or self-quenching processes actually limits the yield of fluorescence in a given solution is not easy to say. If the pigment is stable in light, and its fluorescence is unchanged after long illumination, "physical" quenching is the likely mechanism. True, even when chemical quenching *does* occur, the dyestuff may be photostable, if the quenching reaction is reversible; and the yield of fluorescence may remain unchanged with time, even when quenching initiates an irreversible sensitized chemical reaction, if the products of this reaction do not quench fluorescence stronger (or weaker) than the originally present molecular species. Usually, however, chemical quenching is *not* entirely reversible, but causes a more or less rapid chemical change of the fluorescent pigment; and sensitized chemical reactions often do lead to the formation of products whose presence changes the intensity of fluorescence. When this is the case, the fluorescence yield must change with time —either because the original fluorescent compound is converted into a new one, with different properties, or because the fluorescence yield of the original species is changed by the accumulation of the products of the sensitized reaction. Therefore, whenever the yield (or the spectrum) of fluorescence changes with time, the indication is strong that chemical factors account for at least part of the quenching (for examples, see page 764).

A systematic study of the effects of solvent, concentration, admixtures, temperature and other factors on the yield of fluorescence is needed to elucidate the quenching problem, which was discussed above on the basis of general possibilities more than on the basis of actual observations with chlorophyll. Studies of this kind would be of particular interest for understanding the mechanism of sensitized photochemical reactions, such as

photosynthesis. Since this summary was written, a number of pertinent data have been collected by Livingston and co-workers. A discussion of these will be found below.

If we consider the few presently available data on the intensity of chlorophyll fluorescence in different media, we acquire the impression that physical energy dissipation *and* chemical quenching must both play a part in these systems; but much remains to be done before their relative roles will become clear.

The possibility that the yield of chlorophyll fluorescence in solution may be limited by photochemical *dissociation* of chlorophyll (*e. g.*, into "monodehydrochlorophyll" and a hydrogen atom) was suggested by Franck and Wood (1936). This hypothesis was discussed in chapter 18 (Vol. I, page 484), and it was pointed out that a quantum of red light (with an energy of about 40 kcal/einstein) is unlikely to disrupt a carbon–hydrogen bond (whose standard energy is about 100 kcal/mole), even if some energy might be gained by the solvation of the hydrogen atom. Thus, if light absorption does cause a reversible photochemical change of chlorophyll, it is more likely to be either *tautomerization*, or *reaction with the solvent.* Dissociation becomes more likely when excitation occurs in the blue-violet band, with quanta of about 60 kcal./einstein; it was mentioned before that this is one possible explanation of the lower yield on fluorescence in this region.

It is not unlikely that energy dissipation by internal conversion is the basic factor limiting the yield of fluorescence of chlorophyll in condensed systems; it is also possible that—as suggested by Franck and Livingston (1941)—tautomerization occurs as a more or less regular intermediary stage in this dissipation (*cf.* Vol. I, p. 490). Chemical interactions with solvent or admixtures, as well as self-quenching, are then to be considered as contributing factors, which further depress the yield of fluorescence under certain conditions. (According to Lewis and Kasha—*cf.* pp. 790–2—formation of a metastable triplet state could play the role ascribed above to tautomerization.)

4. Influence of Solvent on Yield of Chlorophyll Fluorescence

Chlorophyll fluoresces in all (or most) organic solvents (as well as in wax and paraffin), but with different intensity. No precise measurements of the fluorescence intensity in different solvents are available, but Franck and Levi (1934), Albers and Knorr (1935), Knorr and Albers (1935), Knorr (1941) and Zscheile and Harris (1943) gave some preliminary results; all these investigators agree that wide differences occur both in the initial intensity of fluorescence in different solvents, and in its change with time (*cf.* Table 23.III).

TABLE 23.III

CHANGES OF FLUORESCENCE INTENSITY OF CHLOROPHYLL WITH TIME IN DIFFERENT SOLVENTS[a] (AFTER ZSCHEILE AND HARRIS 1943)

Time of illumination, min.	Fluorescence intensity (relative units)					
	Ethyl ether	Isopropyl ether	Acetone	Cyclo-hexane	Benzene	CCl_4
0.25	57.5	144	178	61.0	110	90.5
1	54.0	139	167	49.0	71.0	44.5
2	51.5	134	163	44.0	55.6	29.0
3	—	130	161	42.8	44.0	21.0

[a] According to Franck and Levi (1934), the fluorescence of chlorophyll is about twice as strong in ethanol as in aniline.

More recently, Livingston and co-workers found that fluorescence is very weak or entirely absent in nonpolar solvents if these are free from traces of water, alcohols or amines.

An effect of solvent on the yield of fluorescence is to be expected whatever the mechanism of quenching. Even the probability of "monomolecular" chemical quenching by dissociation (or tautomerization) of the excited molecule probably depends on the degree of stabilization of the dissociation products (or of the tautomeric form) by solvation. (Well known is the effect of solvent on enolization—a tautomeric transformation that can occur in chlorophyll; *cf.* Vol. I, page 444.) The occurrence of self-quenching, too, may differ strongly depending on the solvent, as indicated, *e. g.*, by the vastly different stabilities of dimeric dyestuff molecules in water and alcohol. The velocity of physical dissipation of excitation energy by "internal conversion" also must depend on the nature of the medium to which the vibrational quanta are to be transferred in order to make dissipation irreversible. Finally, the probability of "bimolecular" chemical quenching by reaction with the medium obviously is a function of the nature and purity of the solvent.

The "physical" quenching of fluorescence by the solvent can be expected to be least efficient in solvents of symmetric, nonpolar nature, such as cyclohexane or carbon tetrachloride. However, recent evidence shows that chlorophyll solutions of this type fluoresce less strongly than those in more polar or polarizable solvents, and this makes it probable that chemical interactions of the pigment molecules may often be at least as important as physical dissipation. The rapid decline of fluorescence with time, found in many chlorophyll solutions (*e. g.*, those in benzene and carbon tetrachloride), also points to chemical interaction; but whether it involves the solvent or impurities (*e. g.*, dissolved oxygen) remains to be established. It is also uncertain whether the rapid decline of fluorescence is caused by a chemical transformation of chlorophyll, or by chlorophyll-sensitized formation of substances with strong quenching properties.

Observations of Albers and Knorr (1934, 1935) and Knorr (1941) concerning the changes in the fluorescence spectra of chlorophylls *a* and *b* with time, in different solvents (ether, acetone, benzene and methanol), and under different atmospheres (air, oxygen, carbon dioxide and nitrogen) revealed a bewildering variety of shifts in positions, shapes and intensities of the fluorescence bands, which do not lend themselves to easy interpretation, but indicate complex chemical changes. Apparently, both the solvent and the dissolved gases participated in chemical reactions with excited chlorophyll molecules. In some systems, these reactions led to a complete disappearance of fluorescence after less than one hour of illumination. One reason for the complexity of the results of Knorr and Albers may have been the use of unfiltered light from a powerful mercury arc. Strong ultraviolet irradiation may have caused chlorophyll to react with substances that would not have affected it in visible (particularly red) light.

One strange observation of Knorr and Albers is that the fluorescence of chlorophyll *a* (but not that of chlorophyll *b*) in acetone (but not in other solvents) is best preserved under an atmosphere of *oxygen* (where it disappears only after twelve hours of illumination, whereas, in nitrogen or carbon dioxide, it vanishes completely in less than one hour). As described in chapter 18 (Vol. I, page 491), this may mean that the quenching of fluorescence is caused, in acetone, by a *reduction* of the pigment (and oxidation of the solvent) and that oxygen restores the reduced pigment to its original fluorescent form (*cf.* chapter 36).

We have considered so far only those changes in the intensity of fluorescence which could follow from the interaction of the chlorophyll molecule *in the excited state* with the medium. It was mentioned, however, in the introduction that another type of fluorescence effects is possible—one in which the state of the chlorophyll molecule is altered already in the dark, prior to excitation. This alteration must manifest itself in a change of the absorption spectrum. One possibility of this type is that chlorophyll may *dimerize* (or polymerize) in some solvents, and remain monomeric in others. Dimerization is known to cause the disappearance of fluorescence of many dyestuffs (such as methylene blue) in aqueous solution. In the case of chlorophyll, no similar effect has as yet been discovered—unless one considers the nonfluorescence of colloidal solutions and solid chlorophyll as the result of "quenching by polymerization." Another type of association, however, appears to be important in this case—association of chlorophyll molecules with hydroxyl groups or amine groups present in solvent molecules; in contrast to the quenching effect of dimerization, association of this type seems to be necessary to bring out the fluorescence of chlorophyll.

This conclusion follows from some remarkable observations described by Livingston, Watson and McArdle (1949). Contrary to what was generally assumed before, they found that chlorophyll solutions in nonpolar organic solvents *do not fluoresce at all* (or only very weakly), but that traces of polar admixtures, such as water or methanol, are sufficient to "activate" their fluorescence.

In this study, Livingston and co-workers used a mercury arc for excitation (mainly the lines 436 and 405 mμ). The fluorescent light was filtered through a deep-red filter, so that only the second fluorescent band of chlorophyll *a* (720 mμ) was measured. This eliminated self-absorption. (It was also stated that the *position* of the second band is less strongly affected by the solvent than that of the first band, which can be shifted by as much as 7.6 mμ, *cf.* table 23.IC; but this seems strange, since one would not

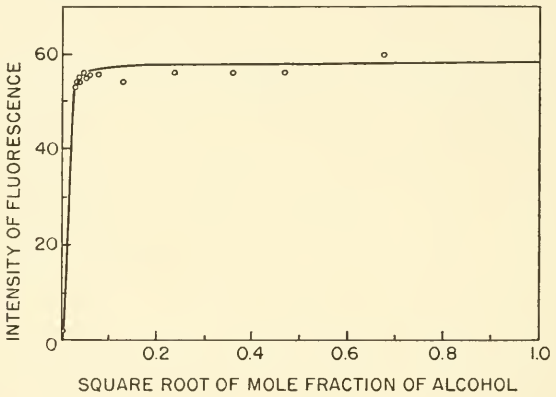


Fig. 23.5. Intensity of fluorescence of chlorophyll *a* in the system octanol-benzene (after Livingston 1948).

anticipate substantial differences in solvent effects on two emission bands originating, presumably, in the same excited state and leading to two adjoining vibrational levels of the same lower state.)

Chlorophyll solutions in dry hydrocarbons were obtained by evaporating in vacuum a solution of chlorophyll *a* or *b*, dissolving the residue in dry hydrocarbon, evaporating again, and repeating this operation until all water (which may have been present in chlorophyll from its preparation) had been removed. (Disappearance of fluorescence was used as criterion of dryness.) Various polar "activators" were then added, dissolved in the same dry hydrocarbon.

In purest dry *benzene*, the intensity of fluorescence (F) was $<3\%$ of that ordinarily observed in the same solvent (F_0); even this weak fluorescence

could perhaps be due to residual moisture (or other polar admixtures). Addition of 0.01% water (6×10^{-5} mole/l. H_2O) brought F back to the usual level, F_0.

Other solutions that proved nonfluorescent were those in *n*-heptane, iso-octane, styrene, chlorobenzene, carbon tetrachloride and diphenyl ether. Solutions in methanol, ethanol, octanol, dimethyl ether and diethyl ether, on the other hand, remained fluorescent even after drying. It was

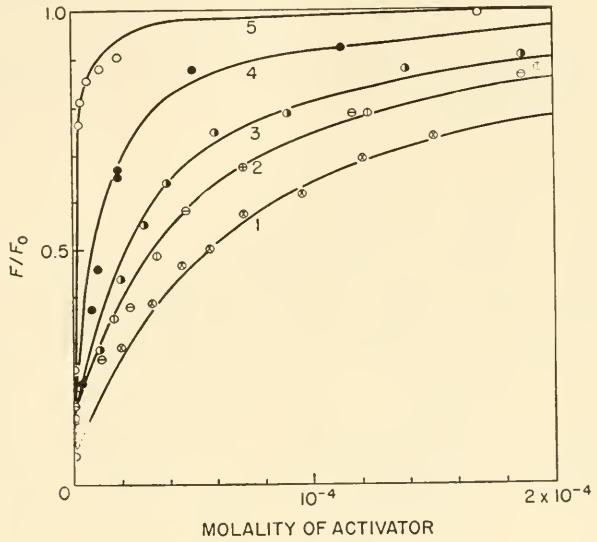


Fig. 23.6. Intensity of fluorescence of chlorophyll *a* in a hydrocarbon as function of the concentration of an activator (after Livingston 1948).

Curve number	1	2	3	4	5
Solvent.....	*n*-Heptane	Benzene	Benzene	Isooctane	Benzene
Activator.....	Phenylhydrazine	Benzyl alcohol	Cetyl alcohol	Methanol	Piperidine
Chlorophyll...	*a*	*a* or *b*	*a*	*a*	*a*

considered possible, however, that the fluorescence in the last two solvents was due to residual impurities.

Figure 23.5 shows the intensity of fluorescence as function of composition in a mixture of benzene and octanol. The fluorescence is completely activated by 0.0016 mole alcohol in a mole of hydrocarbon, corresponding to a concentration of about 10^{-3} mole/l. Similar relationships were found in mixtures of benzene with other polar solvents—alcohols and amines. Figure 23.6 shows the initial parts of five activation curves. Table 23.IIIA gives, under $[Ac]_{1/2}$, the molar concentrations of the activators needed to raise the fluorescence intensity to $^1/_2$ F_0; they range from 6.8×10^{-2}

mole/l. for dimethylamine in benzene, down to only 6.5×10^{-6} mole/l. for piperidine in benzene. Water is about half as effective as piperidine.

Although amines generally are the strongest activators, diphenylamine and diphenylhydrazine are without effect. It is noteworthy that phenylhydrazine acts as activator in low concentration, and as quencher in high concentrations.

The maximum intensity of fluorescence to which chlorophyll in a given nonfluorescent solution can be raised by activators is independent of the specific activator used, at least in the first approximation.

Activation seems to be completely reversible; in other words, fluorescence disappears again if the activator is distilled away, and reappears upon its renewed addition.

TABLE 23.IIIA

EFFICIENCY OF ACTIVATION OF FLUORESCENCE OF A 5×10^{-6} MOLE PER LITER SOLUTION OF CHLOROPHYLL *a*

(AFTER LIVINGSTON, WATSON AND MCARDLE, 1949)

Solvent	Activator	$[Ac]_{\frac{1}{2}}$	K_1
Benzene	Dimethylaniline	6.8×10^{-2}	1.05×10
Benzene	Phenol	6.0×10^{-2}	1.55×10
Benzene	Aniline	2.75×10^{-2}	4.55×10
n-Heptane	Phenylhydrazine	8.0×10^{-4}	1.70×10^3
Benzene	Phenylhydrazine	6.5×10^{-4}	1.78×10^3
Benzene	Formamide	4.3×10^{-4}	2.50×10^3
Benzene	Benzyl alcohol	4.2×10^{-4}	2.90×10^3
Benzene	Benzyl alcohol[a]	4.2×10^{-4}	2.90×10^3
Benzene	Benzoic acid	3.6×10^{-4}	3.15×10^3
Benzene	Cetyl alcohol	2.9×10^{-4}	4.15×10^3
Benzene	Octyl alcohol	2.0×10^{-4}	4.57×10^3
Iso-octane	Methyl alcohol	1.1×10^{-4}	1.03×10^4
Benzene	Benzylamine	3.9×10^{-5}	2.67×10^4
Benzene	Benzylamine[a]	3.3×10^{-5}	3.00×10^4
Benzene	Water	3.5×10^{-5}	2.95×10^4
Benzene	*n*-Heptylamine	7.5×10^{-6}	1.36×10^5
Benzene	Piperidine	6.5×10^{-6}	1.56×10^5

[a] Chlorophyll *b*.

The fluorescence *spectrum* does not change significantly with progressive activation. At least, in benzene–water mixtures, the positions of the two band peaks are the same at $F/F_0 = 0.2$, 0.8 and 1.0. As described earlier, the *absorption spectrum* of chlorophyll does change with increasing admixture of the activator (*cf.* fig. 21.26,A and B). The absorption spectrum of a fully activated solution, although it is different from that of the

nonactivated one, is independent of the nature of the activator, and bears no relation to the absorption spectrum of chlorophyll in pure activator.

The fluorescence of activated solutions is quenched by rising temperature (15–70° C.), particularly strongly in the region of partial activation. The maximum intensity, F_0, is a linear function of temperature over a comparatively wide range (a similar relationship was found also by Zscheile and Harris, 1943). In partly activated solutions, the temperature curve is not only steeper than in fully activated ones, but also shows—with some activators at least—a definite curvature.

The concentration of the strongest known activators, required to achieve complete activation, is of the same order of magnitude as that of chlorophyll itself, which in the experiments of Livingston *et al.* was 5×10^{-6} mole/l. This shows that the effect cannot be due to kinetic encounters between chlorophyll and activator (which are too rare at such low concentrations); nor can it be ascribed to a change in properties (such as dielectric constant) of the solvent as a whole. Rather, the effect must be caused by the association of chlorophyll molecules with the molecules of the activator. The change in absorption spectrum supports this assumption. If the nonassociated form is totally nonfluorescent, the intensity of fluorescence can be used to calculate the proportion of chlorophyll molecules in the associated form—assuming that the absorption coefficient of associated chlorophyll is the same as that of the nonassociated pigment. Figure 21.26.A shows that this is not quite true for chlorophyll *a* at 436 mμ; but Livingston neglected this difference. Assuming a one-to-one complex [ChlAc] the equilibrium constant of association:

$$K = \frac{[\mathrm{ChlAc}]}{[\mathrm{Chl}][\mathrm{Ac}]} \tag{23.4A}$$

can be calculated from the half-activating concentration $[\mathrm{Ac}]_{1/2}$:

$$[\mathrm{ChlAc}] = \frac{K[\mathrm{Chl}]_0[\mathrm{Ac}]_0}{1 + K[\mathrm{Ac}]_0} \tag{23.4B}$$

$$\frac{F}{F_0} = \frac{K[\mathrm{Ac}]_0}{1 + K[\mathrm{Ac}]_0} \tag{23.4C}$$

$$K = \frac{1}{[\mathrm{Ac}]_{1/2}} \tag{23.4D}$$

(*cf.* chapter 27, eq. 27.12). This is a simplified solution, based on the assumption $[\mathrm{Ac}] \simeq [\mathrm{Ac}]_0$; in other words, it assumes that the amount of activator bound in the complex is small compared to the total amount added to obtain activation. In Table 23.IIIA, the concentration [AcChl] at half-activation is 2.5×10^{-6} mole/l. for piperidine; since $[\mathrm{Ac}]_0 = 6.5 \times 10^{-6}$, $[\mathrm{Ac}]_{1/2} = 4 \times 10^{-6}$ mole/l. In other words, the simplified equations are not quite applicable to piperidine (and four other systems

at the bottom of Table 23.IIA. In this case the approximate equation (23.4D) should be replaced by the exact equation:

$$K = \frac{1}{[\mathrm{Ac}]_{1/2} - 0.5[\mathrm{Chl}]_0} \tag{23.4E}$$

Livingston used, however, the simplified equation for all the systems studied by him. Furthermore, he calculated the constants K, not from the value of $[\mathrm{Ac}]_{1/2}$, but from the average slope of the activation curve. He obtained in this way the K-values given in Table 23.IIIA, which differ somewhat (although not in the order of magnitude) from values that could be calculated by means of equation (23.4E). (For example, the latter would give, for piperidine, $K = 2.2 \times 10^5$, instead of 1.6×10^5).

Deviations from straight line in the plot of log $[(F_0/F) - 1]$ against $[\mathrm{Ac}]_0$ were noted by Livingston and co-workers at low concentrations of the activator, and were ascribed by them to the presence of an "adventitious" activator, Ac′ (probably water). They held the latter responsible for the weak fluorescence still noticeable even at $[\mathrm{Ac}]_0 = 0$ (*e. g.*, $F = 0.03F_0$ in purest benzene). By using the same assumptions as before ($\alpha_{\mathrm{ChlAc}} = \alpha_{\mathrm{Chl}}$ for excitation light and $[\mathrm{Ac}']_0 \gg [\mathrm{ChlAc}']$), and assuming the adventitious activator to be water, Livingston and co-workers obtained theoretical curves fitting well the experimental results.

From the equilibrium constant K and its change with temperature, values of ΔF, ΔH and ΔS were calculated for three systems shown in Table 23.IIIB.

Table 23.IIIB

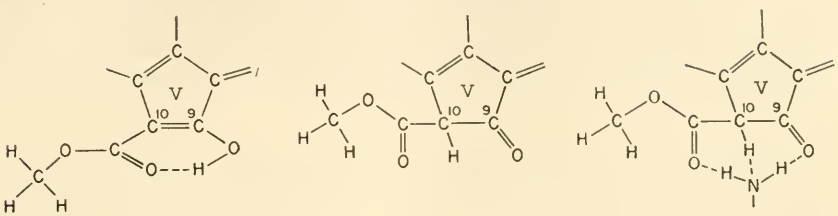
Thermodynamic Constants for the Association of Chlorophyll *a* with "Activators" in Benzene

Activator	ΔF_0, kcal./mole	ΔH, kcal./mole	ΔS_0, E.U.
Heptylamine	−7.1	−6.0	3.3
Cetyl alcohol	−5.0	−2.5	5.0
Aniline	−2.3	−1.5	2.7

Since F_0 seems to be independent of the nature of the activator, Livingston suggested that the essential effect of association is *isomerization* (or, rather *tautomerization*) of chlorophyll, *e. g.*, conversion of the keto form into an enol. If—as assumed by Livingston—this is the transformation discussed in Volume I, page 459, allomerized chlorophyll should be incapable of it, and should therefore remain fluorescent in pure hydrocarbons. This consequence remains to be tested.

Livingston attributed the postulated stabilization of the keto form by

alcohols and amines to the formation of hydrogen bonds with the amino or hydroxy group, shown in formula 23.I.



Chelated Enol Form (nonfluorescent, stable in pure hydrocarbons) | Keto Form (unstabilized) | Stabilized Keto Form (fluorescent, stable in the presence of amine or alcohol)

Formula 23.I. Tautomerization and fluorescence of chlorophyll (after Livingston, Watson and McArdle, 1949).

The fluorescence of ethereal chlorophyll solutions is not covered by this hypothesis, and further experiments are needed to show whether this fluorescence would persist upon more stringent purification. As an alternative explanation of the phenomenon of activation, Livingston considered the possibility that chlorophyll forms nonfluorescent dimers in pure hydrocarbons, and that these dimers are dissociated into fluorescent monomers by association with polar molecules. If this were the case, however, one would expect the extent of activation to depend on concentration of chlorophyll; while a few—admittedly preliminary—experiments showed no difference in the values of F/F_0 between partially activated chlorophyll solutions in benzene containing 4.8×10^{-5}, 1.5×10^{-4} and 2.3×10^{-4} mole/l. chlorophyll, respectively.

Observations bearing obvious relation to those of Livingston and co-workers, have been made also by Evstigneev, Gavrilova and Krasnovsky (1949[1]). In studying the effect of oxygen on the absorption spectrum and fluorescence of chlorophyll, they first found this effect to depend on the solvent. In toluene, heptane and carbon tetrachloride, oxygen *increased* the absorption (page 648) and *activated* the fluorescence, while in pyridine, ethanol, ethyl acetate, acetone and (commercial) benzene, it had no effect on absorption, and quenched fluorescence. Later (1949[2]) the same investigators found that these effects were due not to oxygen, but to water vapor. In *moist* toluene, oxygen quenched fluorescence in the same way as in ethanol or other polar solvents.

Evstigneev and co-workers also discussed two conceivable mechanisms of the action of polar solvents. One was the same as Livingston's alternative hypothesis—solvent effect on dimerization. The other differed from Livingston's preferred hypothesis: it assumed attachment of polar molecules to the free co-ordination places at the central magnesium atom. This

last hypothesis is supported by the observation that polar molecules do not affect the absorption spectrum and fluorescence of pheophytin; even more convincing is the fact that a similar difference in behavior occurs between phthalocyanine and its magnesium complex, although these compounds contain no cyclopentanone structure, so that Livingston's interpretation cannot be applied to them.

5. Influence of Concentration on Yield of Fluorescence. Self-Quenching. Fluorescence of Chlorophyll in Colloids and Adsorbates

The self-quenching of fluorescence in chlorophyll solutions was studied by Weiss and Weil-Malherbe (1944). They found that with a constant intensity of illumination, the intensity of fluorescence of an ethyl chlorophyllide solution in ethanol *increased* with increased concentration, between 1×10^{-7} and 1×10^{-5} mole/l., due to increased absorption. The latter became practically complete above 1×10^{-5} mole/l.; instead of becoming constant from there on, the fluorescence intensity declined rapidly, dropping at 1×10^{-4} mole/l. to one sixth of its maximum value. Six points were measured between [Chl] $= 2 \times 10^{-5}$ and 1×10^{-4} mole/l., and found to lie on a hyperbola:

$$\underline{F} = C/(1 + k[\mathrm{Chl}]) \tag{23.5}$$

where $\underline{F}$ is fluorescence intensity and k and C are constants. The constant k was evaluated graphically and $k = 2 \times 10^5$ sec.$^{-1}$ was found. Making the—unjustified—assumption that the intensity of fluorescence is limited *exclusively* by self-quenching, one can easily show (*cf.* equations on page 546, Vol. I) that the constant k has the meaning:

$$k = k_e/k_f \tag{23.6}$$

where k_e is the bimolecular rate constant of self-quenching:

$$\mathrm{Chl} + \mathrm{Chl}^* \xrightarrow{k_e} \mathrm{Chl}_2 \; (\longrightarrow 2\ \mathrm{Chl}) \tag{23.7}$$

and k_f the monomolecular rate constant of fluorescence·

$$\mathrm{Chl}^* \xrightarrow{k_f} \mathrm{Chl} + h\nu \tag{23.8}$$

The reciprocal, $1/k_f$, is the average life-time of the excited molecule when limited only by fluorescence. According to page 634, $k_f \simeq 10^7$ sec.$^{-1}$; with $k = 2 \times 10^5$ sec.$^{-1}$, this gives, according to (23.6):

$$k_e \simeq 2 \times 10^{12}\ \mathrm{sec.}^{-1} \tag{23.9}$$

This calculation is based on the assumption that reaction (23.7) is the *only* one that limits the yield of fluorescence. However, equation (23.5) would remain correct, *i. e.*, self-quenching would follow a hyperbolic curve,

also if fluorescence were competing, not only with the bimolecular reaction (23.7), but also with one (or several) monomolecular (or pseudomonomolecular) transformations of the excited molecule, such as energy dissipation by internal conversion, or tautomerization, or a reaction with the solvent. In this case, the constant k in (23.5), instead of meaning (23.6), would have the meaning (23.10):

(23.10) $$k = k_c/(k_f + k_d)$$

where k_d is the monomolecular rate constant of the competing energy-dissipating process (or processes), (corresponding to the sum $k_i + k_t$, internal conversion plus tautomerization, in equation 19.5 in Vol. I, page 546).

Furthermore, in this case, the constant C would not be equal to F_0 (the fluorescence intensity with quantum yield 1), as assumed by Weiss and Weil-Malherbe, but would have the meaning:

(23.11) $$C = \underline{F}_0 k_f/(k_f + k_d)$$

With the assumption $C = F_0$, the data of Weiss and Weil-Malherbe indicate a yield of fluorescence ($\varphi = F/F_0 = F/C$) of 4% at 1×10^{-4} mole/l. and 21% at 2×10^{-5} mole/l. (and, of course, 100% at infinite dilution); but since no check was made by an experimental determination of $\underline{F}_0$, the true value of $\underline{F}_0$ may have been $> C$, *i. e.*, the quantum yield of fluorescence may have been correspondingly lower (not reaching 100% at infinite dilution).

Comparison of (23.10) with (23.6) shows that by correcting the derivations of Weiss and Weil-Malherbe for the possibility of internal conversion or other monomolecular deactivation processes, one would obtain, from their value of k, a value of k_c even larger than the one given in (23.9):

(23.12) $$k_c > 2 \times 10^{12}$$

The extraordinarily large value of this bimolecular rate constant makes one skeptical about the correctness of Weiss and Weil-Malherbe's experiments, or, at least, of their interpretation. Assuming that Chl* is deactivated by the first encounter with Chl—which is not implausible—the above value of k_c gives for the average interval between two encounters (at [Chl] = 1 mole/l.):

(23.13) $$t = 5 \times 10^{-3} \text{ sec.}$$

This is at least one whole order of magnitude less than can be estimated from the formula for the frequency of collisions *in gases*, and two or three orders of magnitude less than the estimate based on the rate of diffusion of dyestuff molecules *in solution*.

The rates of those reactions in solution that occur by the first (or one of the first) molecular collision are determined by the rate of diffusion (which brings the molecules

together in an "encounter") rather than by the frequency of collisions. The distinction between *encounters* (or "co-ordinations") and *collisions*, and its importance for reaction kinetics of condensed systems was discussed by Rabinowitch (1937).

The unusual magnitude of the constant (23.12) is illustrated by the fact that for nine other dyes, investigated by the same authors, the self-quenching constants were at least 2000 times smaller (and thus of the order of magnitude to be expected from diffusion constants).

It seemed thus that the conclusions of Weiss and Weil-Malherbe were in need of confirmation. Their results might have been vitiated by self-absorption (which the authors dismissed as unimportant "because there was no evidence of surface fluorescence"). In their set-up, the fluorescent beam was in line with the (ultraviolet) exciting beam—an arrangement that favors self-absorption. Less mutual overlapping of the fluorescence band and absorption band may explain why self-absorption did not affect equally strongly the results obtained with the other nine dyes.

That the observation of Weiss and Weil-Malherbe was due to self-absorption was later confirmed by Livingston and co-workers (1948), who showed experimentally that, if care is taken to avoid self-absorption, no concentration quenching of chlorophyll fluorescence can be noted up to 1.5×10^{-3} mole/l. Weiss (1948) agreed with this, but stated that concentration quenching *does* occur at still higher concentrations. Livingston and co-workers (1948), using a cell 1 mm. thick, extended their measurements up to 0.10 mole/l. of chlorophyll in butyl ether and found in fact a strong concentration quenching above 2×10^{-3} mole/l. (fig. 23.7); at 7×10^{-2} mole/l., the fluorescence yield was only 7% of that in dilute solution. The insert in fig. 23.7 shows the sigmoid shape of the curve.

The *absorption spectrum* of chlorophyll *a* in butyl ether, measured at 2×10^{-2} mole/l. (a concentration at which self-quenching reduces the fluorescence to about 40% of the maximum), appeared not to differ significantly from the spectrum of dilute solution.

A further pertinent observation was that the partly quenched fluorescence of a concentrated chlorophyll solution was more strongly depressed by an increase in temperature than the fluorescence of a dilute solution.

These observations—the unchanged absorption spectrum, the sigmoid quenching curve and the enhancing effect of temperature on concentration quenching—can all be fitted into the picture of quenching as the result of dissipation of excitation energy in a "weak link" in the energy exchange chain, such as a dimeric molecule (Förster) or a "hot" molecule of the monomer (Franck and Livingston). The concentration at which the quenching becomes noticeable (2×10^{-3} mole/l.) is of the same order of magnitude as that calculated by Förster for the onset of the energy exchange (*cf.* chapter 32).

Numerous observations are known showing that the close packing of chlorophyll molecules in *solid* chlorophyll, or in chlorophyll *monolayers* on water, causes a complete disappearance of fluorescence. Chlorophyll *colloids* too are, as a rule, nonfluorescent (Willstätter and Stoll 1918; Stern 1920, 1921; Albers 1935; Meyer 1939). Meyer described certain fluorescent solutions of chlorophyll in ethanol as "colloidal," but no reasons for this description were given (*cf.* Smith 1941). Meyer's *aqueous* chlorophyll colloids, in which the density of the particles was similar to that in the chloroplast grana, were nonfluorescent. Chlorophyll is non-

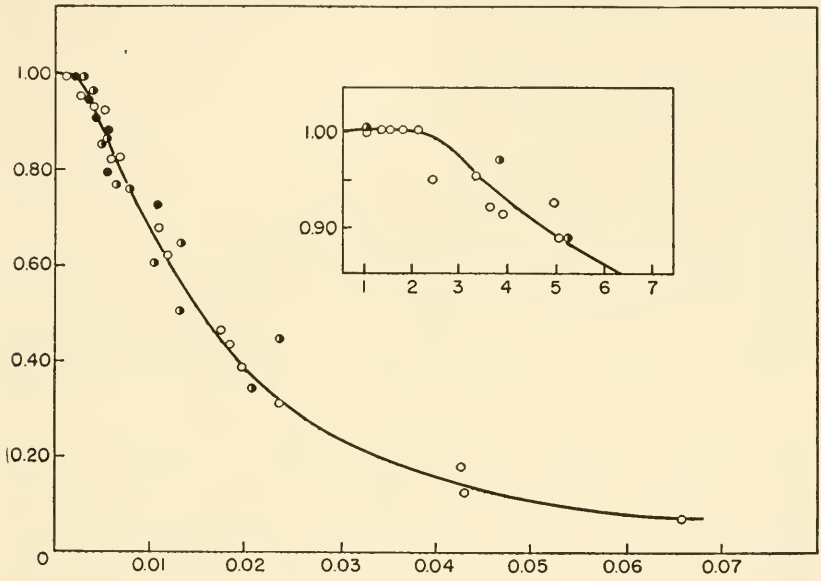


Fig. 23.7. Concentration quenching of fluorescence of chlorophyll *a* in butyl ether (after Livingston and Ke, 1949). Ordinate, F/F_0. Abscissa, [Chl] in mole/l.

fluorescent also in the adsorbed state, *e. g.*, in starch columns used for chromatographic separation (Seybold and Egle 1940).

If we attribute the nonfluorescence of solid, colloidal and adsorbed chlorophyll to self-quenching, and consider it a consequence of close packing, the restoration of fluorescence by certain "protective" substances can be attributed either to simple *dilution* of the pigment, or to effective interruption of the interaction between neighboring pigment molecules. Among the compounds reportedly capable of protecting the fluorescence of chlorophyll, we find first of all *lipides* and lipophilic solvents. Stern (1920.

1921) found, for example, that chlorophyll–lipide emulsions in water are fluorescent, while pure aqueous chlorophyll sols are not. The fluorescence of chlorophyll colloids in the presence of lecithin was confirmed by Bakker (1934). This may be either a true case of protection, or merely a *dilution* effect, since the concentration of chlorophyll molecules in the lipide drops may be lower than in the particles of the hydrosol.

Seybold and Egle (1940) found that chlorophyll on filter paper is fluorescent only if the solution from which it was adsorbed contained lipides, waxes or other lipophilic organic materials. (They even used the absence of a fluorescent rim on a filter paper strip dipped into a chlorophyll solution as a control of the purity of both solution and paper.) Nonfluorescent chlorophyll adsorbates on starch "light up" if they are wetted by organic solvents, or if ether vapor is blown at them.

Wakkie (1935) prepared a series of colloidal chlorophyll solutions (by diluting alcoholic solutions with increasing amounts of water), with and without a lipide (sodium oleate). If no oleate was added, the shift of the absorption band from 660 to 672 mμ (completed within a narrow concentration range, and considered indicative of the transition from a monomolecularly dispersed to an aggregated state) was accompanied by complete disappearance of fluorescence. In the presence of oleate, the absorption band began to shift at the same dilution, but the displacement ceased at 670 instead of 672 mμ; and, at the same time, fluorescence reappeared. By salting out, fluorescent, birefringent chlorophyll oleate "coacervates" could be precipitated from these fluorescent colloidal solutions.

From these and similar observations, it appears that lipophilic molecules can protect the fluorescence of chlorophyll from self-quenching even without diluting the pigment, and without disrupting the chlorophyll–protein or chlorophyll–cellulose bond. One can visualize the protecting molecules as enveloping the lipophilic parts of the adsorbed pigment molecules (*e. g.*, in the case of chlorophyll, the phytol "tails"), and thus interrupting their mutual interaction. The "wrapping up" of flexible parts of the molecule may stiffen the latter and interfere with the internal conversion of electronic into vibrational energy. This stabilization effect may become manifest in a single pigment molecule, as well as within a complex of several such molecules. In the light of the above-described, more recent experiments by Livingston, one has also to consider the possibility that tautomeric transformations from a nonfluorescent into a fluorescent form of chlorophyll could be responsible (or coresponsible) for effects of this type.

It does not seem that the association of chlorophyll with *proteins* can in itself protect fluorescence. True, natural "chloroplastin" preparations apparently *are* fluorescent. Although Smith (1938) called aqueous chlorophyll–protein extracts from spinach leaves "nonfluorescent," Noack

(1927), Stoll and Wiedemann (1938) and Fishman and Moyer (1942) found that they fluoresce weakly, and that fluorescence is preserved also in precipitates prepared from such extracts by salting out. Wassink, Katz and Dorrestein (1942) observed that the yield of fluorescence was about the same ($\sim$0.1%) in live purple bacteria and in aqueous, colloidal bacteriochlorophyll–protein suspensions prepared from them. However, chloroplastin fluorescence probably must be attributed to the presence of as much as 30% lipophilic material. Artificial complexes containing *only* chlorophyll and proteins do not fluoresce. According to Noack, adsorbates of chlorophyll on globin sometimes fluoresce faintly; but Seybold and Egle (1940) suggested—probably with justification—that this must be ascribed to the presence of impurities of a lipide nature.

The bearing of these results on the problem of the state of chlorophyll in the living cell was discussed in chapter 14 (Vol. I). It was argued there that the nonfluorescence of pure chlorophyll adsorbates on proteins does not prove Seybold's hypothesis that the chlorophyll fluorescence *in vivo* is caused by a small fraction of chlorophyll dissolved in a lipide phase; more probably, *all* chlorophyll in the plants is weakly fluorescent (*despite* its high density and *irrespective* of its association with proteins) because of its simultaneous association with protective substances such as fats or phospholipides (Hubert, 1935). Livingston's experiments, (p. 766), indicate association with "activating" groups (such as OH, NH, or SH) as another possible explanation of fluorescence.

6. Quenching and Activation of Chlorophyll Fluorescence by Admixtures

While the limitation of chlorophyll fluorescence in *pure* solutions may be caused equally well by physical dissipation or by photochemical reactions (tautomerization or reaction with the solvent), strong quenching by small amounts ($<10^{-3}$ mole/l.) of foreign substances must be attributed to chemical interactions, since the rate of physical energy dissipation is not likely to be affected by the comparatively rare encounters of excited dyestuff molecules with the molecules of the "quencher," or by changes in the average properties of the solvent caused by the presence of the latter. (An exception may be the case of resonance—to be discussed below.)

As mentioned on page 757, the two most likely mechanisms of chemical quenching are oxidation-reduction (equations 23.1), and complex formation (equation 23.2). The second one is particularly probable when the quencher is another dyestuff with overlapping bands, so that self-quenching conditions are closely approximated. In this case, permanent association of the quencher with the fluorescent molecule also becomes a likely possi-

bility (like the permanent dimerization discussed on page 761). Study of the effect of the quencher on the absorption spectrum of the fluorescent material, and of the dependence of quenching on the concentration of the quencher, may help to distinguish between "quenching by complex formation" and "quenching by kinetic encounters"; but, at present, very few such data are available for chlorophyll solutions. Finally, in analogy to the above-discussed mechanisms of self-quenching (page 759), still another possibility of quenching should exist when the quencher is "in resonance" with the fluorescent molecule: quenching *without* complex formation and *without* kinetic encounters, through transfer of excitation energy over distances considerably larger than the collision diameters. If the molecules of the quencher are nonfluorescent, they will serve as "traps" in the same way as was postulated by Förster for the dimers. Vavilov and co-workers (Pekerman 1947, Vavilov *et al.* 1949[1,2]) found convincing examples of this type in the quencing of dye fluorescence by resonating nonfluorescent dyes. However, strong quenchers in Table 23.IIIC (p. 782) certainly do not owe their effectiveness to a resonance transfer mechanism. They are all oxidants and this points to chemical interaction rather than physical energy transfer. In the second place, they have no absorption bands in the red, and are thus not in resonance with excited chlorophyll molecules. Their lowest excited states must be considerably higher than the fluorescent state A of chlorophyll.

Among the substances whose quenching effect on the fluorescence of chlorophyll has been investigated in some detail are the reaction partners in chlorophyll-sensitized autoxidations—molecular oxygen and oxidation substrates such as benzidine, allyl thiourea etc. Because of the importance of these results for the analysis of the mechanism of sensitized autoxidation, they have already been anticipated in part in Volume I (*cf.* chapter 18, pages 483 and 518, and chapter 19, page 546).

The quenching action of *oxygen* on the fluorescence of different dyestuffs (including chlorophyll) was first investigated by Kautsky and co-workers. Kautsky and Hirsch (1931) had discovered that the fluorescence of certain dyestuffs *adsorbed on silica gel* is considerably weakened by oxygen at pressures of several hundred millimeters, and that their afterglow (phosphorescence) is completely destroyed even by very much lower pressures of this gas. Later, a similar quenching was observed in fluorescent dyestuff *solutions*, including chlorophyll solutions in acetone. According to Kautsky, Hirsch and Flesch (1935), the quenching of chlorophyll fluorescence is proportional to the partial pressure of oxygen, and shows no "saturation effect" even under a partial pressure of one atmosphere.

Franck and Levi (1934) and Weil-Malherbe and Weiss (1942) found that the fluorescence of chlorophyll or ethyl chlorophillide solutions in

ethanol is reduced under one atmosphere oxygen by 30–35% (compared with its intensity in the absence of oxygen). This indicates that 50% quenching must require about two atmospheres oxygen, corresponding to a concentration of about 10^{-2} mole/l. (*cf.* table 23.IIIC). Life-time of the fluorescent state of chlorophyll in ethanol solution was estimated on page 634 as 8×10^{-8} sec. The average time that an excited chlorophyll molecule has available before its first encounter with a molecule of a solute whose concentration is of the order of 10^{-2} mole/l. also is of the order of 10^{-8} sec. (No exact formulae for the calculation of encounter intervals in solutions are available, but it appears likely that these intervals are somewhat—perhaps 10 or 100 times—longer than the collision intervals in gases of the same concentration.) It thus seems that excited chlorophyll molecules in the fluorescent state A undergo a quenching reaction by the very first, or one of the first, encounters with an oxygen molecule.

The nature of this interaction is not known, but it is most likely to be the *autoxidation* of chlorophyll. A different hypothesis was suggested by Kautsky and maintained by him despite much criticism. According to this hypothesis, the interaction is a *bulk transfer of electronic excitation energy* from Chl* to O_2. This concept originated in certain observations made by Kautsky and de Bruijn (1931) and by Kautsky, de Bruijn, Neuwirth and Baumeister (1933) in the study of the autoxidation of leuco malachite green, adsorbed on silica gel. They found that this reaction can be sensitized, in an atmosphere of oxygen of very low pressure (10^{-4} mm.), by the dyestuff trypaflavine, *adsorbed on separate particles of the gel.* Kautsky explained the "transmission" of the sensitizing action across the air gaps separating the dyestuff from the acceptor by the assumption that *excited dyestuff molecules transfer their energy to oxygen molecules*:

(23.14) $$D^* + O_2 \longrightarrow D + O_2^*$$

This process he also made responsible for the quenching of fluorescence. Energy transfer was supposed by him to convert ordinary oxygen into a metastable active form, which Kautsky identified as the state $^1\Sigma$, known from spectroscopic data to be situated 37.3 kcal/mole above the ground state $^3\Pi$. After Gaffron remarked that infrared excitation of bacteriochlorophyll provides <37 kcal, Kautsky (1937) suggested that the state $^1\Delta$ (23 kcal/mole) could serve the same purpose. Both states are metastable because their multiplicity (singlet) is different from that of the ground state (triplet). The same principle underlies Lewis and Kasha's more recent theory of metastable triplet states in molecules with singlet ground states (*cf.* p. 730).

An alternative chemical explanation of the mechanism of quenching

by oxygen was suggested by Weiss (1935). He postulated an autoxidation (dehydrogenation) of the dye:

(23.15) $$D^* + O_2 \longrightarrow HO_2 + oD$$

(o signifies oxidized). The radical HO_2 can diffuse across air gaps and cause the oxidation effects ascribed by Kautsky to metastable oxygen molecules.

A reaction of the type (23.15) could be responsible not only for the quenching of the fluorescence of adsorbed dyes, but also for that of the fluorescence of dyestuffs dissolved in organic solvents. Since the quantum yield of irreversible photoxidation of chlorophyll in pure organic solvents is very low (Vol. I, p. 496), reaction (23.15), if it is responsible for quenching, must be practically completely reversible (at least as far as the chemical composition of chlorophyll is concerned). The restoration of oxidized chlorophyll may be brought about either by direct reversal of (23.15), or—if the HO_2 radicals are partly consumed by dismutation or side reactions—by interaction with the solvent. In the latter case, the net result is sensitized autoxidation of the solvent S:

(23.16a) $$Chl^* + O_2 \longrightarrow oChl + HO_2$$

(23.16b) $$oChl + S \longrightarrow oS + Chl$$

(23.16c) $$HO_2 \longrightarrow \tfrac{1}{2} H_2O + \tfrac{3}{4} O_2$$

(23.16) $$S + \tfrac{1}{4} O_2 \longrightarrow oS + \tfrac{1}{2} H_2O$$

For the solvent, one may substitute an oxidizable substrate—*e. g.*' benzidine, or potassium iodide—thus obtaining a mechanism of chlorophyll-sensitized autoxidation of such substrates. (This mechanism was discussed in Volume I, chapter 18, *cf.* equations 18.33, and chapter 19, *cf.* scheme 19.II; there, tautomerization was added as a preliminary step.)

An alternative interpretation of sensitized autoxidation, also discussed in Volume I, chapter 18 (*cf.* equations 18.40), envisages a primary reaction between excited chlorophyll molecules and the *oxidation substrate* (rather than oxygen). Whenever this mechanism operates, *oxidation substrates* should quench the fluorescence of chlorophyll more effectively than does oxygen. Franck and Levi (1934) measured the quenching of chlorophyll fluorescence by benzidine and potassium iodide. Their quenching curves are not labeled and therefore do not permit reading off the half-quenching concentration, but the authors state that, under the conditions of Noack experiments on the chlorophyll-sensitized autoxidation of benzidine (*cf.* page 528), quenching by benzidine must have been many times more efficient than that by oxygen. If this is true, then the mechanism of this reaction must be different from that of chlorophyll-sensitized autoxidation of substrates such as allylthiourea (*cf.* below).

Livingston and co-workers (Livingston 1948, Livingston and Ke, 1949) made the first systematic investigation of changes in the intensity of chlorophyll fluorescence, produced by small admixtures. They used various organic compounds, certain salts and several gases.

As long as the fluorescence remains the only property measured, *all* the observed changes can be described as quenching (if fluorescence becomes weaker) or stimulation (if fluorescence becomes stronger—as it actually does upon addition of traces of an alcohol or amine to a chlorophyll solution in dry hydrocarbon, or upon the addition of iodine to an alcoholic solution of chlorophyll *b*). It would be best, however, to restrict the terms "quenching" and "stimulation" to cases in which the admixture does not affect the composition or state of the light-absorbing molecules in the dark, but acts only on molecules which have been excited by the absorption of light. We may refer to these phenomena as "true quenching" (or "true stimulation"—if the latter does exist at all, which is doubtful). True quenching can be due to physical or chemical processes. In the first case (*physical quenching*), kinetic encounters of light-excited, fluorescent molecules with the molecules of the quencher, or mutual proximity of these molecules, lead to accelerated conversion of electronic excitation energy into vibrational energy and, ultimately, into heat. The accelerated dissipation can occur within the excited molecule itself (because its configuration or charge distribution change under the influence of the quencher), or in a complex formed by the excited molecule and the quencher, or even within the quencher molecule alone—which in this case, must first take over the electronic excitation energy "in bulk" and then dissipate it, by internal conversion to vibrational energy. (It is also possible for this energy to be re-emitted by the quencher as sensitized fluorescence.) In the second case (*chemical quenching*), either the excited molecule or the molecule of the quencher (or both) are changed chemically in the process of quenching. In this case, a kinetic encounter of the two molecules is needed. If the photochemical reaction responsible for quenching is completely reversible by a dark reaction, the net result is the same as in physical quenching—conversion of light energy into heat. Otherwise, a net photochemical change remains; and only if this change does not involve the fluorescent species is a steady yield of fluorescence observable in the presence of the quencher.

Contrasted to true quenching can be the changes in the yield of fluorescence which are caused by alterations in the composition or structure of the light-absorbing molecules produced by the addition of the admixture. (All cases of "stimulation" probably belong to this class.) These processes can be distinguished from true quenching by the fact that the absorption spectrum of the solution also is changed by the presence of the quencher

(or stimulant). The absorption changes may be major or minor, depending on the character of the interaction (complexing, tautomerization, dissociation, oxidation, reduction, etc.), but should never be entirely absent. Furthermore, chemical changes in the dark will often (albeit not always) proceed at a measurable rate, thus causing the yield of fluorescence to depend on the length of time between the preparation of the mixture and the illumination. Finally, the dependence of quenching on the concentration of the quencher should be different in the case when the absorbing molecule and the quencher combine (or react) in the dark than when they interact only after the absorption of light.

TABLE 23.IIIC

QUENCHING DATA FOR CHLOROPHYLL *a* IN METHANOL, ETHANOL AND ACETONE (AFTER LIVINGSTON AND KE, 1949)

Quencher	Solvent	λ (excit.), mμ	$[Q]_{1/2}$ [a] mole/l.	k_1 [b]
Chloranil	Me_2CO	645	0.0050	175
Quinone	Me_2CO	645	0.0081	143
" "	MeOH	645	0.0096	120
Methyl red	MeOH	645	(0.0088)	101
Trinitrotoluene	MeOH	645	0.0100	90
" "	MeOH	435.8	0.0098	113
m-Dinitrobenzene	MeOH	645	0.0122	68
" "	MeOH	435.8	0.0110	77
Duroquinone	MeOH	645	0.0116	81
β-Nitroso-α-naphthol	MeOH	645	0.0140	62
β-Nitrostyrene	MeOH	435.8	0.0170	59
Nitric oxide	EtOH	435.8	(0.0178)	43
β-Nitro-β-methylstyrene	MeOH	435.8	0.022	41
Oxygen	EtOH	435.8	(0.023)	35
Nitrobenzene	MeOH	435.8	0.034	36
β-Nitro-β,γ-hexene	MeOH	435.8	0.064	12
o-Aminophenol	MeOH	435.8	0.138	7.2
Phenylhydrazine	Et_2O	435.8	0.151	8.4
" "	MeOH	435.8	0.31	3.7
Dimethylaniline	MeOH	435.8	(0.42)	2.4
2-Phenyl-3-nitrobicyclo-[1,2,2]-heptene-5	MeOH	435.8	(0.61)	1.6
2,6-Diaminopyridine	MeOH	435.8	(0.62)	1.4

[a] Half-quenching concentration. [b] *Cf.* equation 23.16C, p. 785.

These general considerations should be kept in mind in the evaluation of the results of quenching experiments. They make it particularly important that measurements of the intensity of fluorescence be combined with measurements of the absorption spectrum (and if possible, also of the fluorescence spectrum) of the light-absorbing species.

The experiments of Livingston and Ke (1949) dealt largely with what appears to be true chemical quenching: changes due to reversible reactions of excited chlorophyll molecules with certain organic and a few inorganic molecules. In some cases, at least, this interpretation was confirmed by observations of the constancy of the absorption spectrum, and by the instantaneous character of the change.

TABLE 23.IIID

NONQUENCHERS OF CHLOROPHYLL FLUORESCENCE IN METHANOL, ETHANOL OR ACETONE[a] (AFTER LIVINGSTON AND KE, 1949)

Reagent	Solvent	[Q], m./l.	F_0/F
Nitropropane	MeOH	0.09	1.00
Nitropropane	MeOH	0.2	1.02
Butyl nitrate	MeOH	0.9	1.02
Butyl nitrite	Me_2CO	0.138	1.01
Phenylhydroxylamine	MeOH	0.021	1.02
Phenylhydroxylamine	MeOH	0.07	1.05
Aniline	Et_2OH	0.16	1.02
Hydrazine	Me_2CO	1.05	1.0
Urethan	Me_2CO	0.07	1.0
Thiourea	Me_2CO	0.02	1.0
2-Aminopyridine	MeOH	0.08	1.00
Phenylurea	MeOH	0.05	1.01
Urea	MeOH	0.19	1.00
Guanidine carbonate	MeOH	(Satd.)	1.0
Phenol	MeOH	0.09	1.0
Hydroquinone[b]	Me_2CO	0.03	1.0
Phenolphthalein	MeOH	0.04	1.0
Dimethylglyoxime	MeOH	0.07	1.0
tert-Hexylmercaptan	MeOH	0.11	1.00
Benzaldehyde	MeOH	0.38	1.0
Benzoic acid	Me_2CO	0.08	1.0
Camphor	MeOH	0.15	1.0
Boric acid	MeOH	(Satd.)	1.00
Sodium methoxide	MeOH	0.05	1.0
Sodium cyanide	MeOH	0.15	1.01
Sodium oxide	MeOH	0.11	1.0
Nitrous oxide	EtOH	(605 mm.)	1.0
Carbon dioxide	EtOH	(576 mm.)	1.0
Carbon monoxide	MeOH	(640 mm.)	1.00
Potassium thiocyanate	MeOH	0.05	1.02
Potassium thiocyanate	Me_2CO	0.004	0.92

[a] Other nonquenchers: ascorbic acid; alkali iodide (Evstigneev and Krasnovsky, 1948); allylthiourea (*cf.* page 789).

[b] The measurements of Evstigneev and Krasnovsky (1948) indicate that hydroquinone at much higher concentrations has some quenching action, but is less efficient than 2-diaminopyridine, the weakest quencher in Table 23.IIIC.

Livingston and Ke used a 1.2×10^{-5} molar solution of chlorophyll *a* in methanol, ethanol, ether or acetone, which they excited by the mercury line 435.8 mμ, or by a red band centered at 645 mμ. Tables 23.IIIC and D show that quenching was similar for both types of excitation. The quenchers are listed in the first table in order of declining efficiency; it is

clear that *oxidants* (quinones, diazo dyes, nitro and nitroso compounds, and oxygen) are strong quenchers, while *reductants* (such as amines) are at best only weak quenchers. Among compounds listed in Table 23.IIID, only phenylhydroxyamine showed any measurable quenching at all, at concentrations up to 0.1 mole/l. See also footnote *b* to Table 23.IIID.

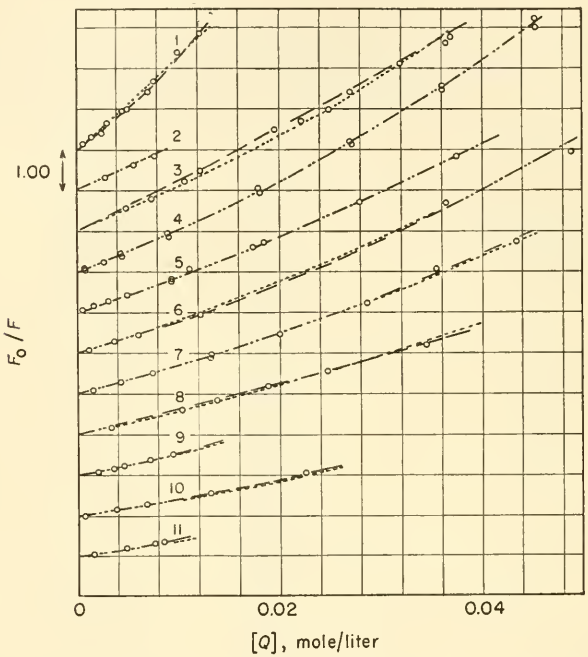


Fig. 23.8. Quenching of fluorescence of chlorophyll *a* in methanol by different quenchers (after Livingston and Ke, 1949). For convenience, a separate zero point of ordinates is used for each compound. Each curve begins with $F_0/F = 1$. 1, chloranil; 2, methyl red; 3, quinone; 4, trinitrotoluene; 5, duroquinone; 6, *m*-dinitrobenzene; 7, β-nitroso-α-naphthol; 8, β-nitrostyrene; 9, nitric oxide; 10, nitric oxide; 11, oxygen. Dotted lines, empirical; broken lines, eqs. (23.1b–c).

As shown by figure 23.8, where F_0/F is plotted as function of $[Q]$ for a number of true quenchers, the quenching follows approximately the Stern-Volmer equation (eq. 23.16B), which we have repeatedly used before. (It applies to all cases of competition between a monomolecular process such as fluorescence, and a bimolecular process, such as quenching, by kinetic encounters):

(23.16A) $$F = F_0/(1 + k[Q]) \qquad \text{or}$$

(23.16B) $$F_0/F = 1 + k[Q]$$

However, deviations from the linear relation required by equation (23.16B) do occur, particularly at the higher values of $[Q]$ (fig. 23.8). Livingston and Ke obtained a better approximation by using an empirically generalized equation:

$$F = \frac{F_0}{1 + k_1[Q] + k_2[Q]^2} \tag{23.16C}$$

The broken lines in figure 23.8 show how this equation can be made to fit the data. The values of k_1 are given in Table 23.IIIC. Livingston compared the empirical two-constant equation (23.16C) with a theoretical equation of Vavilov and Frank:

$$F = \frac{F_0}{e^{[Q]\rho + (AT/\eta)[Q]}} \tag{23.16D}$$

Here, the first exponential term in the denominator represents "static quenching," *i. e.*, quenching determined by *average distance* between quencher and fluorescent molecule (ρ = effective radius of energy exchange). The second term accounts for "kinetic quenching" (A = const., η = viscosity of the medium). Quenching by resonance exchange of energy is a possible mechanism of static quenching; chemical reaction by the first, or one of the first, kinetic encounters (the frequency of which is determined by the rate of diffusion, and thus indirectly by viscosity), can be suggested as one mechanism of "kinetic" quenching.

Developing the exponential in (23.16D) and retaining only the first term, one can obtain an equation of the type (23.16C). Livingston and Ke calculated from their empirical constants (k_1 and k_2) the "action radii", ρ for different quenchers, and obtained values between 23 and 7 Å—which they considered as plausible in view of Förster's calculations of the range of energy exchange (chap. 32). However, Förster's calculations were for the case of *resonance* between the two molecules taking part in the energy exchange, while the molecules listed in Table 23.IIIC have no absorption bands which could resonate with the red fluorescence band of chlorophyll. Therefore, no exchange of excitation energy over distances wider than a molecular collision diameter appears possible—unless one resorts to theoretically feasible, but experimentally as yet unsupported hypotheses.

Resonance between two separately "prohibited"—and therefore spectroscopically unknown—transitions, one in the excited molecule and one in the quencher, which become "permitted" by being coupled together, was suggested by Rollefson; the total spin of the system can be preserved if one molecule goes from a triplet into a singlet state, while the other simultaneously undergoes a reverse change.

Testing equation (23.16D) by comparing quenching efficiencies in different solvents did not give satisfactory results. For example, the k_1

values obtained with nitrophenol and chlorophyll *a*, in a series of alcohols (from methanol to octanol), were *not* inversely proportional to the viscosities of these solvents. This may mean that, although k_1 is a measure of the probability of quenching by kinetic encounters, the frequency of such encounters is not the simple function of viscosity assumed in eq. 23.16D.

Livingston and Ke found that the absorption spectrum of chlorophyll *a* was not markedly affected by the presence of even a large amount of the strongest quenchers listed in Table 23.IIIC (*e. g.*, 0.3 mole/l. of $C_6H_5NO_2$), thus indicating that quenching was not due to a preliminary dark chemical reaction—such as complex formation—between chlorophyll *a* and the quencher.

The considerable effect, which the comparatively weak quencher, phenylhydrazine, has on the absorption spectrum of chlorophyll *b*, was noted before (chapter 21, page 649); no similar effect was observed with chlorophyll *a*.

Reversibility of quenching could be conclusively proved only for the gaseous quencher, oxygen, since it could be easily removed. (It will be recalled in this connection that the fluorescence of *allomerized* chlorophyll *a* in methanol is only about one-half as intense as that of the original solution; in other words, a slow *irreversible* change is superimposed on the reversible quenching by oxygen.) Dilution experiments with one weak organic quencher, phenylhydrazine, indicated that quenching probably is reversible in this case, too.

Franck and Livingston (1941) had discussed the possible existence of a residual fluorescence which could not be further reduced by quenchers. (Such a "nonquenchable" fluorescence is to be expected if the electronically excited molecule requires a certain time to assume the configuration suitable for chemical quenching; the fraction of total fluorescence, emitted between excitation and the attainment of this configuration, would then be "unquenchable".) In the system (chlorophyll *a* in CH_3OH + nitrobenzene), a weak fluorescence appeared even in a 4 *M* solution of the quencher; however it was too weak ($F = 0.003F_0$) to consider it as a definite confirmation of this prediction.

Whatever the interpretation of the quadratic term in the denominator of equation (23.16C), the large values of the linear term found with oxidizing molecules and the low values found with reducing molecules (including the substrates whose autoxidation is sensitized by chlorophyll, such as allylthiourea) undoubtedly are significant. Livingston and Ke stressed a detailed similarity of the list of strong quenchers in table 23.IIIC with the list of substances known to inhibit certain polymerization processes; but rather than looking for a causative relation between the two effects, one should perhaps consider both as consequences of the same oxidative properties of the quenchers (or inhibitors).

Quenching of chlorophyll fluorescence by kinetic encounters with oxi-

dants can be attributed, with a fair degree of certainty, to a (reversible) *oxidation* of the excited chlorophyll molecule by the quencher—perhaps the same reaction which causes the reversible bleaching of chlorophyll in light (Vol. I, page 486, and chapter 35). Thus, quenching experiments confirm the repeatedly noted capacity of chlorophyll to serve as a photochemical *reductant*. (In chapter 35, we will present evidence that it can act also as a photochemical *oxidant*.)

Some of the effects described by Livingston (1948) clearly belong to a different type—which we may call "pseudo-quenching." These are fluorescence changes caused by chemical reactions between nonexcited chlorophyll molecules and the quencher (or stimulant). The most striking results of this type were obtained with iodine. When traces of iodine were added to a solution of chlorophyll, fluorescence began to change gradually; minutes or even hours were needed to reach a steady state. This points to a slow chemical conversion of chlorophyll to a compound with a different capacity for fluorescence. Probably, the reaction is an irreversible oxidation; perhaps, preceded by transient complex formation. As discussed elsewhere (*cf.* page 613), the product appears to be similar to (or identical with) *allomerized chlorophyll*, as obtained by slow oxidation of alcoholic chlorophyll solutions in air. According to Fischer (*cf.* Vol. I, page 459), this is chlorophyll oxidized at carbon atom 10. Similar to allomerization in air, the reaction with iodine (and a similar one with bromine) occurs only in alcoholic solution (methanol or ethanol) but not in ether or carbon tetrachloride. In the case of chlorophyll *a*, the final product has about 55% of the fluorescence intensity of the nonallomerized solution; in the case of chlorophyll *b*, it fluoresces twice as strongly as the initial compound.

Admixture of carbon tetrachloride to methanol lengthens the time needed to complete the allomerization by iodine, from about 5 minutes for chlorophyll *a* in pure methanol, to over an hour in a mixture of equal parts of methanol and carbon tetrachloride.

With chlorophyll *b* in methanol, fluorescence declines at first upon the addition of iodine; but later (in about 30 minutes) it begins to increase again and reaches, after 10–15 hours, a steady level about twice as high as the original one. With bromine and chlorophyll *b*, the minimum is reached faster (in about one minute), and the high steady level is approached in about an hour. The initial dip in fluorescence may be taken as sign of complex formation—the subsequent increase, as indication of the conversion of chlorophyll to the allomerized form.

Higher amounts of iodine, and particularly of bromine, quench the fluorescence of chlorophyll more or less completely, by causing a deeper chemical change in the chlorophyll molecule.

Effects similar to those caused by small quantities of iodine or bromine

can be produced also by small amounts of salts, such as $LaCl_3$ or $CeCl_3$ *in the presence of air.* These salts probably catalyze the allomerization of chlorophyll by air, with the concomitant changes in the intensity of fluorescence (decrease with chlorophyll *a*, increase with chlorophyll *b*). These catalyzed reactions, too, occur only in alcoholic solutions. In ethereal solution, chlorophyll *a* is converted by $LaCl_3$ into a yellow compound, and all fluorescence soon vanishes.

Evstigneev and Krasnovsky (1948) and Evstigneev, Gavrilova and Krasnovsky (1949[7]) also have investigated the quenching of chlorophyll fluorescence by several substances (see footnotes to Table 23.IIID), in particular by oxygen. Their peculiar initial result already was mentioned on page 648: they found the effect of oxygen to be strongly dependent on the solvent. In polar solvents—pyridine, ethanol, ethyl acetate, acetone (and also in commercial benzene)—the effect of oxygen was as previously described: moderately strong quenching. In nonpolar solvents—heptane, toluene, carbon tetrachloride—on the other hand, an entirely different effect was found; fluorescence (of chlorophyll *a* or *a* + *b*) *decreased* upon removal of oxygen (evacuation of the vessel by an oil pump) by as much as a factor of two; it increased to approximately the original level after readmission of air. (The fact that benzene behaved like a polar solvent probably was due to an impurity.) Repeated evacuation and aeration produced a gradually weakening effect—a result which could be attributed to superposition, upon reversible association of chlorophyll with oxygen, of an irreversible oxidation (allomerization). Later (1949[2]), the same investigators reported that what they first took for an activating effect of oxygen was in fact an activating effect of water vapor contained in the admitted air. Admission of air caused no activation of fluorescence in moist solvents. As described on p. 771, Livingston attributed activation to an enol-ketone transformation in the cyclopentanone ring, enhanced by the formation of hydrogen bonds, while Evstigneev and co-workers thought that polar solvent molecules might have an affinity for the magnesium atom (which, in the chlorophyll molecule, has two free coordination places). Saturation of these affinities could stiffen the molecule and delay internal dissipation of excitation energy. In agreement with this concept, no activation was obtained with Mg-free pheophytin, or phthalocyanin (*cf.* p. 772).

Coe (1941) suggested that "chemical" quenching of chlorophyll fluorescence by "antioxidants" present in oils and fats, could be used practically as a measure of their rancidity; but French and Lundberg (1944) found no evidence of such quenching by cottonseed oil.

Kautsky, Hirsch and Flesch (1935) found, and Franck and Livingston (1941) confirmed, that certain substrates whose photoxidation is sensitized

by chlorophyll, *e. g.*, isoamylamine and allylthiourea, do *not* cause any weakening of chlorophyll fluorescence. According to Kautsky, chlorophyll solutions in acetone, saturated with isoamylamine, fluoresce brightly; chlorophyll solutions in pure isoamylamine are not only fluorescent, but also show a red afterglow, lasting for about 0.01 sec. According to Franck and Livingston, the fluorescence of a 10^{-5} *M* chlorophyll solution in acetone, saturated with air and containing 0.5 mole/l. of allylthiourea, is only 15–20% weaker than the fluorescence of the same solution free of both oxygen and allylthiourea—despite the fact that the quantum yield of sensitized photoxidation is, under these conditions, of the order of unity. This shows that the sensitization of the reaction between allylthiourea (and similar oxidizable substrates) and oxygen cannot be attributed to the interaction of excited chlorophyll molecules *in the fluorescent state* with either oxygen *or* the oxidation substrate. In other words, sensitization is brought about—in this particular case—predominantly or exclusively by molecules whose energy would otherwise be dissipated without fluorescence (for similar observations with other sensitizers, see Shpolskij and Sheremetev 1936).

To explain this phenomenon, one has to assume that the majority of excited chlorophyll molecules do not fluoresce because they undergo transformation into a still energy-rich but comparatively long-lived form. In this form, they retain some of their original excitation energy as electronic or chemical energy. Because of their long life, these activated molecules have a good change of encountering oxygen molecules (or substrate molecules, A), even when the latter are present in a very low concentration. This explains why a high yield of sensitized autoxidation was sometimes observed even at very low values of $[O_2]$ and [A].

Thus, experiments on the quenching of chlorophyll fluorescence by oxygen and autoxidizable substrates bring us back to the problem of long-lived activation state (or states) of chlorophyll, which we have discussed once before when dealing with the mechanism of photochemical sensitization by chlorophyll *in vitro* and *in vivo* (*cf.* Vol. I, chapters 18, page 483, and 19, page 544).

Weiss and Weil-Malherbe (1944) suggested that self-quenching may explain the nonquenching of chlorophyll fluorescence by isoamylamine, without the assumption of long-lived active states. On page 774, we noted that the high efficiency of self-quenching, reported by these observers, turned out to be an error, caused by self-absorption. But even if it were as high as they suggested, it could not prevent sensitization from competing with fluorescence. If, in the absence of the sensitization substrate, 90% of excited chlorophyll molecules undergo self-quenching by encounters with nonexcited chlorophyll molecules (Chl* + Chl), and 10%

emit fluorescence, the addition of a substrate that is oxidized with a quantum yield of 50% should reduce both self-quenching *and* fluorescence in the same proportion—*i. e.*, to 45 and 5%, respectively.

Contrary to the suggestion of Weiss and Weil-Malherbe, self-quenching could not explain also the dependence of the yield of sensitized autoxidation on chlorophyll concentration (eq. 18.32).

The statement that stimulation of fluorescence indicates chemical change (p. 781) does not apply to *sensitization*. That energy absorbed by other pigments can be utilized for chlorophyll fluorescence, was first observed *in vivo* (chapter 24). According to Duysens (1951), in a 10^{-3} *M* solution of chlorophyll $a + b$ in acetone, one-half of the quanta absorbed by b are available for the fluorescence of a (by the same token, a must quench the fluorescence of b).

7. Long-Lived Active States and Afterglow of Chlorophyll

As stated in chapter 18, "long-lived activations" can sometimes be due to chemical changes (as well as to the formation of metastable electronic states—an explanation advocated by Kautsky and more recently by G. N. Lewis). Several of the quenching processes discussed earlier in this chapter may lead to transient formation of unstable products. Metastable active products may occur in the course of physical energy dissipation as well as in that of chemical quenching. In the first case, strong vibrations, excited during "internal conversion," can induce internal chemical changes—*e. g.*, one or two hydrogen atoms may be transferred to a different position in the molecule, thus creating a metastable, tautomeric form. In the second case—that of chemical quenching—metastable states may be produced by reversible photochemical reaction with the solvent—*e. g.*, an exchange of electrons or hydrogen atoms. In this case, the long-lived, metastable state of the pigment is an oxidized or reduced (rather than a tautomeric) state. The active, oxidized or reduced product can be reconverted to the original pigment either by reversal of the reaction by which it was formed—thus leaving no net photochemical change at all—or by other reactions (*e. g.*, with the solvent, or with dissolved oxygen), thus leaving a sensitized photochemical change. All these possibilities were discussed in some detail in Volume I (chapter 18).

The hypothesis that long-lived activated molecules are molecules in metastable electronic states (Kautsky, G. N. Lewis) was dismissed as implausible in Volume I, chapter 18 (page 486). Subsequent development of this concept, supported by extensive experiments by Kasha and other workers at Berkeley, makes it necessary to bring the subject up again here.

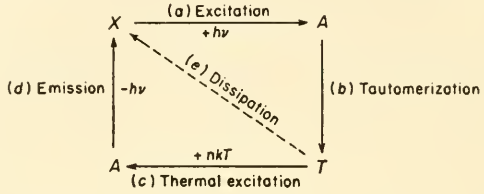
Both the ground state of a valence-saturated molecule, and the excited states corresponding to intense absorption bands, usually are *singlet*

states (*i. e.*, states with total electronic spin zero). Triplet states, with a total electronic spin of one unit, can be obtained by reversing the spin of one electron; but to do this in a closed shell, it is necessary to change also its orbital eigenfunction, thus converting it from a bonding into an antibonding electron—in other words, dissociating or weakening a chemical bond (*e. g.*, breaking the second bond in a C=C, C=O or C=N double bond). The molecule in the triplet state thus partakes of the character of a biradical.* The radiative return of such a molecule into the ground state, with the emission of fluorescence, is "prohibited," because of the requirement that the spin must be conserved; the light quantum has no mechanism for carrying the spin away. This makes "activated" molecules of the triplet biradical type metastable—at least as far as termination of activation by fluorescence is concerned. The singlet-triplet prohibition applies, however, strictly only when the electron spin does not interact with other modes of motion of the electrons in the system, since such interactions give the chance of disposing of the spin momentum by converting it into other rotational momenta. One interaction, which is always there, is the coupling of spin momentum with the rotational momentum of the electron movement around the nucleus (orbital momentum). This interaction is weak in light elements and increases with increasing atomic number. Other interactions arise when the triplet molecule is exposed to external fields of force (electric or magnetic). This is the case in condensed phases, where each molecule finds itself in the fields of force of the adjoining molecules. Thus, the theoretical life-time of the metastable triplet molecules, as calculated for an isolated molecule by considering only the coupling with the orbital momentum, must be considerably reduced in condensed systems in consequence of coupling with the medium. Furthermore, in addition to radiative transfer into the ground state (fluorescence), metastable molecules are exposed also to energy dissipation by internal conversion into vibrational energy—made irreparable, in a condensed phase, by loss of vibrational quanta to the surrounding molecules. This is the same kind of process by which metastable molecules are produced from the excited molecules in the fluorescent singlet state (transition $A \rightarrow T$ in schemes 23.IA and 23.IB). The latter transition occurs within $<10^{-8}$ sec. (since it successfully interferes with fluorescences); could it be that a similar transition, $T \rightarrow X$, is delayed for a second or even a minute? (This is the lifetime of long-lived activation in some dyestuffs, as derived from the decay curves of phosphorescence.)

These were the considerations which have caused us in Volume I to consider the hypothesis of metastable *tautomeric* states as more likely to explain long-lived activation than the hypothesis of metastable *elec-*

* We mean by this term a radical with two free valencies, not a combination of two radicals.

tronic states. The difference between the two hypotheses is that, in the second one, the position of the atomic nuclei in the metastable state is supposed to be the same as in the normal state—or, at least, not separated from the normal position by a potential barrier. In the tautomeric state, on the other hand, the atomic nuclei are rearranged so that a return into the ground state is prevented by a potential barrier, and thus requires a certain activation energy. For the rest, the tautomeric state, too, may



Scheme 23.1B

have the chemical nature of a biradical, with two free valencies, and the spectroscopical nature of a triplet state, with a corresponding paramagnetic moment.

The following two elementary transformations illustrate the difference between pure electronic excitation to a triplet state and electronic excitation coupled with tautomeric rearrangement:

(23.16E) $R_1-CH=CH-R_2 \rightleftharpoons R_1-\underset{|}{CH}-\underset{|}{CH}-R_2$ (electronic tautomerization)

(23.16F) $R_1-CH=CH-R_2 \rightleftharpoons R_1-\underset{H}{\overset{|}{C}}-\underset{|}{CH}-R_2$ (atomic tautomerization)

The return into the normal state requires, in the second case, that the H atom moves over to a neighboring carbon atom, swinging from one potential minimum into another over a barrier. This type of metastability may therefore be longer lived than the first one, in which the return into the ground state can be achieved by electronic rearrangement alone.

The concept of metastable triplet states as the origin of long-lived fluorescence (phosphorescence) of dyestuff solutions has been further developed in several papers from the Berkeley laboratories by Lewis, Kasha, McClure and Calvin (1945, 1947, 1948). One interesting result was the experimental confirmation of the paramagnetism of the phosphorescent

state (at least in one case—that of fluorescein in a rigid solvent). The observed paramagnetic moment corresponds to that of the spin of two unpaired electrons, as expected for a triplet spectroscopic state, and it was argued that this provides a clinching argument for the Lewis-Kasha theory. However, according to what we said above, paramagnetism does not prove that the biradical is of a purely electronic nature, and not a tautomer of the normal molecule. (Perhaps, one should call the metastable molecules envisaged by Lewis and Kasha "electronic tautomers" and contrast them with ordinary or "atomic tautomers"; the term "mesomers" usually is applied only to electronic structures of equal, or nearly equal, energy.)

Life-time calculations of metastable organic molecules have been made in three ways—theoretically, on the basis of spin-orbit interaction alone (this should give a high upper limit for actual life times in condensed systems!), and experimentally, either from the duration of phosphorescence, or from the intensity of the (weak) absorption bands which have been found to correspond to the phosphorescence bands in some organic compounds. In general, the actual life-times of phosphorescence were not shorter, but longer (by factors of the order of 10, 10^2, or even 10^3) than the theoretical life-times, particularly in the case of aromatic compounds. The life-times derived from the intensity of the absorption bands also often were shorter than those observed by the phosphoroscopic method. Whether these results indicate that in some molecules, at least, the metastable state corresponds to an atomic, rather than an electronic, tautomer remains to be seen. A possible alternative explanation is that the life-time calculations on the basis of the triplet-singlet exclusion rule alone give too small values because this exclusion rule is reinforced, particularly in aromatic systems, by additional symmetry considerations.

In the present chapter, we are concerned particularly with one aspect of the problem of long-lived active states—that of the "afterglow." The photochemically produced tautomeric products or the metastable triplet molecules may have such high energy that, with the help of thermal energy fluctuations, they can return, after a certain interval of time, into the original electronically excited state and cause the emission of "delayed fluorescence" (also designated as "afterglow" or "phosphorescence"). This cycle (*cf.* schemes 23.1A and B) provides the most plausible explanation of phosphoresence of many dyestuff solutions. (Solutions of eosin, erythrosin, rose bengal and many other dyes all show an afterglow lasting for 10^{-3} to 10^{-1} sec.) According to Kautsky, Hirsch and Flesch (1935), who studied this effect in numerous dyes, the afterglow is extremely sensitive to oxygen; a few millimeters pressure of this gas suffice to suppress it.

In the case of chlorophyll solutions in organic solvent, the same authors could find no afterglow, *except when isoamylamine was used as solvent.* The interpretation of this result is uncertain. Perhaps, the tautomeric state, T, does occur in chlorophyll, as in other dyestuffs, but the relative probability of its termination by direct transition into the ground state X (shown by broken line in scheme 23.IB) is much larger than that of the return into the fluorescent state A. Thus, the intensity of red phosphorescence is practically zero—except in certain cases (such as that of solution in isoamylamine) where, for an as yet unknown reason, the relative probability of the two competing processes is changed in favor of phosphorescence.

It is hardly a coincidence that isoamylamine—the only medium in which red chlorophyll afterglow was observed—is a compound whose photoxidation is sensitized by chlorophyll with a high quantum yield. It was pointed out above that the sensitization process does not compete with fluorescence (since neither isoamylamine nor oxygen, in the low concentrations used, has a marked quenching effect on chlorophyll fluorescence). Therefore, sensitization must be brought about by long-lived active forms of chlorophyll. If the same forms were also responsible for (red) phosphorescence, then clearly this phosphorescence and sensitization would be competing; this is obviously not the case, since red phosphorescence occurs only in a medium (isoamylamine) where sensitization also takes place. This is indeed a paradoxical result! One may try to find a solution by assuming that the phosphorescence of chlorophyll in isoamylamine is a *photochemiluminescence,* with the light-emitting pigment molecules being formed in the process of restoration of chlorophyll after its reversible oxidation (or reduction). For example, using the sensitization mechanism (18.40) one could write (with A for amine, and t for tautomer):

(23.17a) $\mathrm{Chl} + h\nu \longrightarrow \mathrm{Chl}^* \left(\longrightarrow \mathrm{tChl}\right)$

(23.17b) $\mathrm{Chl}^*\ (\text{or tChl}) + \mathrm{A} \longrightarrow \mathrm{oA} + \mathrm{rChl}$

(23.17c) $\mathrm{rChl} + \tfrac{1}{4}\,\mathrm{O_2} \longrightarrow \mathrm{Chl}^*\left(+\tfrac{1}{2}\,\mathrm{H_2O}\right)$

(23.17d) $\mathrm{Chl}^* \longrightarrow \mathrm{Chl} + h\nu$

(23.17) $\mathrm{A} + \mathrm{O_2} \longrightarrow \mathrm{oA}\left(+\tfrac{1}{2}\,\mathrm{H_2O}\right)$

Reaction (23.17c) is an exception from the rule that back reactions in photochemical sensitizations occur *without* chemiluminescence. In chapter 24, we will quote evidence of a chemiluminescence accompanying the back reactions in photosynthesis.

Another possible cause of phosphorescence—with a spectrum somewhat different from that of instantaneous fluorescence—can be the direct, radiative return of the metastable molecules from state T into the ground state X, with the emission of a quantum. This is an alternative to the above-mentioned, *nonradiative* return by internal conversion of electronic into vibrational energy. The assumption that this type of phosphorescence alone limits the life-time of the metastable state is the basis of the above-mentioned calculations of Lewis, Kasha and McClure. Long-lived luminescence, with a frequency 2000–20,000 cm.$^{-1}$ lower than that of direct fluorescence, actually is known for many dyestuffs and other fluorescent organic compounds, particularly at low temperatures in glassy solvents. In the case of chlorophyll, a phosphorescence of this type would have to be sought in the infrared.

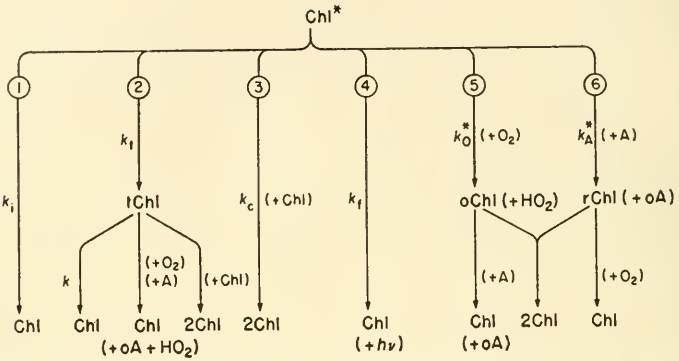
Calvin and Dorough (1947) reported that in a mixture of chlorophyll *a* and *b*, dissolved in a "rigid solvent" (EPA = mixture of ether, pentane and alcohol solidified $< -100°$ C. without crystallization), an afterglow lasting 0.2 sec. can be observed after illumination. Spectroscopic observation revealed a band beginning at 780–800 mμ and stretching into the infrared. Similar results were obtained with zinc tetraphenylchlorin, but not with copper tetraphenylchlorin—a difference ascribed by Calvin to weakening, by the paramagnetic Cu^{2+} ion, of the metastability of the triplet state.

Livingston and co-workers (1948) found no such afterglow, at $-180°$ or $-150°$ C. (2×10^{-7} mole/l. chlorophyll *a* in EPA). Experiments in other solvents and at other temperatures also gave negative results—with chlorophyll $a + b$ as well as *a*, and at concentrations from 10^{-2} to 5×10^{-6} mole/l., in air or in vacuum. According to Livingston, a personal communication from the Berkeley group confirmed that the luminescence of Chl($a + b$) at 800 mμ, reported by Calvin and Dorough, probably had been due to impurities. However, Berkeley observers asserted that chlorophyll *b* does have a weak infrared afterglow ($\tau = 0.02$ sec.), starting at 860 mμ. This phosphorescence, if it exists, should be in direct competition with photochemical sensitization by chlorophyll (Weiss, 1948).

8. Summary—A Scheme of Fluorescence and Sensitization

In summing up the discussion, we may go back to Volume I (chapter 19) and reproduce again, in a somewhat amplified form, scheme 19.II

given there to describe the most likely mechanism of fluorescence and sensitized photoxidation in chlorophyll solutions.* This scheme, amplified to include also internal conversion, self-quenching by collisions and photochemical reaction with the oxidation substrate A—*e. g.*, an amine—(but simplified as far as the mechanism of sensitized photoxidation is concerned), is reproduced in scheme 23.II. To prevent the scheme from becoming too



Scheme 23.II. Fate of excitation energy in chlorophyll solutions containing oxygen and an autoxidizable substrate A

complicated, it does not include reactions with the solvent; it must, however, be kept in mind that, as shown repeatedly in chapter 18, "pseudomonomolecular" reactions with the solvent may nearly always serve as alternatives for truly monomolecular tautomerizations. The meaning of the arrows in the scheme is: (1) internal conversion, (2) tautomerization (followed by sensitized autoxidation of the substrate A, or by dissipative return into the normal state Chl), (3) self-quenching, (4) fluorescence, (5) sensitized autoxidation of A through primary reaction with O_2, (6) sensitized autoxidation of A through primary reaction with A. According to the Lewis-Kasha theory, "tautomerization" may mean an electronic (rather than nuclear) rearrangement, and "dissipation" may be achieved by emission of phosphorescence.

Scheme 23.II shows eight different ways by which normal Chl can be re-formed after excitation, and four ways by which substrate A can be oxi-

*In the last two lines of page 546 in Volume I (first printing), a misprint and an omission must be corrected: The lines should read: "If, at $[O_2]$ = 10-1000 mm., $k_0^*[O_2]$ is not $\ll k$, the fluorescence yield, φ, must depend on oxygen pressure in this range; and if, at $[O_2] = 10^{-4}$ to 10^{-6} mole/l., $k_0^*[O_2]$ is $\ll k$, and $k_0'[O_2]$ is $\gg k_f'$, . . ."

dized to oA (two alternative direct reactions of Chl* and two alternative reactions of the metastable tChl). It neglects all resonance effects.

The yield of fluorescence, φ, is according to scheme 23.II:

$$\varphi = \frac{k_f}{(k_f + k_i + k_t) + k_c[\mathrm{Chl}] + k_0^*[\mathrm{O_2}] + k_A^*[\mathrm{A}]} \tag{23.18}$$

(This equation is to replace equation 19.5.)

Some of the constants in (23.18) can be estimated.

k_f is 1.2×10^7 (inverse of the natural life-time of Chl*, as calculated from the intensity of the red absorption band on page 634).

k_i may be small compared to k_t—in other words, tautomerization may be a normal intermediate step of internal conversion. This is, however not certain. The quantum yield $\gamma \simeq 1$ was found (*cf.* Tables 18.II and 18.III) at $[\mathrm{A}] \simeq 5 \times 10^{-4}$—*i. e.*, concentrations at which $k_A^*[\mathrm{A}]$ may well be high enough to make k_i insignificant even if it is *not* small compared to k_t.

$k_i + k_t$ must be about 10^8, if one assumes a fluorescence yield of $\sim 10\%$ in the absence of quenching by O_2 or A.

k_c should be $>10^{12}$ according to Weiss and Weil-Malherbe (*cf.* page 773), but more probably is about 10^9 (the value obtained by Weiss and Weil-Malherbe for dyes other than chlorophyll). Figure 23.7 indicates half-quenching at $[\mathrm{Chl}] = 1.5 \times 10^{-2}$ mole/l., and thus $k_i = 67$ and $k_c \simeq 7 \times 10^9$. However, the sigmoid shape of the curve points to resonance transfer rather than collisions as the main quenching mechanism.

k_0^* is about 5×10^9, calculated from a "half-quenching" concentration of 2×10^{-2} mole/l. (*cf.* Table 23.IIIC).

k_A^* could be calculated from Franck and Levi's curves for the quenching of fluorescence by benzidine, if the concentration units used were known.

(The k_1 values in Table 23.IIIC are bimolecular rate constants in sec. $\times$ l. $\times$ mole^{-1}, the k_c, k^* and k_A values in equation 25.18 are products of these constants and the rate constant of monomolecular deactivation, $k_f + k_i + k_t \simeq 10^8$ sec.$^{-1}$.)

We refrain from an attempt to deduce from scheme 23.II an equation for the quantum yield of sensitized autoxidation (to replace equation 19.6), because the result is much too complicated to allow comparison with the experimental data, *e. g.*, with Gaffron's equation (18.32) for the sensitized autoxidation of allylthiourea. The "self-quenching" reaction of the tautomer tChl:

$$\mathrm{tChl} + \mathrm{Chl} \longrightarrow 2\ \mathrm{Chl} \tag{23.19}$$

has been added to account for the decrease in the yield of chlorophyll-sensitized photoxidation with increased pigment concentration [Chl], in

the [Chl] region where self-quenching of the short-lived state Chl* cannot be significant (*cf.* Table 18.III, page 513, Vol. I). This "deactivation" of tautomeric chlorophyll by normal chlorophyll can perhaps occur via dismutation:

(23.20) $$\text{tChl} + \text{Chl} \longrightarrow \text{oChl} + \text{rChl}$$

(as suggested, *e. g.*, in equation 18.42b, page 519, Vol. I) followed by back reaction:

(23.21) $$\text{oChl} + \text{rChl} \longrightarrow 2\ \text{Chl}$$

B. Fluorescence of Carotenoids and Phycobilins *in Vitro**

1. Fluorescence of Carotenoids

Carotenoids are usually described as nonfluorescent. According to Willstätter and Stoll (1918), this is true both of the leaf carotenoids and of the carotenoids of brown algae (*e. g.*, fucoxanthol). However, de Rogovski (1912) asserted that he has observed a fluorescence of carotene in petroleum ether, in the region 505–600 mμ, and Dhéré and Castelli (*cf.* Dhéré 1939) stated that, at $-180°$, three separate fluorescence bands can be observed in carotene solution in xylene. Klein and Linser (1930) mentioned a green fluorescence of carotene solutions in alcohol. Strain (1936) found, in the chromatograms of leaf extracts in petroleum ether, a fluorescent layer situated below that of carotene α, and belonging to an unknown colorless substance, probably a hydrocarbon without sharp absorption bands in the visible or near ultraviolet. Zechmeister and co-workers (see, for example, Zechmeister and Sandoval 1945, 1946) found a fluorescent, colorless polyene hydrocarbon with sharp absorption bands at 331, 348 and 367 mμ (in petroleum ether) to be present in great abundance in extracts from various plant organs (fruits, stems etc., but not chlorophyllous organs, such as grass, leaves or green needles). This hydrocarbon, called *phytofluene* (probably $C_{40}H_{64}$), may be a hydrogenation product (or precursor) of the carotenes; it contains seven double bonds, with probably only five of them conjugated.

Absence of fluorescence indicates, according to page 799, that the excitation energy of carotenoid molecules in solution is dissipated within less than 10^{-11} sec.

* Bibliography, page 804.

2. Fluorescence of Phycobilins

The phycobilins are usually described as brilliantly fluorescent. However, since no exact determinations of the yield of fluorescence exist, it is impossible to judge whether the fluorescence is so much stronger than that of chlorophyll, or whether its greater brilliancy is due to the fact that the fluorescence bands of the phycobilins lie near the region of the greatest sensitivity of the human eye, whereas those of chlorophyll are in the far red, and partly in the infrared.

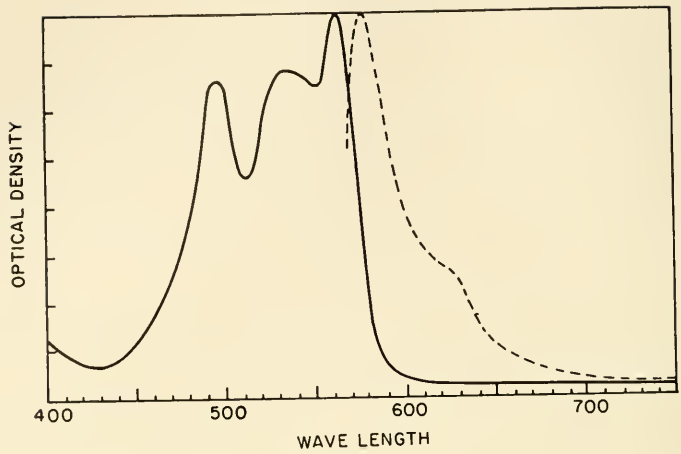
Association with proteins does not impair the fluorescence of phycobilins. On the contrary, according to Lemberg (1930), the isolated pigments fluoresce less strongly than the chromoproteids.

The fluorescence of red algae was first described by Stokes, in the same paper in which he also reported the discovery of the fluorescence of green leaves (*cf.* page 805). Since then, the fluorescence of both the living algae, and of their aqueous extracts, has repeatedly been observed, *e. g.*, by Schütt (1888), Hanson (1909), Turner (1916), Lemberg (1928), Dhéré, and Fontaine (1931), Roche (1933), Dhéré and Raffy (1935), Van Norman. French and Macdowall (1948), Arnold and Oppenheimer (1949) and French (1951).

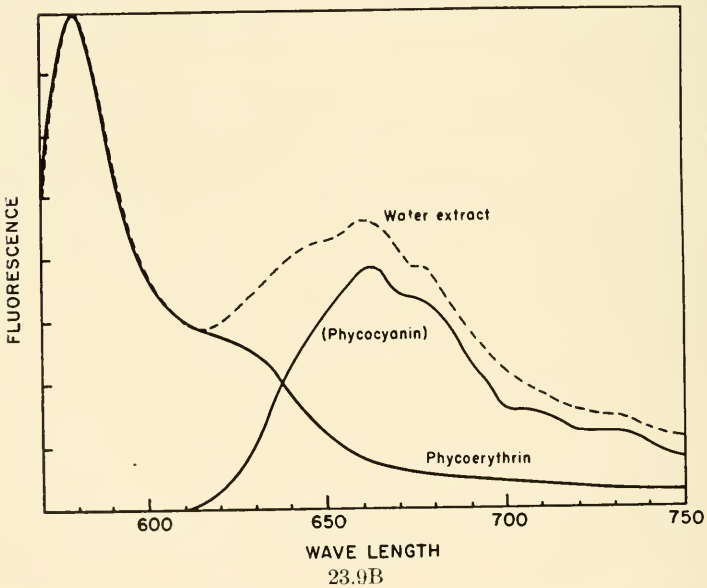
Van Norman, French and Macdowall (1948) gave a photometric curve for the fluorescence of an extract obtained by grinding a species of the red alga *Iridaea* under water and centrifuging at high speed. It shows a sharp peak at about 580 mμ, clearly related to the first long-wave absorption band of phycoerythrin at 566 mμ (*cf.* fig. 23.9A, from French 1951).

A shoulder appears on the long-wave side of the 580 mμ fluorescence band, indicating the presence of a second maximum at about 630 mμ. This probably is the second ($0 \rightarrow 1$) fluorescence band of phycoerythrin (leading to a vibrational ground state). The first fluorescence band of phycocyanin (correlated with the first absorption band of this chromoproteid, the peak of which appears, in the same extract, at about 615 mμ) lies at 660 mμ, according to the curve obtained by French (1951) by subtraction of the phycoerythrin fluorescence from the fluorescence spectrum of the crude aqueous extract from a red alga (fig. 23.9B). Earlier, Dhéré and Fontaine (1931) gave 578 and 648 mμ, respectively, as the axes of the fluorescence bands of the two phycobilins in aqueous extract. Dhéré and Raffy (1935) noted a second phycocyanin band at about 728.5 mμ.

According to French's figures, the fluorescence bands of the phycobilins are shifted to the red of the absorption bands, by 14 mμ in the case of phycoerythrin, and by 45 mμ in that of phycocyanin.



23.9A



23.9B

Fig. 23.9A. Absorption (–) and fluorescence (--) spectra of pure phycoerythrin Courtesy C. S. French (1951).

Fig. 23.9B. Fluorescence spectrum of phycocyanin as derived from the spectrum of a crude water extract of a red alga by subtracting the fluorescence of phycoerythrin. Courtesy C. S. French (1951).

Arnold and Oppenheimer (1950) broke blue-green algae, *Chroococcus* by squeezing through a syringe (*cf.* chapter 35) and separated the phycobilin chromoproteid from the chlorophyll chromoproteid by fractionation with ammonium sulfate. The phycobilin fraction, resuspended in water, showed a fluorescence band at 620–655 mμ. An estimate of the *yield* of fluorescence gave a value of about 20%. The yield of fluorescence is much lower (about 1.5%) in *living Chroococcus* cells. It increases upon grinding of the cells under water, even without the separation of the phycobilin from chlorophyll (*cf.* chapter 24, page 816). This is interesting because the blue-green algae contain no chloroplasts; therefore, the "cell juice," obtained by their grinding, should not differ so strongly in its properties from the contents of the living cell, as do the products of mechanical destruction of chloroplast-bearing green cells. Arnold and Oppenheimer suggested that the approximately tenfold increase in the yield of fluorescence upon grinding could be a pure dilution effect: In live cells, close proximity of the average phycobilin molecule to one or several chlorophyll molecules, permits a highly efficient resonance transfer of excitation energy from phycobilin to chlorophyll (accounting for a high quantum yield of photosynthesis in light absorbed by the phycobilins; *cf.* chapter 30, section 6). This leads to quenching of phycobilin fluorescence and substitution of the (less intense) sensitized chlorophyll fluorescence. Dilution decreases the rate of resonance transfer and thus preserves the fluorescence of the phycobilins.

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Fluorescence of Pigments *in Vitro*

A. Fluorescence of Chlorophyll in Vitro

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B. Fluorescence of Carotenoids and Phycobilins in Vitro

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Chapter 24

FLUORESCENCE OF PIGMENTS *IN VIVO* *

Because of close relationship that exists between fluorescence and sensitization (*cf.* chapters 18 and 19 in Volume I, and chapter 23 in this volume), the study of fluorescence of chlorophyll in the living plant can make an important contribution toward the understanding of the mechanism of photocatalytic action of this pigment in photosynthesis. Fluorescence is a property of chlorophyll that can be—and has been—observed simultaneously with the measurement of photosynthetic activity. By measuring the *yield of fluorescence*, one can obtain insight into the energy exchange and dissipation processes in photosynthesizing cells, without interfering with their life processes. No attempts have been made to observe changes in the *fluorescence spectrum* (or, for that matter, in the *absorption spectrum*) of chlorophyll during photosynthesis, but this, too, may prove possible and useful in future.

Many plant tissues fluoresce in *ultraviolet* light; but only those containing chlorophyll, bacteriochlorophyll or the phycobilins show a rather weak, red or orange fluorescence when illuminated with *visible* light. The fluorescence of the phycobilins (in blue-green and red algae) is more vivid than that of chlorophyll, because it is stronger and the eye is more sensitive to orange than to red light. Among green plants, the algae show fluorescence more clearly than land plants, because light scattering (which obscures fluorescence) is much weaker in their water-filled thalli than in air-filled leaves. The fluorescence of leaves is so difficult to observe, that after it was discovered by Stokes in 1852, and also described by Simmler in 1862 and Askenasy in 1867, other investigators, notably Lommel (1871), Hagenbach (1870, 1872) and Reinke (1883), were unable to confirm its existence. Although Hagenbach (1874) and Reinke (1884) revised their views later, the reality of leaf fluorescence remained subject to occasional doubts for another quarter of a century, until the invention of the fluorescence microscope permitted the observation of the fluorescence of single chloroplasts. (As early as 1883, Engelmann had tried, unsuccessfully, to observe the fluorescence of chloroplasts in ultraviolet light under an ordinary microscope.) The picture of chloroplasts glowing with a crimson light on a faint milky background so thrilled the investigators who first

* Bibliography, page 826.

saw it—Tswett (1911), Lehmann (1914), Wilschke (1914) and Gicklhorn (1914)—that they gave enthusiastic descriptions of this phenomenon. More recently, green leaves, green and colored algae and diatoms have all been studied under the fluorescence microscope, by Lloyd (1923, 1924), Testi Dragone (1927), Klein and Linser (1930) and Metzner (1937).

In the meantime, methods of *macroscopic* observation of plant fluorescence also have been improved, and Stokes' original results confirmed and expanded. Notably Dhéré and co-workers (see Dhéré 1937, 1939) have carried out numerous *spectrophotographic* investigations of plant fluorescence: Dhéré and Fontaine (1931), Fontaine (1934) and Dhéré and Raffy (1935) studied brown, green and blue algae; Bachrach and Dhéré (1931), diatoms; and Dhéré and Raffy (1935) and Dhéré and Biermacher (1936), green leaves. The investigations of Kautsky and co-workers (1932–1943), McAlister and Myers (1940), Franck, French and Puck (1941), Shiau and Franck (1947) and of the "Dutch group" (Wassink, Katz, Dorrestein *et al.* (1939, 1942, 1945) dealt mainly with the alterations in the *intensity* of fluorescence that accompany changes in the rate of photosynthesis.

French and co-workers (1948, 1951) and Duysens (1951) initiated a very promising *spectrophotometric* investigation of fluorescence, particularly of red algae.

1. Fluorescence Spectra of Plants

If we leave aside the phycobilin-carrying algae, the spectroscopic pattern of the fluorescence of living plants is very simple. In solutions, both chlorophyll *a* and chlorophyll *b* have a two-band fluorescence spectrum in the visible region (*cf.* Table 23.I). In the living cell, these bands are shifted so far toward longer waves that only one band of chlorophyll *a* and one of chlorophyll *b* remain within the visible spectrum. Thus, the visible fluorescence spectrum of green leaves and green algae consists of only two bands.

Brown algae and diatoms contain no chlorophyll *b*; their fluorescence spectrum therefore shows only one visible band. It was mentioned on page 406 that Wilschke (1914) and Dhéré and Fontaine (1931) observed a second band in the fluorescence spectrum of heat-killed brown algae and extracts from these organisms, and attributed it to a "chlorophyll *c*" (chlorofucin); but no corresponding band was found by Dhéré and Raffy (1935) in the fluorescence spectra of living brown algae. Manning and Strain (*cf.* page 614) concluded more recently that a chlorophyll *c* actually exists in live diatoms; but they did not attempt to confirm this by observation of the fluorescence band of this pigment in the spectrum of the algae.

The positions of the visible fluorescence bands of the two chlorophylls in

living cells are listed in Table 24.I. The two different figures given in the table for the leaves of *Pelargonium* illustrate the statement made on page 744 that the position of the band "axis" depends on the sensitivity curve of the photographic plate.

TABLE 24.I

FLUORESCENCE BANDS OF CHLOROPHYLL IN PLANTS

Plant		Chlorophyll *a*				Chl. *b*	Observer
		First band		Second band axis	Third band axis	First band axis	
		Axis	Max.				
Brown algae...	*Fucus virsoides*	670	—	—	—	Absent	Wilschke (1914)
Green algae..	*Ulva lactuca*	670	—	—	—	656.5	Wilschke (1914)
	Ulva lactuca	684.7	—	—	—	655.5	Dhéré, Fontaine (1931)
	Chlorella	684.5	681	—	—	—	Stern (1921)
	Chlorella	—	685	—	—	—	Vermeulen, Wassink, Reman (1937)
	Hormidium flaccidum	686	—	—	—	—	Baas-Becking, Koning (1934)
Higher plants..	*Elodea canadensis*	670	—	—	—	657.5	Wilschke (1914)
	Elodea canadensis	677.5	—	—	—	655	Tswett (1911)
	Tradescantia	685	681	—	—	—	Stern (1921)
	Pelargonium zonale	{680.5[a] 686[b]}	—	738	—	—	Dhéré, Raffy (1935[1])
	Pelargonium zonale	684	—	740	812	—	Dhéré, Biermacher (1936[2])
Red algae..	*Gigartina; Iridaea*	—	707[c]	—	—	—	Van Norman *et al.* (1948)
	Porphyridium	—	605	—	—	—	French (1951)
	Porphyra laciniata	—	690[d]	—	—	—	Duysens (1951)

[a] Ilford Panchromatic Plate. [b] Agfa Infrared 730 plate. [c] Probably chlorophyll (*a* + *d*). Other peaks at 575 mμ (phycoerythrin) and 655 mμ (phycocyanin). [d] Other peaks at 725 mμ (chlorophyll *d*?) and 660 mμ (phycocyanin).

Figure 24.1 shows the fluorescence spectrum of a *Pelargonium* leaf (No. 7 on panchromatic plate, and Nos. 2 and 3 on Agfa Infrared 730 plate), compared with the absorption spectrum of the same leaf (No. 6) and with the fluorescence spectrum of chlorophyll in ether (No. 4). We note the

close coincidence of the fluorescence band *in vivo* (No. 7) with the corresponding absorption band (No. 6), and the "red shift" of the fluorescence band in the living cell compared to its position in solution (Nos. 2 and 3 compared to No. 4).

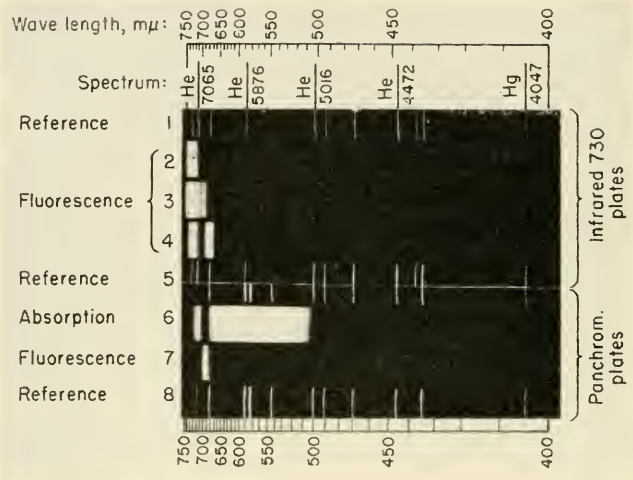

Fig. 24.1. Fluorescence spectrum of living leaves of *Pelargonium* (Nos. 2, 3, 7) and of chlorophyll in ether (No. 4), compared with the absorption spectrum (No. 6) (after Dhéré and Raffy 1935).

Dhéré and Raffy (1935) suggested that the second fluorescence bands of chlorophylls *a* and *b* *in vivo*, situated in the near infrared, may account for the striking brightness that green vegetation exhibits on landscape photographs on infrared-sensitive plates (*cf.* fig. 22.31A). However, the fluorescence of living leaves is much too weak to produce such a spectacular effect. Mecke and Baldwin (1937) disproved Dhéré's theory by showing that the vegetation remains dark when illuminated with infrared-free light and photographed through a filter that transmits only the infrared. The brightness of green plants in infrared light is thus due to lack of absorption, and not to fluorescence.

Dhéré and Biermacher (1936) photographed the fluorescence of *Pelargonium* leaves on plates whose sensitivity extended far into the infrared, and found a new band, with an axis at 812 mμ, extending to 830 mμ. In Table 24.II, the wave lengths of the peaks of the four known fluorescence bands of the chlorophylls *a* and *b* in living cells are compared with the wave lengths of the corresponding bands in ethereal solution.

Table 24.II shows that, in the living cell, the fluorescence bands are shifted by 5–15 mμ toward the infrared from their positions in ethereal solution—*i. e.*, by about the same distance as the corresponding absorption

TABLE 24.II

FLUORESCENCE BANDS OF CHLOROPHYLL IN PLANTS AND IN ETHEREAL SOLUTION[a]

Band		Axis, mμ[b]		Maximum, mμ	
		Plant	Soln.	Plant	Soln.[c]
Chlorophyll *a*	I....	680, 685	672[d]	681–685	665
	II....	740	736	—	720
	III....	812	801	—	—
Chlorophyll *b*	I....	656	651[d]	—	649
	II....	—	713	—	708
	III....	—	789	—	—

[a] On Ilford Infrared Plates.
[b] Biermacher (1936).
[c] *cf.* table 23. I.
[d] The difference between these values and those in Table 23.IB is attributed by Biermacher to the decrease of the sensitivity of the infrared-sensitive plate in the orange. The values in Table 23.IB are supposed to be the correct ones; they are not used here, to preserve the consistency of Table 24.II.

bands. The strong shift of peak I is due to self-absorption. French (1954) found it at 675 mμ in a faintly green, and at 685 mμ in a dark-green leaf. The intensity ratio I:II was 5:1 in the first, and 1:1 in the second, case.

Vermeulen, Wassink and Reman (1937) gave spectrophotometric curves of the fluorescence of the alga *Chlorella* (chlorophyll *a* + *b*) and the bacterium *Chromatium* (bacteriochlorophyll), which are reproduced in figures 24.2 and 24.3. *Chlorella* shows a peak at 680 mμ and only a shoulder at 740 mμ. Duysens (1951) found that, even in light absorbed by chlorophyll *b*, *Chlorella* shows only the fluorescence of chlorophyll *a*—indicating efficient energy transfer from *b* to *a*.

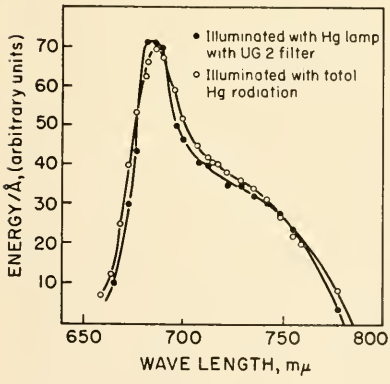

Fig. 24.2. Fluorescence spectrum of *Chlorella* suspension (after Vermeulen, Wassink and Reman 1937).

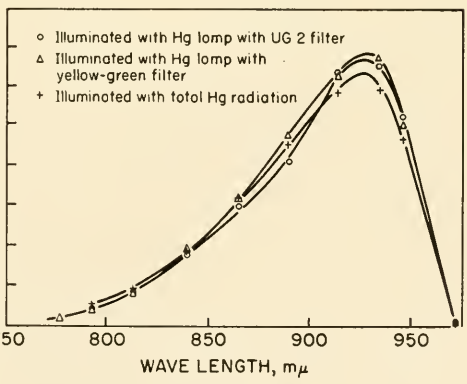

Fig. 24.3. Fluorescence spectrum of *Chromatium* suspension (after Vermeulen, Wassink and Reman 1937).

Comparison of the figures 24.3 and 23.4 shows that the displacement of the fluorescence band is much stronger in the purple bacteria than in green plants. The fluorescence spectrum of live *Chromatium* shows only one band, at 926 mμ, while that of the extract contains *two* bands, at 806 and 695 mμ, respectively. It was mentioned on page 751 that the first and more intense of these two bands can be correlated with the main absorption band of extracted bacteriochlorophyll, at 770 mμ, but that the correlation of the 695 mμ fluorescence band with the 605 mμ absorption band is doubtful. Absorption spectra of live purple bacteria show *two* (or three) absorption bands, at 860–870 and 800 mμ, respectively (*cf.* p. 702); but here again, only the first one can be identified with the main $X \rightarrow Z$ absorption band of dissolved bacteriochlorophyll (at 770 mμ), while the identification of the second one—which is comparatively weak and variable in intensity—with the $X \rightarrow Y$ band at 605 mμ is uncertain (*cf.* page 702). The relation between the fluorescence and the absorption bands of bacteriochlorophyll is illustrated by Table 24.IIA. The table shows that the fluorescence band I *in vivo* is shifted toward the infrared by as much as 120 mμ, compared to the position of the fluorescence band *in vitro*, and by about 60 mμ compared to the position of the corresponding absorption band *in vivo*.

TABLE 24.IIA

SPECTRA OF BACTERIOCHLOROPHYLL *in Vitro* AND *in Vivo*

Band	I, mμ ($X \rightarrow Z$)	II, mμ ($X \rightarrow Y$)	III, mμ ?
Absorption			
Cell	860–870	—	800
Extract	770	605	—
Fluorescence			
Cell	926	—	—
Extract	806	695 (?)	—

This tabulation indicates that, in the living cell, bacteriochlorophyll fails to show one absorption band and one fluorescence band that are found in extracts, but shows one (or two) extra absorption bands, without corresponding fluorescence bands, which have no counterpart in the solution spectrum. Duysens (1951) confirmed that *Chromatium* and *Rhodospirillum* show only one fluorescence band, correlated with the absorption band at 890 mμ. Absorption in the 800 and 850 mμ bands contributes to the excitation of this fluorescence band; so does—with a 50–70% lower efficiency—the absorption by cartenoids. Excitation energy is thus transferred from all pigments of the bacteria to the bacteriochlorophyll form having the lowest excitation energy.

The fluorescence of *green bacteria* (*Chlorobium mirable*), probably due to "bacterioviridin" (*cf.* Vol. I, p. 407), was observed by Buder (1913).

A fluorescence band at 635 mμ, noted by French (1951) in a partially green leaf could be due to *photochlorophyll*, whose absorption band lies at 620–630 mμ in ether. French estimated its position *in vivo* as 650 mμ (from the action spectrum of chlorophyll formation), but noted that this is incompatible with the location of the fluorescence at 635 mμ.

The fluorescence of the *phycobilins* in red and blue-green algae is of great interest, because it permits a study of the interaction of two different fluorescent pigments in one cell. The first photograph of the fluorescence spectrum of *Rhodymenia*, made by Dhéré and Fontaine (1931), showed one band in the orange (phycoerythrin) and one in the red (chlorophyll and phycocyanin).

Van Norman, French and Macdowall (1948) determined fluorescence curves of two red algae—*Gigartina* and *Iridaea*; both showed three peaks, at 575 mμ (phycoerythrin, *cf.* p. 799), 655 mμ (phycocyanin, *cf.* p. 800) and 700 mμ (chlorophyll, probably *a* + *d*, *cf.* below). French (1951) gave fig. 24.4 for *Porphyridium*: Only chlorophyll fluoresces when cells are excited with λ 436 mμ or 450 mμ, *i.e.*, by light absorbed by chlorophyll and carotenoids only. *Phycobilin* bands develop with excitation by 470, 490, and 546 mμ but, even though most of the incident light is now absorbed by phycoerythrin, chlorophyll fluorescence remains strong.

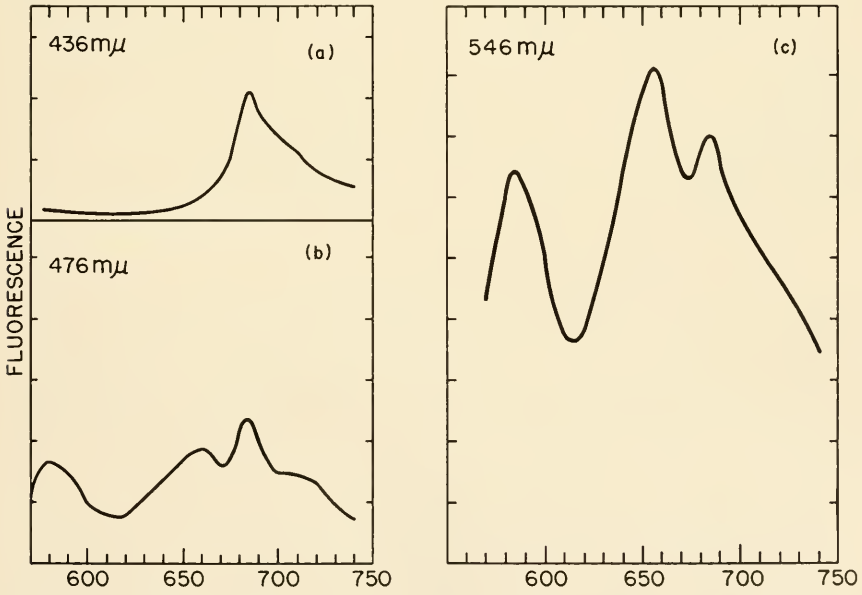

Fig. 24.4. Fluorescence spectra of a red alga when illuminated with equal energies of different wavelengths. Courtesy L. N. M. Duysens.

The chlorophyll *a* band in fig. 24.4 is in the usual position—at 685 mμ. However, an additional band is indicated at 730 mμ; it can be attributed to chlorophyll *d*. The weakness of chlorophyll *d* absorption (mere ripple in fig. 22.20!) makes one suspect that chlorophyll *d* fluorescence is excited mostly by energy transfer from other pigments. This is strikingly confirmed by the observation, reported by Duysens (1951), that an "unidentified pigment"—presumably chlorophyll *d*— whose absorption, in *Por-*

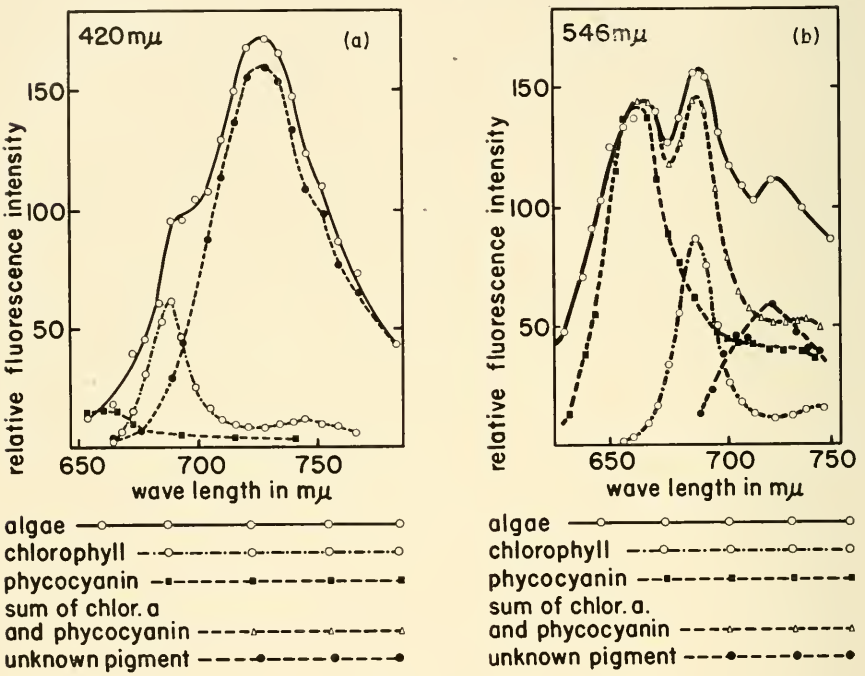

Figure 24.5

phyra lacineata, is < 0.1% of that of chlorophyll *a*, emits, when 420 mμ is used for excitation, ten times more fluorescence than chlorophyll *a* (fig. 24.5). With excitation by 546 mμ, the chlorophyll *a* band at 685 mμ is slightly higher than the "chlorophyll *d*" band at 730 mμ. In agreement with French (fig. 24.4) the phycocyanin fluorescence band at 665 mμ is very weak in violet exciting light but becomes strong in green light.

2. Fluorescence Yield and Sensitized Fluorescence *in Vivo*

All observers concur that the fluorescence of chlorophyll in the living cells is "weak," but absolute measurements of its yield are very few. Vermeulen, Wassink and Reman (1937) determined the quantum yields of fluorescence in four *Chlorella* suspensions, and found values between 0.15 and 0.30%; Wassink and Kersten (1944) found a yield of about 0.15%

in a suspension of diatoms. In *Chromatium* (a purple bacterium) Vermeulen, Wassink and Reman (1937) at first found a much smaller yield—between 0.005 and 0.01%. These values are incredibly low for a "fluorescent" material. (Theoretically, the lowest yield to which fluorescence could sink even in a "nonfluorescent" pigment is about 0.001%, since the ratio of the period of a molecular vibration and the life-time of electronic excitation is $10^{-12.5}/10^{-7.5} = 10^{-5}$.) A redetermination of the yield of fluorescence of *Chromatium* by Wassink, Katz and Dorrestein (1942) in fact gave considerably larger figures—of the order of 0.1%, *i. e.*, similar to those found in algae. Self-absorption may have been the cause—or at least, one cause—of the error of the earlier determinations.

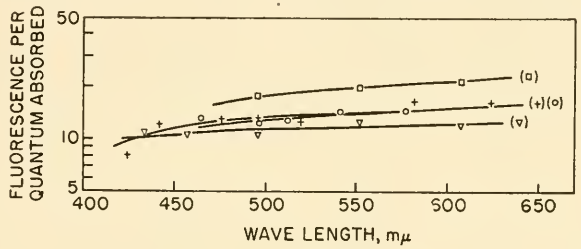

Fig. 24.5A. Yield of fluorescence of *Chlorella* suspensions in relation to wave length of exciting light (after Vermeulen, Wassink and Reman 1937). 4 sets of measurements.

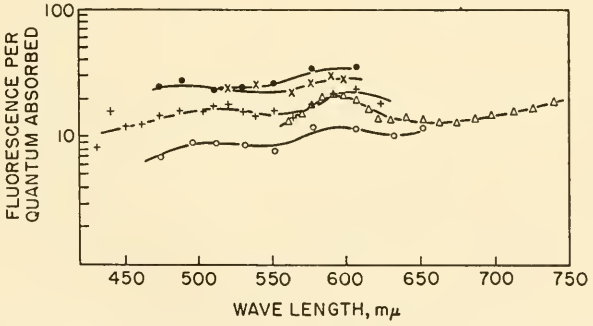

Fig. 24.5B. Yield of fluorescence of *Chromatium* suspensions in relation to wave length of exciting light (after Vermeulen, Wassink and Reman 1937). 4 sets of measurements.

The *yield* of fluorescence in live blue-green algae (*Chroococcus*) was estimated by Arnold and Oppenheimer (1950) by a rather crude method (visual comparison with the intensity of light scattered by a block of magnesium); they found it to be of the order of 1.5%—about ten times higher than the yield of fluorescence in green cells. Presumably, this fluorescence originates predominantly in phycocyanin, although chlorophyll, too, may contribute to it.

Interesting results were obtained in the study of the effect of wave

length of the exciting light on the fluorescence of live cells. Vermeulen, Wassink and Reman (1937) found that the *spectral distribution* of the fluorescent light of *Chlorella* and *Chromatium* is independent of the wave length of exciting radiation. The *quantum yield* of fluorescence (φ) also was approximately constant, between 442 and 624 mμ in *Chlorella*, and between 450 and 750 mμ in *Chromatium*. However, a slow systematic decrease of φ was observed in *Chlorella* at the shorter waves—a trend that became accelerated below 424 mμ (fig. 24.5A). In *Chromatium*, maxima and minima of φ were observed in two or three places in the visible spectrum (fig. 24.5B). The Dutch investigators concluded from these observations that the quantum yield of chlorophyll fluorescence *in vivo* does not depend on wave length except when carotenoids interfere with the light absorption by chlorophyll or bacteriochlorophyll. In green plants, this occurs only below 520 mμ; the carotenoids of purple bacteria, on the other hand, have absorption bands in the green, yellow and orange (*cf.* Table 21.IX and fig. 22.27), and these bands could perhaps account at least for the first minimum of the fluorescence yield noticeable in figure 24.5B at about 550 mμ.

While qualitatively the conclusions of Vermeulen and co-workers appear plausible, quantitative considerations lead to some interesting complications. In *Chlorella*, for example, φ declined, in the violet, by only 10 or 20%, while figure 22.43 indicates that the carotenoids must account for at least one third the total absorption in this region! It thus appears as if the chlorophyll fluorescence can be excited, with considerable probability, also by the light absorbed by carotenoids! This hypothesis has been strikingly confirmed by experiments with fucoxanthol-containing diatoms.

The results of these experiments, carried out by Dutton, Manning and Duggar (1943), are shown in Table 24.III. They indicate that the yield of chlorophyll fluorescence is the same, whether it is excited by red light, absorbed exclusively by chlorophyll, or by blue-green light (470 mμ), three quarters of which probably is absorbed by carotenoids, mainly fucoxanthol (see fig. 22.46 and figs. 30.9B and C. Table 24.III also contains new results with *Chlorella*, which confirm the conclusions drawn above from the earlier work of the Dutch observers. These results indicate that the light absorbed by carotene and luteol is almost—but not quite—as efficient in the excitation of chlorophyll fluorescence in green algae as the light absorbed by fucoxanthol in diatoms. In striking contrast is the result of the experiment with an acetonic *extract* from *Nitzschia*; here, light absorbed by the carotenoids is completely lost for fluorescence. The experiment with acetonic solutions of chlorophylls *a* and *b* shows that the quantum yield of chlorophyll fluorescence in solution does not increase with

the wave length of exciting light between 436 and 578 mμ. This proves that an increased yield of *nonsensitized* fluorescence of chlorophyll in violet light cannot be offered as alternative explanation of the results of the first two experiments. Excitation transfer from carotenoids to chlorophyll might become possible in sufficiently concentrated solutions; we mentioned on p. 790 that Duysens (1951) reported excitation transfer from chlorophyll *b* to chlorophyll *a* in 10^{-3} *M* solution in acetone.

TABLE 24.III. DIRECT AND CAROTENOID-SENSITIZED FLUORESCENCE OF CHLOROPHYLL IN DIATOMS (AFTER DUTTON, MANNING AND DUGGAR 1943)

Material	Excitation wave lengths compared, mμ	Fraction of absorbed energy absorbed by chlorophyll[a] %	Ratios of fluorescence yields excited by the two wave lengths	
			Expected (without energy transfer)	Observed
Nitzschia closterium....	470 *vs.* 578 or 600	26 *vs.* 95 or 99	0.27	1.2 ± 0.2
	436 *vs.* 578 or 600	51 *vs.* 95 or 99	0.53	1.1 ± 0.2
Chlorella pyrenoidosa...	470 *vs.* 578 or 600	52 *vs.* 100	0.52	1.05 ± 0.04
	436 *vs.* 578 or 600	81 *vs.* 100	0.81	0.93 ± 0.18
N. closterium, acetone extract.....	470 *vs.* 600	19 *vs.* 100	0.19	0.22 ± 0.02
	436 *vs.* 578	40 *vs.* 99	0.40	0.49 ± 0.07
Acetone soln. of Chl *a* and *b*.....	436 *vs.* 578	100	1.0[b]	0.97 ± 0.06[b]

[a] These estimates are rather crude approximations (*cf.* chapter 22, page 726), but it seems improbable that errors could be large enough to account for all the differences between the calculated and observed ratios in the two last columns.

[b] The proportion of light absorbed by these two chlorophyll components is nearly equal at 436 and 578 mμ. Hence the yield ratio should be approximately 1.0 despite differences in the fluorescence spectra of chlorophylls *a* and *b*.

The experiments of Dutton and Manning are of importance for the theory of photosynthesis. They indicate that the energy absorbed by some carotenoids may become available to chlorophyll in almost the same measure as that absorbed directly by the green pigment. Thus, chlorophyll can play the part of a photocatalyst in photosynthesis, even when another pigment acts as a "primary" sensitizer. Results similar to those in Table 24.III have also been obtained by Wassink and Kersten (1946) with *Nitzschia dissipata*.

Even more interesting are the results obtained with red algae. Van Norman, French, and Macdowall (1948) first found indications that chlorophyll fluorescence in *Gigartina* and *Iridaea* can be excited with equal (if not higher) intensity by light absorbed by phycoerythrin (at 560 mμ) as by light absorbed by chlorophyll (at 650 mμ). French's (1951) fig. 24.4 clearly shows that light absorbed by chlorophyll (and, partly, by

carotenoids) at 436 mμ, does *not* excite the fluorescence phycoerythrin or phycocyanin (curve *a*). Light absorbed mainly by phycoerythrin, on the other hand, excites the fluorescence not only of both phycobilins, but also of chlorophyll curves *a*, *b*, *c*. The hump on the red side of the chlorophyll *a* band probably is due to chlorophyll *d* (*cf.* below).

Duysens' (1951) fluorescence spectra of *Porphyra lacineata* (fig. 24.5) indicate that after excitation with 420 mμ (absorbed by chlorophyll *a* and the carotenoids) over 90% of excitation energy is transferred to an "unknown pigment" (probably chlorophyll *d*); less than 10% of total fluorescence is emitted by chlorophyll *a*, and only a negligible proportion by phycocyanin. Excitation with 546 mμ (light absorbed mainly by phycoerythrin) causes strong fluorescence of both phycocyanin and chlorophyll *a*, and a comparatively weak fluorescence of "chlorophyll *d*." Duysens interpreted these results as indicating the existence, in red algae, of two kinds of pigment complexes: the largest part of chlorophyll *a* he suggested, must be coupled with chlorophyll *d*, and transfer practically all excitation energy to the latter pigment, although it is present in such a small amount as to be hardly noticeable in the absorption spectrum at all (*cf.* p. 812). A small part of chlorophyll *a*, not coupled with chlorophyll *d*, appears to be associated with the phycobilins, and serves as ultimate recipient of the major part of quanta absorbed by them. French's results indicate that the extent of energy "leak" into chlorophyll *d* must vary widely from species to species. In chapter 29, this hypothesis will be tied up with the results of quantum yield determinations by Haxo and Blinks, who noted a low photosynthetic efficiency of light absorbed by chlorophyll in some red algae. In chapter 32, we shall explore whether the paradoxical fact that fluorescence of chlorophyll *a* can be excited more strongly by light absorbed by phycobilins than by light absorbed by chlorophyll *a* itself, could be explained without Duysens' assumption of two different pigment complexes in red algae.

The general rule, indicated by the above-described fluorescence experiments, is that plant cells contain one (sometimes, perhaps, two) pigment complex in which light energy absorbed by any one component tends to flow into the component with the lowest excitation level, and is therefore remitted mainly as fluorescence of the latter—even if it is present in a very low relative concentration.

This picture is supported by Duysens' observations (p. 810) that all energy absorbed, in purple bacteria, by some carotenoids or by different forms of bacteriochlorophyll, flows into the form of bacteriochlorophyll that has the lowest excited level.

On page 801, we mentioned the increase in the intensity of the phycobilin fluorescence observed by Arnold and Oppenheimer (1950) upon

breaking *Croococcus* cells under water, and their interpretation of this effect as a consequence of suppression of energy transfer from excited phycobilin molecules to chlorophyll molecules, caused by dilution.

If we assume that all chlorophyll in the cells is present in the same form, then a fluorescence yield φ means the shortening of the normal life-time of the excited state, τ_0, to $\tau = \varphi\tau_0$. In the case of chlorophyll in *Chlorella*, assuming $\tau_0 = 8 \times 10^{-8}$ sec. (page 634), we obtain:

$$\tau = (1.5 \text{ to } 3 \times 10^{-3}) \times 8 \times 10^{-8} = (1.2 \text{ to } 2.4) \times 10^{-10} \text{ sec.} \tag{24.1}$$

and in the case of bacteriochlorophyll in *Chromatium*, assuming the same value of τ_0:

$$\tau = 7 \times 10^{-3} \times 8 \times 10^{-8} = 0.6 \times 10^{-10} \text{ sec.} \tag{24.2}$$

Two factors may determine the life-time of excited chlorophyll molecules in live cells, and thus account for the above-calculated small values of τ: "normal" energy dissipation in the pigment–protein–lipide complex (chloroplastin) and quenching (or stimulation) of fluorescence by metabolic processes. The latter phenomena may themselves be of two kinds: direct "photochemical quenching" by competition between sensitized photochemical reaction and fluorescence, and indirect quenching (or stimulation) of fluorescence due to the metabolic formation of substances that diminish (or enhance) the fluorescence of chlorophyll. Observations described in section 3 can be interpreted as revealing changes in the general structure of the chloroplastin complex, while in section 4—and, in more detail, in chapters 27, 28 and 33—we will discuss variations in intensity of fluorescence closely associated with participation of chlorophyll in photosynthesis.

3. Effects of Heat and Humidity on Chlorophyll Fluorescence *in Vivo*

It has been found that, when chloroplast sediments (Noack 1927) or live leaves (Seybold and Egle 1940) are placed in *hot water*, their fluorescence vanishes almost immediately; at the same time, the red absorption band is shifted toward the shorter waves. This transformation occurs at a temperature of 64–72° C. If the leaves are kept in hot water for several minutes, fluorescence reappears, but the absorption band remains in the shifted position. Metzner (1937) probably dealt with the same phenomenon when he described a "burst" of fluorescence caused by heating the chloroplasts under the fluorescence microscope.

According to Seybold and Egle (1940), *drying* extinguishes the fluorescence of fresh leaves, but not that of leaves killed by boiling. The fluores-

cence of some plants is highly sensitive even to minor changes in humidity: for example the fluorescence of *Pleurococcus* colonies on wood bark vanished after one or two hours in an atmosphere of less than 80% relative humidity (at 25° C.); while the fluorescence of *Mnium punctatum* disappeared when the humidity declined below 85%. The fluorescence of the leaves of *Adiatum* and *Paretaria* was found to be somewhat less sensitive, but it, too, ceased to be visible after one or two days in an atmosphere of 75% relative humidity.

The fluorescence of sharply dried leaves cannot be restored by simple wetting, but returns upon immersion into boiling water. A similar transformation of the "sensitive" fluorescence of live cells into the "stable" fluorescence of dead cells can be achieved by freezing or immersion into ether. In the latter case, the fluorescence after the treatment is considerably stronger than it was in the living state.

Seybold and Egle interpreted these results as indication that practically all chlorophyll in leaves is present in a nonfluorescent (probably, protein-bound) state, but that a small fraction of the pigment is dissolved in a lipide phase, and therefore capable of fluorescence. They suggested that, upon drying, the fraction of chlorophyll normally present in the lipide phase is transferred into the colloidal aqueous phase, while, upon heating, chlorophyll is first extracted from the lipide phase into the colloidal proteinaceous phase (thus causing the fluorescence to disappear), but later returns into the lipophilic material (concomitantly with the denaturation of the proteins and melting of lipides), and thus again becomes fluorescent. (Metzner 1937 also had attributed the "burst" of fluorescence caused by heating to the melting of the lipides.) Underlying this "two-phase" hypothesis of Seybold and Egle was the conviction that all chlorophyll–protein complexes are nonfluorescent. However, while this seems to be true enough of pure chlorophyll–protein precipitates (*cf.* page 775), it does not apply to complexes which contain both proteins *and* lipides (*e. g.*, to "coacervates" of the type described by Hubert and Frey-Wyssling; *cf.* chapter 23, page 777). Seybold and Egle's argument is therefore not convincing. The effects of heating and drying on chlorophyll fluorescence *in vivo* can be explained in a much simpler way than suggested by Seybold and Egle: by assuming that the pigments normally contained in a weakly fluorescent protein–chlorophyll–lipide complex lose the protection against self-quenching (and therefore become nonfluorescent), when the lipides melt in the heat and form a separate phase, but diffuse into this new phase if the pigment–protein link is broken by denaturation (*e. g.*, by somewhat more prolonged heating) and thus again become fluorescent. The displacement of the fluorescence bands of chlorophyll in living cells (by 5–15 $m\mu$ toward longer waves from their position in organic solvents) agrees with the assumption that fluorescence is emitted by the same chlorophyll molecules responsible for the (similarly displaced) absorption bands.

Seybold and Egle, on the other hand, had to attribute the fluorescence bands to the fraction of chlorophyll dissolved in a lipide, and the absorption bands to the bulk of chlorophyll present in a protein-bound colloidal state. Therefore their theory was predicated on the contention that the fluorescence band of chlorophyll is shifted in lipides toward the longer waves much more strongly than the corresponding absorption band. This hypothesis was characterized as implausible on page 746.

To sum up, there seems to be no reason to attribute the fluorescence of living plants to a small fraction of chlorophyll molecules, present in a strongly fluorescent solution, rather than to the whole mass of the pigment forming a weakly fluorescent complex with proteins and lipides (including the carotenoids). The close relationship between fluorescence intensity and rate of photosynthesis, which will be discussed in the next section, also indicates that fluorescence is a property not of a small fraction but of the bulk of chlorophyll in the cell.

4. Variations of Chlorophyll Fluorescence Related to Photosynthesis

In the preceding section, we discussed chlorophyll fluorescence *in vivo* in relation to what may be called the gross state of the green pigment in the living cell—its high concentration and its simultaneous association, in the "chloroplastin," with proteins and lipides. The effects of drying, heating, boiling or immersion in ether, described in that section, can be assumed to be indicative of a partial or complete disintegration of the chloroplastin.

In the present section, we will deal with reversible changes in the yield of fluorescence that are more or less closely associated with photosensitizing activity and can be assumed to occur without essential changes in the composition and structure of chloroplastin.

Kautsky discovered in 1931 that rapid changes in the intensity of fluorescence of leaves occur during the first seconds and minutes of illumination after a period of darkness, and bear definite relation to the previously known changes of the rate of photosynthesis during this "induction period." Subsequent investigations by Kautsky and co-workers (1931–1948), Franck and co-workers (1934–1949), McAlister and Myers (1940) and of the Dutch group of investigators (Ornstein, Wassink, Katz, Dorrestein *et al.* (1937–1949) have revealed many striking examples of close interrelation between the intensity of fluorescence and the momentary rate of photosynthesis. This relationship can be observed not only during the induction period, but also in the steady state. Factors such as light intensity, temperature, concentration of reactants that take part in photosynthesis, presence of oxygen and various poisons and narcotics are found to affect significantly the yields of both fluorescence and photosynthesis.

The close interrelation of the fluorescence of chlorophyll and its photo-

sensitizing activity (revealed through parallel measurements of the yields of photosynthesis and fluorescence) has made fluorescence measurements an important tool in the kinetic analysis of photosynthesis. We will therefore restrict ourselves in the present chapter to some general considerations of this relationship, postponing more detailed description of experimental results and their interpretation to the several chapters in part IV dealing with the effects of light intensity, temperature, carbon dioxide and other external factors, on the kinetics of photosynthesis.

Fluorescence is one of the several ways in which excited chlorophyll molecules can dispose of their energy. Others include energy *dissipation* (conversion into vibrational energy and ultimately into heat), and *photochemical reactions* (either involving the chlorophyll molecule itself, or sensitized by it). The intrinsic capacity of the excited chlorophyll molecule to fluoresce (the monomolecular fluorescence constant k_f, or its reciprocal, the "natural life time" of the excited state, τ_f) can be considered as constant as long as the absorption spectrum of the chlorophyll molecule remains essentially unchanged. The intrinsic capacity for energy dissipation (the monomolecular dissipation constant k_i) and the rate of energy loss through chemical reactions (rate constants k_i^*, $k_A^* \ldots$, which can be monomolecular or bimolecular) are, on the other hand, subject to changes depending on the association of the chlorophyll molecule with other molecules before excitation, and on its encounters with other molecules during excitation (as discussed in the sections of chapter 23 dealing with the quenching and self-quenching of chlorophyll fluorescence *in vitro*). The variations of chlorophyll fluorescence *in vivo* associated with variations in the rate of photosynthesis must therefore be attributed to changes in the composition or structure of the chlorophyll-bearing molecular complex (and consequent alterations in the values of monomolecular constants of dissipation and chemical quenching), and to changes in the probability of the chlorophyll complex encountering, during the excitation time, molecules capable of serving as effective "physical" or "chemical" quenchers (and consequent alterations in the values of bimolecular constants of quenching).

The several more or less detailed interpretations of fluorescence changes in photosynthesizing plants, which have been suggested, all are based on these general ideas but differ in emphasis laid on one or the other specific mechanism of quenching. Some (Kautsky; Wassink and Katz) attribute the main function to "chemical quenching" by the reactants taking part in photosynthesis, and consider each increase in fluorescence as evidence of a decrease in the efficiency of the sensitized photochemical process (and consequent decline of chemical quenching), and each decrease in fluorescence as evidence of increased efficiency of utilization of excitation energy for the sensitized photochemical reactions (and consequent increase of chemical quenching). Others (Franck) see the most important cause of

changes in fluorescence intensity in the formation of chlorophyll complexes with surface-active substances ("narcotics") which slow down energy dissipation, and at the same time inhibit photochemical sensitization by preventing photosensitive substrates from reaching the chlorophyll. This amounts to a weakening of *both* processes (sensitization *and* dissipation) which compete with fluorescence; whereas in theories of the first-mentioned type, only one competing process (sensitization) is affected, while the other two (dissipation and fluorescence), profit equally by the elimination of a common competitor.

We will now describe more specifically the several suggested mechanisms of interrelation of fluorescence and photosynthesis beginning with the picture used in Volume 1 (chapter 19).

In scheme 19.III (Vol. I, page 547) we attempted to represent the probable relationship between sensitization and fluorescence of chlorophyll *in vivo*. This scheme was formulated primarily for the interpretation of sensitized *photoxidation*, but essentially similar conditions may be assumed to prevail in photosynthesis as well. The primary process was assumed in chapter 19 to be a "tautomerization" of the complex X.Chl.HZ (formed by association of chlorophyll with oxidant X and reductant HZ):

$$\text{X}\cdot\text{Chl}\cdot\text{HZ} \xrightarrow{h\nu} \text{X}\cdot\text{Chl}^{*}\cdot\text{HZ} \longrightarrow \text{HX}\cdot\text{Chl}\cdot\text{Z} \qquad (24.3)$$

In photosynthesis, this primary process must be followed by secondary, catalytic reactions, in which HX is oxidized back to X (directly or indirectly) by the carbon dioxide-acceptor compound, $\{CO_2\}$*, and Z is reduced back to HZ (directly or indirectly) either by water (in ordinary photosynthesis of green plants) or by reductants such as H_2, H_2S or thiosulfate (in the photosynthesis of purple bacteria).

In this picture, variations in fluorescence can be related to those in photosynthesis in both the above-mentioned ways—by means of primary changes in the probability of sensitized chemical reaction, and by means of primary change in the rate of dissipation of energy in the chlorophyll-bearing complex. If the dissipation rate is constant, fluorescence is an indicator of the efficiency with which the excitation energy of chlorophyll is used for the primary photochemical process (equation 24.3): Whenever the latter process is retarded for one reason or another, the sum of the probabilities of the two competing processes—fluorescence and internal dissipation of the excitation energy—increases correspondingly. Since fluorescence and internal energy dissipation are two alternative monomolecular processes, the yield of both will be changed in the same proportion. Fluorescence thus becomes an index of the yield of the primary photochemical process, even though the absolute yield of fluorescence

* If X = $\{CO_2$, the secondary reaction is the replacement of a reduced by a fresh molecule $\{CO_2\}$.

(<1%) is much too small to make it a significant competitor of this process. For example, if the yield of the primary photochemical process drops from 80 to 40%, and the sum of the yields of dissipation and fluorescence therefore increases from 20 to 60%, the yield of each of these two processes will increase by a factor of 3. If the yield of fluorescence was $\varphi = 0.2\%$ before it will become 0.6% afterward.

The occurrence of antiparallel changes of the yields of fluorescence and photosynthesis was discovered by Kautsky in his investigations of the induction period (for an example, *cf.* fig. 33.19A), and we now know that this type of correlation is quite common, both in induction phenomena and in the steady state. However, the relation between the yields of photosynthesis and fluorescence is not always that of antiparallelism. Sometimes, yield of photosynthesis changes strongly without an appreciable change in yield of fluorescence (*cf.*, for example, fig. 28.24 in which "light saturation" of photosynthesis has no counterpart in the—steadily increasing—intensity of fluorescence). In other cases, *e. g.*, in some types of induction phenomena, photosynthesis and fluorescence both change in the same direction (*cf.* fig. 33.22C). The picture of the mechanism of photosynthesis used above to explain the usual antiparallelism of photosynthesis and fluorescence can, however, be used also to explain how exceptions from this antiparallelism can arise.

In the first place, fluorescence competes only with the *primary* photochemical reaction—*not* with the over-all process of photosynthesis. The rate of photosynthesis, as measured by the liberation of oxygen or consumption of carbon dioxide, often is determined, not (or not only) by the efficiency of the primary photoprocess, but also by the rate of one or several of the associated dark, catalytic reactions. Among these are reactions that convert the primary photoproducts into the stable end products of photosynthesis. When these "finishing" reactions are too slow to keep pace with the primary photochemical process (a situation that may arise, for example, in excessively strong light, or at low temperature, or in the presence of certain poisons), the primary photoproducts will accumulate to a certain extent, but will then disappear by back reactions. The quantum yield of photosynthesis will thus be reduced, but that of fluorescence need not be affected at all, since the primary photochemical process—which alone competes with fluorescence—continues at full speed. This can explain the occurrence of light saturation of photosynthesis without simultaneous increase in the yield of fluorescence (a phenomenon to which we have referred above).

In Volume I (*cf.*, for example, chapter 7) we have considered, in addition to "finishing" dark reactions (which, as just stated, are likely to have no effect on fluorescence at all), also catalytic reactions of "preparatory" character,

such as the binding of carbon dioxide by an "acceptor," to form a compound designated as $\{CO_2\}$. These reactions "prepare" the reactants for their participation in the photochemical reaction proper. If one of the preparatory reactions ceases to keep pace with the primary photochemical process (equation 24.3), the conversion of the primary photoproduct HX·Chl·Z back into the photosensitive form X·Chl·HZ will be retarded. If the supply of the oxidant, $\{CO_2\}$, is too small, while that of the reductant, $\{H_2O\}$ (or of substitute reductants in bacterial metabolism), is ample, the chlorophyll–oxidant–reductant complex may accumulate in the reduced form, HX·Chl·HZ. If the supply of the oxidant is ample, but that of the reductant is limited (a situation which can easily be realized in experiments with bacteria), the complex will accumulate in the reduced form, X·Chl·HZ. Both forms are *stable*, because they cannot be transformed into the photosensitive form X·Chl·HZ by simple back reaction, and *photostable* because they cannot undergo the primary photochemical process (equation 24.3). The accumulation of either of them is likely to enhance fluorescence. It seems, in fact, that all factors (such as CO_2-starvation or cyanide poisoning) which limit severely the carboxylation reaction $CO_2 \rightarrow [CO_2]$ normally increase the yield of fluorescence, and that the same is true of the factors limiting the supply of the reductant (H_2, H_2S or thiosulfate) in purple bacteria.

Considerations of this kind could explain why light saturation of photosynthesis is accompanied by an increase in the yield of fluorescence in some cases (namely, when saturation is caused by the limited velocity of a "preparatory" reaction), and has no effect on the yield of fluorescence in others (namely, when it is due to the limited rate of a "finishing" reaction).

Closer consideration of the picture also could explain why, when changes in the yield of photosynthesis *are* correlated with changes of fluorescence, not only does the absolute extent of the latter vary within wide limits, but sometimes, even the sign of the effect is reversed, the usual antiparallelism being replaced by a parallel increase (or decrease) of the quantum yields of both photosynthesis and fluorescence:

Fluorescence and internal dissipation are affected in the same proportion by a change in the rate of the primary photoprocess only *if the factor that caused this change does not affect the rate constant of internal dissipation.* There is no reason why this should always be true. The *a priori* probability (rate constant) of internal conversion may be quite different in the complexes X·Chl·HZ, HX·Chl·Z, HX·Chl·HZ and X·Chl·Z. If one of the "photostable" complexes, such as HX·Chl·HZ, dissipates the excitation energy much more efficiently than the "photosensitive" complex X·Chl·HZ, the fluorescence-quenching effect of its accumulation may overcompensate the fluorescence-stimulating effect of the suppression

of the primary photochemical process, and the net result will be a simultaneous decline in the yields of both fluorescence and photosynthesis. (In other words, fluorescence, freed of one of its two competitors—the primary photoprocess—will face a stronger second competitor—internal dissipation—and will suffer a net loss.) The energy-dissipating properties of the chlorophyll-containing complexes may differ somewhat in different species and even strains (otherwise, the yield of fluorescence would be exactly the same in all plants); this may explain why the rationing of carbon dioxide (or outright starvation) apparently has a different effect on fluorescence in leaves (investigated by Franck, French and Puck 1941 and McAlister and Myers 1940), purple bacteria (investigated by Wassink, Katz and Dorrestein 1942) and diatoms (investigated by Wassink and Kersten 1944). In the first case, denial of carbon dioxide caused a considerable increase of φ at high light intensities (*cf.* figs. 28.25, page 1048); in the second case, it caused a slight increase of φ at moderate intensities and a decrease at high intensities (fig. 28.30); in the third case, φ declined in strong light in the presence of carbon dioxide, but remained constant in the absence of carbon dioxide (fig. 28.28).

The primary photochemical process can be retarded not only by a deficiency of reactants, *e. g.*, $\{CO_2\}$ or thiosulfate, which are needed to restore the photosensitive complex, but also by *narcotization*, that blankets the complex and prevents its contact with the reactants. In this case, too, one can expect a simultaneous effect on the probability of internal dissipation—this time in the direction of making the dissipation *slower*. Narcotics are therefore likely to enhance fluorescence in a twofold way: by preventing "chemical quenching" by the primary photochemical process, and by exercising a "protective" action of the type discussed in chapter 23, (page 776), *i. e.*, by "wrapping in" the excited molecules, and weakening in this way all energy-dissipating interactions with neighboring molecules.

According to Franck *et al.* (1941,1947,1949), formation of protective "narcotic" layers can be brought about by supplying plants externally with substances such as chloroform or urethan, and also by metabolic reactions—particularly those occurring under anaerobic conditions. Such "internal" narcotization effects are supposed by Franck and co-workers to be responsible for the induction effects after an extended period of darkness (see later in chapter 33), for the inhibition of photosynthesis by anaerobic incubation (*cf.* chapter 13, Vol. I, and chapter 33 in Part 2) and for the "midday depression" (*cf.* chapter 26, page 873). In all these cases, cessation or retardation of photosynthesis is accompanied by enhancement of fluorescence, and the effect on fluorescence often is considerably stronger than could be attributed to the limitation of the primary photochemical process.

In the case of inhibition of photosynthesis by anaerobic incubation in the dark, the "narcotic" appears to be a fermentation product, an acid, since its effect can be destroyed by neutralization. The "long" induction period, and the midday depression, may be due to the accumulation of similar acids. The "short" induction period, on the other hand, must be associated more directly with the intrinsic mechanism of photosynthesis. It has been suggested (by Gaffron and Franck, see chapter 33) that, in this case, a "narcotic" is produced as a result of partial oxidation of a metabolite (sugar?) by the first oxidation product of photosynthesis ("photoperoxide" or, more generally, "oxygen precursor"); according to Gaffron and Franck, a transient accumulation of this product occurs in the first seconds of illumination, because of inactivation in the dark of the enzyme (or enzymes, which we called E_c and E_o in chapter 7), required to convert the oxygen precursor into free oxygen. Franck attributes to similar "internal narcotization" also most, if not all, the fluorescence changes produced by depriving purple bacteria of their specific reductants (H_2, $H_2S_2O_3$, etc.). He sees the reason for the enhancement of fluorescence caused by this treatment, not (or not primarily) in the stoppage of the primary photochemical process by lack of a reactant (and consequent increase in both internal dissipation and fluorescence as suggested on p. 823), but in the accumulation of photoperoxides (which in the normal course of bacterial photosynthesis, are destroyed by the specific reductants), and consequent production and deposition on chlorophyll of surface-active (narcotic) substances. In this way, the rates of both the primary photochemical process and the internal dissipation are reduced, and the chances of fluorescence are correspondingly increased.

The effect on the yield of fluorescence of the deprivation of green plants (or purple bacteria) of *carbon dioxide* (or of cyanide poisoning—both treatments have the same primary result, namely reduction in the supply of $\{CO_2\}$ to the photochemical system) is less pronounced than the effect of the scarcity of the reductants; but, contrary to the belief of Wassink and his co-workers, such an effect undoubtedly occurs (*cf.* chapter 27, p. 940. Here, again, the enhancement of fluorescence could be attributed either to declining rate of the primary photochemical process (and consequent increase in the probability of both internal dissipation and fluorescence); or (as suggested by Franck and co-workers) to the formation of an internal "narcotic" by *photoxidation*, which is known to set in when plants deprived of carbon dioxide are exposed to light. In anaerobic purple bacteria, photoxidation cannot occur and CO_2 has less effect on fluorescence.

Franck sees in the formation of a protective layer of an internal narcotic an important safety device the plants have developed to prevent destructive photochemical reactions from being sensitized by chlorophyll, when

the absence of reactants, or poisoning, prevents the use of absorbed energy for constructive purposes (*i. e.*, for photosynthesis).

While Franck's theory emphasizes the *indirect* mechanism of fluorescence changes associated with photosynthesis, the Dutch group (see Wassink, Katz *et al.* 1938, 1942, 1949) use the simple concept of competition between the primary photochemical process and fluorescence (+ dissipation). Because of the stronger effect on φ, in purple bacteria, of the presence or absence of reductants (as compared to the presence or absence of the oxidant, CO_2) they assume that the primary process involves excited chlorophyll and the (enzymatically "prepared") reductants, but does *not* involve CO_2 (or the $\{CO_2\}$-complex).

There is no doubt that simple competition between fluorescence and photochemical primary process is insufficient to interpret all the experimental evidence. Franck's concept of indirect action, on the other hand, has been successfully applied to a wide range of phenomena and has thus acquired a considerable degree of probability.

We will end this discussion here and leave the more detailed presentation and interpretation of experimental results to chapters 27–33 in part IV, dealing with the kinetics of photosynthesis. Measurement of fluorescence has become a tool in the study of the reaction kinetics of photosynthesis, and it would be inconvenient to separate the discussion of the effects of light intensity, or temperature, or carbon dioxide concentration on the yield of fluorescence, from the discussion of the influence of the same factors on the yield of photosynthesis.

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PART FOUR

KINETICS OF PHOTOSYNTHESIS

INTRODUCTION

The investigation of reaction kinetics, *i. e.*, of the dependence of rate on various external factors, has acquired a greater importance in the study of photosynthesis than in that of other biochemical processes. Faced with the failure of the usual qualitative methods of biochemistry to disentangle the complex mechanism of photosynthesis, many investigators turned to rate measurements, hoping that the number, sequence and character of the partial processes involved in photosynthesis could perhaps be derived from such studies. The results did not quite satisfy expectations, for reasons not difficult to understand. What one measures as the "yield of photosynthesis" is the net result of the operation of a complex mechanism. No simple kinetic equation can account for all the factors that influence this yield. It is comparatively easy, after having made a series of kinetic measurements on a selected object, to invent a model that would interpret these particular observations, or even to write down equations fitting the experimental results more or less closely. The literature on photosynthesis abounds in such formulations. Their limited significance is illustrated by the fact that practically nobody ever uses equations derived by somebody else; instead, every investigator starts anew, often without as much as referring to his predecessors, and—what is even more unfortunate—without making an attempt to correlate his formulae with any experimental results but his own.

Photosynthesis is such a complex and heterogeneous process that it is probably impossible to make a complete analysis of its mechanism merely by measuring the rate of the over-all process under different conditions. However, this does not mean that kinetic measurements of photosynthesis are useless; but rather that they are most useful when combined with other biochemical and biophysical methods of approach, such as the use of poisons and narcotics, provision of substitute reductants and oxidants, or partial tearing down of the photosynthetic apparatus (*e. g.*, by the separation of chloroplasts from cytoplasm). Several investigators have already used measurements of the intensity of chlorophyll fluorescence as an auxiliary physical tool to supplement chemical rate measurements; it may prove possible to make similar use of absorption and fluorescence spectra, of radiochemical and perhaps also electrochemical measurements, to provide "running comment" on the state of the photosynthetic apparatus during

photosynthesis. In such an integrated study, kinetic measurements will remain one of the most important components; but the ambition to obtain, by their means alone, a quantitative interpretation of the whole phenomenon of photosynthesis will be replaced by more limited aims.

Chapter 25

METHODS OF KINETIC MEASUREMENTS*

It is not our purpose to give here a detailed description and appreciation of the experimental methods used in the quantitative study of photosynthesis, but merely to indicate the principles on which these measurements have been (or can be) based.

Photosynthesis by green plants consumes *carbon dioxide*, *water* and *light*; it produces *oxygen*, *carbohydrates* and *chemical energy*. This gives six possible objects of quantitative study. However, one of the reaction components—water—is present so abundantly in living organisms that the determination of its consumption is practically impossible (except, perhaps, by means of isotopic tracers). In the photosynthesis of bacteria and "adapted" algae (*cf.* Vol. I, chapters 5 and 6), on the other hand, the consumption of the reductant (H_2, H_2S, $H_2S_2O_3$ etc.) can be measured as easily as that of the oxidant (CO_2).

1. Material

Since Warburg's fundamental investigations (1919, 1920), unicellular algae, particularly the two species *Chlorella vulgaris* and *Chlorella pyrenoidosa*, have become the favorite objects of quantitative studies, because of the ease with which they can be cultured and handled in the form of suspensions. The methods of culture of *Chlorella* and similar algae cannot be discussed here; reference must be made to special literature such as Küster (1921), Pringsheim (1924, 1926), Pearsall and co-workers (1937, 1940) and Myers *et al.* (1944, 1946). One observation, however, must be mentioned: It has been found (Pratt and co-workers 1940 to 1945) that growing *Chlorella* cultures produces a substance that acts as an inhibitor of further growth (and incidentally also as an inhibitor of photosynthesis; *cf.* chapter 26, page 880).

Chlorella suspensions have provided the material for the studies by Warburg and his school, and by Noddack and co-workers in Germany, as well as by Emerson, Franck, Gaffron, Daniels, Manning and their co-workers in America. The algae used are green, roughly spherical, unicellular organisms, about 5 μ in diameter, containing a single, bell-shaped chloro-

* Bibliography, page 855.

TABLE 25.I
CHARACTERISTICS OF TWO *Chlorella pyrenoidosa* SUSPENSIONS
(AFTER NODDACK AND EICHHOFF 1939)

Type	Cells/ml.	Dry weight, g./ml.	Chlorophyll: % of dry wt.	Chlorophyll: molecules/cell	$P_{max.}$, cc. O_2/min.[a]: per cc. of cells	$P_{max.}$, cc. O_2/min.[a]: per g. Chl[b]
"Shade"	1.92×10^6	4.6×10^{-5}	4.9	8×10^8	4.9×10^{-5}	22
"Light"	3.84×10^6	5.1×10^{-5}	2.7	2.5×10^8	5.4×10^{-5}	39

[a] Maximum photosynthesis, as obtained with an illumination of 30,000 lux (white light), in a carbonate–bicarbonate buffer solution.
[b] "Assimilation number."

plast, which almost encloses the central part of the cell with the nucleus. Their chlorophyll content is remarkably high—from 2.5 to 5% of the dry weight, the higher concentrations occurring in cells grown in weak light. Table 25.I shows, as an example, the characteristics of two *Chlorella pyrenoidosa* suspensions used by Noddack and Eichhoff—one grown in weak light and the other in strong light. According to the table, the "shade cells" are two times larger than the "light cells," and contain almost twice as much chlorophyll (in per cent of dry weight), which means three to four times more chlorophyll molecules per cell. Despite their lower chlorophyll content, the light cells can produce as much oxygen per milliliter of cell material as the shade cells, so that their oxygen production per unit weight of chlorophyll (the "assimilation number," *cf.* chapter 28) is about twice as large as that of shade cells.

Sargent (1940) gave 3.3% and 6.6% of dry weight as the chlorophyll contents of light cells and shade cells of *Chlorella*, respectively. Myers' results (1946[1]) agree with those of Noddack and Eichhoff and of Sargent in showing a higher *concentration* of chlorophyll in cells grown in weak light, but deviate from those of Noddack and Eichoff in so far as *amount* of the green pigment in a single cell is concerned. In Myers' cultures, the average volume of cells *increased* rather than decreased with increasing light intensity of cultivation (from 28×10^9 cells per milliliter at 6 foot candles to 6×10^9 cells per milliliter at 360 foot candles). The growth in cell *volume* almost exactly balanced the decrease in cellular pigment *concentration*, so that the total number of chlorophyll molecules per cell remained practically constant, independently of the intensity of illumination.

The maximum capacity for photosynthesis of Myers' cultures (related to one milliliter of cell material) showed a sharp increase with increasing intensity of illumination during cultivation, between 5 and 25 foot candles, remained constant between 25 and 60 foot candles and then declined. Since at the same time the concentration of chlorophyll declined steadily, the "assimilation number" (maximum photosynthesis per gram chloro-

phyll) increased rapidly at first—in agreement with Noddack and Eichhoff's observations—and then approached constancy (*cf.* chapter 31 for more detailed discussion of the relation of the rate of photosynthesis to chlorophyll content).

Suspensions like those in Table 25.I, placed in vessels 1–3 cm. deep, absorb 20–50% of incident white light. If complete absorption of visible light is desired (as in Warburg and Negelein's procedure), suspensions containing more than 10^7 cells per milliliter must be used in vessels of the same depth.

The photosynthetic efficiency of *Chlorella* suspensions has been related to many factors, such as the intensity of light (Warburg) and the rate of

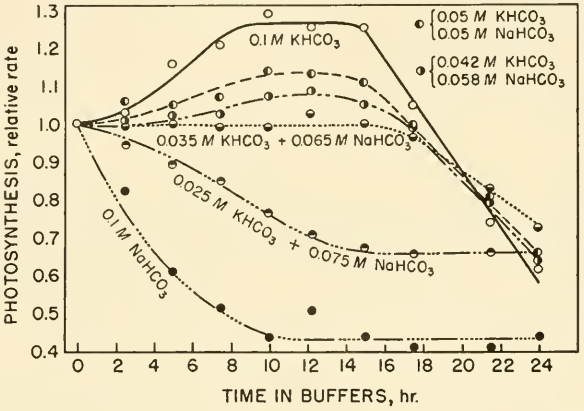

Fig. 25.1. Relative rate of photosynthesis in *Chlorella vulgaris* as a function of the length of exposure of the cells to 0.1 *M* solutions of $KHCO_3$, $NaHCO_3$, or varying mixtures of the two bicarbonates (after Pratt 1943).

gas supply during growth (Burk), or the "oligodynamic" effects of small quantities of rare elements (*cf.* Emerson and Lewis 1939). However, some investigations on the quantum yield of photosynthesis (*cf.* chapter 29) indicate that these conditions may influence mainly the gas exchange in the first few minutes of exposure to light, and have little effect on the steady rate of photosynthesis (*cf.* Table 25.I).

According to Emerson and Green (1938) the photosynthesis in *Chlorella* is insensitive to wide variations in *p*H (Vol. I, p. 339); consequently, these algae can be used in acid as well as in alkaline solutions (Concerning the difference between quantum yields of *Chlorella* in acid and alkaline buffers, see pp. 1096, 1107.) Pratt (1943[2]) observed that the rate of photosynthesis of *Chlorella* in strong light *declined* to 40% of its initial value after nine hours in a 0.1 *M* $NaHCO_3$ solution and then remained

steady; while, in a 0.1 *M* $KHCO_3$ solution, there resulted an *increase* in rate by about 30% within six hours, followed by several hours of steady oxygen production and then a rapid decline. In a mixture of 0.035 *M* $KHCO_3$ and 0.065 *M* $NaHCO_3$, the rate remained unchanged for about fifteen hours, after which the stimulating effect of the potassium salt apparently wore off, and the rate declined rapidly (fig. 25.1). In all these experiments, the cells spent about one half of the total time in light and one half in darkness.

Among other algae used in quantitative photosynthetic studies were the *Chlorophyceae*: *Scenedesmus* (*cf.*, for example, Gaffron 1942), *Hormidium flaccidum* (van der Honert 1930 and van der Paauw 1932), *Stichococcus bacillaris* (Aufdemgarten 1939); the *Rhodophycea*, *Gigartina harveyana* (Emerson and Green 1934); the *Cyanophycea*, *Chroococcus* (Emerson and Lewis 1942); the brown alga, *Fucus serratus* (Steemann-Nielsen 1942); and the diatoms, *Nitzschia closterium* (Barker 1935, Dutton and Manning 1941), *N. dissipata* (Wassink, Kersten 1945), *Navicula minima* (Tanada 1951).

Advantages of algae as material for photosynthetic measurements (*e. g.*, the convenient use of manometric methods) are shared to some extent by the *higher aquatic plants*. Among them, *Elodea* has been the most popular in photosynthetic studies, because of its widespread occurrence in stagnant waters. Other aquatic plants used in photosynthetic work were *Cabomba caroliniana* (Smith 1937) and *Potomageton* (Gessner 1937).

Detached leaves provided the material for most of the earlier investigations of photosynthesis; they were used by Blackman and co-workers, by Brown and Escombe (1905) and by Willstätter and Stoll (1918) in their pioneer investigations of the quantitative aspects of photosynthesis. Because of the interruption of natural translocation processes, the time course of photosynthesis in detached leaves may differ from that in similar leaves attached to the stem (Vol. I, p. 332).

Whole land plants, enclosed in glass vessels, were long used in investigations of the rate of photosynthesis under field conditions. A group of workers at the Smithsonian Institution in Washington (*cf.* Hoover, Johnston and Brackett 1933 and McAlister 1937) showed that this method can give results equivalent, as to precision and consistency, to those derived from experiments with algae. The material used in their studies were single young wheat plants.

The culture of *purple bacteria* that can be used for studies of bacterial photosynthesis has been described by van Niel and co-workers (1931, 1944), French (1937), Gaffron (1933–1935) and the Dutch group (Eymers, Wassink, Katz, Dorrestein *et al.* 1938, 1942); the bacteria used included *Rhodospirillum rubrum*, *Streptococcus varians*, and strains of *Chromatium* and *Rhodovibrio*.

2. Light Measurements

In many older investigations, the influence of light intensity on photosynthesis was studied by illuminating the plants with "white light" (of the sun, or of an incandescent lamp) and introducing gray filters, or altering the distance between light source and plant. The intensity was then given in relative units (*e. g.*, "$^1/_{10}$ of full sunlight"; or "lamp at 30 cm. distance"). Other observers determined the intensity of illumination by visual comparison with a standard light source, and expressed it in *meter candles* (also called *lux*, or lumen per square meter) or *foot candles* (1 foot candle = 10.764 meter candles). These figures cannot be used for the calculation of the incident *energy*, unless the spectral distribution is known. A knowledge of the so-called "color temperature" of the light source (the temperature that a black body must have to produce radiation of the same color) provides some helpful information. However, no light source is a black body; and, even if it were, the spectral distribution of the light it supplies is modified by passage through air, glass or other material media. The figures given below can therefore be used only for approximate calculations.

The maximum illumination from direct *sunlight* at noon in summer (at sea level at the latitude 42° N) is about 85,000 lux* (8000 foot candles), its color temperature (*cf.* Taylor and Kerr 1941), about 5400° K. Under these conditions, the average light flux is 2.0×10^{-2} cal. or 8.5×10^5 erg/(cm.^{2} sec.) (including the infrared) or 10 erg/(cm.^{2} sec.lux). The diffuse light from the clear blue sky increases the illumination by about 21,000 lux (2000 foot candles). The *sky light* has a much higher color temperature than the sunlight—over 10,000° K. for clear blue sky, decreasing to 7000° K. for hazy or overcast sky (see fig. 22.52). At sea level, when the sun is in the zenith, about 40% of the incident radiation belongs to the spectral region below 700 mμ (photosynthetically active radiation) and about 60% to the region above 700 mμ (photosynthetically inactive, extreme red and infrared radiation). Thus, in sunlight, one lux is equivalent to 4 erg/(cm.^{2} sec.) of photosynthetically active light.

The characteristics of *incandescent lamps* (gas-filled tungsten filament lamps) in table 25.II are taken from Hardy and Perrin's *The Principles of Optics* (1932).

About 30% of the lamp light below 760 mμ belongs to the region 700–760 mμ, which is scarcely used at all by green plants, so that the proportion of photosynthetically active energy is only 7–8% in medium power lamps (100–200 watts) and 9–10% in high power lamps (500–1000 watts). In other words, an illumination of one lux from a 100 watt lamp corresponds to about 5.0 erg/(cm.^{2} sec.) and the same illumination from a 500 watt lamp, to about 4.5 erg./(cm.^{2} sec.) of photosynthetically active light.

* We will use the abbreviation klux for 1000 lux.

TABLE 25.II
CHARACTERISTICS OF INCANDESCENT LAMPS

Watts	Color temp., °K.	Erg cm.² sec. lux (including infrared)	Fraction of energy Below 760 mμ	Fraction of energy Above 760 mμ
100	2740	72	0.100	0.900
200	2810	66	0.111	0.898
500	2920	55	0.122	0.878
1000	2980	50	0.135	0.865

White fluorescent lamps emit, as visible light, ~18.5% of energy input. Their brightness corresponds to 6–10 lux/sq. cm. Their spectral distribution peaks are at 480 mμ ("blue-white"), 490 and 585 mμ ("standard cool white"), 585 mμ ("standard warm white"), 520 and 640 mμ ("deluxe cool white") and 530 and 620 mμ ("de luxe warm white"); *cf.* Barr (1950). The two "standard whites" emit little energy > 650 mμ; the two "warm whites," little energy <500 mμ.

The quanta of visible light are from 5×10^{-12} to 2.5×10^{-12} erg each. In sunlight, the intensity maximum lies at about 575 mμ, corresponding to $\overline{h\nu} = 3.5 \times 10^{-12}$ erg. Thus an illumination of 1 lux from the sun corresponds to 1.2×10^{12} quanta or 2.0×10^{-12} einstein/(cm.² sec.) of photosynthetically active light. In artificial light, $\bar{\lambda}$ is much nearer the red end of the spectrum. If one assumes, for this light, $\overline{h\nu} = 3.2 \times 10^{-12}$ erg, 1 lux becomes equivalent to 1.4×10^{12} quanta or 2.3×10^{-12} einstein/(cm.² sec.) of photosynthetically active light.

In direct sunlight at noon, about 10^{17} visible quanta impinge per second on one square centimeter of horizontal surface. A pigment molecule situated on this surface, whose average molar absorption coefficient for visible light is of the order of 3×10^4, will absorb about twelve quanta every second.

The frequency of absorption acts is determined, for a molecule with the molar absorption coefficient α, by the equation:

$$n = 4 \times 10^{-21} \alpha N_{h\nu} \tag{25.1}$$

where $N_{h\nu}$ is the light flux in quanta/(sec. cm.²).

Instead of calculating, as above, the energy flux and the number of quanta impinging on the illuminated surface from the intensity of illumination in lux, it is of course much better to measure this flux directly, by means of a thermoelement, bolometer, photocell or actinometer. This is also the only way to define the intensity of *colored* light, which cannot be measured in lux. The energy flux can be expressed in ergs or calories (per unit surface and unit time) or watts (per unit surface). The relation be-

tween these units is shown in Table 25.III. However, without information as to spectral composition, the indication of the energy flux in ergs, or calories, is even less revealing than that of the intensity of illumination in lux, because 60% of direct sunlight and about 95% of the energy flux from incandescent lamps belong to the far red and infrared, and are not used by plants for photosynthesis. Unless the proportion of these radiations is known, quoting the energy flux may easily give an entirely erroneous concept of the quantity of light available for photosynthesis.

TABLE 25.III
ENERGY FLUX UNITS*

Units	Watt/cm.^{2}	Erg/(cm.^{2} sec.)	Cal/(cm.^{2} sec.)
Watt/cm.^{2}	1	10^7	0.239
Erg/(cm.^{2} sec.)	10^{-7}	1	2.39×10^{-8}
Cal./(cm.^{2} sec.)	4.19	4.19×10^7	1

Of the common photometric devices, only thermoelements and bolometers react uniformly to radiations of all wave lengths. All other instruments—vacuum photocells, barrier layer cells, actinometers—possess a selective spectral sensitivity. Some investigators suggested that instruments insensitive to infrared light, *e. g.*, selenium barrier layer cells ("photronic cells"), should be used in preference to thermopiles or bolometers in the measurement of light intensities in the work on photosynthesis, in order to avoid measuring infrared together with visible light. However, this is almost equivalent to a return to visual photometry, since the eye, too, can be described as an infrared-insensitive photometer. In fact, the sensitivity curve of the selenium barrier layer cell is quite similar to that of the human eye. Both drop rapidly above 600 mμ—right in the middle of the main red absorption band of chlorophyll (*cf.* fig. 25.2A). Vacuum type photocells also show strong variations in sensitivity with wave length (fig. 25.2B). No photocells, actinometers or photographic plates possess uniform sensitivity throughout the visible spectrum (although some of them are reasonably constant in the region of the shorter waves). Therefore, no instruments of these types can give reliable photometric data in nonmonochromatic light, (*cf.* Mestre 1935).

Warburg and Schocken (1949) have developed an actinometer based on Gaffron's earlier observations in Warburg's laboratory of sensitized autoxidation of allyl thiourea (chapter 18, page 509). Thiourea was substituted for allyl thiourea as oxidation substrate, and pyridine for acetone as solvent; it was found that, with ethyl chlorophyllide (or protoporphyrin) as sensitizer, a quantum yield equal to 1.0 ± 0.1 (molecules oxygen con-

* We will use the abbreviation kerg for 1000 erg.

sumed per quantum absorbed) can be obtained over a considerable range of wave lengths and intensities. The convenience of this actinometer is the possibility of using it in conjunction with Warburg reaction vessels in a manometric system. Assuming equal absorption of light in the reaction

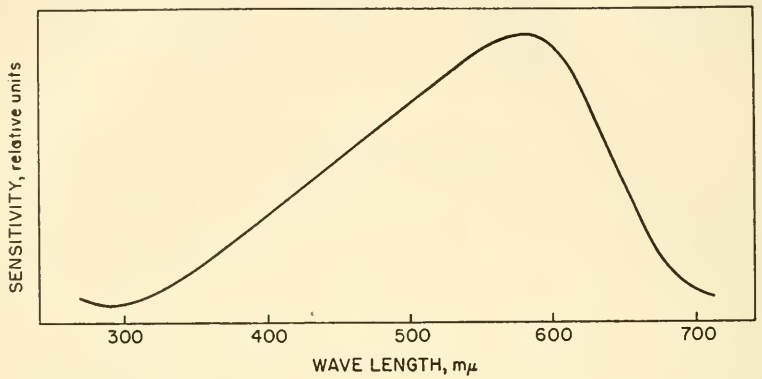

Fig. 25.2A. Spectral sensitivity curve of a selenium barrier layer cell (Weston Photronic Cell).

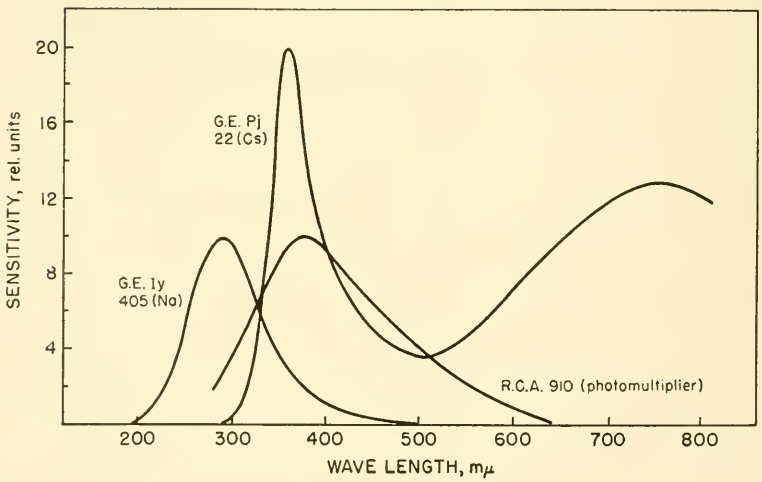

Fig. 25.2B. Spectral sensitivity curves of three typical vacuum-type photocells.

vessel and in the actinometer vessel, and arranging for identical light fluxes to reach both vessels (*e. g.*, by placing them side-by-side in a uniform light field, or by alternating the reaction vessel and the actinometer vessel in

the same position), quantum yield determinations can be made simply by comparing the pressure changes in the two vessels. *Average* quantum yields can be determined in this way for prolonged periods of illumination, or for illumination with diffuse light, more easily than with instruments which measure *momentary* light intensity, and require a collimated beam. This actinometer promises to become a very useful tool in the study of photosynthesis; but despite its convenience it, too, must be used with caution, and checked from time to time against a physical light-measuring instrument such as a thermopile or bolometer. The nature and mechanism of the reaction in the Warburg-Gaffron-Shocken actinometer is unknown, and the exact dependence of its rate on light intensity, wave length, nature of the solvent, presence of impurities, oxygen pressure, concentrations of the reductant and the sensitizer, and rate of stirring, remain to be investigated. Available measurements indicate that the quantum yield declines slowly with increasing illumination in the "middle range" ($3–7 \times 10^{-10}$ einstein/(sec. cm.^{2})), but changes faster both at the higher light intensities ($>10 \times 10^{-10}$ einstein/(sec. cm.^{2})) and at very low light intensities ($<3 \times 10^{-10}$ einstein/(sec. cm.^{2})). It remains to be seen, however, whether the factor determining the change is *intensity* of the beam—*i. e.*, energy per unit cross section—or its *energy* (more probably, energy absorbed in unit volume of the liquid). Some decline in quantum yield with increasing illumination may be caused by exhaustion of oxygen in the illuminated layer; however, this is likely to account only for a part of the observed trend. Another possible cause of this decline is competition of back reactions between the intermediate oxidation and reduction products, with the "forward" reaction which leads to the consumption of oxygen. If they are bimolecular in respect to the intermediates, the back reactions must be favored by a higher concentration of the latter and therefore can become more effective at light intensity increases.

It must be recalled that photometers with selective spectral sensitivity cannot be relied upon not only in the determination of *absolute* light intensities, but also in the comparison of two light sources or in the determination of the proportion of light absorbed by passage through a colored system. Unless the absorption is very weak throughout the spectrum, the spectral composition of the transmitted light will be different from that of incident light, and the selectively sensitive instrument will react differently to these two fluxes. It will tend to exaggerate the absorption if the latter takes place in the region of maximum sensitivity, and underestimate it if it occurs in the region of low sensitivity. The steeper the spectral sensitivity curve, the larger will be the errors that occur in absorption measurements in nonmonochromatic light. Selenium barrier layer cells, for example, cannot be used for such measurements even in "monochro-

matic" red light, isolated by filters (or monochromators with wide slits), since their sensitivity drops by a factor of 10 between 600 and 700 mμ (fig. 25.2A).

For all these reasons, if white light is used for photosynthetic work, the best way of characterizing its intensity is to measure it by means of a thermopile protected from infrared light by a suitable filter. This will give an adequate picture of the quantity of light available for photosynthesis, and enable one to determine correctly the proportion of this light absorbed by the plants.

The desire for greater sensitivity often will force the investigator to use a photoelectric cell, instead of a thermopile, despite all the shortcomings associated with its selective sensitivity; this should be done only in full realization of the errors that can be introduced in this way. Only in work with truly monochromatic light are the photocells entirely reliable (assuming that the linearity of their response has been ascertained by frequent comparison with a thermoelement).

In addition to the problem of a reliable photometric instrument, difficulty arises in the determination of the quantity of light absorbed by leaves, algae or cell suspensions, because of the scattering phenomena discussed in chapter 22.

The scattering by cell suspensions is comparatively weak, and that by leaves can be reduced by injection with water, or—still better—with glycerol (by evacuation under the liquid), thus eliminating the most effective source of scattering—the liquid-air interfaces (*cf.* fig. 22.8 and 9). However, figure 22.2 shows that, even in *Chlorella* suspensions, enough scattering is present to cause a marked error in the determination of absorbed light energy—an error of only a few per cent in the region of strong absorption, but of 100% or more in green or in the far red, where true absorption is very weak.

In measurements in these spectral regions, as well as in precision experiments in other parts of the spectrum, it is necessary to measure the total scattered flux (*i. e.*, the diffusely transmitted and diffusely reflected fluxes, T_d and R_d, together with directly transmitted and specularly reflected fluxes T_s and R_s) and to use the complete formula:

$$A = I_0 - T_s - T_d - R_s - R_d = I_0 - S \tag{25.2}$$

for the determination of the absorbed light energy, A.

The neglect of both T_d and R_d in the determination of A must lead to entirely erroneous results, because, for all leaves and thalli, T_s is only a fraction—and often a small one—of the total transmitted flux.

The fact that optical work with leaves requires a consideration of scattering was clear to Maquenne (1860) and Simmler (1862); but some investigators—not only botanists like Sachs (1864) and Detlefsen (1888), but

even physicists like Vierordt (1871) and Lazarev (1924, 1927)—thought that they could neglect it. In most measurements, however, an attempt was made to include at least the diffusely transmitted light, T_d, by the simple device of placing a large collecting surface immediately behind the absorbing system. Seybold (1932) pointed out that this procedure brings the risk of measuring the thermal radiation of the tissue together with the transmitted flux. (A similar error could be caused by fluorescence, but the latter usually can be neglected.) To avoid errors, one may interpose an infrared-absorbing filter between the leaf and the collecting thermopile.

The measurement of the diffusely reflected flux R_d requires more elaborate devices and has often been omitted. The resulting error in the determination of the absorbed intensity can be considerable, since leaves of land plants reflect about as much, or more, light as they transmit—namely, from 10 to 15% of (infrared-free) white light (*cf.* page 683). Submerged algae or water-filled leaves have a lower reflectance—they transmit about 20% and reflect from 5 to 10% of white light. However, diffuse reflection cannot be entirely neglected even when working with algal suspensions, as shown by the results of Noddack and Eichhoff (1939) in figure 22.2. The sharp reflection peak at 180° is due to the walls of the vessel; but, in addition to this specular reflection, the figure shows a small, but not negligible, *diffuse* reflection; integrated over all angles, it adds 3 or 5% to the transmitted flux and reduces correspondingly the absorbed energy, A.

In work with cell suspensions in spherical or cylindrical vessels, the distinction between reflected and transmitted light becomes irrelevant, and an integral measurement of light scattered in all directions, S, can be substituted for the separate measurements of R and T. A small vessel containing the suspension can be placed inside an "integrating" box or cell, or in the focal point of a mirror, illuminated by a narrow beam of light entering through a hole in the mirror, and the light scattered in all directions can be collected and measured. For the determination of I, a "white" scatterer can be substituted for the suspension cell. A device of this type was Noddack and Eichhoff's (1939) "ellipsoid photometer," in which the light scattered by a small cell was collected on a thermopile sensitive to light falling from all directions. The scatterer was placed at one focus of an ellipsoidal mirror, and the collector at the other.

In speaking of the methods of determining the light energy absorbed by cell suspensions, we must also mention Warburg and Negelein's method of total absorption (1922, 1923). These authors used a very concentrated *Chlorella* suspension; the vessel had a silvered back wall so that no light was transmitted; and the absence of diffusely reflected light was ascertained by experiment. (The correctness of this last assertion was questioned by Mestre 1935, and this criticism is supported by the above-mentioned results of Noddack and Eichhoff.) Thus, Warburg and Negelein assumed, simply, $A = I$

It was mentioned on page 673 that, in working with solutions, the necessity of estimating R is usually avoided by using a blank cell, whose reflection is assumed to be equal to that of the solution cell. Many authors have hoped to get around the necessity of measuring R_d for leaves or thalli in a similar way, by using as "blanks" plant tissues deprived of pigments. This idea has been carried out in different ways: Reinke (1886) used algal thalli from which the pigment had been extracted by alcohol; Linsbauer (1901), Brown and Escombe (1905), Seybold (1932, 1933[1,2]) and Meyer (1939) compared the transmission by green parts with that by white parts of variegated leaves; Wurmser (1921) determined the transmission of thalli before and after bleaching by prolonged illumination. However, the interpretation of results obtained in this way presents considerable difficulties. It has already been said (page 673) that equation (22.2b) is only an approximation, although a satisfactory one, even in the work with transparent media. Weigert (1911) thought that it could also be used, as such, for leaves, and applied it to the data of Brown and Escombe; but his calculation led to absurdly low values of A, and its fallacy has been pointed out by Willstätter and Stoll (1918) and Warburg (1925).

In all precision experiments on light absorption by plants, measurements of the *three* quantities I, T and R cannot be avoided. The determination of T and R can be carried out either by means of *integrating* devices that collect the reflected and the transmitted light, or by differential "goniophotometric" methods, *i. e.*, by determining scattering as a function of the angle between the incident and the scattered beam.

3. Measurements of Oxygen Evolution

Oxygen produced in photosynthesis can be identified and measured by different chemical or physicochemical methods, either in the liquid phase containing the aquatic plants, or in the gas phase. Because of the low solubility of oxygen in water, methods of the first kind (*e. g.*, the potentiometric determination of the oxygen concentration in solution) are suitable only for the measurement of small effects, *e. g.*, for the observation of the photosynthetic activity in the first minutes of illumination (*cf.* chapter 33).

We cannot deal here with the analytical technique of the determination of oxygen. *White phosphorus, organic oxygen absorbers* (such as pyrogallol or the leuco dyes), *copper, sodium hyposulfite* and *chromous chloride* are among the reagents used for this purpose. For solutions, Winkler's method is perhaps the most popular one; it utilizes oxygen (through the intermediary of the system manganous chloride–manganic chloride) for the liberation of an equivalent quantity of chlorine, which can easily be de-

termined by titration with potassium iodide and thiosulfate. If pyrogallol or the leuco dyes (indigo white, leuco methylene blue) are used for oxygen determination, the progress of oxygen liberation can be followed colorimetrically or spectrophotometrically. The same methods are applicable to the conversion of hemoglobin into oxyhemoglobin—a method of oxygen determination first introduced into photosynthetic studies by Hoppe-Seyler (1879) and used more recently by Hill (1937, 1939). Osterhout (1918) suggested the use of hemocyanine-containing crab blood (which becomes blue in the presence of oxygen) for the same purpose.

Two *physiological* methods of oxygen detection were discovered by Beijerinck (1901) and Engelmann (1881, 1886 and 1894), respectively. Beijerinck's method utilizes the bioluminescence of certain bacteria (*e. g., Micrococcus phosphorens*), which becomes visible in the presence of extremely small quantities of oxygen (5×10^{-3} mm. O_2 pressure above the liquid, or 1×10^{-8} m./l. in water, according to Harvey and Morrison 1923). Engelmann's method utilizes motile bacteria (*e. g., Proteus vulgaris*), which come to rest in oxygen-free medium, but begin to move about in the presence of traces of oxygen. This method has been sharply criticized by Pringsheim (1886); for an answer to this criticism, see Engelmann (1887). With the help of motile bacteria, the photosynthetic activity of a single cell can be observed under the microscope. Luminescent bacteria have been used, *e. g.*, in attempts to decide whether single isolated chloroplasts liberate oxygen in light (*cf.* Vol. I, chapter 4, page 62).

The chemical and biochemical methods are difficult to adapt to a continuous control of the rate of photosynthesis. Physicochemical methods therefore early attracted the interest of workers in this field. In modern quantitative studies of metabolic processes, *manometric* measurements have acquired a predominant importance; biochemists have found that almost every biochemical reaction can be conducted so as to cause absorption or liberation of a gas and this often provides the best means of measuring its rate. The reactions of hemoglobin with oxygen and carbon monoxide were the first for which this method was developed by Haldane and Barcroft; applications to respiration and photosynthesis came next. Since the time of Sachs (1864), a crude method of measuring the volume of liberated oxygen was known and widely used—"bubble counting." In quiet solutions of a given surface tension, the gas bubbles detaching themselves from the leaves have an approximately uniform size, so that the rate of gas formation can be calculated by multiplication of the number of bubbles formed per unit time by the volume of a single bubble. This method is simple and sensitive, but obviously fraught with errors, caused by the differences in wettability of leaf surfaces, coalescence of small

bubbles to larger ones, effect of convection currents or stirring on the size of bubbles and similar complications. A number of authors (Kohl 1897 Kniep 1915, Wilmott 1921, Bose 1924, Arnold 1931, among others) have

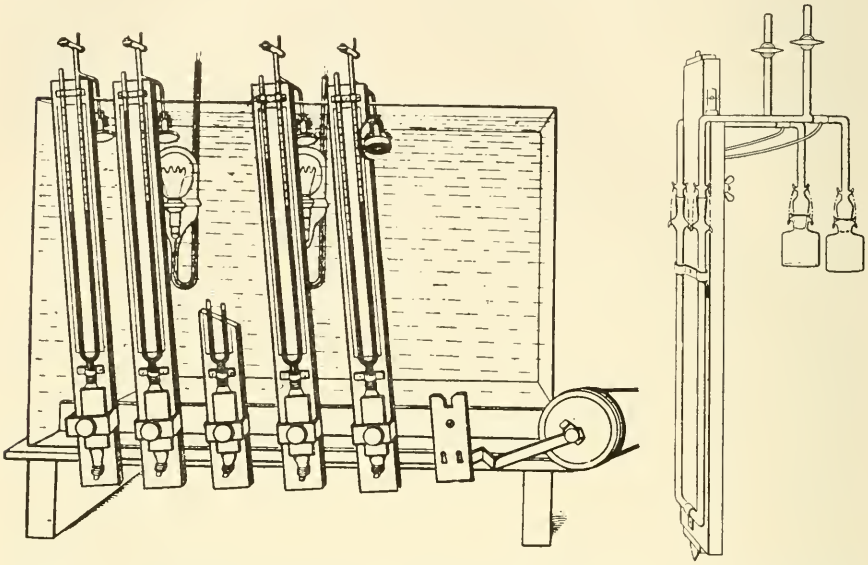

Fig. 25.3A. "Warburg apparatus" for manometric measurement of photosynthesis.

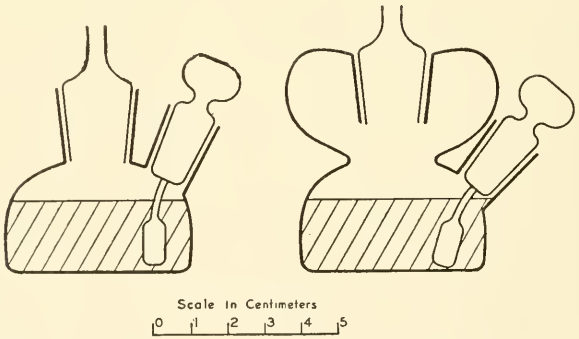

Fig. 25.3B. Two "Warburg vessels" with equal liquid volume but different gas volume (after Emerson and Lewis 1941).

strived to improve this method and to make the bubble counting automatic; a discussion of these attempts can be found in Spoehr's book (1926, page 231). An important objection has been raised by Gessner (1937): Constant size bubbles can only be formed in quiet water, where a photosynthe-

sizing plant rapidly becomes surrounded by a layer of water that is alkaline, deficient in carbonic acid and supersaturated with oxygen—three factors each of which may strongly affect the rate of photosynthesis.

Perhaps the most serious source of error is the fact, studied by Kniep and Minder (1909), that the gas bubbles emerging from cut stems of water plants contain not pure oxygen produced by photosynthesis but a mixture of oxygen with air from intercellular spaces. The nitrogen content of the bubbles varies with the rate of bubbling. As the air is drawn from the intercellular spaces by the stream of photosynthetic oxygen, new air diffuses into them from the medium, so that even after an extended period of illumination the bubbles still contain some nitrogen.

The precise manometric method was introduced into the investigation of photosynthesis by Warburg in 1919 (see figure 25.3A). Since one mole of carbon dioxide is consumed for each mole of oxygen liberated, the manometric method can only be used if the carbon dioxide consumption is completely or partially eliminated as a source of pressure changes. This can be achieved by the use of a large volume of liquid in contact with a small volume of gas, or by means of an alkaline buffer. In the first case, the comparatively high solubility of carbon dioxide in water enables the plants to use dissolved gas for a considerable time while only slowly reducing the pressure of carbon dioxide in the gas phase. The use of buffers enhances this stabilizing effect of the aqueous phase, though at the cost of introducing a "nonphysiological" *p*H.

In the application of manometric technique to *respiration*, practically complete elimination of carbon dioxide as source of pressure changes could be achieved by binding this gas chemically outside the cell suspension. For this purpose, the reaction vessels were provided with a central well (or side tube) containing alkali. A similar procedure cannot be applied to photosynthesis because the latter requires the presence of carbon dioxide in the suspension. The alternative of eliminating *oxygen* from the gas phase (for example, by placing white phosphorus, or pyrogallol, in the side tube) and measuring pressure changes due to carbon dioxide alone, also does not recommend itself, because the rate of photosynthesis is sensitive to oxygen pressure and often is strongly reduced by anaerobic conditions (*cf.* chapter 13, page 326). Furthermore, elimination of oxygen makes the application of the respiration correction difficult if not impossible since in the dark, respiration is totally suppressed, whereas, in light, some oxygen produced by photosynthesis is drawn into the respiration cycle before reaching the external absorber.

We are thus left with the choice of using cells suspended in a carbonate buffer, thus working at a high "unphysiological" *p*H, or employing an acid solution with an unphysiologically high carbon dioxide content (*e. g.*, phos-

phate buffer equilibrated with air containing 1–5% CO_2; without this high initial concentration, the carbon dioxide supply in the carbonate-free solution will be rapidly exhausted in light).

From the point of view of interpretation of manometric data, alkaline-buffered solutions are more convenient than acid solutions, since oxygen alone is responsible for all the pressure changes observed above these solutions.

Ordinarily, mixtures of 0.1 molar solutions of sodium bicarbonate and potassium carbonate are employed (Table 8.V). Using one sodium and one potassium salt seems to be somewhat preferable to using two sodium or two potassium salts (*cf.* page 835). A mixture of 85 parts bicarbonate and 15 parts carbonate at 20° C. is in equilibrium with free carbon dioxide at a partial pressure of about 1.9 mm. (*cf.* Tables 8.II and 8.V; it has to be taken into account that the solubility of CO_2 in 0.1 molar salt solution is ~10% lower than in distilled water). Ten cubic millimeters of carbon dioxide can be withdrawn from 1 cc. of this mixture without appreciably altering the partial pressure of CO_2 in the gas. Mixtures containing more carbonate and less bicarbonate have higher buffering capacities, and lower concentrations of carbonic acid.

As mentioned before, the drawback of carbonate buffers is their high *p*H (about *p*H 9 for the above-mentioned buffer No. 9). Some algae are damaged by short exposure to even less alkaline buffer mixtures (*p*H 8.5). *Chlorella pyrenoidosa*, on the other hand, can be kept even in the more alkaline carbonate mixtures for many hours without signs of damage to the photosynthetic apparatus. Respiration in carbonate mixtures is somewhat slower than respiration in neutral or acid media. Despite absence of visible damage, the maximum quantum yield of *Chlorella* photosynthesis seems to be somewhat lower in alkaline carbonate than in acid (phosphate) buffers; whether the reduction is <20% (Rieke, Emerson and co-workers) or as high as 50% (as suggested by Warburg) is a matter of controversy (*cf.* chapter 29, pages 1096 and 1107).

When it is definitely desirable to avoid alkalinity, measurements of photosynthesis usually are made in slightly acid medium (*e. g.*, *p*H $\simeq$ 5, obtained with KH_2PO_4), saturating this medium with air enriched with from 1 to 5% carbon dioxide. Both oxygen and carbon dioxide are exchanged with the gas phase above these solutions, and the observed pressure changes must be apportioned to the two gases by means of a rather delicate calculation.

For each individual "Warburg vessel," filled to a certain mark with water, a "vessel constant," K_{O_2}, can be determined, giving the increase in pressure caused by the production in this vessel of one cubic centimeter of oxygen; a similar constant, K_{CO_2}, can be calculated for carbon dioxide.

(If the liquid volume is sufficiently large compared with the gas volume, K_{CO_2} will be much smaller than K_{O_2}.)

The knowledge of these constants and assumption of a photosynthetic quotient, $Q_P = \Delta O_2/-\Delta CO_2$ (compare chapter 3, Vol. I), permit one to calculate the rate of photosynthesis from manometric readings in a single vessel. If doubts arise as to the value of the photosynthetic quotient, the latter can be treated as a second unknown, and an additional equation for its determination can be obtained by using a second Warburg vessel of different dimensions (*e. g.*, the same vessel filled to a different level, or a vessel with equal liquid volume but an enlarged gas volume; *cf.* fig. 25.3B). The pressure increase in the first vessel is:

$$\Delta p' = K'_{O_2}\Delta O_2 + K'_{CO_2}\Delta CO_2 = \Delta O_2 \left(K'_{O_2} - \frac{K'_{CO_2}}{Q_P}\right) \tag{25.3a}$$

and that in the second vessel:

$$\Delta p'' = \Delta O_2 \left(K''_{O_2} - \frac{K''_{CO_2}}{Q_P}\right) \tag{25.3b}$$

If the K values are known, the two equations permit the calculation of the two unknowns, ΔO_2 and Q_P (Warburg).

Of course, this purely manometric determination of the rate of photosynthesis is only reliable if it is definitely known that no other gas except oxygen and carbon dioxide is involved in the gas exchange. After Gaffron (1942) became suspicious that this is not the case with certain anaerobically incubated algae, he introduced chemical absorbers for oxygen, carbon dioxide and hydrogen into the side arms of the manometer, and proved in this way the occurrence of hydrogen fermentation and photochemical hydrogen consumption by these algae (*cf.* Vol. I, chapter 6).

Two conditions must be strictly fulfilled if manometric measurements are to be reliable. In the first place, when pressure changes of the order of 0.1 mm. Hg (1 mm. Brodie solution) are to be measured, while the total pressure is of the order of 100–1000 mm. Hg, maintenance of constant temperature becomes very important. The reaction vessels must be placed in a precision thermostat, and shaken vigorously to ensure continuous thermal equilibrium. Vigorous shaking is needed also to satisfy a second condition—rapid establishment of equilibrium between free and dissolved gases. This is essential both to prevent a lag in the manometric readings and to make sure that no depletion of carbon dioxide occurs in the lowest layer of the reaction vessel, where illumination (through the flat bottom!) is strongest, and carbon dioxide consumption by photosynthesis is fastest. It is thus necessary both to mix the liquid rapidly (to equalize carbon dioxide concentration) and to splash it vigorously (to

increase and renew the liquid–air interface), thus accelerating the release of oxygen into the gas space.

Because of these complications, direct *chemical* methods for determination of carbon dioxide and oxygen appeal to some investigators as more satisfactory than pressure measurements; the latter give only indirect evidence as to the identity of the gases causing the pressure changes. In spite of this, the manometric techniques have been resorted to again and again because of certain advantages not available with chemical analysis. The manometer measures *change* in pressure, regardless of *total* pressure of the gas in question. Thus, 10 cu. mm. of carbon dioxide can be determined with equal precision (assuming constancy of temperature), regardless of whether the total amount of gas present is 50 or 500 cu. mm. This is not true of direct chemical methods. The manometer has a fast response, so that measurements can be made over short periods of light or darkness.

The two-vessel method further requires exact identity of physiological processes and identical time course of pressure equilibration in the two vessels.

For the most precise manometric work, a *differential manometer* may be substituted for the usual open-type manometer (Warburg 1926). This eliminates the disturbing influence of barometric pressure. Differential manometers can be conveniently read to 1/100 of a millimeter with a cathetometer. This technique has been especially developed for measurements of the quantum requirement of photosynthesis (*cf.* chapter 29).

Several methods of *magnetometric* oxygen determination (based on paramagnetism of the O_2 molecule) have been developed. Pauling's magnetic oxygen meter (Pauling, Wood and Sturdivant 1946) is fabricated by Beckman, Inc. Its range (0–1 atm. O_2) makes it not directly applicable to precision measurements of photosynthesis.

A new method for continuous determination of oxygen content in solution was introduced in 1938, based on the measurement of conductivity. It is a form of the so-called *polarographic analysis*, which has found numerous applications in modern analytical chemistry. The essential device is a small-surface cathode, in a solution such that the passage of the current involves cathodic reduction of dissolved oxygen to H_2O_2. The maximum current that can pass through a cell with such an anode is determined by the supply of oxygen to the anode by diffusion, and is therefore proportional to the oxygen concentration.

An apparatus for polarographic oxygen determination in biological studies was developed by Petering and Daniels (1938) and applied by Petering, Duggar, and Daniels (1939) to the determination of the quantum yield of photosynthesis in *Chlorella*. The range of determinable concentrations is from about 5×10^{-5} m./l. upward to saturation. (Water saturated with air at 25° contains 2.4×10^{-4} m./l. O_2.) A similar method was used by Blinks and Skow (1938[2]) for the investigation of induction phenomena in photosynthesis. They found it advisable to replace the usual

dropping mercury electrode by a stationary mercury electrode, or by a Pt point electrode. (Concerning a rotating electrode, see Kolthoff 1940.)

Kautsky discovered the extreme sensitivity of the phosphorescence of certain dyestuffs, *e. g.*, trypaflavine adsorbed on silica gel, to traces of oxygen. Franck and Pringsheim (1943) investigated this quenching effect quantitatively and found that 50% quenching is obtained at a partial pressure of 5×10^{-5} mm. O_2. Pollack, Pringsheim and Terwood (1944) and Franck, Pringsheim and Lad (1945) applied this method to some problems of photosynthesis, *e. g.*, oxygen production by single flashes of light. Figure 25.4 shows the results obtained with a 2 second flash and several 0.03 second flashes of illumination in a suspension of *Chlorella*. The reason why the "oxygen bursts" recorded in the figure last for 1 minute or more is that the oxygen produced durng the flash is only gradually

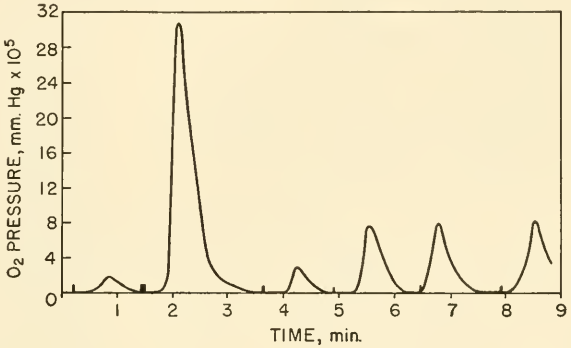

Fig. 25.4. Oxygen production by *Chlorella* in single light flashes (measured by the phosphorescence method) (after Pollack, Pringsheim and Terwood 1944). Large peak: 2 sec. illumination; small peaks: 0.03 sec. illumination.

carried away by the stream of nitrogen from the cell suspension into the vessel containing the phosphorescent gel. The apparatus, as constructed by Pringsheim and co-workers, could be used for the measurement of rates down to 5×10^{-8} cc. O_2/min. However, the method is only useful under anaerobic, or almost anaerobic conditions, since, in the presence of more than 10^{-3} mm. O_2, the quenching becomes practically complete.

4. Measurements of Carbon Dioxide Consumption

Like the liberation of oxygen, the consumption of carbon dioxide in photosynthesis can be measured either by the traditional methods of chemical analysis, or by physicochemical methods. Absorbers for carbon dioxide are well known; any alkaline material (*e. g.*, lime or baryta) can be used for this purpose, and the quantity of absorbed carbon dioxide

can be determined gravimetrically, titrimetrically, electrometrically or manometrically. The tests are made either in samples of the air or solution taken from the reaction vessel before and after a period of photosynthesis, or, more conveniently, in circulating gas, before and after its passage through the reaction chamber—a method first introduced by Kreusler

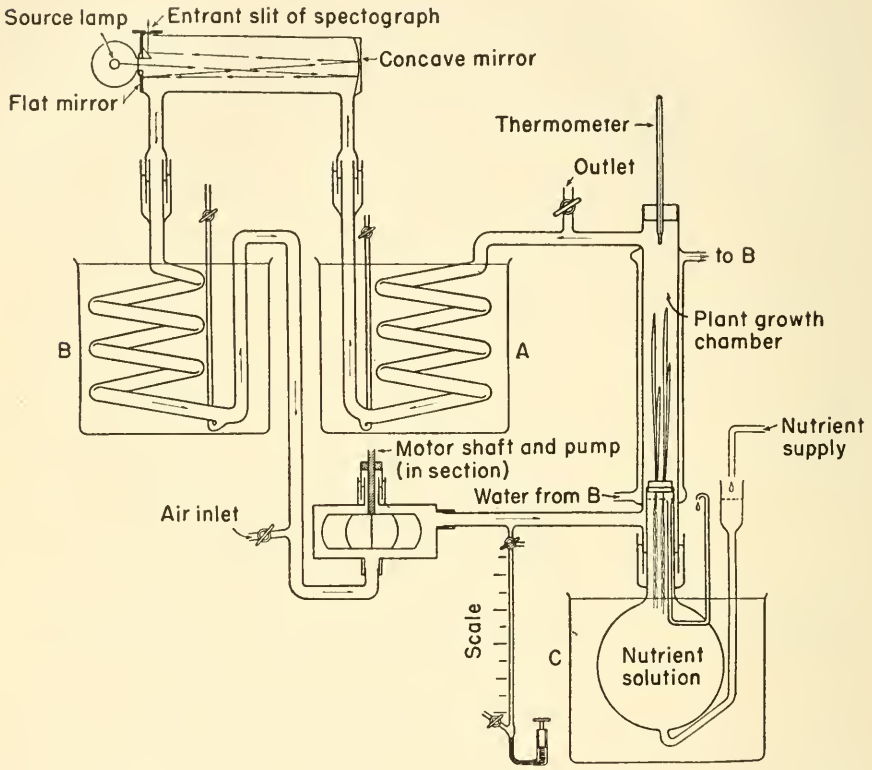

Fig. 25.5. Apparatus for continuous spectroscopic measurement of carbon dioxide exchange in plants (after McAlister 1937).

(1885), and used also in the classical work of Willstätter and Stoll (1918) It is convenient to use methods of analysis not requiring the taking of samples, *e. g.*, to measure the conductivity of the absorbing solution in equilibrium with the gas (*cf.* Newton 1935, and Clark, Shafer and Curtis 1941). The smallest amounts of carbon dioxide that can be determined in this way are of the order of 10^{-7} g.

Several methods of continuous determination of carbon dioxide in solution have been suggested. In working with (unbuffered) solutions, the

consumption of carbon dioxide can be followed acidimetrically, *e. g.*, by means of an appropriate color indicator (Osterhout and Haas 1918; Osterhout 1918), or a glass electrode (Blinks and Skow 1938).

Two sensitive, purely physical methods have been used for the determination of carbon dioxide in the gas phase. They were based on spectrophotometry and thermal conductivity, respectively. McAlister (1937) built an apparatus (fig. 25.5) in which the carbon dioxide content of circulating gas was recorded continuously by means of an infrared-sensitive spectrophotometer, using the strong carbon dioxide absorption band at 4.2 to 4.3 μ. The apparatus was later altered to register *changes* in carbon dioxide concentration (instead of concentration itself); *cf.* McAlister and Myers (1940).

A simplified method of estimation of small quantities of carbon dioxide in the air by infrared absorption, without the use of a monochromator (after eliminating water vapor as the only other infrared-absorbing component), was described by Dingle and Pryce (1940). Scarth, Loewy and Shaw (1948) improved this apparatus, increasing considerably its sensitivity, and adapting it for the determination of both carbon dioxide and water. They used it to follow the course of photosynthesis and of transpiration of leaves.

Harder and Aufdemgarten (1938) and Aufdemgarten (1939) described an automatic carbon dioxide recorder, in which the rate of cooling of an electrically heated wire was used to determine the content of the ambient gas in carbon dioxide. (Changes in the concentration of carbon dioxide affect the heat conductivity more strongly than equivalent changes in oxygen concentration.) Some registration curves obtained with this instrument are reproduced in figs. 33.10A,B and 11A,B. An improved apparatus was described by van der Veen (1949).

5. Measurements of Carbohydrate Production and Energy Conversion

Two methods—analytical determination of the carbohydrate production, and determination of the heat of combustion of the synthesized organic material—were much used in the earlier work on photosynthesis. They are, however, hardly suitable for exact kinetic investigations. As described in chapter 3 (Vol. I, page 35) the amount of analytically determinable carbohydrates found in the plant after a prolonged period of photosynthesis often is considerably smaller than was expected from the rate of consumption of carbon dioxide—a result attributable to rapid secondary transformations of the primary product, and possibly also to direct photosynthetic production of compounds other than carbohydrates.

The combustion of organic material is an appropriate method for the

determination of total yield of photosynthesis over the whole growth period, and is widely used in field experiments; but it is obviously unsuitable for exact kinetic determinations, in which the organic material present at the beginning of the experiment, cannot be neglected.

However, a different method of measurement of photosynthesis, also based on the determination of energy conversion, appears feasible, although it probably cannot compete in simplicity and exactness with the methods based on the determination of carbon dioxide or oxygen:

Of the total light energy (ΔH) absorbed by the plants, one part (ΔH_c) is transformed into chemical energy (latent heat of combustion of the synthesized organic material) and the rest (ΔH_t) is converted into heat. We stated above that the exact determination of ΔH_c is difficult because of the initial weight of organic material; but the determination of ΔH_t is possible. It can be carried out, without affecting the plants, by placing them in a sensitive calorimeter; ΔH_c can then be calculated by subtracting ΔH_t from ΔH. Magee, DeWitt, Smith and Daniels (1939) measured the heat produced in a small quartz vessel in a thermostated container in consequence of the absorption of a certain amount of light by a suspension of *Chlorella* cells, and compared it with the amount of heat produced in the same apparatus when the light is absorbed by India ink or another inert absorber. In these experiments, the rate of energy absorption was of the order of 1000 erg/sec., and ΔH_1 was found to be smaller than ΔH by about 20%. Similar methods were developed by Arnold and by Tonnelat (*cf.* chapter 29, page 1122).

6. Application of Isotopic Indicators

The use of *isotopic indicators*—stable isotopes such as H^2, C^{13} or O^{18}, and radioactive isotopes such as H^3, C^{11} and C^{14}—may make possible the development of several new sensitive methods for measuring the yield of photosynthesis. All that seems to be needed is to supply the plants with carbon dioxide or water enriched in one of these isotopes and measure either the accumulation of this isotope in the plant cells, or its disappearance from the supplied material. However, the phenomena of isotopic *exchange* and isotopic *discrimination* make the task less simple than it may appear at first.

Applications of the isotopes H^3, C^{11}, C^{14} and O^{18} to the solution of some *qualitative* problems in the chemistry of photosynthesis (such as the formation of a carbon dioxide acceptor complex, and the origin of the oxygen evolved in photosynthesis) were described in Volume I (see particularly pages 54, 202 and 241). New investigations with C^{14} as tracer, aimed at the identification of the organic intermediates of the reduction of carbon dioxide, will be described in chapter 36.

The techniques of labeling and counting have been described in several reviews, for example, in the monographs by Kamen (1947) and Calvin, Heidelberger, Reid, Tolbert and Yankwich (1949).

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Chapter 26

EXTERNAL AND INTERNAL FACTORS IN PHOTOSYNTHESIS

1. The "Cardinal Points" and the "Limiting Factors"

Even when studying simple reactions *in vitro*, the physical chemist is rarely able to control *all* the conditions that affect the reaction rate. Consequently, seldom will two investigations of the velocity of a reaction result in agreement in more than the order of magnitude. Beside the readily controllable external factors, such as temperature, pressure and light intensity, the rate often depends on factors as elusive as the state of the walls of the vessel, or the presence of minute impurities. It is therefore easy to judge the difficulties encountered in the kinetic study of a complex chemical process in a living organism. The rate of such a process depends on many physiological factors that do not enter ostensibly into the kinetic equations. Among all life phenomena, photosynthesis is perhaps the most sensitive to slight variations in the structure and composition of the biocatalytic apparatus. No wonder that doubts have been expressed as to the very possibility of deriving significant kinetic relationships from the quantitative study of this phenomenon.

At first, considerable optimism prevailed in this respect. The development of natural science in the nineteenth century led to the belief that biological processes follow relatively simple mathematical laws, and several attempts have been made to formulate such laws for the production of organic matter by plants.

The earliest discussions of the dependence of photosynthesis on external factors were based on the concept of the three "cardinal points" (Sachs 1860). According to this concept, which was widely accepted until the turn of the century, biological processes get under way at certain *minimum* values of the relevant external variables (such as temperature, pressure, humidity, light intensity etc.), reach the highest rate at certain *optimum* values of these variables, then decline, and cease altogether after the *maximum* tolerable values have been exceeded.

Twenty years before the enunciation of the principle of the three cardinal points, Liebig (1840, 1843) had formulated a simple rule for the effect of various elements on the yield of field crops. He postulated that these

yields are determined by the quantity of the one nutrient element (such as potassium, phosphorus or nitrogen) present in the lowest concentration (relative to its optimum quantity). This postulate became known as the "law of the minimum."

Sixty years later, Blackman (1905) suggested that a generalized form of this law can be applied to photosynthesis, and can explain the observations in this field better than the concept of the three cardinal points. He took from Liebig the idea that the rate of a biological process (in this case, photosynthesis) is determined, under given conditions, by a single "limiting" factor; but, in addition to the supply of material ingredients (the only kind of factors with which Liebig was concerned), Blackman considered also *temperature* and *light intensity* as potential "limiting factors."

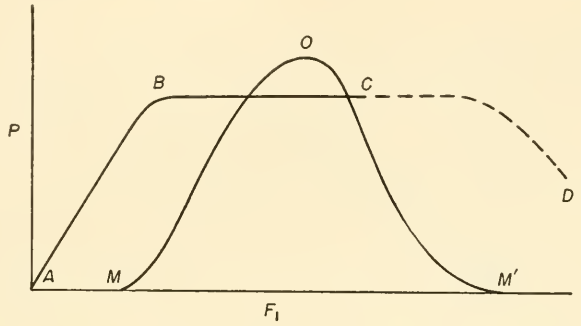


Fig. 26.1. Photosynthesis according to the concepts of "cardinal points" (*MOM'*) and "limiting factors" (*ABCD*).

He suggested that the rate of photosynthesis increases with the increase in value of any one of these factors (F_1) as long as this factor is "slowest" and ceases to be dependent on F_1 when one of the other factors (F_2, F_3, ...) becomes limiting. In other words, the plot of the yield of photosynthesis, P, versus a variable F_1 (at constant values of all the other kinetic factors) was postulated by Blackman to have the shape *ABC* (fig. 26.1), instead of the shape *MOM'* required by the optimum theory. Blackman could not deny that extreme conditions generally inhibit biological processes—in other words, that the horizontal plateau *BC* in figure 26.1 cannot extend indefinitely, but that sooner or later, P must begin to decline (as indicated by the broken line in the figure). However, he attributed this decline to destructive phenomena (*e. g.*, freezing of cell water, or denaturation of proteins) not inherent in the kinetic mechanism of photosynthesis itself. According to Blackman, these inhibitions merely limit the range in which the "law of limiting factors" can be verified; their superimposed character is indicated by the fact that, whenever they come into play, the rate, instead

of being determined uniquely by the momentary values of the variables F_1, F_2, ..., becomes *time-dependent*, thus revealing a progressive (and often irreversible) destruction of the biochemical apparatus.

Compared with the hypothesis of the three cardinal points, Blackman's principle of limiting factors represented substantial progress in the interpretation of the kinetics of photosynthesis. Since its first enunciation in 1905, it has been accepted and widely used by students of photosynthesis. Unfortunately, Blackman was not satisfied with the improvement of the general qualitative picture: In the belief that the efficiency of biological processes must be subject to simple quantitative rules, he insisted on treating the concept of "limiting factors" as an exact law of nature. He postulated that the functions $P = f(F_1)$ must have precisely the shape shown in

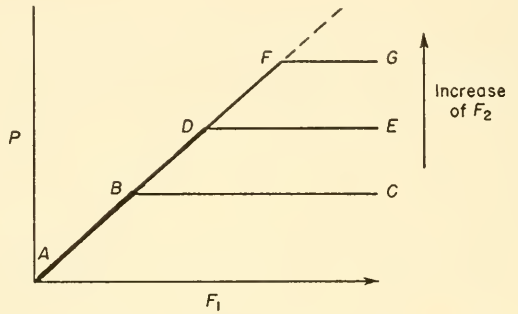


Fig. 26.2. Kinetic curves of the first type (Blackman type).

figure 26.1, *i. e.*, consist of a *linear* ascending part, terminated by a sharp break and followed by a *horizontal plateau*. In order that the same rule be true also for the dependence of photosynthesis on all the other factors, F_2, F_3, ..., he had to assume that for different values of a parameter, F_2, the yield P as a function of F_1 is represented by a set of broken lines—such as ABC, $ABDE$, $ABDFG$...in figure 26.2—which coincide at the low F_1 values (part AB in the figure) and are distinguished only by the position of the break, where the ascending part goes over into the horizontal plateau. This means that the rate of photosynthesis was assumed to be *proportional* to the one factor that is limiting under the given conditions, and *entirely independent* of all the other factors.

Only in the case of temperature as the "limiting factor" did Blackman admit the possibility of a different shape of the ascending part of the curve, an exponential (instead of a linear) rise, in agreement with the general experience in the field of temperature dependence of chemical reactions.

In the belief that the law of limiting factors must be strictly obeyed,

Blackman and his pupils made attempts to fit the experimental data into this oversimplified theoretical picture. Others objected to this, and a controversy arose, with the result that articles "for" or "against" Blackman's theory have been appearing in botanical journals for now over forty years. This protracted and largely unnecessary controversy has hampered rather than helped the penetration into plant physiology of the general principles of reaction kinetics and photochemistry (such as the law of mass action, Boltzman's and Arrhenius' activation equations and the quantum principle of photochemistry), which alone can provide adequate basis for the kinetic treatment of *any* chemical reaction, whether *in vitro* or *in vivo*. It will be shown below that, from the point of view of these principles, Blackman's "law" is only an idealization, which can be more or less closely approximated under certain special conditions.

Brown and Heise (1917) and Brown (1918) were among the first to criticize the way in which Blackman supported the law of limiting factors by reinterpretation of the observations of earlier investigators, and to point out that even Blackman's own measurements did not conform strictly to the type of figure 26.2. Some subsequent investigations, *e. g.*, that of van der Honert (1930), produced curves that so closely approached the "Blackman type" (*cf.*, for example, fig. 27.2) that the authors believed the law of limiting factors to be strictly valid under ideal experimental conditions (*e.g.*, uniform illumination of all cells; *cf.* page 864). Other, equally reliable measurements gave, however, an entirely different picture—families of $P = f(F_1)$ curves that diverged from the very origin (*cf.*, for example, fig. 27.1). On the basis of measurements of this type, Bose (1924) went to another extreme and suggested, as an alternative to Blackman's postulate, that the effect of a certain change in a factor, F_1, on the yield of photosynthesis, is *independent* of the prevailing values of all the other factors, F_2, F_3 . . . (while according to Blackman this effect should depend entirely on whether F_1 is the "limiting factor" or not). Bose's postulate requires that the curves representing the yield of photosynthesis in relation to a factor F_1 at different values of F_2 have shapes of the type shown in figure 26.3. Bose's "law" was derived from a very small number of measurements, and is merely another approximation, applicable to certain conditions opposite to those that favor the "Blackman behavior."

"Blackman type" curve systems have widely separated saturation plateaus, but coincident initial slopes; "Bose type" curve systems also have separated saturation plateaus, but distinct initial slopes. We will often refer to Blackman type curve systems as the "first type," and to Bose type curve systems as the "second type." A third type of kinetic curves, also encountered in photosynthesis, is characterized by initial divergence, but final convergence in a common saturation plateau (as in fig. 26.4).

The conditions that bring about the three types of curves will be discussed later in this chapter.

While the limitations of Blackman's law of limiting factors were debated, Liebig's notion of the "absolute minimum" of one nutritive factor, from which Blackman's concept was derived, also was found to be too rigid, and attempts were made to change it so as to admit the possibility of simultaneous sensitivity of a crop to several nutrient factors, each of which was said to be in a "relative minimum." New analytical formulations of the minimum law, suggested, *e. g.*, by Mitscherlich (1909, 1916, 1919, 1921) and Baule (1918, 1920), led to yield curves that approached the maximum asymptotically, without a sudden break.

Similar compromise solutions were suggested for photosynthesis. Harder (1921), Lundegårdh (1921, 1924) and Singh and Lal (1935) con-

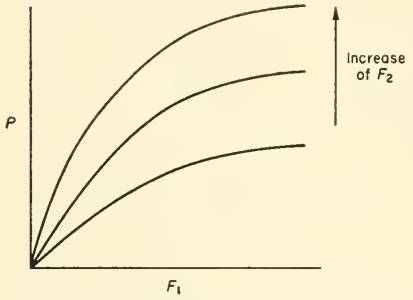


Fig. 26.3. Kinetic curves of the second type (Bose type).

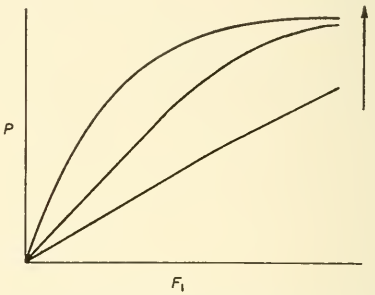


Fig. 26.4. Kinetic curves of the third type. Arrow indicates increase of F_2.

cluded from their measurements that the rate of photosynthesis may depend on several factors at the same time; when one "factor" gradually ceases to be "limiting," the influence of another one increases. This concept stands midway between the two postulates of Blackman and Bose; attempts have been made to use it for a general formulation of the kinetic relationships in photosynthesis.

However, because of the diversity of factors and conditions encountered in photosynthesis, it is unlikely that *any* analytical approximation will prove equally satisfactory for all the cases studied. Liebig's law of the minimum applied to one kind of factor only—nutrient elements; it was not unreasonable to suggest that it represents an equally good first approximation for all such factors, and that an improved mathematical formulation may provide a generally useful second approximation. Such hopes are much less justified in the case of photosynthesis, where Blackman included under the heading of "limiting factors" such heterogeneous mag-

nitudes as the concentration of a reactant (CO_2), the amount of the sensitizer (chlorophyll), the rate of supply of light energy, and temperature.

Maskell (1928[2]), in a paper from Blackman's laboratory, made one more attempt to justify Blackman's law in its original form. He suggested that the cause of the apparent deviations from this law lies in "mutual interaction of factors." Here is one example of what he meant: In the "carbon dioxide-limited state," the rate-determining carbon dioxide concentration must be that prevailing in the immediate neighborhood of the chloroplasts. Because of the consumption of carbon dioxide by photosynthesis, this concentration is not necessarily identical with the outside concentration (in the atmosphere or in the ambient solution), but depends on the rate of diffusion of carbon dioxide from the medium to the chloroplasts, and thus on the aperture of the stomata. Consequently, if illumination causes changes in this aperture, it may indirectly affect the rate of photosynthesis even in the "carbon dioxide-limited" state, not because the rate is "truly" sensitive to both light intensity, I, *and* carbon dioxide concentration, $[CO_2]$, at the same time, but because illumination influences the effective value of the factor $[CO_2]$!

However, the insistence that a "true" limiting factor must exist under all conditions is alien to reaction kinetics. The relation between the "law of limiting factors" and the general concepts of reaction kinetics was first clearly stated by Romell in 1926. He pointed out that Blackman's term "slowest factor" is meaningless, and that one can only speak of a *slowest process* in a sequence of processes. The rate of a simple homogeneous reaction usually is a function of *all* the relevant factors, *e. g.*, the concentrations of all reactants, temperature and (in a photochemical process) light intensity. "Limitation" effects of the type suggested by Blackman can occur only if the reaction, the over-all rate of which is being measured, consists of several successive steps, with one step supplying the reactants for the next one. If the supply process is slow, it becomes a "bottleneck," and the velocity of the over-all reaction may become independent of all factors which do not affect this one "limiting" or "rate-determining" step. A simple example is provided by many photochemical reactions, where the supply of light-activated molecules is the "bottleneck," or limiting process. Whenever "Blackman behavior" is observed in practice, it can be assumed that one is dealing with a *series* of consecutive reactions that includes (at least) one step of limited maximum efficiency. The rate of the over-all process then cannot exceed the maximum rate of passage of the system through this "bottleneck."

The bottleneck stage of photosynthesis may be the *supply of light energy*, or the *supply of a reactant* or the *removal of a reaction product*. We will consider these three cases more closely in section 3 of this chapter. We will

see there that, even when a "bottleneck" reaction does exist, the relationships postulated by Blackman are only *approximated*, but never exactly fulfilled.

2. Photosynthesis Not a Homogeneous Reaction

In the kinetic treatment of photosynthesis, it is necessary to keep in mind that photosynthesis is not a homogeneous reaction. This statement is true in a twofold sense: In the first place, the photosynthetic apparatus is a colloidal system with a definite structure—probably containing rows of oriented pigment molecules adsorbed on interfaces between proteinaceous and lipide layers (*cf.* the discussion in chapter 12, Vol. I). In the second place, the supply of light energy and of the reactants to different parts of this structure is not uniform, particularly when photosynthesis proceeds at a high rate.

Let us consider these two aspects of the problem more closely.

The reactions involved in photosynthesis are, at least in part, *surface reactions.* It is furthermore likely that they can be called *topochemical reactions*—meaning that they occur not by encounters of molecules moving about at random in two-dimensional adsorption layers, but by a more purposeful mechanism, which requires the reacting molecules to take definite paths past a number of catalytic "reaction centers." The products formed in one reaction center are directed to the next one, so that back reactions of unstable, intermediate products of oxidation and reduction products are prevented by their spatial separation. Equations based on the law of mass action can be applied to this type of reaction only with considerable reservations.

The second aspect of the inhomogeneous character of photosynthesis—the nonuniform supply of reactants and energy to the different regions of the photosynthetic apparatus—was repeatedly discussed in the literature, for example, by Schanderl and Kaempfert (1932) and Katz, Wassink and Dorrestein (1942). Even if the chlorophyll molecules and the catalyst molecules participating in photosynthesis were available uniformly in all parts of a chloroplast—which is in itself uncertain—the supply of *carbon dioxide,* as well as that of *light quanta,* is likely to vary from place to place. In strong light, and with a limited supply of carbon dioxide, the chlorophyll molecules closer to the source of supply may use up most of the available reduction substrate and leave only little for the less favorably situated pigment particles; in other words, the numerical value of the concentration factor $[CO_2]$ in kinetic equations may vary from place to place. A similar consideration applies to the light intensity factor, I: Because of the high optical density of chloroplasts—particularly for red and blue-

violet light (*cf.* figure 22.35, p. 698)—the rate of light absorption is likely to be much slower for chlorophyll molecules situated deep in the body of a chloroplast (or on the "shady side" of it) than for those located on the light-exposed surface. Thus, inhomogeneity cannot be avoided even by using dilute cell suspensions in which illumination is the same for all cells, but not for all chlorophyll molecules in them. In denser suspensions, only a uniform *time average* of illumination of all cells can be achieved, and this only by very intense stirring. In the thalli of multicellular algae, or in the leaves of the higher plants, the disparity between the rates of light absorption in different cells cannot be corrected at all. The absorption in the

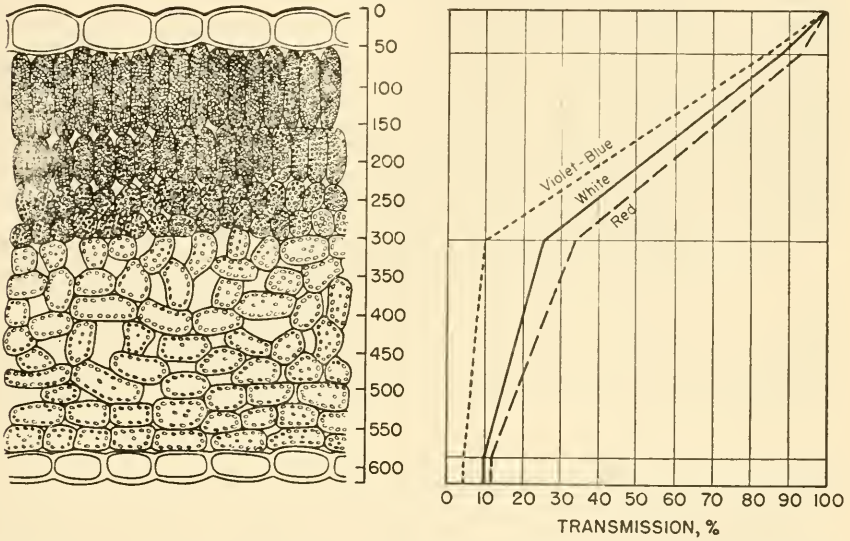


Fig. 26.5. Weakening of light in transmission through a leaf of *Cyclamen persicum* (after Schanderl and Kaempfert 1933). Scale at right of leaf cross-section, depth in microns.

spongy parenchyma cells, for example, is under all circumstances considerably weaker than that in the palisade cells (*cf.* fig. 26.5). Thus, in curves representing the rate of photosynthesis (P) as a function of carbon dioxide concentration or light intensity, the abscissae are *mean* values (averaged over the whole cell or over many cells). This alone must prevent these curves from following the course suggested by Blackman, even if the law of limiting factors were exactly valid for the ideal case of a uniformly illuminated and uniformly supplied homogeneous system. Katz, Wassink and Dorrestein (1942) derived an equation by means of which the experimentally obtained "light curves," $P = f(I_0)$ (where I_0 is the intensity of the light falling on the front wall of a vessel with a suspension of algae or bac-

teria), can be recalculated to represent P in relation to the average light intensity, I, actually falling on an individual algal or bacterial cell (see p. 1009 and figs. 28.22A, B and C).

3. Some General Kinetic Considerations

Some investigators, who realized the inevitable distortion of light curves and carbon dioxide curves of photosynthesis by the "depth effects" discussed in the preceding section, assumed that in the measure in which these effects can be eliminated (experimentally, by the use of very dilute systems, or mathematically, by applying adequate corrections for inhomogeneity) the kinetic curves would approach the ideal "Blackman type." Undoubtedly, the elimination of depth effects shortens the transitional region between the ascending part of the curves and the saturation plateau; but figure 28.22C shows that it does not make the breaks sharp. Only a fraction of deviations from "Blackman behavior" can be attributed to inhomogeneity; even with all "depth effects" eliminated, the kinetic analysis of photosynthesis will still have to contend with kinetic curve systems of all three types exemplified by figures 26.2, 26.3 and 26.4.

The *common feature* of all these curves is the occurrence of *saturation*, *i. e.*, of states in which the rate of photosynthesis is *independent* of the variable F_1. The saturation level may depend on the parameter F_2—as in the Blackman type and Bose type curve systems (figs. 23.2 and 23.3)—or it may be independent of F_2, in which case curve systems of the "third type" (fig. 23.4) are observed.

(*a*) *Sources of Saturation in Photosynthesis*

The over-all rate of a process consisting of a series of successive chemical or physical stages—sometimes referred to as a *catenary series*—cannot exceed the rate of any of its individual steps. "Saturation" of such a process with respect to a given kinetic variable, F_1, is therefore reached whenever the over-all rate becomes equal to the maximum rate of a single step (a step which in itself must be independent of this variable). For example, under given conditions of external carbon dioxide pressure and temperature (perhaps also humidity and other factors affecting the colloidal structure of the cell), carbon dioxide can be supplied to the photosynthesizing cells at not more than a certain maximum rate, which is reached when the stationary concentration, $[CO_2]$, at the site of photosynthesis is zero, and the diffusion gradient between the medium and the chloroplast has therefore the maximum possible value. This maximum rate of carbon dioxide supply by diffusion is independent of illumination (except for possible indirect relations of the type mentioned on page 863). Therefore, "light saturation"

should occur whenever the over-all rate of photosynthesis approaches the maximum rate of carbon dioxide supply by diffusion. In this consideration, the diffusion of carbon dioxide could be replaced by a preliminary chemical reaction the rate of which is proportional to the concentration, $[CO_2]$, *e. g.*, the formation of the compound $\{CO_2\}$ from carbon dioxide and an "acceptor," which was postulated in chapter 8 (Vol. I). In this case, light saturation is determined by the maximum possible rate of formation of $\{CO_2\}$ that is reached when all acceptor molecules are free, (*i. e.*, when all complexes $\{CO_2\}$ are utilized for photosynthesis practically instantaneously after their formation). Similarly, "carbon dioxide saturation" must occur whenever the over-all rate of photosynthesis approaches the rate of supply of light energy, and the quantum yield assumes its highest possible value.

There are other factors, besides carbon dioxide supply and the supply of light energy, which also can impose "ceilings" on the over-all rate of photosynthesis and thus cause saturation phenomena. This role can be played, *e. g.*, by the concentration of any one of the several catalysts participating in photosynthesis (including the "photocatalyst" chlorophyll). For example, if one reaction step in the "catenary series" of photosynthesis is the monomolecular transformation of a catalyst–substrate complex:

$$(\text{Catalyst} + \text{Substrate}) \longrightarrow \text{Catalyst} + \text{Product}$$

the maximum rate of this reaction step is reached when all the available catalyst molecules are loaded with substrate molecules. When photosynthesis proceeds at a rate that requires such maximum utilization of one catalyst, variations in most kinetic variables (such as the concentrations of the reaction partners or of the other catalysts, or light intensity) cannot increase the rate any further. Only a rise of temperature can lift the "ceiling" imposed by the maximum velocity of such a catalytic transformation. The part of a "rate-limiting" catalyst can be assumed by any of the several catalysts the existence of which was postulated in Volume I (*cf.* chapters 6, 7 and 9)—for example, the "carboxylase" E_A, the "stabilizing catalyst" (a "mutase" ?) E_B, or the "deoxygenases" E_C and E_O. Chlorophyll can play a similar role, *e. g.*, if the primary photochemical process involves a chemical change of this pigment, and a certain time is required for its restoration. "Acceptors" or "carriers," such as the carbon dioxide acceptor postulated in chapter 8 (Vol. I) are catalysts, too, and the available quantity of any such auxiliary compound also can serve as a "limiting factor" in photosynthesis.

All these factors can—and most of them probably do—contribute to the limitation of the rate of photosynthesis under different conditions, thus causing the "saturation" of this rate with respect to vacious kinetic vari-

ables. There is no theoretical reason to expect, and no actual experience to indicate, that saturation with respect to, say, light intensity, or carbon dioxide concentration, is always caused by the same "limiting factor." To the contrary, clear indications can be found of saturation phenomena caused by slow diffusion, slow carboxylation, various catalytic deficiencies, limited light supply and limited supply of reductants (in bacterial photosynthesis). One may consider this as evidence of good adjustment of the photosynthetic process as a whole, since it means that the different parts of the complex mechanism of photosynthesis have approximately the same maximum capacity. One understands, in the light of this multiplicity of possible rate-limiting steps, why repeated attempts to represent the kinetics of photosynthesis by means of models consisting of a small number of reactions, *e. g.*, of one light reaction and one dark reaction only, could not have led to more than very limited success.

Saturation with carbon dioxide is reached at pressures three or four times higher than the partial pressure of carbon dioxide in the free atmosphere (*cf.* Table 27.I); while *saturation with light* is reached at light intensities equivalent to 10–100% of full midday sunlight (*cf.* Table 28.I) and thus about equal to the average light intensity to which freely growing plants are exposed in nature. The *optimum temperature* of photosynthesis is somewhat above the average summer temperature, at least in temperate zones. An approximate adjustment of the photosynthetic mechanism to natural conditions is thus obvious. Perhaps, this adjustment was achieved in times when both the average temperature and the carbon dioxide content of the atmosphere were somewhat higher than they are now. However, this inference is by no means certain; nature has seldom been able to develop ideal solutions of its adaptation problems, and is usually satisfied with more or less rough approximations. The present kinetics of photosynthesis may have been the best plants were able to evolve in response to the now prevailing climatic conditions.

(*b*) *Origin of Kinetic Curve Systems of Different Types*

The distinctive feature of "Blackman type curves," described on page 860, are closely coincident initial, linear parts of the curves. This characteristic distinguishes them from the two other types of curve systems, represented in figures 26.3 and 26.4. On the other hand, Blackman curves (fig. 26.2) and Bose curves (fig. 26.3) have in common a wide spacing of saturation plateaus, while curves of the "third type" in figure 26.4 all approach a common saturation level.

Coincidence or separation of the saturation levels depends on whether saturation is imposed by the same parameter F_2, to which the set of curves

refers, or by some other parameter, F_3, F_4 ... For example, in a set of curves, $P = f[CO_2]$, for various values of light intensity, I, the saturation levels will be well separated (as in figs. 26.2 and 26.3) if saturation is imposed by the rate of light supply, but will coincide (as in fig. 26.4) if saturation is due to the limited rate of a dark, catalytic reaction.

Coincidence or divergence of the initial slopes depends on the qualitative and quantitative relationships between the variables F_1 and F_2. If both these variables affect the rate of the same reaction step, the rate will generally depend on both of them. For example, if F_1 is the concentration, $[CO_2]$, and F_2 is temperature, and if the slope of the ascending part of the curves $P = f[CO_2]$ is determined by the velocity of carbon dioxide supply by diffusion, this slope will depend on temperature. If, on the other hand (*a*) the factors F_1 and F_2 affect *different* steps in the "catenary series" (*e. g.*, if F_1 is light intensity and F_2 is temperature) and (*b*) the relative values of F_1 and F_2 are such that the process affected by F_2 is far below its maximum rate when that affected by F_1 approaches its maximum rate—then (and only then) the reaction rate will be a function of F_1 alone, and practically independent of F_2.

The second condition (*b*) is important. Contrary to the way in which the concept of "limiting factors" or "rate-determining reaction steps" often is used, the existence of a reaction step of limited maximum efficiency affects the rate of the over-all reaction long before the rate actually "hits the ceiling." Therefore, if several reaction steps have maximum rates which are not too different in their order of magnitude, the rate of the over-all reaction must be affected by all these "potential bottlenecks" and not only by the "narrowest" one.

This fact was recognized in the alterations of Liebig's "law of the minimum," which we have mentioned on page 862, and some quantitative illustrations of it will be given later in our analytical discussions. To make it plausible, we may use a mechanical analogy; the flow of water through a system of pipes with several strictions depends on the diameter and length of all of them, and not only on the one that has the greatest flow resistance.

In the plot of P against F_1, for different values of F_2, we can expect all curves to be to the right of, and below, two "limiting" lines: one a slanting "roof" imposed by the maximum rate of the "slowest" reaction step the rate of which is *proportional* to F_1 (or, more generally, is a function of F_1); and the second a horizontal "ceiling" imposed by the maximum rate of the slowest reaction step the maximum rate of which is *independent* of F_1. For example, in the case of curves $P = f[CO_2]$, for different temperatures, the two limiting curves may be determined by the maximum rate of diffusion of carbon dioxide and the rate of light absorption, respectively (*cf.* fig. 26.6). These two limiting lines together form a typical "Blackman

curve"; but they are merely *limits* within which the actual kinetic curves are confined. The point we are trying to make—in advance of its analytical proof—is that it would be wrong to imagine that the existence of the "roof" OA and the "ceiling" BC will leave unaffected the curves situated entirely within the "permitted area" OXC, and merely force back into this area the curves (or section of curves) that would otherwise cross the limit-

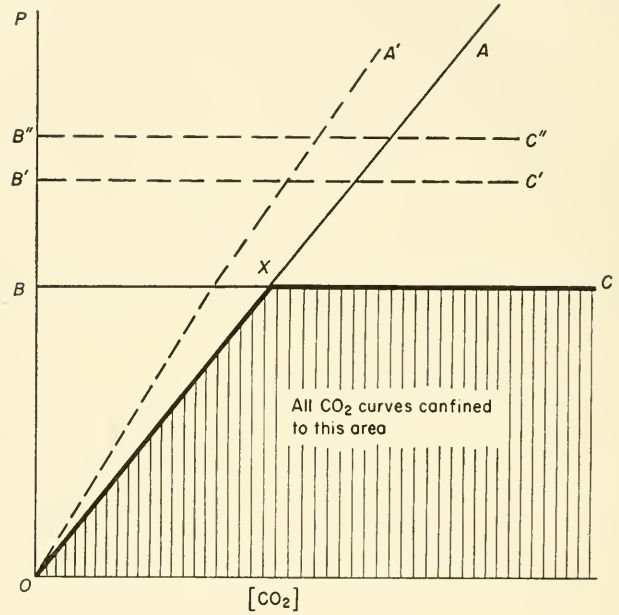


Fig. 26.6. "Roof" and "ceiling." OA, maximum rate of a diffusion step (proportional to $[CO_2]$); OA', maximum rate of carboxylation (proportional to $[CO_2]$); BC, maximum rate of primary photochemical process (= rate of light absorption) (independent of $[CO_2]$); $B'C'$, $B''C''$, maximum rates of catalytic reactions.

ing lines. To the contrary, the existence of the "roof" and the "ceiling"—as well as that of the potential, higher "roofs" and "ceilings" (OA', $B'C'$, $B''C''$ in fig. 26.6)—will push down and toward the right even the kinetic curves that would not have approached the limiting values if the limitations were absent.

We will have opportunity for analytical proof of these statements in subsequent chapters. For example, in chapter 27 we will derive "carbon dioxide curves" of photosynthesis from simple models of the mechanism of entry of carbon dioxide into the photosynthetic reaction; and we will find there that the sequence of the two reactions:

$$\text{(26.1)} \qquad CO_2 + A \underset{k_{a'}}{\overset{k_a}{\rightleftharpoons}} ACO_2 \xrightarrow{+\{H\}} A + \{HCO_2\}$$

(*i. e.*, a reversible binding of carbon dioxide by an acceptor A followed by reduction of the complex ACO_2 by a photochemically produced reductant $\{H\}$) leads to a "Bose type" system of curves $P = f[CO_2]$ (where P is the rate of the over-all reaction). If carboxylation is so rapid (compared with photosynthesis) that its equilibrium is not disturbed even in strong light, these "carbon dioxide curves" will be hyperbolae, which diverge from the origin, and remain in a constant ratio up to saturation. Saturation corresponds, in this case, to complete carboxylation of all the available acceptor, and is therefore reached at the same value of the variable $[CO_2]$ in all curves. The saturation rate rises with increasing concentration of the reductant $\{H\}$ (and therefore also with light intensity, since we assume that $\{H\}$ is produced by light).

We will further see that, if carboxylation is a slow process the rate of which is proportional to $[CO_2]$, a $[CO_2]$-proportional "roof" is imposed on the rate of the over-all reaction, namely:

$$\text{(26.2)} \qquad P_{\max.} = k_a A_0 [CO_2]$$

where A_0 is the total concentration of the acceptor*, and k_a the rate constant of carboxylation (*cf.* equation 26.1). We will show that, because of this "roof," the curves $P = f[CO_2]$, which would otherwise begin with the slope $k_a A_0$ (*i. e.*, which would stay just inside the "permitted area") will be reduced to an initial slope *half as large*, *i. e.*, $k_a A_0/2$. More generally, curves that, without limitation, would have begun with a slope $\alpha k_a A_0$ will be reduced to an initial slope $k_a A_0 \alpha/(\alpha + 1)$. Thus curves that in the case of rapid carboxylation would begin with slopes between 10 $k_a A_0$ and 100 $k_a A_0$, would all be confined, in consequence of slow carboxylation, to slopes between 10/11 $k_a A_0$ and 100/101 $k_a A_0$, and would thus present a "Blackman picture." If the carboxylation product, ACO_2, in equation (26.1) has to undergo a monomolecular transformation before it can react with $\{H\}$, this would impose a $[CO_2]$-independent *ceiling* on the rate of the overall reaction, namely:

$$\text{(26.3)} \qquad P_{\max.} = k_1 A_0$$

where k_1 is the rate constant of the postulated monomolecular transformation, and A_0 the total available quantity of A. As a result of this "ceiling," the curves $P = f[CO_2]$, which would otherwise reach a saturation value of $P = k_1 A_0$, will be reduced to a saturation level half as high, $P = k_1 A_0/2$.

* We omit square brackets in the designation of *constant* concentration, *i. e.*, we write A_0, Chl_0, etc., instead of $[A]_0$, $[Chl]_0$, etc.

The curves that would otherwise reach a saturation value high above k_1A_0 will be crowded into a narrow space immediately below k_1A_0, and the curves with saturation values below k_1A_0, will all be depressed. A curve the saturation level of which would otherwise be βk_1A_0 will be reduced by the imposition of the ceiling to a saturation level of $\beta k_1A_0/(\beta + 1)$. It follows from this formula that, even if the saturation rate without the limitation were only one tenth of the maximum rate of the postulated monomolecular transformation of ACO_3, it would nevertheless be reduced by 10% by this "potential bottleneck."

4. Internal Factors and the "Physiological Concept" of Photosynthesis

The belief, referred to on page 860, that the rate of photosynthesis *must* obey a simple and rigid kinetic law has been partly responsible for the conclusion, reached by some plant physiologists, that it does not obey any recognizable kinetic law at all. These physiologists, disillusioned by the over-simplifications in which the preceding generation had indulged, turned their attention to the complex relations between photosynthesis and other phenomena of plant life, such as nutrition, respiration, growth or aging. Factors often referred to as "internal" or "plasmatic" are supposed to be responsible for these relations. Stressing the prime importance of these factors in the regulation of photosynthesis, Kostychev and his pupils minimized or denied the direct influence of the easily regulated "external" factors, such as light intensity, CO_2 concentration and temperature.

Their revolt against the application of kinetic laws to photosynthesis and similar processes would perhaps be less violent if these physiologists would have realized that the "law of limiting factors" is by no means the last word in the physicochemical approach to photosynthesis, that, in fact, the concept of "limiting factors" is foreign to reaction kinetics. The belief that no other kinetic laws are possible led Chesnokov and Bazyrina (1930) to argue that since, according to Harder and Lundegårdh, the factors "carbon dioxide concentration" and "light intensity" are *not* "truly limiting" (*i. e.*, that often the change in either of these factors can affect the rate) the "true" limiting factors must be sought inside the plant. It did not occur to them that photosynthesis may have no "true" limiting factor at all.

Kostychev, in an article called "A New Concept of Photosynthesis" (1931), suggested that "external" factors affect photosynthesis mainly, if not exclusively, in an indirect way, by stimulating or inhibiting certain unknown plasmatic activities. All the conclusions obtained by Blackman (and others before and after him) on the basis of the "physicochemical" approach were rejected by Kostychev as spurious. A similar point of view was taken by van der Paauw (1932) and by Kostychev's co-workers, Chesnokov and Bazyrina (1930, 1932), who sought to prove by experiments that two external factors—carbon dioxide concentration and temperature—have no direct effect on the rate of photosynthesis at all, and that the third

one—light intensity—affects this process only partly by direct action, and partly through plasmatic stimulation.

In the U.S.S.R., there is a tendency now to consider this point of view as the only one in accord with dialectic materialism, and attempts to isolate photosynthesis from other functions of the living organism and study it as an independent photochemical reaction are criticized as "mechanistic."* Such dogmatic assertions, practically banished from physics and chemistry, but still recurring in biological sciences, particularly in the U.S.S.R., are strangely beside the point. A reaction in a living organism is distinguished from that in a test tube by the complexity of the system in which it takes place. This complexity is due to three causes: the *impossibility of separating* the reacting system from other components of the organism; its *inhomogeneous structure*; and its complex and largely unknown *chemical composition*. Unprejudiced experiments alone can prove whether, despite these handicaps, direct relationships can be established between external kinetic variables and the rate of the specific process under investigation. If this proves possible, a promising approach to the understanding of the process is opened, and it would be foolish to refuse to use it because of dogmatic objections.

5. Rate of Photosynthesis under Constant Conditions. Midday Depression and Adaptation Phenomena

Observations that lend support to the "physiological" concept of photosynthesis include, among others: the difference in photosynthetic activity, under identical external conditions, of plants grown in various habitats; the adaptation of photosynthetic activity to changed conditions (stronger or weaker light, higher or lower temperature—*cf.* for example, Harder 1933 and Brilliant 1940); the effects of aging; and the changes of photosynthetic activity under constant external conditions (fatigue, midday depression etc.). Not only do plants of different species behave differently under identical external conditions, but variations are found also between "sun plants" and "shade plants" of the same species, "sun leaves" and "shade leaves" on the same branch and even between different parts of the same leaf (*cf.* Drautz 1935). The photosynthetic activity of a plant often changes strongly in the course of a single day, not to speak of a whole season. Observations of diurnal changes have played an especially important role in the development of Kostychev's "new concept" of photosynthesis.

Offhand, one would expect photosynthesis to increase steadily after sunrise until the light intensity has reached the saturating value, and then remain more or less constant, unless cloudiness decreases the illumination below the saturating intensity, until the evening decline sets in. The actual behavior of the plants often follows, however, a much more complicated

* An interesting monograph, *Photosynthesis as Life Process of the Plant*, by Miss Brilliant (1947) seems to be the only comprehensive review of photosynthesis published in Russian in recent years. It contains a survey of about 200 Russian and 350 other papers, many of them not utilized in the present book.

pattern. Thoday (1910) discovered that the production of organic material by leaves may show a temporary decline in the middle of the day; the plant takes an "afternoon nap" (*cf.* fig. 26.7). McLean (1920) observed that this decline may even result in the *release* of carbon dioxide.

The gas exchange measurements of Kostychev and co-workers (1926–1931) (*cf.* also Chesnokov, Bazyrina and co-workers 1932), carried out under a large variety of climatic conditions, from Central Asia to the Arctic Sea, and with algae as well as with land plants, showed that the phenomenon of "midday rest" is widespread in the plant world, but that it assumes

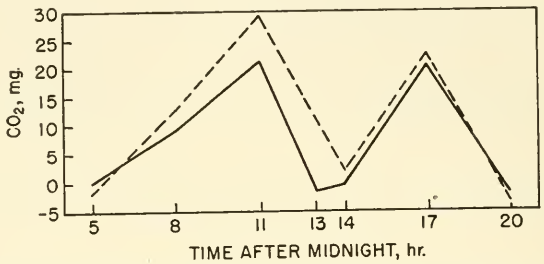


Fig. 26.7. Diurnal course of photosynthesis of two leaves of *Eriobotrya japonica* under natural conditions (after Kursanov 1933): solid line, May 30; broken line, June 6.

the extreme form of a reversal of photosynthesis and evolution of carbon dioxide only under special conditions, particularly in hot climates.

The diurnal course of photosynthesis has received the attention also of numerous other investigators, among whom we may mention Geiger (1927), Montfort and Neydel (1928), Maskell (1928[1]), Hiramatsu (1932), Harder, Filzer and Lorenz (1932), Bosian (1933), Kursanov (1933), Stålfelt (1935), von Guttenberg and Buhr (1935), Mönch (1937), Filzer (1938), Neuwohner (1938), Neubauer (1938), B. S. Meyer (1939) and Böhning (1949).

Harder (1930) and co-workers, Schoder (1932) and Drautz (1935), as well as Neuwohner (1938), have attempted to explain the diurnal curves by combined variations of several external factors (light intensity, humidity and temperature). They succeeded only partially, and had to admit that a considerable part of the observed variations remained unexplained and had to be attributed to unknown "plasmatic" factors. This is particularly clearly demonstrated by the observations of Filzer (1938), who found that leaves, picked from trees at different times of the day and then investigated under constant conditions in the laboratory, showed the same periodic changes in photosynthetic production as did leaves left attached to the plant and exposed to the natural change of night and day. Similarly, Mas-

kell (1928[1]) found that detached cherry laurel leaves, illuminated with constant light for 24 hours, showed a deep depression of photosynthesis during the night hours; thus, not only the "midday nap," but also the "night sleep" appears to be influenced by internal factors.

Geiger (1927), Maskell (1928[1,2]) and Stålfelt (1935) considered the *closure of the stomata* as the immediate cause of the midday depression. Maskell (1928[1]) observed that the nightly depression of photosynthetic activity of steadily illuminated leaves can be avoided by increasing the partial pressure of carbon dioxide ("pressing carbon dioxide through half-closed stomata"), and that steadily illuminated leaves of *Hydrangea* (the stomata of which are almost rigid) showed only a slight decline of photosynthesis during the night hours. Both Maskell (1928[2]) and Stålfelt (1935) found a parallelism between the average aperture of the stomata and the rate of photosynthesis (*cf.* chapter 27, page 910).

It thus seems plausible that the diurnal rhythm of photosynthesis of the higher land plants is to a large extent conditioned by stomatal movements. The question remains, however, what causes the stomata to close at certain times of the day, even though the illumination and the carbon dioxide supply are kept constant?

One "internal factor" that has been much discussed in connection with this problem is the accumulation of (soluble or insoluble) *carbohydrates.* (Concerning the effect of excess carbohydrates on the rate of photosynthesis, see chapter 13, Vol. I.) The midday depression may be a pause during which these materials are translocated or partially combusted. This explanation, first accepted by Kostychev, Kudriavtseva, Moisejeva and Smirnova (1926) and Kostychev, Bazyrina and Chesnokov (1928), was later rejected by Chesnokov and Bazyrina (1930[2]), who found that plants with entirely different diurnal course of translocation may nevertheless show the same diurnal course of photosynthesis. It was on the basis of results such as this that Kostychev (1931) finally reached his extreme conclusion concerning the purely physiological regulation of photosynthesis.

Against these findings of Kostychev and co-workers, Kursanov (1933), von Guttenberg and Buhr (1935) and Mönch (1937) confirmed the existence of a relation between the accumulation of sugars and starch and the diurnal rhythm of photosynthesis. However, according to von Guttenberg and Buhr (1935) and Neuwohner (1938), no single explanation can be made to fit *all* cases of midday depression. In some cases (*e. g.*, in young leaves in spring) it is clearly traceable to the choking of the photosynthetic apparatus with carbohydrates. In other cases (*e. g.*, in summer leaves on hot days) the loss of water and the ensuing closure of stomata provide the most plausible explanation. Accumulation of half-oxidized products which "narcotize" the photosynthetic apparatus, as suggested in Franck's theory

of induction (chapter 33), is another type of mechanism which must be taken into consideration. Drop of the CO_2 content of the air (Böhning 1949) and enhanced CO_2 supply through the roots (p. 910) also have been blamed for the midday depression.

The phenomenon of midday depression was found by Montfort and Neydel (1928) also in stomata-free ferns, and by Kostychev and Soldatenkov (1926), Kursanov (1933) and Neubauer (1938) in algae.

Gessner (1938) found no pronounced midday decline of photosynthesis in the higher aquatic plants (*Elodea*, *Potomageton* etc.), except with shade-adapted species or individual plants in which it could be interpreted as "inhibition by excess light" (*cf.* Volume I, page 535). Although minor fluctuations of the rate remained unexplained, the rate of photosynthesis in Gessner's submerged plants generally followed the changes in the intensity of illumination. The maximum of photosynthesis was often found in the early afternoon rather than at noon, but this could be explained as a temperature effect.

Chesnokov, Grechikhina and Jermolayeva (1932) found that respiration, too, has a complicated diurnal rhythm. Because of this, the true rate of photosynthesis cannot be obtained by applying a uniform respiration correction to the net rate of oxygen liberation measured at different times of the day. They also found that the respiration of leaves often is much stronger than was generally assumed before. In young leaves, in particular, the rate of respiration may approach that of photosynthesis. This explains why the rate of carbon dioxide *liberation* by some plants during the midday depression was found to be almost as large as the rate of the carbon dioxide *consumption* by photosynthesis before aud after this rest period.

However interesting the phenomena of the diurnal rhythm of photosynthesis, and similar "physiological" effects (such as aging, fatigue, etc.) may be, the primary question for a kinetic study of photosynthesis is not whether these variations can be *explaincd*, but whether they can be *eliminated*, and photosynthesis made to proceed at an even and reproducible rate. It is difficult to realize such steadiness in field experiments. Boysen-Jensen and Müller (1929), Boysen-Jensen (1933) and Mitchell (1936) said that, if conditions are reasonably constant, rate of photosynthesis in natural surroundings remains steady; but Maximov and Krasnosselskaja-Maximova (1928) and Waugh (1939) observed that the photosynthetic production of leaves on the tree fluctuated, under constant external conditions, in successive four minute periods, by as much as $\pm 100\%$ of the hourly average. Kostychev (1931) concluded from these observations that measurements of photosynthesis over short periods have no meaning, and that the minimum time over which photosynthesis should be measured is a whole day!

Stocker, Rehm and Paetzold (1938) found that rapid changes of the rate of photosynthesis under natural conditions (fluctuation period:

several minutes) are associated with similar fluctuations of the carbon dioxide concentration in the air.

Scarth, Loewy and Shaw (1948) observed that the photosynthesis of detached leaves, determined by measuring the infrared absorption of carbon dioxide, occasionally showed unexplained, regular fluctuations (with a period of the order of 1 hour).

When plants are investigated under natural conditions, the apparently erratic behavior can be attributed to the difficulty of controlling all the relevant factors. However, considerable doubt has also been expressed as to the capacity of plants to carry out photosynthesis at a constant rate under controlled conditions in the laboratory. Experiments with lower plants, *e. g.*, unicellular algae, such as *Chlorella*, have given comparatively satisfactory results: If certain prescriptions concerning culture and treatment were adhered to, these algae could be relied upon to maintain a constant and reproducible rate of oxygen production for several hours (leaving aside the short time induction phenomena to be discussed in chapter 33). According to Pratt (1943[3]), when alkaline buffers are used, the constancy of the rate depends on the nature of the cation present: Thus, in 0.1 *M* $NaHCO_3$, the rate declined during the first 10 hours and then became steady; in *M* $KHCO_3$, it increased during the first 10 hours, remained steady for the next 5 hours and then declined rapidly; in 0.065 *M* $NaHCO_3$ + 0.035 *M* $KHCO_3$, the rate remained steady for the first 15 hours and then began to decline (see fig. 25.1).

Noddack and Eichhoff (1939) stated that for a given suspension of *Chlorella*, the rate of photosynthesis is reproducible within $\pm 10\%$, and that these variations can be further reduced by preliminary adaptation of the cells to the light intensity in which they are to be studied.

Experiments with higher land plants or aquatic plants have been contradictory, and at first rather discouraging. True, Willstätter and Stoll (1918) found that detached leaves, properly supplied with water and carbon dioxide, maintain a constant rate of photosynthesis (within a few per cent) for 4 or 6 hours, even in strong light (40,000 lux); but Harder (1930, 1933), Arnold (1931) and Jaccard and Jaag (1932) asserted that strong trends as well as irregular changes develop in the photosynthesis of aquatic plants kept under constant external conditions. Arnold observed, *e. g.*, that in moderately strong light (18,000 lux) the rate of photosynthesis of *Elodea* dropped, in 2 or 3 hours, to one fifth or one tenth of its original value; at 4000–6000 lux, it increased for the first 2 or 3 hours and then decreased slowly; only at 2000–3000 lux did it remain approximately constant for several hours. Harder (1930, 1933) made similar observations and stated that light intensity must be measured relative to the intensity to which the plants have been "acclimated" before the experiment. By

changing the ratio of these two intensities, he obtained the family of curves represented in figure 26.8—some showing a steady increase in photosynthesis with time, others exhibiting an equally steady decline. He interpreted these time phenomena in terms of three plasmatic effects: "activation,"

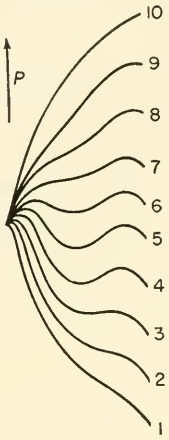


Fig. 26.8. Changes of photosynthesis with time in aquatic plants under constant conditions. Numbers indicate increasing ratios between the conditioning and the illuminating intensity (after Harder 1930).

"deactivation" and "exhaustion" (the last two being distinguished by the duration of the dark period required for recovery).

Experiments by Gessner (1937) and Steemann-Nielsen (1942) indicated, however, that only the phenomenon of induction (Harder's "activation") is of fundamental nature (it will be dealt with in chapter 33), whereas "deactivation" and "exhaustion" can be avoided—at least as far as water plants in light of about 40,000 lux are concerned—by preventing the stagnation of water, and the consequent dwindling of the carbon dioxide supply.

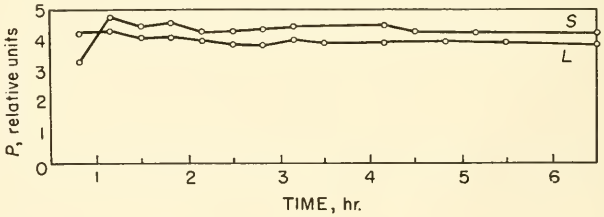


Fig. 26.9. Constant photosynthesis of a light-adapted plant (*L*) and a shade-adapted plant (*S*) of *Elodea canadensis* (after Gessner 1937).

That stagnant water in the immediate neighborhood of assimilating plants can easily be depleted of carbon dioxide (even if it contains reserves in the form of carbonates and bicarbonates) was first proved by the calculations of Romell (1927). Figure 26.9, taken from Gessner, shows the time course

of photosynthesis of two twigs of *Elodea canadensis*, one adapted to strong light and another to weak light, in a steadily renewed medium. Both show good constancy for many hours of uninterrupted illumination (after an initial induction period in the shade plant). Some of Gessner's experiments were extended over 6 days, with rate variations remaining within ±25%.

If to these results of Gessner with aquatic plants we add those of Warburg and his successors with unicellular algae, and of Hoover, Brackett, and Johnston (1933), Mitchell (1936) and Böhning (1949) with higher land plants, there appears to be no fundamental difference between plant

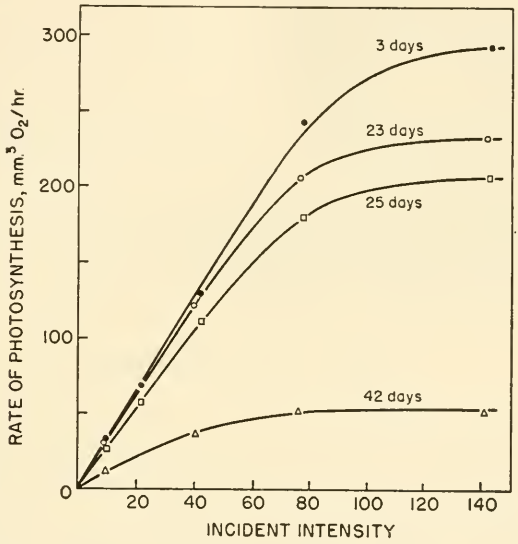


Fig. 26.10. Gas exchange of *Chlorella* cells from cultures of different ages (in air, temperature 29° C.) (after Wassink and Katz 1939).

classes with respect to their capacity to carry out uniform photosynthesis for considerable periods of time.

Experiments with carefully treated plant material show a simple, direct and reversible response of the rate of photosynthesis to at least two external factors, *light intensity* and *concentration of carbon dioxide* (and within certain narrow limits, also to changes in *temperature*).

Obviously the "internal factors" are in no way eliminated, even in such selected material. Their importance is revealed in the induction phenomena, in the permanent effects of age and previous treatment and in the fluctuations of the rate, by as much as 10 or 20%, which occur without any apparent external reason. Different plants or algal suspensions of the same

species (even when grown under apparently identical conditions) may differ in photosynthetic production by a factor of 2 or 3. Effects of age appear not only in the higher plants (see the comparison of young leaves with mature leaves in table 28.V, after Willstätter and Stoll, 1918) but also in cultures of unicellular algae (*cf.* fig. 26.10; van Hille 1937, 1938, Wassink and Katz 1939 and Pratt 1943). We cannot hope to *eliminate* these internal factors in the study of photosynthesis, but it *is* possible to keep them approximately constant, at least for the duration of an experiment. Furthermore, there is not much point in treating these internal factors as mysterious "plasmatic stimulations" or "inhibitions." It is reasonable to expect that some of them will be traced to accumulations or eliminations of certain *chemical compounds*—catalysts, poisons or metabolites—while others will be found to be connected with changes in the *physical structure* of the photosynthetic apparatus, for example, with the swelling or shrinking of the protoplasm or changes in permeability of cell membranes. In chapter 33 we will discuss the most extensively studied example of the action of "internal factors"—the induction phenomena, and will attempt to interpret them as a result of deactivation, in the dark, of certain catalysts required for photosynthesis, combined with the accumulation of metabolites possessing narcotizing properties.

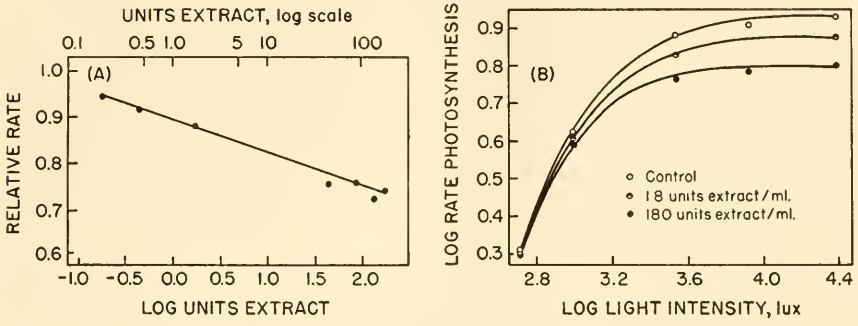
6. Aging and Self-Inhibition

The aging effect, in *Chlorella* in particular, shown in figure 26.10, has been correlated by Pratt (1943[1,2]) with the gradual accumulation of a growth-inhibiting substance of definite chemical and biological properties. Since this is the first case in which an "internal factor" in photosynthesis was identified as a chemical entity, the observations of Pratt will be described in some detail.

The production of a growth-inhibiting factor in *Chlorella* cultures was discovered by Pratt in 1940 (*cf.* chapter 25, page 833). Later (1942) he observed that this "factor" is a substance that can be extracted from "aged" cells, and whose action can then be demonstrated on young cultures. Its molecules are ~15 Å in diameter; it is soluble in 95% ethanol, ether, petroleum ether and water and is destroyed by heat. It is more effective in neutral than in acid solution, and can more easily be extracted from alkaline than from acid aqueous solutions. It has considerable antibiotic effect on bacteria.

The water-soluble growth-inhibiting substance (prepared by extracting dried cells of 50–60 day old *Chlorella vulgaris* cultures with 95% ethanol, evaporating to dryness and extracting with water) was tested for its effect on photosynthesis of *Chlorella* cells from 4 day old cultures (Pratt 1943).

Figure 26.11A shows that the rate declined linearly with the logarithm of the amount of extract added. Experiments at various light intensities (fig. 26.11B) showed that the inhibitor affects the saturation rate in strong light rather than the quantum yield in weak light, *i. e.*, it acts like a catalyst



A B

Fig. 26.11. Effect of extract from aged *Chlorella* cells on photosynthesis of young cells in 0.1 *M* $KHCO_3$ (one unit = extract from 10^6 dried cells) (after Pratt 1943).

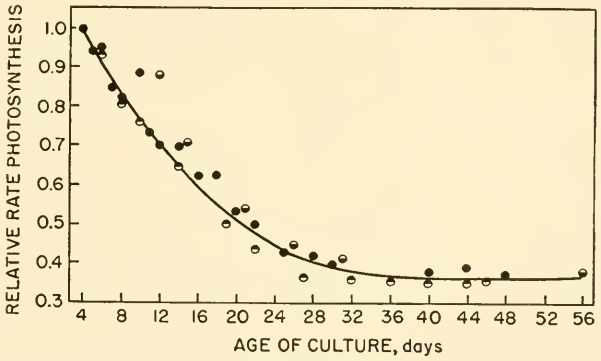


Fig. 26.12. Decline of photosynthesis in *Chlorella vulgaris* with age (after Pratt 1943). The rate in 4 day old cultures (taken as unity in the figure) was 8.5 to 10 mm.^{3} O_2/(10^8 cells × min.).

poison rather than like a narcotic (the difference between these two types of poisoning was explained in chapter 12, Vol. I).

In a logical development of these observations, Pratt (1943[2]) then studied the decline of photosynthetic activity of aging *Chlorella* cultures, and found complete parallelism between this phenomenon and the accumulation of the growth-inhibiting substance. The study was made in 5% carbon dioxide in air, with 15,000 lux illumination (which, according to

fig. 26.11B, is sufficient for saturation). Figure 26.12 shows the decline of the rate with advancing age; after 30 days, the rate was only one third the original—in fair agreement with Wassink and Katz's results in figure 26.10. Respiration also declined during the same period, but a little less than photosynthesis (to about one half the initial rate). The concentration of chlorophyll and the size of the cells showed no marked change.

The influence of the inhibitor produced by aged cultures can be recognized even in the "second generation". The cultures prepared by inoculation with a 3 day old culture showed, after 5 days' growth, a 10% higher rate of photosynthesis than a similar culture prepared by inoculation with material from a 23 day old culture. During the 5 days' growth, the number of cells has increased by a factor of 20, but the effect of the inhibitor was noticeable even after this dilution.

Pratt, Oneto and Pratt (1945) found that in *Chlorella* cultures grown in continuous light in inorganic medium, the inhibiting agent ("chlorellin") accumulated in the medium rapidly in the first 3–4 days; its concentration decreased sharply in the next 2–4 days, and then grew again, reaching a constant level in about 2 weeks. The decline in chlorellin concentration occurs at the period when the increase in the number of cells per unit volume is most rapid. Possibly, rapidly dividing cells use up all the available chlorellin for their own metabolism (assuming it is a useful product); alternatively (if chlorellin is merely a poison, not only for bacteria but also for the algae themselves), these cells may produce an antidote to this poison.

"Chlorellin" production was also studied, in co-operation with Pratt and his group, by Spoehr and co-workers (1944, 1945, 1946). They worked on improvement of the methods of cultivation of algae and extraction of the antibiotic, first using cell-free culture medium, and later, the cells themselves. The quantity of antibiotic extractable from dried cells was found to increase by allowing the latter to undergo oxidation (by grinding and exposing to light in air). Brown, partly crystalline material, extracted from dried cells with 80% methanol containing 2% KOH, and transferred into petroleum ether after acidification, is nonantibiotic; it becomes colorless and antibiotically active by oxidation in light. It thus seems that living cells contain no antibiotic material; the latter is formed by oxidation, both in the medium and in dried and ground cells (it remains to be proved that the two antibiotic products are identical). The cell-derived antibiotic is a lipoid—probably a mixture of unsaturated fatty acids. Pure acids of this type (linoleic, elaidic, etc.) also show no antibiotic activity before exposure to oxygen and light, but acquire antibiotic properties after such exposure. Autoxidation of unsaturated compounds is known to produce peroxides whose bactericidal effect is well established.

However, the antibiotic activity of "chlorellin" is not affected by thiourea and potassium iodide, which destroy labile peroxides. Spoehr and coworkers noted considerable similarity between "chlorellin" and the first antibiotic, discovered fifty years ago (but never chemically identified)—the pyocyanase from *Pseudomonas ruginosa*. In both cases, the material seemes to be a mixture of several unsaturated fatty acids.

Further chemical study showed that the mixture obtained by photoxidation of the fatty acids from *Chlorella* contained short-chain acids (about C_{11}) separable by distillation, and showing a relatively strong antibacterial activity.

The studies of Pratt and Spoehr offer a promising approach to the understanding of at least some of the phenomena in photosynthesis usually attributed to not further identifiable "plasmatic" or "internal" factors.

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Chapter 27

CONCENTRATION FACTORS

In this chapter, we will describe how the rate of photosynthesis and the yield of chlorophyll fluorescence depend on the concentration of the reactants. In the ordinary photosynthesis of green plants, the only reactant the amount of which can be varied freely is the oxidant, *carbon dioxide*. True, the activity of the reductant, *water*, also can be changed within certain limits (*cf.* Vol. I, chapter 13, page 333); but the effect of such variations is in the main an indirect one: Changes in hydration affect the colloidal state of the protoplasm, which in turn influences all the activities of the living cell. In bacterial photosynthesis ("photoreduction," *cf.* chapter 5, Vol. I), where *hydrogen*, *hydrogen sulfide*, *thiosulfate* or another inorganic or organic reductant takes the place of water, its concentration can be varied as easily as that of the oxidant, carbon dioxide. Thus, bacteria (and "hydrogen adapted" algae, *cf.* chapter 6) open a new approach to the kinetic study of photosynthesis. Finally, the "Hill reaction" (chapter 6, page 63, and also chapter 35, in whole cells or in isolated chloroplast material, permits one to measure the influence of concentration of substitute oxidants (Fe^{+++}, quinone, chromate, etc.) on the rate of liberation of oxygen.

We will also describe in this chapter the effect on photosynthesis and fluorescence of varying amounts of additions such as catalyst poisons, narcotics and inorganic ions. This section (part D) forms a quantitative elaboration (and contains, inevitably, some repetition) of the qualitative information presented in chapters 12 and 13 in Volume I.

A. Experimental Carbon Dioxide Curves*

1. Carbon Dioxide Molecules and Carbonic Acid Ions

In the "carbon dioxide curves," which will be discussed in this chapter, the rate of photosynthesis is plotted as a function of the concentration of carbon dioxide, while all the other kinetic conditions are assumed to be constant. The concentration of *free*, neutral carbon dioxide molecules, $[CO_2]$, will be used as the independent variable, whether the experiments

* Bibliography, page 960.

were carried out with land plants, in an atmosphere containing gaseous carbon dioxide, or with aquatic plants, in either acid or alkaline solutions, despite the fact that in the last-named case carbonic acid was present mainly in the form of carbonate and bicarbonate *ions*. This presentation is chosen because the neutral molecular species CO_2 easily enters and leaves the cell, while ions appear to encounter a much greater difficulty in diffusing through the cell membrane. Consequently, the intracellular concentrations of all molecular species of carbonic acid, including the ions HCO_3^- and CO_3^{2-}, probably are determined mainly by the extracellular concentration of the species CO_2 and largely independent of the concentration of the carbonate ions (and thus also of the *p*H of the medium, since at a given value of $[CO_2]$, variations of $[HCO_3^-]$ and $[CO_3^{2-}]$ are uniquely correlated with changes in the *p*H).

This simplification is convenient, but is likely to prove an oversimplification. In chapter 8 (Vol. I, page 195), we described the controversy between Natansohn (1907, 1910), Wilmott (1921), Romell (1927) and James (1928), on the one hand, and Angelstein (1911) and Arens (1930, 1933, 1936), on the other. Natansohn believed that only the concentration of neutral carbon dioxide molecules in the medium is of importance for the photosynthesis of aquatic plants; the occasionally observed rate-enhancing effect of bicarbonate ions (quoted by Angelstein as proof of their availability for photosynthesis) was interpreted by Wilmott and Romell as a buffering effect. (The dissociation of HCO_3^- ions into OH^- and CO_2 provides ample replacement for the carbon dioxide molecules used up by photosynthesis.) James found, in fact, that, if care is taken to avoid exhaustion of carbon dioxide by strong circulation, the influence of bicarbonate ions on the rate of photosynthesis tends to disappear. In many plants, excess carbonates may even produce an inhibition attributable either to alkaline reaction (*cf.* Vol. I, page 339) or to the damaging effect of the cations (as indicated by the different influences of the bicarbonates of sodium and potassium; *cf.* Vol. I, page 340, and p. 835). Experimental support of Natansohn's concept was provided by the experiments of Osterhout and Dorcas (1926), in which the rate of penetration of carbonic acid into the interior of giant *Valonia* cells was proved to be proportional to the external concentration of the carbon dioxide molecules and independent of the concentration of the anions of carbonic acid.

However, Arens (1930, 1933, 1936) observed that, in light, HCO_3^- ions were taken up by the lower surface of leaves of aquatic plants (such as *Elodea*), while CO_3^{2-} or OH^- ions were set free at the upper surface. He interpreted this observation as proof that HCO_3^- ions actually penetrate into cells and are used there for photosynthesis, either completely, according to the equation:

$$HCO_3^- \longrightarrow CO_2 + OH^-$$

or partially, according to the equation:

$$2\ HCO_3^- \longrightarrow CO_3^{2-} + H_2O + CO_2$$

In Volume I (page 157), we said that Arens' results are in need of experimental verification, and that, if they prove to be correct, they may be explained (*a*) by the diffusion of ions through the leaf without penetration into the interior of cells, and (*b*) by cell wall penetration by neutral salt molecules, such as $KHCO_3$. In connection with the latter possibility, it would be important to obtain *quantitative* information on the rate of penetration of bicarbonate as compared to that of free carbon dioxide; conceivably, the observations of Arens could be explained even if the first rate is only one hundredth or one thousandth of the second one, and thus negligible from the point of view of the kinetics of photosynthesis.

By considering the penetration problem as a quantitative rather than qualitative one, we can anticipate that the influence of the external concentrations $[HCO_3^-]$ and $[CO_3^{2-}]$ on the carbonic acid system inside the cell will depend on whether we deal with an approximate equilibrium (*i. e.*, work in the dark, or in low light), or with a photostationary state in which carbonic acid is rapidly consumed by photosynthesis. In the first case, even a very slow penetration of salt molecules may result in considerable changes in the composition of the cell fluids, while, in the second case, the effect of such slow penetration may be completely negligible in comparison with that of the much more rapid flow of CO_2 molecules.

More recently, Steemann-Nielsen (1946) revived Angelstein's (1911) argument. He studied the rate of photosynthesis as a function of the concentration $[CO_2]$ in the medium, using the two aquatic plants *Myriophyllum spicatum* and *Fontinalis antipyretica*. In *Fontinalis*, the rates of oxygen liberation found in alkaline solutions (*p*H 8.3, containing from 0.5×10^{-3} to 5×10^{-3} mole HCO_3^- per liter, together with from 0.5×10^{-5} to 5×10^{-5} mole CO_2 per liter) were hardly different from those observed in acid solutions with the same amount of CO_2, but practically no HCO_3^- ions. A significantly different result was obtained with *Myriophyllum:* In this plant, the yield of photosynthesis in alkaline bicarbonate solutions was ten times higher than in acid solutions with the same content of CO_2 molecules! In some alkaline solutions the rate of oxygen liberation by *Myriophyllum* was as much as one third of the rate found in acid solutions with the same *total* concentration ($[CO_2] + [HCO_3^-]$). This was interpreted by Steemann-Nielsen as indication that *Myriophyllum* uses HCO_3^- ions *directly* with about one third the efficiency with which it uses neutral CO_2 molecules. The difference between the two species, *Fontinalis* and *Myriophyllum*, was tentatively related by Steemann-Nielsen to the fact that

Myriophyllum grew in a locality where the *p*H in summer was as high as 9–10 (corresponding to a ratio of 100 HCO_3^- to 1 CO_2), while *Fontinalis* was gathered in a locality where the water contained very little bicarbonate, but as much as 30×10^{-5} mole per liter of free CO_2 molecules (a remarkably high figure, if one recalls that water equilibrated with the free atmosphere contains only about 1×10^{-5} mole per liter CO_2).

The behavior of the two species is illustrated by figures 27.1A and B. One can either accept these figures as evidence of direct participation of

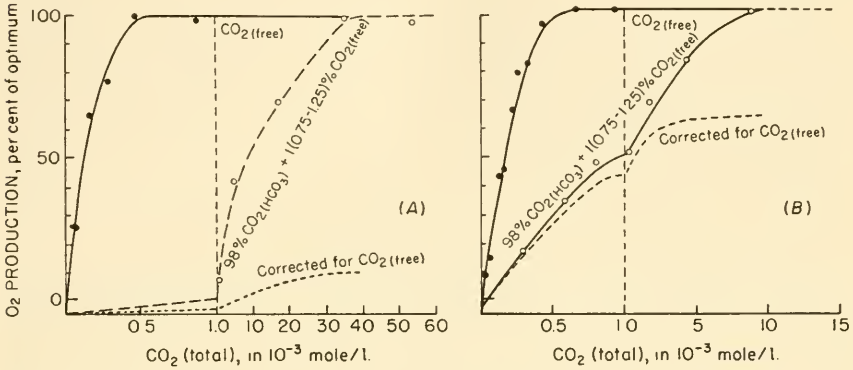

Fig. 27.1. (A) Photosynthesis in *Fontinalis antipyretica*, at 15,000 lux, 22° C., as function of total carbonate concentration; (B) Photosynthesis in *Myriophyllum spicatum*, at 37,000 lux, 20° C., as function of total carbonate concentration (after Steemann-Nielsen 1946).

HCO_3^- ions in the photosynthesis of *Myriophyllum* (or, more exactly, of comparatively easy penetration of HCO_3^- ions into the cells of this plant), or one must assume that, for some reason yet unknown, the buffering action of bicarbonate was much more effective with *Myriophyllum* than with *Fontinalis*.

It will be noted that the "free carbon dioxide" curves are practically identical for both species; in other words, if we attribute the shapes of these curves to carbon dioxide depletion, we must assume that the latter has been equally strong in both experiments. We must then assume that addition of about 100 HCO_3^- to 1 CO_2 has had very little effect on depletion in the case of *Fontinalis*, but had reduced it by about one third in that of *Myriophyllum*. Whether this explanation is plausible is difficult to say without the knowledge of various relevant factors, such as the absolute rate of photosynthesis per unit area, shapes of the two plants (ratio of volume to surface; *cf.* page 905) and the efficiency of stirring.

In chapter 13 (Vol. I) we mentioned the *effect of cations* on the rate of photosynthesis, in particular the difference between the rates in sodium carbonate and potassium carbonate buffers, as observed by Pirson and later by Pratt. Some additional information on the unfavorable effect of sodium bicarbonate was given in chapter 25. Steemann-Nielsen

(1946) enlarged these observations by determining the carbon dioxide curves of photosynthesis in solutions containing different cations. He found that, with *Myriophyllum spicatum*, in acid solution, the presence of sodium or calcium (in the form of chlorides) had no effect on the rate of photosynthesis. In alkaline solutions, on the other hand, the rate was lowest in sodium bicarbonate, higher in potassium bicarbonates and still higher in calcium bicarbonate. The highest rate could be obtained in a solution containing K^+, Na^+, Ca^{2+}, Cl^-, and SO_4^{2-} ions in the same proportion as the water of the lake that was the natural habitat of the plants. In lake water, the rate was independent of *p*H between 8.5 and 10.5, while in potassium carbonate–bicarbonate buffer (10^{-3} mole HCO_3^- per liter) the rate increased slowly between *p*H 8.4 and 10.5 and dropped sharply to zero at *p*H 11.

These results can be interpreted in terms of Steemann-Nielsen's concept of direct participation of bicarbonate ions in photosynthesis (for example, by assuming different rates of penetration of different neutral molecules, $MeHCO_3$, through the cell membrane; *cf.* Vol. I, page 197), but they may also be of a more indirect and complex origin. According to Pratt (*cf.* fig. 25.1) the cation effects are largely irreversible, a complication not considered by Steemann-Nielsen.

Tseng and Sweeney (1946) studied the red alga *Gelidinium cartilagineum* and found that the rate of its photosynthesis was determined exclusively by the concentration of free carbon dioxide molecules, $[CO_2]$, and *not* affected by the simultaneous presence of a large number of bicarbonate ions, $[HCO_3^-] \geq 10\ [CO_2]$.

Ruttner (1947) compared the limiting *p*H values established in water as the result of prolonged photosynthesis of different aquatic plants. *Elodea* (*canadensis* or *densa*), *Photomageton*, *Myriophillum prismatum*, *Lemma trisulia* and several other aquatic phanerogams continued to reduce carbon dioxide until the *p*H rose considerably beyond *p*H 9; while several mosses (such as *Fontinalis antipyretica*) ceased to assimilate carbon dioxide when *p*H reached 9.0. At the latter *p*H, $[CO_2] = 0.4 \times 10^{-5}$ mole/l.; this is the region in which the "carbon dioxide compensation point" was found previously with land plants (*cf.* page 899). Ruttner suggested that the capacity of aquatic higher plants to carry out net positive photosynthesis at $[CO_2]$ equilibrium values $\ll 1 \times 10^{-5}$ mole/l., if bicarbonate is present, indicates their capacity to utilize bicarbonate ions *directly*, and not merely as source of CO_2 molecules in the medium. In a second paper, Ruttner (1948) gave evidence supporting the assumption that the cessation of photosynthesis of *Fontinalis* at *p*H 9 is the result of low CO_2 concentration (0.4×10^{-5} mole/l., corresponding to about 0.01 vol.%), and not of excess alkalinity. Earlier observations of Shutov (1926), Bode (1926) and Dahm (1926), who found that many aquatic plants can raise the *p*H of the medium to values as high as 11.8 (*Spirogyra*), can then be interpreted as meaning that these plants, too, can use bicarbonate ions directly as source of carbon for photosynthesis (or, more exactly, as a vehicle to transport carbon dioxide from the medium into the cells).

Österlind (1948,1949) went even further than Steeman-Nielsen and Ruttner, and asserted that certain plants use bicarbonate ions more effectively than carbon dioxide molecules. He noted that the alga *Scenedesmus quadricauda* did not grow at all at *p*H 5.5 (in a solution aerated with ordinary air). It reached a high rate of growth at about *p*H 6.5, and increased it slowly up to *p*H 9. According to Österlind, this increase is not an effect of alkalinity, but a consequence of the presence of bicarbonate ions. He based this conclusion on the observation that, in air containing 5% CO_2, good growth could be obtained even at *p*H 3–4. The maximum rate of growth was reached when $[HCO_3^-]$ exceeded 9×10^{-6} mole/l.; between 2 and 8×10^{-6} mole/l., the rate was proportional to $[HCO_3^-]$. Österlind thought that, with the cell populations used, no

carbon dioxide exhaustion could occur even in the solutions which contained no bicarbonate ions. He also noted that, with 10×10^{-6} mole/l. of HCO_3^- present, growth was as much as twenty-five times faster than with 10×10^{-6} mole/l. of carbon dioxide; and concluded that *Scenedesmus quadricauda* uses bicarbonate ions (for growth, and thus presumably also for photosynthesis) twenty-five times more efficiently than free carbon dioxide molecules. Later (1950[b,2]) Österlind found that *Chlorella pyrenoidosa* does not use bicarbonates; since he found no difference in the carbonic anhydrase content of the two species, he suggested that their cell membranes are different.

Pending further analysis concerning the role of carbonate ions (a question which the above-described experiments have reopened), we will proceed on the old assumption that the rate of photosynthesis is primarily a function of the concentration of the molecular species CO_2 in the immediate surroundings of the cells, and that the main effect of the presence of HCO_3^- ions is to prevent this concentration from depletion during photosynthesis.

Figures collected in Table 27.I, apart from those given for *Myriophyllum* by Steemann-Nielsen, give no indication of a large, direct contribution of carbonate ions to photosynthesis. We note, for example, that Emerson and Green (1938) were able to achieve carbon dioxide saturation of photosynthesis in an acid phosphate buffer when the medium contained only 0.7×10^{-5} mole/l. CO_2. In experiments with carbonate buffers, in which each carbon dioxide molecule was accompanied by 1000 bicarbonate ions (and as many or more carbonate ions), saturation usually was observed either at approximately the same or at an even higher value of $[CO_2]$.

2. General Review of Carbon Dioxide Curves

The necessity of carbon dioxide ("fixed air") for photosynthesis was discovered by Senebier in 1782 (*cf.* Vol. I, chapter 2). The earliest quantitative studies of the relation of the rate of photosynthesis to the concentration of carbon dioxide were made by Kreusler in 1885 and 1887, Brown and Escombe in 1902 and Treboux, and Pantanelli, both in 1903. Since these observations showed an increase of the rate with increasing $[CO_2]$ in the region of low concentrations, and a decline at high concentrations, they were interpreted on the basis of the then popular "optimum theory" (fig. 26.1), until Blackman and Smith suggested in 1911, that they can better be explained by the concept of "limiting factors." Blackman pointed out that no evidence existed of a "minimum" $[CO_2]$ required for the beginning of photosynthesis, or of a sharp "optimum"; instead of the latter, experiments showed a wide range of $[CO_2]$ values over which the rate remained approximately constant. As described in chapter 26, Blackman claimed that correctly determined carbon dioxide curves must be broken lines of the shape shown in figure 26.2; and many investigations have been carried out with the expressed purpose of "proving" or "disproving" this "law."

TABLE 27.I
DETERMINATIONS OF CARBON DIOXIDE CURVES IN PHOTOSYNTHESIS

Observer	Plant species	Medium	Light intensity (in klux if not otherwise designated)	$[CO_2]$ in 10^{-5} mole/l. required for: Half saturation	Full saturation
		HIGHER LAND PLANTS			
Hoover, Johnston, Brackett (1933)	*Triticum vulgare* (wheat)	CO_2–air circulated	0.8	0.6	2
			4.0	0.8	3.2
			9.5	1.6	5.6
Singh, Kumar (1935)	*Raphanus sativus* (radish)	CO_2–air circulated	5.73 28.65 68.76	≦100	≦200
Singh, Lal (1935)	*Saccharum* (sugar cane)	CO_2–air circulated	3.75[a]	—	≦4
			15.0	—	≦≦4
			18.75	—	≦≦6
	Triticum (wheat)	CO_2–air circulated	18.75	—	≦≦6
	Linum (flax)	CO_2–air circulated	18.75	—	≦6
Stocker, Rehm, Paetzold (1938)	*Impatiens sultani*	CO_2–air (held)	Sun	—	7
	Plectranthris fruticosus			—	7
	Codineum hybridum			—	3.5
		AQUATIC HIGHER PLANTS			
Blackman, Smith (1911)	*Fontinalis antipyretica*	CO_2 in water	Gas burner	200	400
	Elodea canadensis	CO_2 in water	Gas burner	100	200
Harder (1921)	*Fontinalis antipyretica*	$KHCO_3$ soln.	0.611	2	
			2	4	>32
			18	15	
	Chladophora	$KHCO_3$ soln.	18	7.7	
James (1928)	*Fontinalis antipyretica*	$KHCO_3$ soln.	20 rel. units	12	>80
			40 rel. units	40	
Smith (1937)	*Cabomba caroliniana*	Carbonate buffers	6.31[b]	1.5	≦10
			21.9	3	>10
			282	8	>30
Smith (1938)	*Cabomba caroliniana*	Carbonate buffers	0.41	<1	≦ 0.5
			1.74	1	7.5
			6.31	2	≦≦20
			21.9	3.5	≦≦30
			123.0	4	>30

Observer	Plant species	Medium	Light intensity (in klux if not otherwise designated)	$[CO_2]$ in 10^{-5} mole/l. required for	
				Half saturation	Full saturation
Steeman-Nielsen (1946)	*Myriophyllum spicatum*	Acid CO_2 soln.	32	17	50
		Alkaline soln. (*p*H 8.4)	32	1	10
	Fontinalis antipyretica	Acid CO_2 soln.	15	17	50
		Alkaline soln. (*p*H 8.4)	15	10	35
		ALGAE			
Warburg (1919)	*Chlorella*	Carbonate buffers	300 W. lamp (20 cm. away)	0.4	9
Emerson, Green (1938)	*Chlorella pyrenoidosa*	Phosphate buffers (*p*H 4.6)	Saturating	0.35	0.7
Emerson, Arnold (1932)	*Chlorella pyrenoidosa*	Carbonate buffer No. 9	Saturating	~1	~10
van der Honert (1930)	*Hormidium flaccidum*	CO_2–air stream over wet cells	2.0 rel units	0.25	2
			6.2 rel. units	0.6	2
van der Paauw (1932)	*Hormidium flaccidum*	CO_2–air stream over wet cells	150 W. lamp (13 cm. away)	0.8	1.6
Emerson, Green (1934)	*Gigartina harveyana* (red) (thick tissue)	Carbonate buffers	60 W. lamp (8 cm. away)	8	35
Tseng, Sweeney (1946)	*Gelidium cartilagineum* (red)	Bicarbonate solutions	5 or 8 fluorescent lamps	~5	≥11
Barker (1935)	*Nitzschia palea* (diatom)	Carbonate buffers	75 W. lamp (9 cm. away)	0.1	0.5
		PURPLE BACTERIA			
Katz, Wassink, Dorrestein (1941)	*Chromatium* D	CO_2 in phosphate buffer (*p*H 6.3), 1% thiosulfate	Yellow light 5 kerg./cm.^{2} sec.	—	<150[c]
Wassink, Katz, Dorrestein (1942)	*Chromatium* D	CO_2 in phosphate buffer (*p*H 6.3), 15% H_2	Same, 30 kerg./cm.^{2} sec.	25[c]	>75[c]
			Same, ~ 25 kerg./cm.^{2} sec.	8[c]	40[c]

[a] Intensity given in "candle powers" probably meaning meter candles.
[b] Intensity measured in klux *before* passage through red filter; only red light (approximately 22% of total white light) used.
[c] Assuming $[CO_2] = 0.5([CO_2] + [HCO_3^-])$ at *p*H 6.3.

It hardly needs repeating that the problem must be treated, not from the point of view of an apodictic "law," but on the basis of the general principles of reaction kinetics, and that these principles admit of "limiting factors" only as approximations, useful under certain extreme conditions.

Table 27.I gives a summary of the most important experimental determinations of the carbon dioxide curves of photosynthesis, since the time of Blackman and Smith. As a general rule, these curves rise rapidly at first, then more slowly and finally go over into "saturation plateaus." At excessively high $[CO_2]$ values, the rate may decline again. Table 27.I gives, in the last two columns, the concentrations found necessary to produce full carbon dioxide saturation and half saturation, respectively. (When the approach to saturation is gradual, the second figure can often be given with more precision than the first one.)

We note that the observed saturating concentrations vary all the way from 0.5×10^{-5}, to 400×10^{-5} mole/l. CO_2. It will be shown in section 5 that the higher values are beyond doubt due to depletion of carbon dioxide in the medium surrounding the plants, and consequent establishment of large external concentration gradients. They can be strongly reduced by accelerated circulation or buffering. The lower values, on the other hand, may be determined either by diffusion resistance which is not affected by buffering or stirring (*e. g.*, that of the stomata, air channels, of adsorption layers, cell walls and cytoplasm), or by intrinsic kinetic characteristics of photosynthesis (such as the carboxylation equilibrium and the rate of carboxylation).

In the general discussion of the kinetic curves of photosynthesis in chapter 26, three types of curve sets, $P = f[F_1]$, with F_2 as parameter, were considered and designated as the first (or "Blackman") type, the second (or "Bose") type, and the third type (see figs. 26.2, 26.3 and 26.4, respectively). It was stated that curves of the *first* type must arise when the parameter, F_2, determines the maximum rate of a partial process that does not depend on the independent variable, F_1, and therefore imposes a horizontal "ceiling" on the curves $P = f(F_1)$, without affecting the initial slope of these curves. Curves of the *third* type are found when the parameter affects only the initial slope of the light curves, for example, if it codetermines the rate of a process that is also proportional to the independent variable, F_1. In curve systems of the *second* type, the parameter affects both the initial slope *and* the saturation level. Carbon dioxide curves of all three types can be expected under appropriate conditions; theoretical examples were given in chapter 26. So far, however, the only experimentally determined carbon dioxide curve sets have been obtained with light intensity as parameter. Four sets of such curves, which appear comparatively reliable as far as the measuring technique is concerned, are reproduced in figures

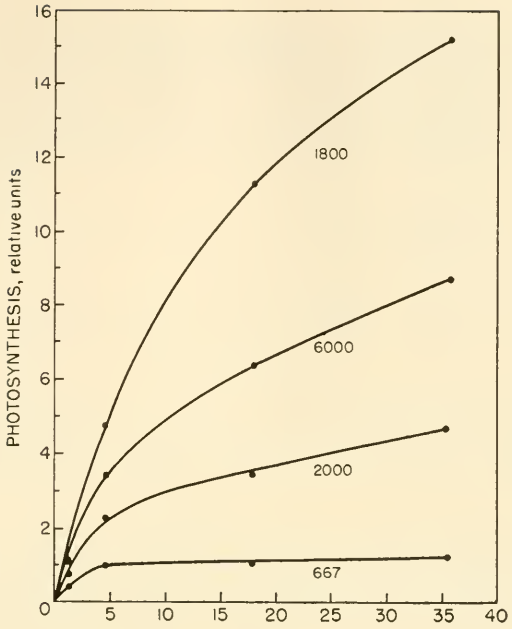

Fig. 27.2A. Carbon dioxide curves of *Fontinalis antipyretica* at various light intensities (in lux) (after Harder 1921). Abscissa, $[CO_2]$ in (mole/l.) $\times 10^5$.

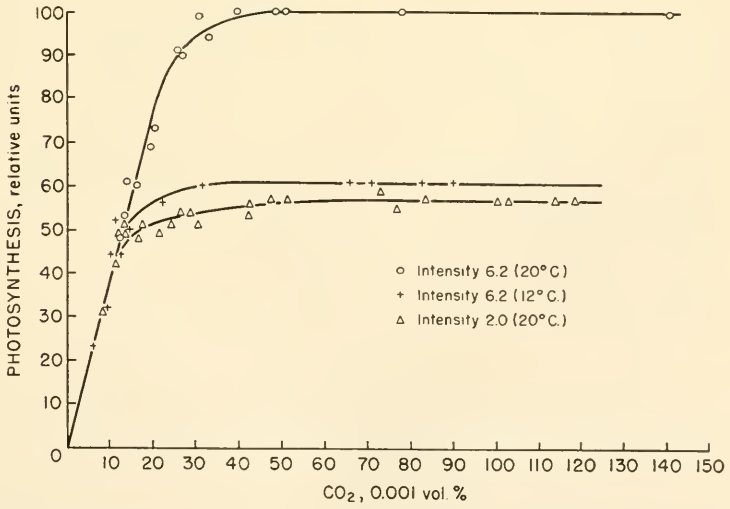

Fig. 27.2B. Carbon dioxide curves of *Hormidium flaccidum* at two light intensities (in relative units), and two temperatures (after van der Honert 1930). 0.01 volume per cent CO_2 corresponds at 20 °C. to 3.37×10^{-6} mole/l. (*cf.* Vol. I, p. 174).

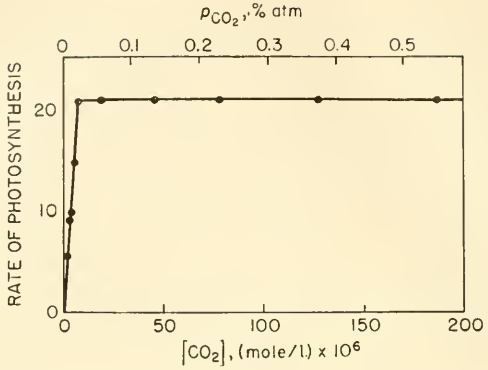

Fig. 27.2C. Carbon dioxide curve of *Chlorella pyrenoidosa* (after Emerson and Green 1938). *M*/25 phosphate buffer; *p*H 4.6; 25° C.; rate in mm.^{3} O_2/hr. per mm.^{3} of cells.

27.2A, 27.2B, 27.3 and 27.4. Figure 27.2B (taken from van der Honert's work on *Hormidium*, 1930) closely resembles Blackman's prototype: At low concentrations, all curves merge into a single straight line; close to saturation they sharply turn horizontal. Emerson and Green (1938) gave a single $[CO_2]$ curve for *Chlorella* in saturating light, which showed an even earlier and more sudden saturation: It rose linearly up to $[CO_2] = 0.7 \times$

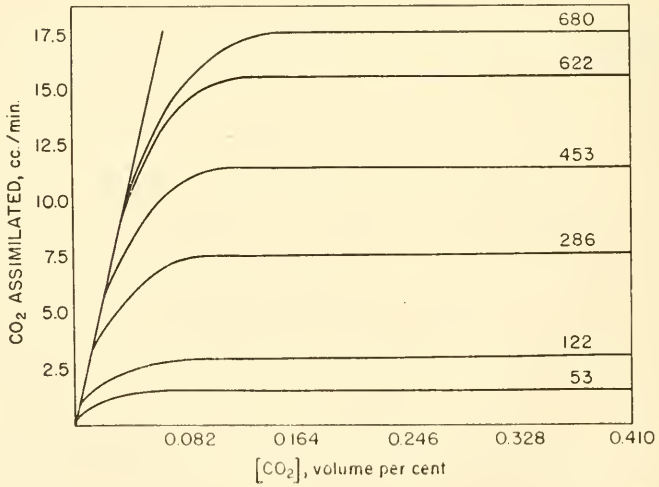

Fig. 27.3. Carbon dioxide curves of whole *Triticum* (wheat) plants at different light intensities (after Hoover, Johnston and Brackett 1933). Parameters in kerg/(cm.^{2} sec.).

10^{-5} mole/l. and then abruptly became horizontal (fig. 27.2C). In this figure the maximum yield corresponds to one volume oxygen per volume of cells each three minutes. The rate values were obtained by admitting a known amount of carbon dioxide into a Warburg vessel, shaking vigorously and measuring the pressure changes at short intervals until all carbon dioxide was used up.

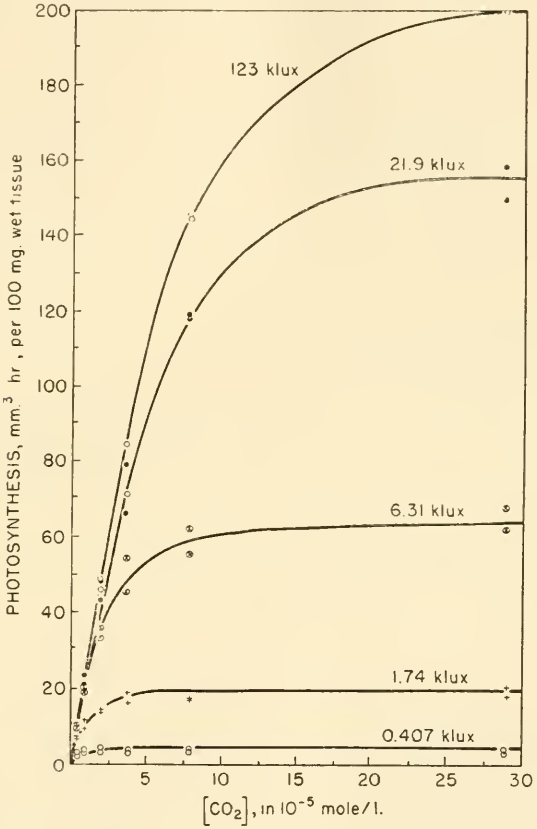

Fig. 27.4. Carbon dioxide curves of *Cabomba caroliniana* (after Smith 1938).

Figures 27.3 and 27.4, obtained with wheat and with the water plant *Cabomba*, respectively, show a more gradual approach to saturation, but they, too, indicate a coincidence of all curves at low [CO_2] values, which is characteristic of the Blackman type. Harder's *Fontinalis* curves (fig. 27.2A), on the other hand, are of a pronounced "Bose type": Curves corresponding to different light intensities diverge from the beginning, and

remain in an approximately constant ratio; saturation is approached very gradually, and is not quite reached at 32×10^{-5} mole/l., even on the curve that corresponds to an illumination of only 2000 lux. Harder's curves indicate that, in his plants, the rate of oxygen liberation did not become limited entirely by carbon dioxide supply even at the lowest used bicarbonate concentrations, and did not become independent of $[CO_2]$ even at the highest used concentrations. The range studied, 0.03 to 0.3% $KHCO_3$, *i. e.*, from 4×10^{-5} to 40×10^{-5} mole/l. CO_2 (*cf.* Vol. I, page 178), was, however, a rather narrow one. The curves in figs. 27.2–4 clearly tend to coincide only below 1×10^{-5} mole/l.

In the theoretical discussion later in this chapter, we will see that carbon dioxide curves that diverge from the origin can be expected if the carbon dioxide–acceptor complex, ACO_2, is not fully saturated with carbon dioxide, at low $[CO_2]$ values, even in the *equilibrium* state; while carbon dioxide curves that coincide at low $[CO_2]$ values can be predicted if the carbon dioxide dependence of photosynthesis is due entirely to the limitation of the *rate* of processes by which carbon dioxide is made available for photosynthesis (such as liberation of CO_2 from HCO_3^-, diffusion, and carboxylation of an "acceptor").

Table 27.I shows that, with increasing light intensity, the "half saturation point," which we will designate by $_{1/2}[CO_2]$, generally shifts toward the higher concentrations; so that, in very intense light, it may fall considerably beyond 10×10^{-5} mole/l. This fact *can* be significant, indicating certain kinetic conditions (as will be shown later in this chapter, see p. 934 ff); often, however, it merely means increasing depletion of carbon dioxide in the neighborhood of the cells when photosynthesis proceeds at a faster rate.

3. Carbon Dioxide Compensation Point

For each light intensity, there must exist a carbon dioxide concentration at which photosynthesis just compensates respiration and the net gas exchange is zero, and below which respiration exceeds photosynthesis. This "carbon dioxide compensation point" has not been studied in the same systematic way as was the "light compensation point" (*cf.* Table 28.III); Miller and Burr (1935) first devoted an investigation to it. In their experiments, a large variety of potted plants were enclosed in vessels filled with gas mixtures of different composition and illuminated with white light of about 20,000 lux, until all observable gas exchange stopped, *i. e.*, until the carbon dioxide concentration had declined to the compensation point. It was found that this occurred—at temperatures from 5° to 35° C.—when the carbon dioxide content was down to about 0.01%. At low

temperatures, this gas composition remained unchanged for several hours; at 35–37° C., after a short period of constancy, the carbon dioxide pressure began to increase again—probably because photosynthesis suffered slow thermal inhibition (*cf.* chapter 31), while respiration remained constant.

Thomas, Hendricks and Hill (1944) found that in beet plants, at 15° C., photosynthesis compensated respiration at $[CO_2] \simeq 0.003\%$—about one third of the value found by Miller and Burr. Gabrielsen (1949) found a value of 0.009 vol.% for the CO_2 compensation point of *Sambucus* leaves at 10 klux.

In submerged plants, one has to distinguish between the compensation point at constant *p*H and the steady state reached after prolonged photosynthesis with limited carbon dioxide supply: In the latter case, both $[CO_2]$ and *p*H change with time (OH^- ions being left behind when CO_2 is withdrawn from HCO_3^-), and the final steady state may be determined by either one or both of these factors. We have referred above to the experiments of Dahm (1926), Shutov (1926) and Ruttner (1947, 1948), which were interpreted by Ruttner (1948) and Österlind (1948, 1949) as indicating that some aquatic phanerogams and algae can use bicarbonate ions so efficiently that the presence of a minimum concentration of free CO_2 molecules is not needed to maintain their photosynthesis; these plants are able to continue -net synthesis even after $[CO_2]$ has been reduced to 10^{-6} mole/l. or less, and *p*H had risen above 10 or 11. (The *p*H of the sap inside the cells remains approximately neutral.) Aquatic mosses, such as *Fontinalis*, on the other hand, cease to liberate O_2 when $[CO_2]$ is reduced to some such value as 0.4×10^{-5} mole/l. (Ruttner 1948). This corresponds to 0.01 vol. % CO_2 in the atmosphere, and indicates a compensation point similar to that found with land plants. In the steady state reached by photosynthesis of aquatic plants of this type, the reaction of the medium is below, or about equal to *p*H 9.

A rather striking observation of Miller and Burr was that the carbon dioxide compensation point did not depend on temperature. This contrasts with the strong dependence on temperature of the light compensation point (*cf.* page 984). The reason for this difference is that temperature has a strong influence on respiration, as well as on photosynthesis in strong light, but only a weak effect (or none at all) on photosynthesis in light of low intensity (*cf.* chapters 29 and 31). In the measurement of the "light compensation point," photosynthesis is in the "light-limited" and therefore temperature-independent state; while in the measurement of the carbon dioxide compensation point, it is in the "carbon dioxide limited" state and therefore depends on temperature. However, the exact coincidence of the temperature coefficients of respiration and photosynthesis, implied in the results of Miller and Burr, is unlikely to be more than an accident.

We assume that the carbon dioxide curves of photosynthesis, if properly corrected for respiration, continue smoothly below the compensation point and reach zero when the carbon dioxide concentration is zero. However, their exact determination in the region of very low carbon dioxide concentrations is difficult because of the production of carbon dioxide by respiration. It is difficult to remove this carbon dioxide completely (*e. g.*, by an alkaline absorber), before some of it is utilized for photosynthesis, since this may occur even before the carbon dioxide has left the interior of the cells (*cf.* Vol. I, chapter 19, page 529). Some intermediates of respiration, such as certain carboxylic acids, may perhaps be utilized for photosynthesis without conversion to free carbon dioxide. One would then obtain small positive values of "true" photosynthesis (*i. e.*, of the difference between the gas exchange in light and in the dark), even when the concentration of carbon dioxide is zero, not only in the medium, but also inside the cell.

Experimental investigation of the relation between photosynthesis and respiration at low $[CO_2]$ values is further complicated by the observation that, if carbon dioxide is removed very effectively, *photoxidation* is apt to occur upon exposure to light, and oxygen consumption becomes larger in light than in the dark (instead of being decreased in consequence of reassimilation of respiration products).

One is thus caught on the horns of a dilemma: (*a*) either the respiratory carbon dioxide is not removed effectively enough—in which case illumination produces an apparent reduction in the volume of respiration, and it appears as if photosynthesis can proceed at a positive rate even at $[CO_2] = 0$ (fig. 28.5 gives an extreme example of this kind); (*b*) or the removal of carbon dioxide is fully effective—then, photoxidation sets in, and the rate of photosynthesis appears to become *negative* at $[CO_2] = 0$.

In chapter 19 (page 528) it was noted that Noack (1925, 1926) had observed mainly the first phenomenon (*i. e.*, an apparent "light inhibition of respiration" in a CO_2 free atmosphere); whereas van der Paauw (1932) had discovered, and Franck and French (1941) further explored, the second effect—the photoxidation in carbon dioxide starved leaves.

Photoxidation can be prevented by using low light intensity and short exposures. Whether it is possible to avoid the reassimilation of a part of respiratory carbon dioxide (or of its precursors) is a controversial matter.

Gabrielsen (1949) noted that in the comparatively thick sun leaves of *Sambucus*, in streaming, carbon dioxide free air, as much as 56% of respiratory carbon dioxide were reassimilated; but that in the thinner shade leaves, reassimilation was negligible. Reassimilation could be reduced by lowering the temperature (*e. g.*, to 5° C.), and by increasing the rate of flow of the gas, *e. g.*, to 33 cc./m.^{2} min. Gabrielsen concluded from these ob-

servations that there is no evidence of photochemical reduction of precursors of free carbon dioxide formed in respiration (since the latter would manifest itself in a general reduction of oxygen consumption in light).

Warburg, Burk and co-workers (1949) reached the same conclusion in experiments with strongly stirred, dense *Chlorella pyrenoidosa* suspensions: With alkali present in the side arm of the reaction vessel, illumination with weak red light (below compensation) had practically no effect on the consumption of oxygen. This showed that all the carbon dioxide produced by respiration was conveyed to the alkali and absorbed there (and none reassimilated in light), and that no intermediate products of respiration were used up as substitute oxidants in the photosynthetic process in the absence of the normal oxidant, external carbon dioxide.

We will have to return to these observations in chapter 29, because of their significance for the calculation of the quantum yield of photosynthesis in weak light. We will note there that the results of Warburg *et al.* may have been associated with the intermittency of illumination, caused in their experiments by the rapid stirring of dense cell suspensions. Most of the respiratory carbon dioxide was produced while the cells were in the shade and could escape into the medium before entering the small illuminated zone.

Supporting Kostychev's concept of indirect, physiological regulation of photosynthesis (*cf.* chapter 25), Chesnokov and Bazyrina (1932) concluded, from experiments on higher plants, that the rate of photosynthesis is not a smooth function of the external carbon dioxide concentration at all. They asserted that photosynthesis drops to zero when the external carbon dioxide concentration declines below 0.2×10^{-5} *M*, while above 1×10^{-5} *M* the rate is not affected by changes in $[CO_2]$. They considered this behavior a proof of the admirable adaptation of plants to natural conditions—a "trigger action," which puts the photosynthetic mechanism to work when conditions are "normal" and stops it completely when the conditions become unfavorable. A direct influence of external carbon dioxide concentration on the reaction rate, subordinated to the law of mass action, could not, they argued, produce such an "all or nothing" response. However, the alleged discontinuity of the carbon dioxide curve, and the consequent assumption of the existence of a "carbon dioxide threshold" of photosynthesis, is not confirmed by kinetic investigations under well-controlled laboratory conditions (*e. g.*, by the measurements presented in figs. 27.2A–27.4).

4. Carbon Dioxide Fertilization and Inhibition

Practically all carbon dioxide curves show that neither the normal carbon dioxide concentration of the air (0.03%, or approximately 1×10^{-5} *M*) nor the content of this gas in water equilibrated with the free atmosphere is sufficient for complete saturation of photosynthesis in moderate or strong light—at least, without exceedingly strong stirring. The curves indicate that it should be possible to improve, perhaps by as much as 50–100%, the yield of photosynthesis under natural conditions, by means of "carbon dioxide fertilization," and one may expect that this will lead to a

proportionate increase in crop. Experiments tend to confirm this conclusion.

Among the earliest attempts at carbon dioxide fertilization were those of Demoussy (1904); the first practical successes were achieved by Klein and Reinau (1914). Among the more recent investigations, those of Lundegårdh (1924), Rippel (1926), White (1930), Harder, Keppler and Reuss (1931), Johnston (1935), Richter (1938) and Katunsky (1939) may be quoted. In numerous experiments, carbon dioxide-fertilized cultures produced crops 50–100% larger than control cultures grown in rooms with the normal concentration of this gas. Carbon dioxide fertilization has found some practical application (*cf.*, for example, Reinau 1927) in the suburban greenhouse cultivation of fruits and vegetables, since greenhouses can be "fertilized" comparatively easily and inexpensively by compressed carbon dioxide from cylinders. The possibility of a similar fertilization on a large scale in open fields depends on the availability of cheap combustion gases rich in carbon dioxide, but free of sulfur dioxide and other plant-damaging components (*cf.* Katunsky 1939).

Thomas and Hill (1949) made improved measurements of the rate of photosynthesis of tomatoes, sugar beet and alfalfa under field conditions, and found continuous increase in rate even at 0.3 or 0.4% CO_2—except in the case of a sulfur-deficient beet culture, which apparently was unable to use an increased carbon dioxide supply. The maximum fertilization effects observed in these experiments were rate increases by a factor of about three.

Possibility of fertilization by bicarbonates plays an important role in speculations on large-scale culturing of unicellular algae as source of fuel or food, for man, animals, or protein and fat-producing microorganisms, such as yeast.

It was mentioned above (see page 901) that Chesnokov and Bazyrina (1932) denied that external carbon dioxide concentration has a direct effect on the rate of photosynthesis at all. Bazyrina and Chesnokov (1930) sought a different explanation of the phenomenon of CO_2 fertilization, and thought they found it in the stimulating action of carbon dioxide on plant growth. They denied that field crops are primarily determined by the intensity of photosynthesis, and pointed out that accelerated photosynthesis sometimes causes an unbalanced or premature development and thus diminishes rather than increases the crop. Granted that the size of the crop depends on many factors, it is certain that photosynthesis is one of them, if not the main one; and it is hardly a coincidence that not only the possibility of crop increase by carbon dioxide fertilization, but also its approximate maximum extent (50–100%) can be anticipated from the shape of the carbon dioxide curves obtained under controlled laboratory conditions.

The question of *how strongly the actual concentration of carbon dioxide surrounding vegetation deviates from the average* (0.03%) has been much discussed, and widely divergent opinions have been expressed on this subject. Undoubtedly, carbon dioxide concentration near the ground in dense vegetation can rise considerably above the average, particularly at the end of night. However, extreme figures such as $[CO_2] \geq 1\%$, given by some investigators, are unlikely. We will quote two examples of more reliable determinations: Verduin and Loomis (1944) found that, in a maize field, the concentration of carbon dioxide 100 cm. above ground, was 0.055–0.080% at night, and rapidly declined to 0.045% in the morning. Fuller (1948) found that CO_2 concentration near the ground (0–1 cm.) reached

(at 1 P.M., in June), 0.13% in a forest, 0.10% in grassland, and 0.18% in a river bottom. The concentration declined steeply with height above ground in all these habitats, dropping to near average (~0.04%) 8 or 10 cm. above ground.

The *decline of photosynthesis at excessively high concentrations of carbon dioxide* (*e. g.*, 10 volumes per cent CO_2 or more, corresponding to over 300 $\times 10^{-5}$ *M*), which, before Blackman, was considered a confirmation of the "optimum theory," was reinterpreted by Blackman as an inhibition effect, alien to the intrinsic kinetic mechanism of photosynthesis. It was discussed as such in chapter 13 (Vol. I) which dealt with various inhibitors and stimulants. Referring the reader to this chapter, we merely repeat here references given there to the work of de Saussure (1804) (who discovered the effect), Boussaingault (1865), Böhm (1873), Ewart (1896), Chapin (1902), Pantanelli (1903), Jaccard and Jaag (1932) and Livingston and Franck (1940). A recent study by Ballard (1941) with leaves of *Ligustrum* can be added to the list. It showed that, at 17° C., inhibition occurred (at 35,000 lux) at $[CO_2] = 2\%$, while at low temperatures (6° C.) no inhibition was noticeable up to 5%. We recall that Chapman, Cook and Thompson (1924) found that high carbon dioxide concentration induces closure of the stomata; it was therefore suggested in chapter 13 that stomata may account for some of the observed carbon dioxide inhibition effects. Other phenomena, which also may contribute to the inhibiting influence of excess carbon dioxide, are its adsorption on catalytic surfaces ("narcotization"), and possibly also acidification of the cell fluids (shift of intercellular buffer equilibria).

That the closure of stomata is not the only reason for carbon dioxide inhibition is illustrated by the observation of Österlind (1949) that it also occurs with algae such as *Scenedesmus quadricauda*. An inhibition of the growth of this alga became noticeable at 2×10^{-3} mole/l., and reached 50% at 10×10^{-3} mole/l. CO_2.

5. External Supply and Exhaustion Effects

In commenting on Table 27.I, we noted wide variations in the numerical values of the saturating carbon dioxide concentration and suggested that these variations may be due largely to the exhaustion of carbon dioxide in the immediate neighborhood of the plants. We will now consider this aspect of the problem more closely.

The experimental results fall roughly into three classes. One group, which includes the results of Blackman and Smith (1911) and Singh and Kumar (1935), the somewhat less extreme data of James (1928) and the figures given by Steemann-Nielsen (1946) for *Fontinalis*, and by Wassink

and co-workers (1941–1942) for purple bacteria, is characterized by continued increase of the rate of photosynthesis with increasing carbon dioxide concentration until the latter has reached 50, 80, 200 (Singh and Kumar) or even 400×10^{-5} mole/l. (Blackman and Smith); the last value corresponds to 12% carbon dioxide in the air! Earlier measurements of Kreusler (1885, 1887) and Brown and Escombe (1902), not included in the table, fall into the same category.

An intermediate group of results, including Harder's (1921) and Smith's (1937, 1938) on higher aquatic plants, and Emerson and Green's (1934) on *Gigartina*, place carbon dioxide saturation at $20–30 \times 10^{-5}$ mole/l. CO_2. Finally, in several careful investigations, the rise of photosynthesis with increasing carbon dioxide concentration was found to cease as early as between 0.5 and 5×10^{-5} mole/l. CO_2 (Hoover and co-workers 1933, and Singh and Lal 1935, higher plants; van der Honert 1930 and van der Paauw 1932, *Hormidium*; Emerson and Green 1938, *Chlorella;* and Barker 1935, diatoms). It will be noted that results of this low order of magnitude have been obtained both with land plants in rapidly circulating gas, and with algae in well-stirred acid or alkaline solutions.

There is little doubt that most if not all results of the first type were due to insufficient circulation and consequent depletion of carbon dioxide in the medium surrounding the plants. It is by no means certain that concentration gradients in the external medium did not affect significantly also the results in group 2, or even in group 3. And, in addition to gradients in the external medium (which can be reduced by intense circulation), we also must consider those in the stomata, air channels, cell walls and cytoplasm.

The importance of rapid circulation can be understood by considering that green cells, such as *Chlorella*, can consume, in strong light, up to one half their own volume in carbon dioxide each minute. In cell suspensions, the volume of the cells usually is from 0.1 to 1% of the volume of the medium. Consequently, the suspension as a whole will use up its own volume in carbon dioxide in from 200 to 2000 min. In other words, the rate of consumption of carbon dioxide will be from 2×10^{-5} to 2×10^{-4} mole CO_2/l. min. Consequently, if the concentration of carbon dioxide in the medium is $x \times 10^{-5}$ *M*, it will be all used up in from 0.05 x to 0.5 x minute, or from 3 x to 30 x second. Consulting Table 8.II, we note that in water equilibrated, at 25° C., with an atmosphere containing 0.01% CO_2, $x = 0.4$; 0.1% CO_2, $x = 4.1$; 1% CO_2, $x = 41$, and so on. Consequently, a cell suspension in an acid medium that contains no significant amounts of HCO_3^- ions, containing 0.1 to 1% cells by volume, will consume all its carbon dioxide in from 1.2 to 12 seconds, if it has been equilibrated with air containing 0.01% CO_2; in from 12 to 120 seconds, if the

atmosphere contained 0.1% CO_2, and so on. Bicarbonate solutions contain, for each CO_2 molecule, about 100 HCO_3^- ions; they should therefore last one hundred times longer than acid solutions with the same value of $[CO_2]$. Finally, 0.1 *M* carbonate–bicarbonate buffers containing from 2×10^5 (buffer No. 1) to 330 (buffer No. 11) carbonate and bicarbonate ions for each CO_2 molecule provide sufficient reserves to maintain full photosynthesis, in suspensions containing 0.1 to 1% cells by volume, for from 500 to 5000 minutes, or 8 to 80 hours.

These figures lead to several conclusions. *First*, measurements of the rate of photosynthesis at low carbon dioxide concentrations (*e. g.*, less than 1% CO_2 in the air, or 30×10^{-5} *M* in solution), if they are to last for more than a few seconds, require an ample supply of carbon dioxide, either *in situ*, in the form of carbonate and bicarbonate ions, or from outside, in the form of large amounts of circulating liquid or gas, which must be kept well supplied with fresh carbon dioxide to replace losses. *Second*, whenever leaves or multicellular algae are used, very intense stirring or circulation is required to prevent the establishment of a carbon dioxide concentration gradient around the plants. The required stirring depends on the ratio of surface to volume. This is well illustrated by the following example: Gessner (1938) measured the oxygen liberation by two varieties of *Proserpinaca palustris*—one with large leaves and one with finely divided, feather-like leaves. In stagnant water, the first variety produced much less oxygen than the second one; stirring improved strongly the efficiency of the large-leafed, but did not affect the oxygen production by the feather-leafed variety. In other words, in the absence of circulation, external carbon dioxide supply must have been the rate-limiting factor for the large-leafed, but not for the feather-leafed, variety.

It seems that, with multicellular objects, even the provision of a strongly stirred bicarbonate-buffered medium does not always guarantee the absence of carbon dioxide exhaustion effects. Wassink (1946) found, for example, that the photosynthesis of 5 mm. discs cut out of leaves, suspended in carbonate buffer No. 9 (7.9×10^{-5} mole/l. CO_2) and shaken in a Warburg apparatus, still was largely "carbon dioxide limited." The equilibrium concentration of carbon dioxide in the atmosphere above this buffer is 0.25% (cf. Tables 8.V and 8.II). By increasing the initial carbon dioxide content in the air space to 2% in some cases, and to as much as 9% in others, Wassink was able to obtain carbon dioxide saturation. Without exact knowledge of the dimensions of the apparatus, it is difficult to estimate the final carbon dioxide concentration and the *p*H of the solutions treated in this way.

Closure of the stomata in punched leaf discs may have been one of the reasons for apparent extreme carbon dioxide requirements observed in

these experiments. From this point of view, and from the point of view of favorable ratio of surface to volume, unicellular algae offer much better conditions. In brief experiments, or in weak light, they can be used in acid solutions previously equilibrated with carbon dioxide of sufficiently high partial pressure ($>1\%$; it was calculated above that a suspension containing 1 volume per cent of cells will use, in saturating light, all the carbon dioxide contained in water equilibrated with 1% CO_2, in 1.5 minutes). If stronger illumination or longer duration of experiments is desired, acid solutions can be used only if the carbon dioxide content is continuously renewed, *e. g.*, by stirring with a gas the carbon dioxide content of which is maintained by contact with an alkaline carbonate buffer.

More efficient should be the provision of carbon dioxide reserves *in situ* by using carbonate buffers directly as suspension media, as first suggested by Warburg. However, a certain difficulty arises from their unphysiological and variable alkalinity. In progressing from $M/10$ buffer No. 1 (0.5×10^{-5} mole/l. CO_2) to $M/10$ buffer No. 11 (29×10^{-5} mole/l. CO_2), we find the *p*H declining from 11 to 8.5. Since all living cells are more or less sensitive to excess alkalinity (even if *Chlorella* appears to be remarkably resistant to it), this drop of *p*H could cause continued increase of the rate of photosynthesis in a range where this rate is intrinsically independent of carbon dioxide concentration. This may explain, for example, the difference between the carbon dioxide curve of *Chlorella* as determined by Warburg (1919) in carbonate buffers, and the same curve obtained by Emerson and Green (1938) in a phosphate buffer. The first one continues to increase up to and beyond 9×10^{-5} mole/l. CO_2, while the second one is perfectly flat above 0.7×10^{-5} mole/l. CO_2.

On the other hand, observations of Ruttner (1947, 1948) and others on the maximum *p*H reached in non-renewed media after prolonged photosynthesis by aquatic plants (*cf.* above page 890), tend to discount the damaging effect of alkalinity on algae and submerged phanerogams (as contrasted to water mosses), by indicating the continuation of photosynthesis up to *p*H 11 or 12; *p*H measurements on cell sap showed it to maintain its approximately neutral reaction even in such highly alkaline media.

(For other possible explanations of the difference between the CO_2 curves of *Chlorella* as observed by Warburg, and by Emerson and Green, see page 908.)

Another pertinent question is whether the rate of conversion of HCO_3^- ions into CO_2 molecules always is high enough to provide effective replenishment of used-up carbon dioxide. In chapter 8 (*cf.* Vol. I, page 175) we discussed the finite rate of hydration and dehydration of carbon dioxide, and estimated that, in acid solution at room temperature, an H_2CO_3 molecule lives *ca.* 0.1 sec. before dissociating—the monomolecular rate constant

of dehydration being about 10 sec.$^{-1}$ at 18° C.; (*cf.* Table 8.III). The rate of dehydration of HCO_3^- *ions* was not given there, but we can estimate it from the rate of addition of OH^- to CO_2, determined experimentally by Brinkman, Margaria and Roughton (1933):

$$CO_2 + OH^- \underset{k'}{\overset{k}{\rightleftharpoons}} HCO_3^-$$

$$k = 2.05 \times 10^3 \text{ (18° C.)}$$

To obtain k', first calculate the equilibrium constant of the above reaction from the known constants of dissociation of water (1.04×10^{-14}), ionic dissociation of H_2CO_3 into H^+ and HCO_3^- (1.8×10^{-4}), and hydration of CO_2 (2.2×10^{-3}), and obtain:

$$K = k/k' = 4.4 \times 10^7$$

This, together with the above value for k, gives (for 18° C.):

$$k' = (2.05 \times 10^3)/(4.4 \times 10^7) = 0.47 \times 10^{-4}$$

This indicates that an HCO_3^- ion lives, on the average, at 18° C., as long as 2.7×10^4 sec. before being dissociated into OH^- and CO_2. A bicarbonate buffer containing y mole HCO_3^-/l. can therefore supply a maximum of 4.7×10^{-5} y mole CO_2/l./sec. by this dehydration process. At pH < 10, dehydration via H_2CO_3 must be added; at pH 9 it can double the rate of conversion of HCO_3^- to CO_2 (assuming the association of HCO_3^- and H^+ to H_2CO_3 to be practically instantaneous). A solution containing $y = 0.02$ mole/l. HCO_3^- (Warburg's $M/10$ buffer No. 2, pH $\simeq$ 10.7) is thus able to supply a maximum of 9×10^{-7} mole CO_2/l. sec. The corresponding figure for buffer No. 9 (0.085 M HCO_3^-, pH $\simeq$ 9.4) is 5×10^{-6} mole CO_2/l. sec. Comparing these figures with the above-estimated maximum rates of photosynthesis in strong light (from 2×10^{-5} to 2×10^{-4} mole CO_2/l. min., or from 3.3×10^{-7} to 3.3×10^{-6} mole CO_2/l. sec. for suspensions containing from 0.1% to 1% cells by volume), we note that the maximum supply exceeds maximum consumption in the 0.1% suspension by a factor of about three in buffer No. 2 and by a factor of about fifteen in buffer No. 9. In 1% suspension, the supply is quite insufficient in buffer No. 2 and barely sufficient in buffer No. 9. Considering the roughness of the calculation (*e. g.*, the use of concentrations instead of activities), the margin is by no means secure even in the dilute suspension. Assuming the calculation to be exact, a supply process with a maximum rate equal to only 3 times the noninhibited rate of reaction is bound to cause a marked inhibition (*cf.* chapter 26). It is therefore an open question whether the limited rate of reproduction of CO_2 molecules from HCO_3^- ions can play a role in the determination of the rate of photosynthesis of dilute suspensions in strong light, at least in the more alkaline carbonate buffers. This

"bottleneck" may well have contributed, *e. g.*, to the decline in rate observed by Warburg (1919) in *Chlorella* at $[CO_2] < 9 \times 10^{-5}$ *M*. (As mentioned before, Emerson and Green have noted no such decline until $[CO_2]$ was down to 0.7×10^{-5} *M*, and have suggested that damage caused by increased alkalinity of the lower carbonate buffers could provide an explanation of Warburg's results.)

Carbon dioxide exhaustion effects are not restricted to experiments in liquid media, but affect also measurements made with land plants in a carbon dioxide atmosphere, if it is stationary (*cf.* Lundegårdh 1921), or insufficiently agitated (Kreusler 1885,1887; Singh and Kumar 1935); this was demonstrated by Kostychev *et al.* (1927) and Chesnokov and Bazyrina (1932). Here again, not only the rate of gas circulation, but also the size and shape of the plants may be of importance and the opening of the stomata constitutes an additional complication.

To sum up it seems safe to assume that, whenever the rate of photosynthesis was found to continue its increase with the external concentration of carbon dioxide much above $[CO_2] = 10 \times 10^{-5}$ *M*, the reason was slow outside supply of carbon dioxide to the photosynthesizing cells, and consequent exhaustion of the reduction substrate. Experiments in vigorously stirred solutions, or in rapidly circulating gas mixtures, regularly showed the photosynthetic apparatus to become saturated with carbon dioxide at concentrations not much higher, or even lower, than 1×10^{-5} *M*. Even in experiments of this type, one cannot be certain whether *all* diffusion effects have been eliminated, particularly in higher plants, where the diffusion resistance of the stomata, epidermis and air channels cannot be destroyed by stirring or gas circulation. The diffusion resistance of the cell walls or protoplasmic layers also remains unaffected by all mechanical means (although it may perhaps be changed by chemical agents).

Another source of distortion of the carbon dioxide curves of photosynthesis was noted by Howles (unpublished) and Whittingham (1949) in Brigg's laboratory. They observed that the photosynthesis of *Chlorella* in carbonate buffers with low $[CO_2]$ values was *time-dependent*, if the cells had been transferred into the CO_2 deficient medium from a culture medium of higher concentration (such as 4% CO_2). The initial rate was low; it increased by a factor of 3 in the course of two or three hours, and then became constant. If the cells were cultured in air (0.03% CO_2), the rate was high and constant from the beginning. Obviously then, with cells "incubated" at high $[CO_2]$, the shape of the carbon dioxide curve will depend on the duration of the measurement.

If the carbon dioxide curve of *Chlorella* is determined at low $[CO_2]$ values, with cells "adapted" to low carbon dioxide concentration, the value of $_1/_2[CO_2]$ is as low as 0.5 to 1.0×10^{-6} mole/l.

The initial inhibition of photosynthesis in low [CO_2], shown by cells previously exposed to high [CO_2] values, could be related to the photoxidation phenomena observed in CO_2 starved plants (*cf.* Vol. I, chapter 19). Using Franck's picture, it can be suggested that CO_2 satiated cells, placed in CO_2 deficient medium and exposed to light, develop a large quantity of a "narcotic" (perhaps, because they were full of metabolites), which settles on chlorophyll and holds photosynthesis down. The autocatalytic removal of this inhibition seems to require 2–3 hours (as against a few minutes in ordinary induction, *cf.* chapter 33). That such cells in fact are inhibited is confirmed by the observation that if, after brief exposure to light in low [CO_2], they are brought back into a medium of high [CO_2] (such as buffer No. 9), they show a reduced rate of photosynthesis in this medium as well.

Since these experiments were carried out in carbonate buffers, the observed effects can be attributed either to changes in [CO_2], or to those in *p*H.

If all carbon dioxide activity gradients between the outside medium and the site of photosynthesis could be avoided, we would still anticipate, on theoretical grounds, that carbon dioxide concentration will retain an influence on the rate of photosynthesis: *first*, because of dissociation, under low partial pressure of carbon dioxide, of the carbon dioxide–acceptor compound that we assume is formed as an intermediate in photosynthesis (*cf.* chapter 8); and *second*, because of the dependence of the rate of formation of this compound ("carboxylation") on the factor [CO_2]. These two relationships will be discussed theoretically in sections 7b and c; but there can be no certainty, until much more precise measurements have been carried out, that any of the observed carbon dioxide curves actually reflect one or both of these intrinsic kinetic relationships, rather than the more incidental diffusion phenomena. As long as a [CO_2] effect can be made to disappear by improved stirring, it reveals itself as due to external diffusion; but, when no further improvement in rate can be achieved in this way, this does not mean that the remaining [CO_2] effect is *not* caused by diffusion in those parts of the gas path were external stirring can do no good.

In estimating the supply of carbon dioxide to plants under natural conditions, the possibility of *carbon dioxide supply through the roots* must not be overlooked. It was mentioned in chapter 2 (Vol. I) that the doctrine of the aerial nourishment of plants was the second accomplishment of the discoverer or, more exactly, co-discoverer of photosynthesis, Ingen-Housz. Since the time of Liebig, this doctrine has become the basis of the science of plant nutrition. However, under certain conditions, Senebier's concept that carbon dioxide can be supplied by the soil water to the roots and thence to the leaves, scorned by Ingen-Housz, may be correct. This may affect field determinations of the rate of photosynthesis, based on measurements of the carbon dioxide consumption from the air, and may also influence the results obtained by other methods, if the observed rates are considered in relation to the external carbon dioxide concentration. For

recent discussions of this question, we refer to Bergamaschi (1929), Livingston and Beall (1934), Suessenguth (1937), Overkott (1938, 1939) and Hartel (1938). The experiments of the two last-named authors, in particular, have confirmed unambiguously that a certain amount of carbon dioxide can be conveyed from roots to leaves by convection (and to a smaller extent by diffusion), and that this supply can be utilized by leaves for the synthesis of carbohydrates. It was even suggested that this "invisible" CO_2 supply, brought about by increased transpiration during the hot hours of the day, may be the cause of the decline of the carbon dioxide absorption from the air, which is often observed at midday (*cf.* page 873). Whether this is a valid hypothesis cannot be judged without quantitative investigations; but in any case, it cannot explain *all* the aspects of the so-called "midday depression," (*a*) because these also include a decline of oxygen liberation, (*b*) because they have been observed not only in the higher land plants but also in aquatics.

6. Role of the Stomata

It was stated above that, in experiments with the leaves of the higher land plants, a special problem is posed by carbon dioxide passage through

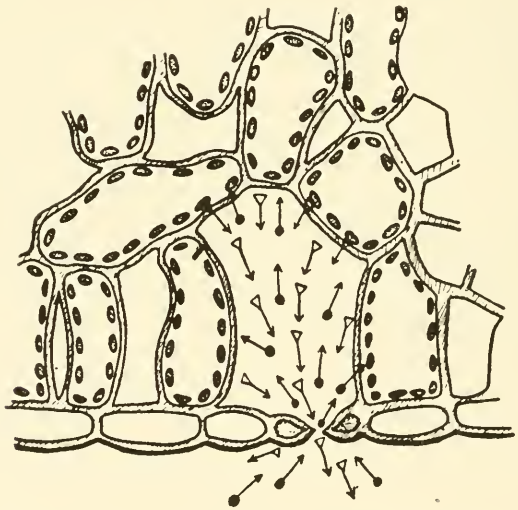

Fig. 27.5. Diagram of a section through stoma and substomatal cavity of a leaf to show direction of diffusion of gases in photosynthesis (after Robbins and Rickett). Arrows with black balls represent carbon dioxide; those with triangles, oxygen.

the stomata and air channels, through which it has to flow in order to reach the photosynthesizing cells of the palisade tissue and of the spongy parenchyma.

A controversy as to whether the carbon dioxide enters the leaf only through the stomata or also through the cuticle was decided by Blackman (1895). He proved, by

experiments with paraffined leaves, that gas exchange takes place almost exclusively through the stomata, in the way indicated in figure 27.5. Only under very high pressure of carbon dioxide did Blackman observe a slight penetration of the gas through the cuticle. According to Stålfelt (1935), in the free atmosphere, carbon dioxide penetrates the cuticle at a rate of only between 3 and 6 $\times$ 10^{-8} mole/cm.^{2} hr.; the gas flow through the stomata may be as much as one hundred times faster, *i. e.*, of the order of 5 $\times$ 10^{-6} mole/cm.^{2} hr., despite the fact that their openings occupy only about 0.1% of the total leaf surface. Freeland (1946) found more recently that, in some leaves, the relative rate of passage of carbon dioxide under pressure through the lower and the upper surface is so low as to suggest predominance of diffusion through the epidermis over passage through the stomata. The thickness of the epidermis may be an important factor in the determination of the relative role of stomata and epidermis as routes for the entry of carbon dioxide into the leaf.

Ferns and other lower land plants possess no stomata, and therefore must receive all their carbon dioxide supply through the epidermis. Stomata also are absent in aquatic plants and algae, where their main function—regulation of evaporation—is not required.

Between 10,000 and 30,000 stomata are present on each square centimeter of the leaf surface, either on both sides or on the lower side only. They are elongated slits, usually from 10 to 15 μ long, flanked by two "guard cells" (*cf.* figs. 27.5 and 27.6), which are capable of changing shape so as to effect the opening or closing of the slit (*cf.* fig. 27.7).

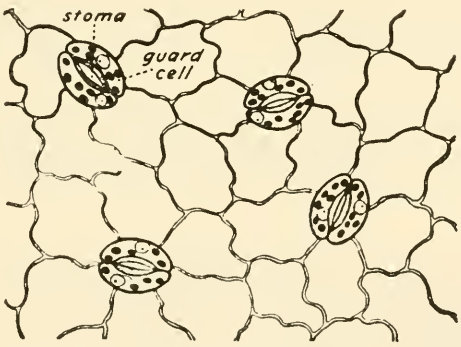

Fig. 27.6. A portion of the lower epidermis of a geranium leaf (after Robbins and Rickett).

This mechanism is brought into operation by shifts in the sugar–starch equilibrium, which increase the turgor when the slits are to be opened, and decrease it when they must be closed.

The problem of the diffusion resistance of stomata has been considered from two points of view: First, it was asked: Is it possible for a diffusion flow of up to 10^{-5} mole (0.24 cc.) CO_2/hr. (*cf.* chapter 28, Table 28.5) to pass through the stomata on 1 cm.^{2} of the leaf surface, when the total open

area is less than 1 mm.^{2} and the concentration drop is not more (and often less) than 1×10^{-5} mole/l. (which is the normal CO_2 concentration in the open air)? The second question was: Granted a remarkably low diffusion resistance of the stomata, is this resistance nevertheless an important "limiting factor" in photosynthesis of higher plants, particularly at low carbon dioxide concentrations?

To understand why the first question had to be asked, suffice it to recall the experiment of Brown and Escombe (1900), who showed that a leaf takes up carbon dioxide from quiet air almost as rapidly as an equally large surface of an alkali solution! It was soon found that this unexpectedly high rate of diffusion has nothing to do with the physiological properties of the leaf but is a general property of multiperforate septa, *i.e.*, barriers containing many small openings. Model experiments on transpiration showed (*cf.* Sierp and Seybold 1929, 1930) that the rate of evaporation from a ves-

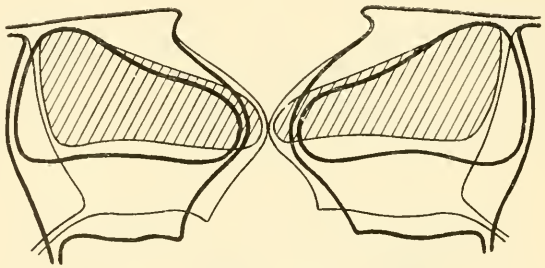

Fig. 27.7. Stoma of *Helleborus* sp. in transverse section. Darker lines show shape assumed by guard cells when stoma is open; lighter lines when stoma is closed (from Strassburger *et al.*, after Schwendener). In closed state, vacuole (shaded area) contracts because of water loss caused by decreased turgor (produced by polymerization of sugars).

sel, covered with a septum, can be as high as three fourths of that from an equally large open vessel—even if the aggregate area of the holes is less than 1% of the total surface of the liquid! The theoretical solution of this apparent paradox was given (for the case of evaporation) as early as 1881 by the Austrian physicist Stefan. He used, for this purpose, the formal similarity of the equations describing the diffusion flow of matter from an extended surface and from a point source, with the equations describing the lines of force in the electrostatic field in front of an extended conducting surface, and around a small conductor. In this formal analogy, the diffusion flow corresponds to the electrostatic capacity of the conductor; and it is known that the capacity of a large flat condenser is determined by the *area* of its plates, while the capacity of a small spherical conductor is determined by its *radius*. In the same way, the amount of

evaporation from an extended surface is proportional to its area, while the amount of evaporation from a small sphere is proportional to its radius; the same applies to the comparison of diffusion across an extended plane (the case usually considered in the derivation of diffusion equations) with the diffusion through a small hole. Diffusion through a multiperforated septum can be treated in the same way as that through a single hole as long as the distance between the holes is large enough (compared with the radius of the holes) for the half-spherical surfaces of equal concentration (and the radial lines of flow, which are normal to these surfaces) to be established around each hole without marked interference by the neighboring holes.

This principle was first applied to the penetration of carbon dioxide through the stomata by Brown and Escombe (1900) upon advice of the physicist Larmor. Renner (1910, 1911), Brown (1918), Freeman (1920), Sierp and Noack (1921), Sierp and Seybold (1927, 1928, 1929), Huber (1928) continued the study, being, however, mainly concerned with the *transpiration* of plants. As a typical result, we reproduce a table from the paper by Sierp and Seybold (1929). Table 27.II shows the rates of evaporation of water through septa with different numbers of holes but a constant total open area. The next-to-last row shows the flow-retarding effect of an inadequate distance between the holes. The table indicates that a maximum rate of diffusion is reached asymptotically when the holes are reduced to 20–10 μ in diameter. Although the aggregate area of the holes (3.14 mm.^{2}) is less than 1% of the total area of the vessel (400 mm.^{2}), the evaporation rate through the septum with holes 10 μ in diameter is as high as 70% of that from the open vessel. These figures indicate that the dimensions of the stomata (5–15 μ) may be appropriate to ensure the desired rate of gas exchange through the smallest possible number of openings.

TABLE 27.II
EVAPORATION THROUGH SEPTA (AFTER SIERP AND SEYBOLD 1929)

Number of holes	Diameter of hole, μ	Total open area, mm.^{2}	Total circumference of all holes, mm.	Distance between holes, multiples of pore diameter	Rate of evaporation in quiet air
1	2000	3.14	6.28	—	1.0
400	100	3.14	125.6	9.5	7.7
1,600	50	3.14	251.2	9.2	11.1
10,000	20	3.14	628.0	9.1	11.7
40,000	10	3.14	1256.0	9.0	12.1
10,000	20	3.14	628.0	4.0	5.5
Open surface		400	—	—	16.3

The theory of gas diffusion through multiperforate septa was further advanced by Verduin (1949), by mathematical analysis of the mutual

interference of the openings. He calculated that interference should be inversely proportional to the square of the distance between pores, d:

$$\log Q/Q_1 = -k/d^2$$

where Q_1 is the diffusion rate at $d = \infty$. This equation agrees well with experimental results of Verduin (1949) and Weishaupt (1935). At a given ratio of pore diameter and pore distance, the interference must be stronger the smaller the pores. The stomata are so small that the diffusion through each of them is reduced significantly by interference—sometimes by > 50% of the theoretical value for an isolated opening of the same size. As stomata close gradually, interference weakens; and the diffusion rate therefore declines slower than proportionally to the open area.

These experiments and their theoretical interpretation explain how the tiny stomata can allow a large volume of carbon dioxide to diffuse into the leaf, thus permitting a high rate of photosynthesis. We now turn to the second question; does the resistance of the stomata impose a significant limit on the carbon dioxide supply and, with it, on the rate of photosynthesis? Closed stomata undoubtedly must curtail photosynthesis drastically (restricting it to the utilization of the carbon dioxide that can reach the chloroplasts by diffusion through the cuticle, or is produced in the leaf by respiration). The question is: How far must the stomata be open to cease exercising a restrictive influence on photosynthesis? May this restriction be significant even when slits are fully open? Are *they* the bottlenecks responsible for the "Blackman features" of many carbon dioxide curves? It will be recalled that, in the preceding chapter, it was shown that the restrictive influence of a reaction step generally becomes felt long before the rate of the over-all process closely approaches the "ceiling" imposed on it by this step. Therefore, the resistance of the stomata may affect the shape of the carbon dioxide curves even when the rate of photosynthesis is not more than one half or one quarter of the maximum possible flow of carbon dioxide through the stomata.

For an experimental study of the influence of stomata on photosynthesis, one must measure the rate of photosynthesis under constant external conditions, but with varying apertures of the stomata. Unfortunately, treatments used to enforce partial closure of the stomata (such as incubation in darkness or in dry air) may also directly affect the efficiency of photosynthesis, so that caution is required in the interpretation of the results. In order to arrive at reliable conclusions, the width of the stomata and the rate of photosynthesis must be determined with the same leaf—a condition that has not always been fulfilled.

The relation between stomatal openings and the rate of photosynthesis

has been the subject of study by several investigators, among them Iljin (1923), Geiger (1927), Maskell (1928), Johansson and Stålfelt (1928), Kostychev, Bazyrina and Chesnokov (1928), Boysen-Jensen (1932), Schoder (1932), Stålfelt (1935), Newton (1936), Heath (1939) and Heath and Penman (1941).

Of these, Kostychev, Bazyrina and Chesnokov (1928), and Schoder (1932) could find no correlation between the two magnitudes. All other observers concluded that, under certain conditions, a clear-cut relationship can be noted between them.

Thus, Maskell (1928) found a parallelism between the diurnal and seasonal course of stomatal apertures (as measured by a porometer) and the

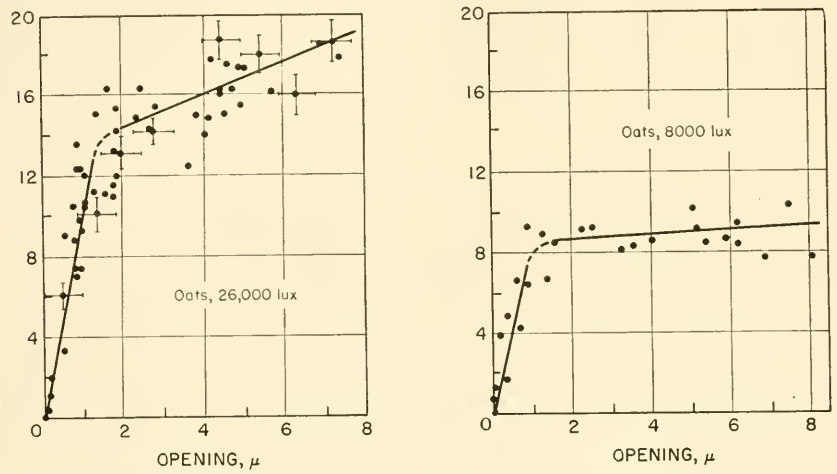

Fig. 27.8. Relation between photosynthesis and stomatal openings in ordinary air (0.03% CO_2) (after Stålfelt 1935). One-sided illumination. Air flow 4 ± 1 m./min. Average errors indicated by crosses. Ordinates, P in mg. CO_2/(100 cm.^{2} hr.).

rate of photosynthesis. He made a theoretical estimate of the maximum rate of carbon dioxide passage through the stomata, and concluded that it is of the correct order of magnitude to operate as a bottleneck in photosynthesis.

Stålfelt (1935) determined the opening of the stomata by microscopic measurements, using excised pieces of leaves of wheat or other cereals. The same leaves also were used for the determination of photosynthesis. The width of the stomata was varied by exposure to dry air, and preillumination by light of varying intensity. Figure 27.8 shows typical results. It will be noted that in strong light (26,000 lux) the limiting effect of stomata does not disappear even when they are fully open; at 8000 lux, on the other hand, this effect already ceases to be noticeable when the stomata are only

one quarter open (2 μ). This difference is understandable since at 8000 lux the maximum rate of photosynthesis is only 8 mg. CO_2/cm.^{2} hr., while in 26,000 lux, it rises to > 20 mg. CO_2/cm.^{2} hr. Stålfelt concluded that stomatal openings easily may limit the carbon dioxide supply in ordinary air, and therefore also the rate of photosynthesis under natural conditions, particularly in strong light. Like Maskell, she supported this view by calculations of the rate of diffusion through the stomata, based on equations of Brown and Escombe (1900). These calculations confirmed that the maximum rate of carbon dioxide flow from ordinary air through wide open stomata is of the same order of magnitude as the maximum rate of photosynthesis.

These results, while clearly showing the possible "bottleneck" role of the stomata, do not mean that other parts of the path between atmosphere and chloroplasts do not contribute commensurable—or even greater—terms to the total diffusion resistance.

In the face of these results, one must disagree with Renner (1910), who thought that the resistance of the stomata represents only a negligible fraction of the total diffusion resistance on the carbon dioxide path from the atmosphere to the chloroplasts, as well as with Schroeder (1924), who attempted to prove that the diffusion resistance of the *air channels* is the rate-limiting influence in the photosynthesis of the higher plants, and in this proof altogether omitted the resistance of the stomata.

Romell (1927) pointed out that Schroeder neglected, not only the flow resistance of the stomata, but also that of the gas–liquid interface, and of the liquid phase between the cell wall and the chloroplasts. Romell calculated that the gradient of the carbon dioxide concentration in the air channels must be smaller than in the protoplasm (between cell wall and chloroplast), and that both these gradients should be negligible in comparison with the drop of concentration at the phase boundary, caused by the relatively small accommodation coefficient of carbon dioxide on water (as calculated from Bohr's measurements of the velocity of escape of carbon dioxide from aqueous solution). The maximum theoretical rate of diffusion, calculated by Romell by taking all these factors into account, proved to be considerably *lower* than the maximum rate of photosynthesis that the leaves actually can reach in open air. One is thus led to assume (*cf.* van der Honert 1930) that the accommodation coefficient of carbon dioxide is larger on the cell wall than on a water–air interface.

7. Interpretation of Carbon Dioxide Curves

The preceding pages show that reliable experimental material for analytical interpretation of the carbon dioxide curves of photosynthesis is hardly available at present, and will not be easy to obtain. We have stated that at least two intrinsic kinetic factors could make the rate of photosynthesis a function of the external carbon dioxide pressure: the probable reversibility of the primary carbon dioxide absorption (carboxylation) step, and a finite rate of carboxylation. The difficulty is to recognize the workings of these "intrinsic" or chemical factors behind the more incidental, physi-

cal flow phenomena outside and inside the plant. We cannot be sure at present whether any of the observed carbon dioxide curves reflect reasonably well the effect of carboxylation equilibrium (or of the rate of carboxylation), or whether practically all carbon dioxide dependence of photosynthesis, known so far, is due to diffusion phenomena, with possible additional distortions by the time effects noted on page 908.

Despite this unsatisfactory state of our experimental knowledge, we will go through with some kinetic derivations leading to general equations for the shape of carbon dioxide curves, as affected by the several factors of slow diffusion, limited rate of carboxylation, reversibility of carboxylation and limited supply of light energy. We will thus obtain a kind of skeleton analytical theory of the carbon dioxide curves, which could prove useful for devising and interpreting future kinetic measurements—if only investigators of the kinetics of photosynthesis would change their present habit of considering only their own limited data, and ignoring all but their own *ad hoc* derived equations.

(*a*) *Carboxylation Equilibrium*

Two steps in photosynthesis, the rate of which depends directly on the factor $[CO_2]$ are: *First,* diffusion of carbon dioxide from the medium to the reaction site, and *second,* the first chemical reaction of carbon dioxide.

In chapter 8 (Vol. I), we decided that this reaction is a nonphotochemical, catalytic carboxylation. We usually described it by the formula $CO_2 \rightarrow \{CO_2\}$, but since the concentration of the "carbon dioxide acceptor" (until now symbolized by braces) enters explicitly into many of the following kinetic equations, we will from now on designate it as A, and the product of carboxylation as ACO_2. (Franck and Herzfeld, 1941, used the more specific symbols RH for acceptor and RCOOH for the product.)

The two $[CO_2]$-dependent steps can then be written as follows:

$$CO_2 \underset{}{\overset{k_d}{\rightleftharpoons}} (CO_2)_a \tag{27.1}$$

(where $(CO_2)_a$ refers to carbon dioxide in the immediate neighborhood of the acceptor, and k_d is a diffusion constant) and:

$$(CO_2)_a + A \underset{k'_a}{\overset{k_a}{\rightleftharpoons}} ACO_2 \left(\longrightarrow \text{reduction}\right) \tag{27.2}$$

The reduction of ACO_2 may be either a direct photochemical process (as assumed by Franck and Herzfeld; *cf.* scheme 7.VA), or a nonphotochemical reaction with an intermediate, as postulated in many other schemes in chapters 7 and 9. Even in the latter case, the rate of reduction is likely to be a function of light intensity, because the partner with which the com-

pound ACO_2 reacts must be a—direct or indirect—product of the primary photochemical process.

Two different premises can be used in the kinetic analysis of the effect of carboxylation. One alternative (indicated by arrows in equation 27.1, is to assume that the carboxylation is markedly *reversible*, *i. e.*, that k_a' is of the same order of magnitude as $k_a[CO_2]_a$. In this case, the association of the acceptor A with carbon dioxide is not complete even without any dislocation of the equilibrium by the consumption of ACO_2 in light. The other alternative, preferred by Frank and Herzfeld, is to assume that the carboxylation equilibrium lies entirely on the side of association (meaning $k_a[CO_2] \gg k_a'$), so that in the dark practically all acceptor is "saturated" by carbon dioxide molecules (at all practically significant partial pressures of carbon dioxide), unless the carbon dioxide molecules are displaced by other association partners, such as reduction intermediates, narcotics, etc. Free molecules A occur in this picture only during (or immediately after) intense photosynthesis, when reduction of ACO_2 is (or has been) too rapid for the recarboxylation to keep step with it.

As described in chapter 8 (vol. I) the known equilibria of carboxylation *in vitro* correspond to practically complete *dissociation*. Only few cases are known in which the carboxyl group is thermodynamically stable with respect to decarboxylation (at least, under sufficiently high carbon dioxide pressures). The "saturation" of photosynthesis with carbon dioxide, which occurs under partial pressures as low as 0.1%, indicates that conditions may be different here, perhaps in consequence of a coupling of carboxylation with another reaction, such as degradation of a "high energy phosphate," or an "endergonic" oxidation-reduction (*cf.* Vol. I, page 201). However, there is no experimental or theoretical reason—except convenience in analytical formulation—to postulate that in photosynthesis the carboxylation equilibrium lies *completely* on the side of synthesis, even at the lowest practically significant carbon dioxide pressures. We will therefore begin our analysis by assuming that the degree of saturation of the acceptor with carbon dioxide *does* depend on the external concentration of carbon dioxide.

If one molecule of carbon dioxide is taken up by one molecule of acceptor, the carboxylation equilibrium is determined by the equation:

$$[ACO_2] = (K_a A_0 [CO_2]_a)/(1 + K_a[CO_2]_a) \tag{27.3}$$

where A_0 is the total available concentration of the acceptor:

$$A_0 = [A] + [ACO_2] \tag{27.4}$$

and K_a is the equilibrium constant of carboxylation:

$$K_a[CO_2]_a[A] = [ACO_2] \tag{27.5}$$

If the carboxylation mechanism is as simple as postulated in (27.1), the equilibrium constant K_a is equal to the ratio of the two rate constants k_a and k'_a.

In equation (27.4) it is assumed that the acceptor, A, is either free or occupied by CO_2. This may not be the complete description for two reasons: In the first place, the first reduction product of ACO_2, designated by us as $AHCO_2$, may require time for its dissociation into A and HCO_2; part of the acceptor is then "blockaded," during photosynthesis, by this reduction product (*cf.* section *f* below). In the second place, the photochemical reduction may have to be repeated several times, *e. g.*:

$$\text{(27.5A)}\quad ACO_2 \xrightarrow{h\nu} AHCO_2 \xrightarrow{h\nu} AH_2CO_2 \xrightarrow{h\nu} AH_3CO_2 \xrightarrow{h\nu} AH_4CO_2 \longrightarrow A + H_2O + \{CH_2O\}$$

before the reduction product can separate itself from the carrier A (as in the Franck-Herzfeld mechanism discussed in section *d* below.) If $AHCO_2$ is assumed to be the only product of photochemical reduction, the completion of its reduction to AH_4CO_2—*i. e.*, to the carbohydrate level—must be ascribed to dismutations:

$$4\ AHCO_2 \longrightarrow AH_4CO_2 + 3\ ACO_2$$

cf. Vol. I, p. 158.

The simplest assumption that can be made in the interpretation of the carbon dioxide curves of photosynthesis is that they are, at least *basically*, saturation curves of the acceptor A. This means that one assumes (*a*) that equilibrium (27.3) is not strongly dislocated during photosynthesis, at least under moderate conditions, and (*b*) that the rate of photosynthesis is given by the rate of reduction of the compound ACO_2, and the latter is proportional to the concentration $[ACO_2]$:

$$\text{(27.6)}\qquad P = nk_r^* \times [ACO_2]$$

where the constant k_r^* depends on the intensity of illumination (as indicated by the asterisk). We will deal with the possible limitations of assumption *b* later (see, *e. g.*, section *e*). The condition for the correctness of *a* (*i. e.*, for the maintenance of equilibrium 27.3 in light) is (*cf.* formula 27.2):

$$\text{(27.7)}\qquad k_r^* \ll k'_a$$

That this condition is not *always* satisfied is demonstrated by the "pick up" phenomena, described in chapter 8 (Vol. I). These observations show that in very intense light (*i. e.*, when k_r^* is very large) or in the presence of certain poisons (when k'_a is very small) the acceptor A becomes "denuded" of carbon dioxide and afterward "picks it up" in the dark.

The factor n in (27.5) is either 1 or a fraction of 1, depending on how many molecules CO_2, at best, can be reduced to the carbohydrate level, $\{CH_2O\}$, for each molecule CO_2 which undergoes the first reduction step (to $AHCO_2$). If the mechanism of reduction involves only one photochemical step ($ACO_2 \rightarrow AHCO_2$), followed by a two-step dismutation:

$$4\ AHCO_2 \xrightarrow{4h\nu} 3\ ACO_2 + H_2O + \{CH_2O\} + A$$

then n is 1/4. If an energy dismutation step of the type discussed in chapter 9 (page 264) also is involved, n is reduced to 1/8. If, on the other hand, CO_2 is reduced to the H_4CO_2 level in a straight series of photochemical reduction steps:

$$ACO_2 \xrightarrow{h\nu} AHCO_2 \xrightarrow{h\nu} \xrightarrow{h\nu} \xrightarrow{h\nu} AH_4CO_2 \longrightarrow A + H_2O + CH_2O$$

then n is equal to 1. (As a compensation, the constant k^* can be equal in the dismuation model to the number of the absorbed light quanta, but must be $1/n$ times smaller in the straight reduction model, where $1/n$ quanta are needed to carry a single CO_2 molecule through all four reduction steps.)

Assuming that the conditions (27.6) and (27.7) are satisfied, we can insert into (27.6) the equilibrium value (27.3) and obtain, for the carbon dioxide curves of photosynthesis, the equation:

$$P = nk_r^* K_a A_0 [CO_2]_a / (1 + K_a [CO_2]_a) \tag{27.8}$$

For various values of k_r^* (*e. g.*, for various light intensities, I), equation (27.8) represents a family of curves of the "Bose type." These curves are hyperbolae:

$$P/(P_{max.} - P) = K_a [CO_2] \tag{27.9}$$

At the saturating concentrations of carbon dioxide, P approaches asymptotically the maximum rate:

$$P_{max.} = nk_r^* A_0 \tag{27.10}$$

All carbon dioxide curves separate from the beginning, their initial slopes being:

$$(dP/d[CO_2]_a)_0 = nk_r^* K_a A_0 \tag{27.11}$$

They all reach "half saturation" at the same carbon dioxide concentration:

$$1/2 [CO_2]_a = 1/K_a \tag{27.12}$$

The empirical carbon dioxide curves deviate more or less widely from this simple type: Even the "Bose type" curves, shown in figure 27.2A, do not all reach half saturation at the same value of $[CO_2]$. We can attempt to consider the curves (27.8), determined exclusively by *static* conditions,

as the "primary" carbon dioxide curves, and treat the empirical curves as if they were basically such curves, deformed by superimposed *kinetic* influences. In the region of low carbon dioxide concentrations (or in the presence of poisons such as hydrogen cyanide), these additional influences comprise supply reactions of limited efficiency—*slow carbon dioxide diffusion* and *slow carboxylation.* These two processes of limited, but $[CO_2]$-proportional, maximum rate tend to impose a slanting "roof" on the $P = f[CO_2]$ curves and thus to convert Bose's several divergent hyperbolae into Blackman's single and almost straight line. In the region of high CO_2 concentrations, the additional kinetic influences must be due to $[CO_2]$-independent factors, such as catalyst deficiencies, which tend to impose a horizontal "ceiling" on the over-all rate, *i. e.*, to produce "carbon dioxide saturation" of photosynthesis even before the acceptor A has become saturated with carbon dioxide.

(*b*) *Diffusion Factors*

When diffusion and carboxylation are slow processes (more exactly, when their maximum rate under the given conditions is not rapid compared with the actual rate of photosynthesis, P) the concentration $[CO_2]_a$ of the carbon dioxide molecules in the immediate neighborhood of the acceptor may decline during photosynthesis considerably below the concentration of the same species in the medium, $[CO_2]$; while the concentration of the carboxylated acceptor, $[ACO_2]$, may decline markedly below the value corresponding to the thermodynamic equilibrium, as determined by equation (27.3). The *stationary* concentrations, $[CO_2]_a$ and $[ACO_2]$, established under such conditions, can be calculated by the application of the law of mass action to the reactions (27.1) and (27.2):

$$[CO_2]_a = (k_d[CO_2] + k'_a[ACO_2])/(k_aA_0 - k_a[ACO_2] + k_d) \tag{27.13}$$

and:

$$[ACO_2] = (k_a[CO_2]_aA_0)/(k^*_r + k'_a + k_a[CO_2]_a) \tag{27.14}$$

Combined, these two equations give a quadratic equation for $[ACO_2]$ (and thus also for P) as a function of $[CO_2]$. Its one physically significant solution is:

$$[ACO_2] = \frac{k_ak^*_rA_0 + k'_ak_d + k^*_rk_d + k_ak_d[CO_2]}{2\,k^*_rk_a} - \sqrt{\left(\frac{k_ak^*_rA_0 + k'_ak_d + k^*_rk_d + k_ak_d[CO_2]}{2\,k^*_rk_a}\right)^2 - \frac{A_0k_d[CO_2]}{k^*_r}} \tag{27.15}$$

Inserting this value of $[ACO_2]$ into (27.6), we obtain:

$$(27.16)\quad \frac{P}{n} = \frac{k_a k_r A_0 + k_a' k_d + k_r k_d + k_a k_d [CO_2]}{2\,k_a} - \sqrt{\left(\frac{k_a k_r^* A_0 + k_a' k_d + k_r^* k_d + k_a k_d [CO_2]}{2\,k_a}\right)^2 - A_0 k_d [CO_2] k_r^*}$$

This equation represents, for different values of the parameter k_r^* (*i. e.*, for different light intensities), a set of hyperbolae. Similarly to the "primary" carbon dioxide curves (27.8), these hyperbolae approach the saturation values (27.10), but, in contrast to the primary curves, they do not all reach half saturation simultaneously. The expression for the half-saturating external carbon dioxide concentration can be derived from (27.15) by assuming $[ACO_2] = \frac{1}{2}A_0$, and is as follows:

$$(27.17)\quad {}_{1/2}[CO_2] = \frac{1}{K_a}\left[1 + k_r^* \frac{(k_a A_0 + 2\,k_d)}{2\,k_d k_a'}\right]$$

This equation shows that, because of delayed diffusion and carboxylation, the half-saturation point advances with increasing light intensity toward higher carbon dioxide concentrations—a behavior actually shown by most if not all of the experimental carbon dioxide curves (*cf.* figs. 27.2A, 27.2B and 27.3).

The *initial slopes* of the curves (27.16) are not proportional to k_r^*, as were the slopes of the primary curves according to equation (27.11), but are given by the equation:

$$(27.18)\quad (dP/d[CO_2])_0 = n k_a A_0 k_d k_r^* / (k_a A_0 k_r^* + k_a' k_d + k_d k_r^*)$$

This equation shows that carbon dioxide curves for all k_r^* values are confined within an angle formed by the axis of the abscissae and a slanting straight line ("roof"):

$$(27.19)\quad P_{\text{lim.}} = \frac{k_a A_0 k_d}{k_a A_0 + k_d}\,[CO_2]$$

and, of course, also under the horizontal ceiling (27.10). Equations (27.16), (27.17), (27.18), and (27.19) represent the *combined effects* of slow diffusion and slow carboxylation. If only one of these factors is operative, the equations can be simplified. Pure diffusion limitation can be assumed if the maximum diffusion supply is much smaller than the maximum rate of carboxylation, while pure carboxylation limitation must prevail under the reverse condition. In the first case:

$$(27.20)\quad k_d \ll k_a A_0$$

and equation (27.16) is reduced to:

$$\frac{P}{n} = \frac{K_a A_0 k_r^* + k_d + K_a k_d [CO_2]}{2 K_a} - \sqrt{\left(\frac{K_a A_0 k_r^* + k_d + K_a k_d [CO_2]}{2 K_a}\right)^2 - A_0 k_d k_r^* [CO_2]} \tag{27.21}$$

(which contains, as expected, only the *equilibrium constant*, K_a, instead of the velocity constants k_a and k_a'). The half-saturating concentration is:

$$_{1/2}[CO_2] = \frac{1}{K_a}\left(1 + \frac{K_a A_0 k_r^*}{2 k_d}\right) \tag{27.22}$$

and the initial slope:

$$(dP/d[CO_2])_0 = nK_a A_0 k_r^* k_d/(K_a A_0 k_r^* + k_d) \tag{27.23}$$

The limiting slanting line (roof") is:

$$P_{lim.} = nk_d[CO_2] \tag{27.24}$$

—an expression obviously representing the maximum possible supply of carbon dioxide by diffusion (multiplied by n).

The initial slope of the particular carbon dioxide curve, which, *without* the diffusion limitation, would have started with the slope equal to that of the limiting line (equation 27.24), *i. e.*, according to equation 27.11, the curve corresponding to $k_r^* = k_d/K_a A_0$, is reduced, by slow diffusion, to *one half* its former value:

$$(dP/d[CO_2])_0 = nk_d/2 \tag{27.25}$$

More generally, a primary curve with an initial slope αk_d is reduced by the diffusion limitation to an initial slope of:

$$\left(\frac{dP}{d[CO_2]}\right)_0 = \left(\frac{\alpha}{\alpha + 1}\right) nk_d \tag{27.26}$$

Thus, primary curves with initial slopes between 10 and 100 k_d, will be confined, in consequence of slow diffusion, between $^{10}/_{11}k_d$ and $^{100}/_{101}k_d$, *i. e.*, their initial parts will practically coincide with the limiting straight line, and thus present a picture of the "Blackman type." On the other hand, primary curves that would have exceeded the limiting rate only by factor of the order of unity, as well as those that would have merely approached, but never exceeded, this limit, will retain their individuality and nonlinear shape, and will show a gradual transition from the "Blackman type" to the "Bose type." A certain depressing effect of diffusion will be felt even in a curve the original slope of which was as low as 0.1 k_d. (The

slope of this curve will be reduced by 10%.) This example of the "advance effect" exercised by a "limiting factor" according to the general laws of reaction kinetics has already been quoted in chapter 26.

(c) Slow Carboxylation

This case is contained in the above-derived general equations (27.16–27.19), if one makes the assumption:

(27.27) $$k_a A_0 \ll k_d$$

This implies another inequality:

(27.28) $$k_d[CO_2] \gg k_a'[ACO_2]$$

(since $k_a A_0[CO_2] > k_a A_0[CO_2]_a > k_a[A][CO_2]_a = k_a'[ACO_2] + k_r^*$, the last equation being the steady state condition for the complex ACO_2).

Conditions (27.27 and 27.28) reduce (27.13) to:

(27.29) $$[CO_2]_a = [CO_2]$$

as it should be when the diffusion supply is ample. Consequently, (27.14) and (27.15) are replaced by:

(27.30) $$[ACO_2] = k_a[CO_2]A_0/(k_a' + k_r^* + k_a[CO_2])$$

and (27.16), by the much simpler equation:

(27.31) $$P = nk_a A_0[CO_2]k_r^*/(k_a' + k_r^* + k_a[CO_2])$$

This equation can be written as:

(27.32) $$P/(P_{max.} - P) = k_a[CO_2]/(k_a' + k_r^*)$$

These hyperbolae reach half saturation at:

(27.33) $$_{1/2}[CO_2] = \frac{1}{K_a}\left(1 + \frac{k_r^*}{k_a'}\right)$$

(as could be derived also directly from equation 27.17). Their initial slopes are (*cf.* 27.18):

(27.34) $$(dP/d[CO_2])_0 = nk_a A_0 k_r^*/(k_a' + k_r^*)$$

All curves (27.31) are confined under the "roof":

(27.35) $$P_{lim.} = nk_a A_0[CO_2]$$

an equation that can also be derived from (27.19), and obviously represents the maximum possible rate of carboxylation. By reasoning similar to that employed just above, it can be shown that a "primary" carbon dioxide curve with an initial slope $\alpha k_a A_0 n$ will have its slope reduced, in consequence of slow carboxylation, to $\alpha/(\alpha + 1)k_a A_0 n$. In other words, in this case, too,

the influence of the limiting process is felt long before the rate approaches the limit.

Carboxylation was treated so far as a one-step reaction, with a rate proportional to the concentration $[CO_2]_a$. It is known, however (*cf.* Vol. I, page 203), that carboxylation in photosynthesis is cyanide-sensitive, and thus undoubtedly a catalytic reaction. It must therefore consist of several steps, such as the formation of a substrate–catalyst complex, and the transformation of this complex. We have no information as to the precise nature of these steps, but it can be assumed that at very low $[CO_2]$ values, the rate-determining step will be one with a rate proportional to $[CO_2]$, *e. g.*, reaction (27.36). Under these conditions, the above-given derivations will be valid for the catalyzed as well as for direct carboxylation. At the higher $[CO_2]$ values, however, other steps or the carboxylation process may become rate-determining—steps limited in their maximum efficiency by the available amount of the relevant catalyst (carboxylase). A $[CO_2]$-independent "ceiling" will thus be imposed on the rate of the overall process, which will be determined by the product of the amount of the catalyst available and the average time a catalyst molecule requires to complete the desired transformation.

The specific form of the corresponding kinetic equations will depend on the postulated mechanism of the catalytic action, and, as stated above, we have at present no reasons to favor any one mechanism among the several compatible with our general knowledge of the mechanism of enzymatic processes.

As an illustration, we will go through a calculation based on the simple mechanism:

$$CO_2 + E_A \underset{k'_e}{\overset{k_e}{\rightleftharpoons}} E_A \cdot CO_2 \tag{27.36}$$

(using E_A, as in Volume I, as symbol for the carboxylase).

$$E_A \cdot CO_2 + A \underset{k'_{ea}}{\overset{k_{ea}}{\rightleftharpoons}} E_A + ACO_2 \tag{27.37}$$

(For the sake of simplicity, we neglect diffusion and use $[CO_2]$ where $[CO_2]_a$ should be used.) The equilibrium constants K_e and K_{ea} are subject to the condition:

$$K_e K_{ea} \left(= \frac{k_e}{k'_e} \times \frac{k_{ea}}{k'_{ea}} \right) = K_a \tag{27.38}$$

We assume that equilibrium (27.36) is established practically instantaneously, and remains undisturbed during photosynthesis, but that equilibrium (27.37) is less rapidly attained, and can therefore be displaced in light.* Designating by E_A^0 the total avail-

* The assumption that (27.36), too, is a slow reaction would lead to more complicated quadratic equations for $[ACO_2]$ and P, similar to those obtained in section *b* for the combined effects of slow diffusion and carboxylation.

able quantity of the enzyme E_A we can derive the following equations for the steady state:

$$[ACO_2] = K_a A_0 [CO_2] \Big/ \left(1 + K_a[CO_2] + \frac{k_r^* K_e}{k'_{ea} E_A^0}[CO_2] + \frac{k_r^*}{k'_{ea} E_A^0}\right) \tag{27.39}$$

$$P = n k_r^* K_a A_0 [CO_2] \Big/ \left(1 + K_a[CO_2] + \frac{k_r^* K_e}{k'_{ea} E_A^0}[CO_2] + \frac{k_r^*}{k'_{ea} E_A^0}\right) \tag{27.40}$$

The term proportional to $k_r^*[CO_2]$ in the denominator imposes on these carbon dioxide curves an "absolute ceiling" (*i. e.*, a maximum rate independent of both $[CO_2]$ and k_r^*, and thus of I):

$$P_{\max.}^{\max.} = n k_{ea} E_A^0 A_0 \tag{27.41}$$

(The lower index refers to $[CO_2]$, the upper to I.) This obviously is n times the maximum possible rate of carboxylation according to the mechanism (27.36 and 27.37).)

For relative saturation, as a function of $[CO_2]$, we obtain:

$$P/(P_{\max.} - P) = \frac{\left(K_a + \frac{k_r^* K_e}{k'_{ea} E_A^0}\right)[CO_2]}{1 + (k_r^*/k'_{ea} E_A^0)} \tag{27.42}$$

and for half saturating carbon dioxide concentration:

$$_{1/2}[CO_2] = \frac{1}{K_a}\left(\frac{k_r^*}{k'_{ea} E_A^0} + 1\right) \Big/ \left(1 + \frac{k_r^* K_e}{K_a k'_{ea} E_A^0}\right) \tag{27.43}$$

which reduces itself to:

$$_{1/2}[CO_2] = 1/K_e \tag{27.44}$$

for high k_r^* values (strong light), and to:

$$_{1/2}[CO_2] = 1/K_a \tag{27.45}$$

for low k_r^* values (weak light).

Depending on whether $K_a < K_e$, or vice versa, $_{1/2}[CO_2]$ will shift upwards or downwards with increasing light intensity. The initial slope of the curves (27.40) is:

$$\left(\frac{dP}{d[CO_2]}\right)_0 = \frac{n k_r^* K_a A_0}{1 - k_r^*/k'_{ea} E_A} \tag{27.46}$$

The reduction in rate caused by E_A deficiency is, by comparison of (27.40) with (27.8):

$$\beta = 1 \Big/ \left(1 + \frac{k_r^*}{E_A^0 k'_{ea}} \times \frac{K_a[CO_2] + 1}{K_a[CO_2] + 1}\right) \tag{27.46a}$$

For saturating $[CO_2]$ values:

$$\beta = 1 \Big/ \left(1 + \frac{k_r^*}{E_A^0 k_{ea}}\right) \tag{27.46b}$$

and is thus equal to ½ for a light intensity $k_r^* = E_A^0 k_{ea}$, which *without* enzyme deficiency would have given the saturation value (equation 27.41). Generally, the saturation

level is reduced from $n\alpha k_{ea}E^0_A A_0$ to $\left(\frac{n\alpha}{\alpha + 1}\right) k_{ea}E^0_A A_0$. Thus, here again, the effect of the limiting process becomes felt when the nonlimiting rate is still far below the imposed "ceiling."

(d) Nondissociable ACO_2 Compound. The Franck-Herzfeld Theory

So far, we have considered the carbon dioxide curves as, basically, ACO_2 saturation isothermals, merely distorted by slow diffusion, slow carboxylation and limited quantity of the carboxylase E_A. An alternative was mentioned several times before. The carboxylation equilibrium may lie practically completely on the side of association, and the effect of the factor $[CO_2]$ on the rate of photosynthesis may be due entirely to kinetic phenomena, such as the limited rates of diffusion and carboxylation. The corresponding kinetic equations can easily be derived from the more general formulae given in sections *b* and *c* by putting $k'_a = 0$, *i. e.*, assuming that the rate of decarboxylation is negligible. For example, if the carbon dioxide limitation comes exclusively from slow carboxylation (while the supply of carbon dioxide by diffusion is ample), we can start directly with equation (27.31); omitting the k'_a term in the denominator of this equation, we obtain:

$$P = nk_a A_0 [CO_2] k^*_r / (k^*_r + k_a [CO_2]) \tag{27.47}$$

(If $k^*_r \ll k_a[CO_2]$, this equation is reduced to $P = nk^*_r A_0$ as expected.)

This equation, too, represents hyperbolic carbon dioxide curves:

$$P/(P_{max.} - P) = k_a [CO_2]/k^*_r \tag{27.48}$$

with half saturation at:

$$_{1/2}[CO_2] = k^*_r / k_a \tag{27.49}$$

and the initial slope:

$$(dP/d[CO_2])_0 = nk_a A_0 \tag{27.50}$$

It will be noted that, in this case, all carbon dioxide curves make the same angle with the $[CO_2]$ axis, independently of k^*_r (*i. e.*, of the light intensity). Half saturation, on the other hand, occurs at $[CO_2]$ values that are proportional to k^*_r (*i. e.*, increase with increasing light intensity).

The same method can be extended, to account also for saturation effects caused by limited amount of the carboxylating catalyst E_A, *e. g.*, by assuming $k'_{ea} = 0$ in formula (27.37). Equation (27.40) is reduced, by this assumption, to:

$$P = nK_e k_{ea} k^*_r A_0 E^0_A [CO_2] / (K_e k_{ea} E^0_A [CO_2] + K_e k^* [CO_2] + k^*_r) \tag{27.51}$$

The "absolute ceiling" of this family of hyperbolae, approached when both

k_r^* and $[CO_2]$ are high, is the same as in formula (27.41), *i. e.*, equal to n times the maximum rate of formation of ACO_2 by reaction (27.37).

The equation of the individual carbon dioxide hyperbolae is:

$$P/(P_{\max.} - P) = (k_{ea}E_A^0 + k_r^*)K_e[CO_2]/k_r^* \tag{27.52}$$

They reach half saturation at:

$$_{1/2}[CO_2] = k_r^*/K_e(k_{ea}E_A^0 + k_r^*) \tag{27.53}$$

and have the following initial slope (independent of k_r^*, and thus of light intensity):

$$(dP/d[CO_2])_0 = nK_ek_{ea}A_0E_A^0 \tag{27.54}$$

The assumption of a stable ACO_2 compound was used by Franck and Herzfeld (1941) in their detailed kinetic theory of photosynthesis—the most elaborate to be found in the literature. The rather complex chemical mechanism on which the kinetic analysis was based was illustrated by scheme 7.VA in Volume I.

It would have been inconvenient for Franck and Herzfeld to use a different postulate concerning the stability of ACO_2, because they assumed that the carrier is associated not only with carbon dioxide molecules, but also with seven reduction intermediates. To consider the equilibrium $ACO_2 \rightleftharpoons A + CO_2$ as reversible, while postulating a firm attachment to the same carrier of the reduction intermediates, would have meant added mathematical complications; while to assume a different reversible association equilibrium for each intermediate (as was once suggested in chapter 9) would have been still more cumbersome. Thus, Franck and Herzfeld chose the simplest way when they assumed the complexes of A with CO_2 as well as with all seven intermediates to be practically undissociable (except by light—a complication discussed in Vol. I, p. 167, and in chapter 29).

Because of the assumption of an undissociable complex, the derivation of Franck and Herzfeld contains no equivalent of section *a*. Since they did not take into account the effects of slow diffusion, it also contains no equivalent of section *b*. The aspects of the carbon dioxide supply problem that Franck and Herzfeld did consider were the phenomena treated in section c, *i. e.*, slow carboxylation, caused either by low carbon dioxide concentration, or by carboxylase deficiency.

Their treatment of these two effects was somewhat different from that given in section c because they postulated a different mechanism of catalysis. Instead of the reaction sequence (27.36, 37), Franck and Herzfeld assumed the following three reactions:

$$CO_2 + A \overset{K}{\rightleftharpoons} A \cdot CO_2 \tag{27.55a}$$

(formation of a "loose" complex)

$$A \cdot CO_2 + E_A \xrightarrow{k_a} ACO_2 + E_A' \tag{27.55b}$$

(catalyzed formation of a "stable" complex and inactivation of the catalyst)

$$E_A' \xrightarrow{k_a'} E_A \tag{27.55c}$$

(reactivation of the catalyst)

Franck and Herzfeld assumed equilibrium (27.55a) to be established practically instantaneously, whereas reaction (27.55b) was assumed to have a finite velocity, and thus to be capable of becoming a "bottleneck" of photosynthesis. This occurs when either the substrate concentration $[A{\cdot}CO_2]$, or the enzyme concentration $[E_A]$ is low, or, more generally, when the product of the two concentrations is small. According to (27.55), the concentration of the "loose" complex is:

$$[A{\cdot}CO_2] = K\,[A][CO_2] \tag{27.56}$$

(For the sake of consistency—*cf.* equation 27.5—we use as equilibrium constant the inverse of Franck and Herzfeld's K). The rate of the bottleneck reaction (27.55b) is:

$$(d[ACO_2]/dt) = k_a[A{\cdot}CO_2][E] = k_aK[A][CO_2][E_A] \tag{27.57}$$

We assume—as we did in all our derivations so far—that no kinetic factors other than those connected with the supply of carbon dioxide affect the rate of photosynthesis. Under these conditions, the equations of the carbon dioxide curves can be derived by calculating the stationary concentrations [A] and $[E_A]$, inserting them into (27.57) and then calculating the stationary concentration $[ACO_2]$ by equalizing the rate of production of $[ACO_2]$ given by equation (27.57) and the rate of reduction of this product by light.

In the Franck-Herzfeld theory, all carrier molecules, A, may be considered, for kinetic purposes, as attached to a molecule of the sensitizer (chlorophyll), so that the substrate molecules bound to A can undergo direct photochemical change; putting it more cautiously, only those A molecules are taken into consideration in kinetic equations that *are* attached to chlorophyll. Their total number can be designated as A_0. Similarly, all chlorophyll molecules are supposed to carry acceptor molecules, A; putting it more cautiously, only the absorption of light by those chlorophyll molecules that *are* associated with A is taken into consideration. We designate the total number of such molecules as Chl_0. This number probably can be reduced by certain inhibitors, such as urethan or other narcotics, that displace the acceptor A from chlorophyll. (The same applies, according to Franck, to "self-narcotization" by the unfinished product of photosynthesis to which reference was made in chapter 24.)

In Franck's picture, the rate constant k_r^* in equation (27.6) can be written as k^*I:

$$P = -n(d[ACO_2]/dt) = k^*I\,[ACO_2] \tag{27.58}$$

Here, $k^*I\,[ACO_2]$ is the rate of absorption of light by the acceptor A in combination with CO_2, k^* being essentially an average absorption coefficient of the specimen under investigation. The factor n is equal to 1 in the Franck-Herzfeld mechanism; but k^*I is, in the steady state, not more than one eighth of the total rate of absorption.

If one assumes, as Franck and Herzfeld did, that this absorption coefficient is the same whether chlorophyll is associated with ACO_2 or with any of the seven reaction intermediates (*cf.* scheme 7.VA), then in the steady state the amounts of $[ACO_2]$ and of seven intermediates must all be the same. This means that:

$$A_0 = (=Chl_0) = [A] + [A{\cdot}CO_2] + 8\,[ACO_2] \tag{27.59}$$

where A_0 is the total available quantity of the acceptor A bound to chlorophyll.

Equalizing (27.57) and (27.58), we obtain:

$$[ACO_2] = (k_aK[A][E_A][CO_2])/k^*I \tag{27.60}$$

The stationary value of $[E_A]$ can be calculated by equalizing the rates of reactions 27.55b) and (27.55c); this gives:

$$[E_A] = k_a'E_A^0/(k_a[A{\cdot}CO_2] + k_a') \tag{27.61}$$

After inserting (27.61) into (27.60), one can use (27.56) and (27.59) to eliminate [A] and [A.CO_2] and to obtain the desired equation for [ACO_2]—and according to (27.58) also for P—in terms of [CO_2], A_0 and E_A^0. Because of the assumed two-step mechanism of formation of [ACO_2] (with the loose complex A·CO_2 as an intermediate), the resulting equation is quadratic. Its solution—which again represents a family of hyperbolae—is a rather complex expression, and we do not need to quote it here.

For high values of both [CO_2] and I, the expression for P approaches asymptotically the value:

$$P_{\text{max.}}^{\text{max.}} = nk_a k_a' E_A^0 A_0/(k_a A_0 + k_a') \tag{27.62}$$

(with the lower index referring to [CO_2] and the upper to I). In the two extreme cases, when either $k_a A_0 \gg k_a'$, or vice versa, expression (27.62) reduces itself either to $nk_a' E_A^0$ (*i. e.*, the maximum rate of reaction 27.55c), or to $nk_a E_A^0 A_0$ (the maximum rate of reaction 27.55b).

The relations are much simpler if the rate of reaction (27.55c) is assumed to be rapid (compared with the rate of photosynthesis P), so that $[E_A]'$ is practically equal to zero, and $[E_A]$ to E_A^0. In this case, one obtains an equation containing only first power of P, the solution of which is:

$$P = k_a K E_A^0 A_0 [CO_2] k^* I/(k^* I + k^* I K [CO_2] + 8\, k_a K E_A^0 [CO_2]) \tag{27.63}$$

This is an equation of a family of hyperbolae (with I as parameter):

$$\frac{P}{P_{\text{max.}} - P} = \left(K + \frac{8\, k_a K E_A^0}{k^* I}\right) [CO_2] \tag{27.64}$$

Half saturation ($P = {}_1/_2 P_{\text{max.}}$) is reached when:

$${}_1/_2[CO_2] = k^* I/(K k^* I + 8\, k_a K E_A^0) = \frac{1}{K}\left(\frac{1}{1 + (8 k_a E_A^0/k^* I)}\right) \tag{27.65}$$

It is shifted, with increasing light intensity, toward the higher [CO_2] values. The initial slope of the curves (27.63) is:

$$(dP/d[CO_2])_0 = nk_a K E_A^0 A_0 \tag{27.66}$$

Because, in formula (27.55b), the formation of ACO_2 was assumed to be irreversible, the slope (27.66) is *independent of light intensity*. (In other words, at very low carbon dioxide concentrations, all ACO_2 formed is reduced by light, whatever the intensity of the latter.) Thus, as stated before, the carbon dioxide curves represented by equations such as (27.47) or (27.63) have more pronounced "Blackman characteristics" than the curves obtained with the assumption of a dissociable ACO_2 complex.

Of course, the hypothesis of a stable association of the acceptor A with chlorophyll is independent of the other postulates of the Franck-Herzfeld theory; the latter can be combined also with the assumption of a dissociable ACO_2 complex.

(*e*) *Back Reactions in the Photosensitive Complex*

So far, we have discussed the carbon dioxide curves only from the point of view of the "preparatory" dark processes at the "reduction end" of photosynthesis, since these are the stages of photosynthesis most closely related to the "carbon dioxide factor."

The influence of the preparatory reactions at the "oxidation end"

(such as the possible preliminary enzymatic binding of water) as well as the influence of "finishing" reactions (such as conversion of $AHCO_2$ to a carbohydrate, and liberation of oxygen) have so far been neglected; while the role of reactions within the photosensitive complex proper was taken into account only by assuming the rate of reduction of ACO_2 to be equal to the product $k_r^*[ACO_2]$, where k_r^* was considered a function of the illumination intensity, I. No new source of carbon dioxide saturation was added by this assumption; saturation remained determined entirely by the four factors treated in detail in sections *a–d* (limited quantity of A, slow diffusion, slow carboxylation and limited quantity of the carboxylase, E_A).

Obviously, however, carbon dioxide saturation can also be produced by limitations of any of the other partial reactions in photosynthesis. For example, insufficient amount of a catalyst needed for the preliminary transformation of the reductant (H_2O, H_2, H_2S...) might have the same "ceiling" effect on the carbon dioxide curves as the limited amount of the enzyme (carboxylase) that catalyzes the preliminary transformation of carbon dioxide itself.

Little could be gained by trying to write out equations for carbon dioxide curves that would include the effect of a limited supply of the reductant. On the other hand, a few words may usefully be said about the influence on the carbon dioxide curves of a limited supply of light.

The kinetic equations that follow from the consideration of the probable forward and back reactions within the photosensitive complex proper will be derived in chapter 28 (p. 1020 ff.). They show, as expected, that the rate of absorption of light by this complex imposes a limit on the rate of photosynthesis that cannot be raised by increased supply of carbon dioxide (or change in any other external factor). Consequently, the "light factor" is in itself capable of producing a saturation effect in the carbon dioxide curves. For example, if we consider equation (28.14), derived from reaction mechanism (28.11), as an equation of carbon dioxide curves (*i. e.*, if we treat I as a parameter), we find that, at high $[CO_2]$ values, the rate approaches the maximum:

$$P_{max.} = nk^*I\mathrm{Chl}_0 \tag{27.67}$$

which is the rate of supply of light quanta multiplied by the number n of carbon dioxide molecules that can be transformed by a single quantum—perhaps $\frac{1}{8}$. Half saturation is reached, according to scheme 28.IA, at:

$$_{1/2}[ACO_2] = k'/k_r \tag{27.68}$$

One could thus ask whether the interpretation of carbon dioxide curves really *requires* the assumption of an acceptor A, supposed to be present in

limited quantities and carboxylated by a slow dark reaction; or whether one could perhaps explain all carbon dioxide saturation phenomena by reference to limited supply of light. The occurrence of phenomena such as carbon dioxide "pick-up" after intense photosynthesis (Vol. I, page 206) provides, however, direct evidence that a dark carboxylation reaction actually does occur, and that it has an effect on the rate of the over-all reaction of photosynthesis. On the other hand, it is undoubtedly true that some, at least, of the carbon dioxide curves, particularly those measured in weak light, owe their hyperbolic shape entirely or preponderantly to the limited rate of supply of light quanta.

Reaction mechanism (28.IA, eqs. 28.20), which we have used, provides that the reaction of the primary photoproduct, $HX \cdot Chl \cdot Z$, with the oxidant, ACO_2 (rate constant, k_r), competes with the "deactivating" reaction that converts $HX \cdot Chl \cdot Z$ back to $X \cdot Chl \cdot HZ$ (rate constant k'). An alternative mechanism (28.IB, eqs. 28.21) also is discussed in chapter 28, in which the "primary" back reaction of the photoproduct, $HX \cdot Chl \cdot Z$, is eliminated by immediate reaction of $HX \cdot Chl \cdot Z$ with (free or bound) water, converting it to $HX \cdot Chl \cdot HZ$. This simplified mechanism will be used in chapter 28 to analyze another possible kinetic effect within the photosensitive complex—the accumulation, during strong photosynthesis, of the chlorophyll complex in the changed, photoinsensitive form ($HX \cdot Chl \cdot HZ$ in the mechanism used). In deriving equation (28.14), the simplification $[HX \cdot Chl \cdot Z] \ll [X \cdot Chl \cdot HZ] \simeq Chl_0$ was made; we do not make a similar assumption in respect to $HX \cdot Chl \cdot HZ$, but postulate that the "reduced" form accumulates and brings about saturation of light curves. (With increasing light intensity more and more chlorophyll complexes will be present, in the steady state, in the photoinsensitive form, $HX \cdot Chl \cdot HZ$.) This assumption leads to equation (28.27) for P as function of I and $[ACO_2]$, and to equation (28.28), if the equilibrium value (27.3) is substituted for $[ACO_2]$ in (28.27). Considering (28.28) as equation of carbon dioxide curves (I = constant), we obtain the following expressions for these curves:

$$\frac{P_{max.} - P}{P_{max.}} = \frac{k^* I}{k^* I(1 + K_a[CO_2]) + k_r K_a A_0 [CO_2]} \tag{27.69}$$

$$_{1/2}[CO_2] = \frac{1}{K_a}\left(\frac{k^* I}{k_r A_0 + k^* I}\right) \tag{27.70}$$

Equation (27.70) shows that the half-saturating carbon dioxide concentration rises with increasing light intensity, and that the equilibrium constant K_a can be obtained by an extrapolation of $_{1/2}[CO_2]$ to *high* light intensities (linear extrapolation with $1/I$ as abscissa). It will be recalled that in the case of the carbon dioxide supply limitation (*cf.* equation 27.22 or 27.33) we had to obtain the same value by extrapolation to *low* light intensities

($I = 0$, and thus $k_r^* = 0$). We will return to the evaluation of K_a from carbon dioxide curves in section *g*.

(*f*) *Acceptor "Blockade"*

In comparing the equations obtained in the preceding sections, with the empirical carbon dioxide curves, one has to keep in mind that, despite the considerable complexity of some of these equations, they all embody certain simplifying assumptions, and therefore cannot be valid except within a limited range of conditions.

The only kinetic factors taken into account so far were slow diffusion of carbon dioxide, slow carboxylation and limited quantities of the acceptor A and of the carboxylase E_A. In section *e* we discussed the additional complications that may be caused by the deactivation of the primary photochemical product, $HX \cdot Chl \cdot Z$, in competition with its reaction with ACO_2, or by the accumulation of the photosensitive complex in the reduced form, $HX \cdot Chl \cdot HZ$.

A single slow preparatory dark reaction with a rate proportional to $[CO_2]$ plus the limited quantity of a single catalytic agent (*e. g.*, E_A, A or Chl) could suffice to account for the increase of P at low values of $[CO_2]$, for the individual saturation of each carbon dioxide curve and for the occurrence of "absolute" saturation (*i. e.*, saturation with respect to both $[CO_2]$ and I). We know, however, that several catalytic steps of limited maximum efficiency play a part in photosynthesis; and, although some of these steps are not directly associated with the assimilation of carbon dioxide, a limitation of the rate of the over-all reaction, whatever its source, must be reflected in the shape of the carbon dioxide curves, particularly in the region where they approach "absolute saturation."

In Volume I, we outlined a general scheme of photosynthesis that includes, in addition to preparatory supply reactions, two other main types of catalytic processes—"finishing" reactions, associated with the conversion of the first reduction products into carbohydrates and with the production of molecular oxygen.

What happens to the first reduction product (which we will now designate as $AHCO_2$) can affect the rate of photosynthesis in various ways. If, for example, this product has to undergo a chemical transformation before it can be separated from the carrier A, this transformation may require a certain time, so that, in intense light, a considerable fraction of the acceptor A can be "blocked" by $AHCO_2$. Or else, the product $AHCO_2$ may require a catalyst (E_B) for its stabilization, and unless this catalyst is available within a sufficiently short time, $AHCO_2$ may react back (with the oxidized photosensitive complex $X \cdot Chl \cdot Z$, or with the intermediates

of the oxidation of water, such as A'HO, or with some other cellular oxidants) to form ACO_2 (or, according to a hypothesis of Franck, $A + CO_2$). The latter possibilities will be discussed in section 7*e* of chapter 28.

We will briefly consider here the effects of the "acceptor blockade." The conservation equation (27.4) must in this case be replaced by:

$$A_0 = [A] + [ACO_2] + [AHCO_2] \tag{27.71}$$

Assuming that the restoration of the acceptor is a monomolecular process

$$AHCO_2 \xrightarrow{k_1'} A + HCO_2 \tag{27.72}$$

we can write the equations for the stationary concentration of ACO_2 and (assuming the validity of 27.6) also for the rate of photosynthesis. Taking the simplest case—that discussed in section *a* (carboxylation equilibrium undisturbed by slow diffusion or slow carboxylation)—we obtain:

$$P = \frac{nA_0K_a[CO_2]k_r^*}{1 + K_a[CO_2]\left(1 + \frac{k_r^*}{k_1'}\right)} \tag{27.73}$$

$$\frac{P}{P_{max.} - P} = K_a[CO_2]\left(1 + \frac{k_r^*}{k_1'}\right) \tag{27.74}$$

$$_1/_2[CO_2] = \frac{1}{K_a\left(1 + \frac{k_r^*}{k_1'}\right)} \tag{27.75}$$

The carbon dioxide curves (27.73) are thus hyperbolae the half saturation of which is shifted, with increasing light intensity, toward the *lower* concentrations of carbon dioxide (a shift opposite to that caused by slow diffusion or slow carboxylation; and apparently not encountered in experimental curves). Because of the occurrence in the denominator of (27.73) of the product $k_r^*[CO_2]$, the rate cannot exceed the "absolute maximum":

$$P_{max.}^{max.} = nk_1'A_0 \tag{27.76}$$

i. e., n times the maximum rate of restoration of the acceptor according to (27.72).

(*g*) *Calculation of Carboxylation Constant from Carbon Dioxide Curves*

If one wants to use the carbon dioxide curves for the determination of the carboxylation constant K_a, as was done in chapter 8 (Vol. I), one should avoid the region of "absolute saturation," and determine systematically, and with a high degree of precision, the carbon dioxide curves in the region where the yield is still proportional to light intensity. In this way, one could perhaps determine which of the several above discussed mechanisms of carbon dioxide supply provides the best interpretation of the facts. The experimental data available at present are neither exact nor systematic enough for such an analysis.

Among the characteristics of the various theoretical equations derived above for the carbon dioxide curves, which might be used for comparison with the experiment, are the relations between half-saturating carbon dioxide concentration and light intensity (*cf.* equations 27.12, 17, 22, 33, 44, 45, 49, 53, 65, and 75), and between the initial slope and light intensity (equations 27.11, 18, 23, 34, 46, 50, 54 and 66).

The value of $_1/_2[CO_2]$ is independent of k_r^* (*i. e.*, of light intensity) and equal to $1/K_a$ only to the extent to which the carbon dioxide curves are proportional to the saturation curves of the ACO_2 complex (eq. 27.12). It has been suggested by some that this assumption is legitimate whenever the carbon dioxide curves are found to be hyperbolic. Thus, Burk and Lineweaver (1935), having satisfied themselves that the carbon dioxide curves of Warburg, Harder, James, van der Paauw, and Emerson and Green (Table 27.I) follow the hyperbolic saturation law, proceeded to calculate from them the carboxylation constants K_a and obtained values ranging from 1×10^{-6} to 10×10^{-6} l./mole (at room temperature). The corresponding free energies of carboxylation, $\Delta F_a (= RT \log_e K_a)$ are between -6.9 and -8.3 kcal/mole.

It was mentioned on page 908 that, according to Whittingham (1949), the half-saturating CO_2 concentration of *Chlorella* is shifted down to 0.5 or 1.0×10^{-6} mole/l. if care is taken to avoid inhibition phenomena at low CO_2 concentration, by allowing 2–3 hour induction period (if the cells were grown in high $[CO_2]$) or, still better, by using cells grown in low $[CO_2]$ (*e. g.*, in air).

The *heat of carboxylation* also was estimated by Burk and Lineweaver. From the absolute rate values found by Emerson and Arnold, at 6° and 24° C. in flashing light, they calculated $\Delta H_a = 1.3$ to 6.2 kcal/mole. However, Table 8.VIII shows that the fixation of carbon dioxide by organic molecules is accompanied—as is natural in reactions in which small molecules are attached to larger ones—by a *decrease* in entropy, $-T\Delta S$ being as large as $+8$ or even $+16$ kcal/mole at room temperature. Thus, if $-\Delta F$ of carboxylation is 7–8 kcal/mole, $-\Delta H$ should be of the order of 15–20 kcal—considerably larger than the estimate of Burk and Lineweaver.

Our derivations in sections b, c and d show that the carbon dioxide curves may be strongly affected by slow diffusion, or slow carboxylation, without losing the hyperbolical shape. Equations (27.17, 22 and 33) show that these two factors cause $_1/_2[CO_2]$ to *increase linearly with increasing light intensity*. The constant K_a can in this case be obtained by linear extrapolation of $_1/_2[CO_2]$ to $I = 0$. Figure 27.9 shows that the data of Harder, Hoover and co-workers, and Smith (compare Table 27.I) extrapolated in this way, give $_1/_2CO_2$ values in the neighborhood of 5×10^{-6} M, and thus K_a values of about 2×10^5, corresponding to $\Delta F = -7.9$ kcal./mole.

However, the linear extrapolation procedure is not always reliable. While slow diffusion and slow carboxylation tend to shift the half-saturating concentration *upward* with increasing light intensity, other influences, such as the "acceptor blockade" (equation 27.75), or the deficiency of catalysts, may shift it *downwards*. Equation (27.43) shows, for example, that a limited amount of the carboxylase E_A may cause $\frac{1}{2}[CO_2]$ either to increase or to decrease with increasing light intensity, depending on whether K_c is smaller or larger than K_a. In this case, extrapolation of $\frac{1}{2}[CO_2]$ to zero light intensity should still give the correct value of K_a, but systematic data will be needed to make the required nonlinear extrapolation possible.

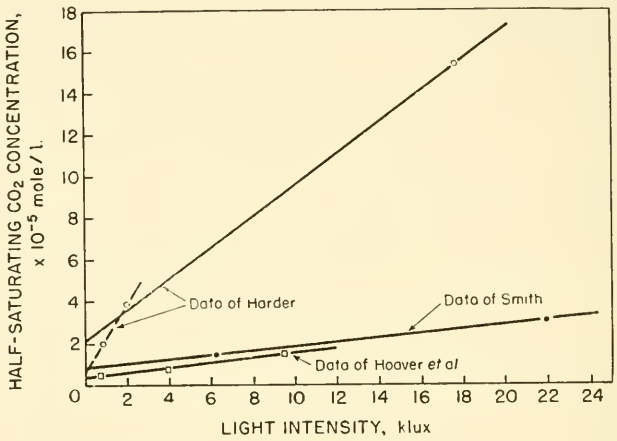

Fig. 27.9. Extrapolation of $\frac{1}{2}[CO_2]$ to $I = 0$.

Finally, under certain conditions the value of $\frac{1}{2}[CO_2]$ may have no relation to the carboxylation constant at all. This is obviously true if the ACO_2 complex is practically undissociable. Equation (27.49), for example, contains only the kinetic constants k_a and k_r^*. Similarly, in section *c*, where we postulated that the carbon dioxide saturation is caused by "detautomerization" of the photocomplex HX·Chl·Z, equation (27.68) showed $\frac{1}{2}[CO_2]$ to be a function of the kinetic constant k_a and k_r only.

To sum up, the constant $1/K_a$ may be $\gg \frac{1}{2}[CO_2]$ or $\ll \frac{1}{2}[CO_2]$, and the carbon dioxide curves may nevertheless be hyperbolae. The observations of the "pick up" and of the uptake of C^*O_2 in the dark speak in favor of a reasonably stable ACO_2 complex, and thus against the second possibility. Consequently, the above calculated value of K_a ($\sim 5 \times 10^{-6}$ mole/l.) and of $\Delta F(-7.9$ kcal/mole) are to be considered as upper, rather than lower, limits; in other words, the ACO_2 complex is either as

stable as or even more stable than indicated by these values. We recall that in chapter 8(Vol. I) a compilation of experimental data showed the known free energies of carboxylation of compounds of the type of ($RH + CO_2 \rightarrow RCOOH$) to be about +10 kcal/mole. It was also mentioned that Ruben had suggested attributing the extraordinary stability of the ACO_2 compound in photosynthesis to a coupling of carboxylation with an exergonic transphosphorylation—the transformation of a "high energy" into a "low energy" phosphate ester. Another explanation of the same type would be to assume the coupling of carboxylation with a parallel or consecutive exergonic *oxidation-reduction*, a coupling that actually occurs in the uptake of carbon dioxide catalyzed by certain enzymes derived from yeast and bacteria (as described by Lipmann and Tuttle 1945; see also Ochoa 1946).

There is one obvious experiment by which the carboxylation constant derived from the carbon dioxide curves of photosynthesis could be checked —precise measurements of the carbon dioxide uptake in the dark as a function of the concentration $[CO_2]$ in the medium. This could be done by means of radioactive carbon, or by ordinary analytical methods. So far, no such measurements have been made. The observations of Ruben and co-workers (*cf.* Vol. I, chapter 8) seem to indicate, however, that the complex ACO_2 *is* dissociated in vacuum, albeit very slowly, and thus possesses a finite, although perhaps very small, dissociation pressure (*cf.*, however, chapter 36 for the interpretation of C*-measurements).

(*h*) *Are Experimental Carbon Dioxide Curves Hyperbolae?*

It will be noted that *all* theoretical carbon dioxide curves discussed in this chapter were hyperbolae. Smith (1937) analyzed the empirical carbon dioxide curves and concluded—in contrast to Burk and Lineweaver (1935)—that many of them reach saturation more rapidly than a hyperbola approaches its asymptote. He has therefore attempted to obtain a better analytical representation of the carbon dioxide curves by changing the exponents in equation (27.9). The empirical equation:

$$P/\sqrt{P_{max.}^2 - P^2} = K[CO_2] \tag{27.77}$$

was found by him to fit satisfactorily his own results, as well as those of Warburg, Emerson and Green, and Hoover, Johnston and Brackett. In the saturation region, where $(P_{max.} - P) \ll P$, equation (27.77) should not differ much from the simpler quadratic relationship:

$$P/(P_{max.} - P) = K'[CO_2]^2 \tag{27.78}$$

Exponential functions often are used in the interpretation of curves that rapidly reach saturation. Harder (1921) tried to represent his results by the formula:

(27.79) $$(P_{\max.} - P)/P_{\max.} = e^{-\text{const}\cdot[CO_2]}$$

(analogous to Mitscherlich's and Baule's revised formulations of Liebig's "minimum law" of fertilization). A similar formulation was suggested by Brackett (1935):

(27.80) $$\frac{P_{\max.} - P}{P_{\max.}} = \text{const. } e^{-\{[CO_2]-(P/b)+c\}}$$

The correction term P/b in the exponent was intended to account for the fact that the carbon dioxide curves approach asymptotically, at low $[CO_2]$ values, a straight line with a finite slope rather than the axis of ordinates. The correction term c was attributed to respiration. The exponential as a whole was supposed to represent the "true amount of CO_2 available at the site of photosynthesis"—the first two terms describing the supply by diffusion from outside, and the third that by respiration. The occurrence of a concentration factor in the exponent, unusual in reaction kinetics, was associated with Beer's law of light absorption. However, since carbon dioxide is not the light-absorbing species in photosynthesis, it is not clear why *its* concentration should appear in the exponent, and the equation of Brackett (as well as that of Harder) is not likely to represent more than an empirical approximation formula fitting some of the experimental results.

If it is true, as asserted by Smith (1937) that the carbon dioxide curves definitely cannot be represented by hyperbolae, the question arises as to what could cause their deviation from the hyperbolic form, and lead to a more sudden saturation. It is easy to show that the relation between P and $[CO_2]$, which was expressed by *quadratic* equations in the several cases when *two* reaction steps were assumed to lead from the external carbon dioxide to the complex ACO_2, will be represented by a *cubic* equation if a third intermediate process is added (*e. g.*, $B + ACO_2 \rightarrow BCO_2 + A$; $BCO_2 +$ light products $\rightarrow$ photosynthesis). Since the equation representing $[BCO_2]$ as a function of $[CO_2]$ will contain higher powers of $[BCO_2]$, but only the first power of $[CO_2]$, the carbon dioxide curves will deviate from hyperbolae in the direction of a *slower* (rather than of a more rapid) approach to saturation.

An obvious way to obtain carbon dioxide curves that approach saturation *more rapidly* than hyperbolae is to assume carboxylation equilibria involving *two* (or more) carbon dioxide molecules, *e. g.*,

(27.81) $$2\ CO_2 + A \underset{k'_a}{\overset{k_a}{\rightleftharpoons}} A(CO_2)_2$$

or more generally:

(27.82) $$nCO_2 + A \underset{k'_a}{\overset{k_a}{\rightleftharpoons}} A(CO_2)_n$$

In this case, the thermodynamic carboxylation equilibrium condition (assuming that the several carbon dioxide molecules in the complex are independent of each other) is:

(27.83) $$[A(CO_2)_n] = K_a A_0 [CO_2]^n/(1 + K_a[CO_2]^n)$$

consequently:

$$\frac{[A(CO_2)^n]}{[A(CO_2)^n]_{max.} - [A(CO_2)]} = K_a[CO_2]^n \tag{27.84}$$

One may use this derivation as starting point for speculations in which the "condensation" of carbon dioxide into a C_n compound (perhaps, with $n = 3$ or 6) is assumed to be achieved, not *after* photochemical reduction (or between successive photochemical reduction steps), but already in the preliminary, nonphotochemical carboxylation stage. One could quote in this connection the observations of Van Rysselberghe and coworkers (1946), who found indications that carbon dioxide is reduced, at the cathode of a polarograph, after preliminary formation of a polymeric adsorption complex containing six CO_2 molecules.

Studies of the Hill reaction (chapter 35) and of the C(14) uptake in light (chapter 36) indicate three possible additional complications of the kinetics of carbon dioxide uptake in photosynthesis:

(*1*) There may be *several carboxylations* involved, *e.g.*, one of the $C_2 \rightarrow C_3$ and one of the $C_3 \rightarrow C_4$ type. Since, in the steady state, both must proceed at the same rate, this complication may not change the kinetic derivations too radically. When, for some reason, one carboxylation becomes a rate-limiting step, the other must be slowed down too, by the blocking action of its accumulated products.

(*2*) *Carboxylation may be coupled with hydrogenation* ("reductive carboxylation," typified by direct conversion of pyruvic to malic acid, and already mentioned on p. 937). In other words, reaction (27.2), instead of being kinetically independent, may be combined with the first step in (27.5A). This, too, should not necessarily change the kinetic derivations in a radical way, since "coupling" probably means a fast sequence of two reaction steps, perhaps catalyzed by two active groups in the same protein molecule.

(*3*) The acceptor, A, *may be a product of photosynthesis*, disappearing in the dark. This would make A_0 a function of the rate of photosynthesis, P, and would necessitate reconsideration of the kinetic equations based on a constant value of A_0.

B. Carbon Dioxide Concentration and Fluorescence*

In chapter 24, when discussing the fluorescence of chlorophyll in the living cell, we mentioned the close relationship often found between the intensity of fluorescence and the rate of photosynthesis; we stated that, because of this relationship, the measurement of fluorescence has become an important tool in the study of the kinetics of photosynthesis. Conse-

* Bibliography, page 963.

quently, we proposed to review the effects of various external factors on the intensity of chlorophyll fluorescence *in vivo* parallel with the presentation of the influence of these factors on the rate of photosynthesis.

In following this plan, we have now to describe the changes in the intensity of fluorescence of living plant cells, associated with variations in the supply of carbon dioxide.

The general finding appears to be that reduction or complete stoppage of the carbon dioxide supply usually affects the yield of fluorescence, φ, in a certain range of illuminations. It has no effect on the yield of fluorescence in very weak light; and probably also none in very strong light, but the latter generalization is in need of experimental confirmation. In purple bacteria, the commonly observed *increase* of φ upon removal of carbon dioxide sometimes gives place, with increasing light intensity, to the opposite effect (*cf.* fig. 28.30). Since no systematic measurements of φ for variable $[CO_2]$ have been carried out, the plotting of "carbon dioxide curves" of fluorescence, $F = f[CO_2]$, is not possible. Instead, "light curves" have been drawn, showing the intensity of fluorescence as a function of *light intensity*, with $[CO_2]$ as a parameter, usually for two different concentrations of carbon dioxide only (or simply "with carbon dioxide" and "without carbon dioxide"). Several such curves will be reproduced in chapter 28 (*cf.* figs. 28.25, 28, 29 and 30).

We anticipate here some of the facts to be presented there: The yield of fluorescence, φ, generally remains constant (φ_1) up to or beyond the intensity region in which photosynthesis becomes saturated with light; but sooner or later, it increases more or less gradually, finally to reach a new steady level, φ_2. We designate the intensity at which the transition begins, as I', and that at which it ends, as I''. The effect of removal of carbon dioxide appears to be a downward shift of this transitional range. Thus, McAlister and Myers (1940) found that in 4% CO_2, the yield of fluorescence of wheat leaves was constant up to 600 kerg/(cm.^{2} × sec.) (kerg = 10^3 erg) while, in 0.03% CO_2, the yield increased (by about 15–20%) between 200 and 600 kerg/(cm.^{2} × sec.) (*cf.* fig. 28.25).

Similarly, Franck, French and Puck (1941) found that the steady state fluorescence of a leaf of *Hydrangea*, at $I = 7$ kerg/cm.^{2} sec. was about 20% higher in carbon dioxide-free air than in air containing 5% CO_2. At 71 kerg/cm.^{2} sec., on the other hand, the yield of fluorescence in 5% CO_2 had increased so strongly that now a change to 0.03% CO_2 had no noticeable effect.

Working with a culture of diatoms (*Nitzschia* sp.), Wassink and Kersten (1945) found the yield of fluorescence in intense light, 50 kerg/cm.^{2} sec., to be higher in the absence than in the presence of carbon dioxide. Figure 28.28 indicates that, in this case, the difference is brought about by a *de-*

cline of φ in the carbon dioxide-supplied algae, rather than by an *increase* of φ in the carbon dioxide-starved cells (as in figs. 28.25 and 28.30A).

Wassink, Katz and Dorrestein (1942) noticed a similar effect of carbon dioxide deficiency on the yield of fluorescence in purple bacteria; however, the change was much less pronounced than that caused by the rationing or denial of the *reductants* (*cf.* below, section D2). In the *absence* of a reductant (a condition that cannot be paralleled in green plants), the removal of carbon dioxide had no effect at all on the fluorescence of *Chromatium* (*cf.* fig. 28.29). In the *presence* of reductants (such as thiosulfate or hydrogen), the fluorescence of carbon dioxide-starved bacteria was, however, considerably stronger than that of carbon dioxide-supplied cells (*cf.* fig. 28.30A). This difference occurred in the range from 2 to 30 kerg./cm.^{2} sec. At light intensities above 30 kerg, the fluorescence curves with and without carbon dioxide again approached, and finally even crossed each other, so that φ became higher in the absence than in the presence of carbon dioxide. In other words, fluorescence was now higher when photosynthesis was possible than when it was suppressed! (This is a good illustration of the fact that the relation between photosynthesis and fluorescence is *not* a simple competition; *cf.* page 826.)

In chap. 28 we will discuss several possible explanations of the change in intensity of chlorophyll fluorescence in strong light. One, is to attribute this change to the accumulation of the photocomplex, X·Chl·HZ (a complex of primary oxidant + chlorophyll + primary reductant) in a changed form due to the incapability of the catalytic dark reactions to keep pace with the primary photochemical process. The changed forms of the photocomplex can have a different fluorescence yield, *first*, because they are photostable (*i. e.*, cannot undergo the primary photochemical photoprocess (*e. g.*, $X\cdot Chl\cdot HZ \xrightarrow{h\nu} XH\cdot Chl\cdot Z$), and, *secondly*, because they have an intrinsically different capacity for dissipating the excitation energy. An alternative or additional cause is, according to Franck, the formation of a "narcotizing" substance, by reaction of excess oxidation intermediates with metabolites; this narcotic displaces the reactants from the photocomplex and thus causes changes in the yield of fluorescence.

Absence of carbon dioxide slows down the restoration of the primary oxidant, HX or ACO_2, after it has been reduced by light. It must thus favor the accumulation of the photocomplex in a form such as HX·Chl·Z or HX·Chl·HZ, and this accumulation will find its expression in a change (usually an increase) in the yield of fluorescence.

Franck and Herzfeld (1941) were inclined to consider the effect of carbon dioxide on fluorescence of plants as proof that carbon dioxide (in the form of a compound with an acceptor, ACO_2) is a direct part of the photosensitive complex (*i. e.*, that X in X·Chl·HZ is identical with ACO_2).

It was argued, however, in Volume I (page 167) that ACO_2 and $X \cdot Chl \cdot HZ$ can be separated by intermediate oxidation-reduction systems, and, nevertheless, an exhaustion of ACO_2 may cause an accumulation of the primary oxidant, X, in the form HX, and consequent change in the intensity of fluorescence. (A strike of longshoremen in America can cause tin ore to accumulate at the pit heads in the East Indies.)

Fluorescence observations on purple bacteria produced new evidence bearing upon the relation of carbon dioxide to the fluorescent pigment complex.

Wassink, Katz and Dorrestein (1942) were strongly impressed by the above-mentioned observation that changes in the concentration of *reductants* affect the fluorescence of purple bacteria more strongly than changes in the concentration of the *oxidant* (CO_2), and that the former effect persists in the absence of carbon dioxide, while the latter disappears when reductants were absent. They concluded that carbon dioxide does not come into (direct or indirect) energy exchange with excited chlorophyll at all, and that the excitation energy is taken up (directly or indirectly) by the reductants (H_2S, $H_2S_2O_3$, H_2 . . . in purple bacteria, H_2O in green plants). They thought that the small observed effects of CO_2 on fluorescence are without real significance—an unjustifiable simplification.

Franck and his co-workers (1947, 1949), on the other hand, interpreted the enhancing effect of the absence of reductants on the chlorophyll fluorescence of purple bacteria as an indirect action caused by accumulation of unreduced oxidation intermediates of photosynthesis ("photoperoxides"), and narcotization of the chlorophyll apparatus by the products of action of these peroxides on cell metabolites. Using this picture, they gave the following interpretation of the effect of $[CO_2]$ on yield of fluorescence, its variability with light intensity, and its disappearance in the absence of reductants (in purple bacteria):

Lowering the CO_2 concentration has an effect on the yield of fluorescence if it makes the rate of photosynthesis $[CO_2]$-limited. When this is the case, the chlorophyll complex becomes free (depleted of the oxidant, ACO_2), and light energy absorbed by it cannot be used for the primary photochemical process. This itself should change the yield of fluorescence (p. 941). However, Franck considers this "denudation" effect as of minor importance, as far as fluorescence is concerned, compared to the "self-narcotization" which follows it. He postulates that whenever the photosensitizer complex is deprived of carbon dioxide, *photoxidation* sets in (*cf.* Vol. I, chapter 19), and produces a "narcotizing" intermediate (probably a carboxylic acid) which settles on chlorophyll.

This mechanism cannot come into play in purple bacteria, since these are studied under anaerobic conditions; this explains why carbon dioxide deprivation has a comparatively slight effect on fluorescence in these

organisms. The deprivation of reductants, on the other hand, has a strong effect, because abundant "narcotic" is produced in light by the action of accumulated, unreduced photoperoxides.

One may ask: why, then, should the fluorescence-stimulating effect of the deficiency of reductants persist also in the absence of carbon dioxide? No photoperoxides should be formed under these conditions. Franck explains this paradox by questioning the efficiency with which CO_2, produced by fermentation, is removed in these experiments. He points out that some hydrogen is taken up by purple bacteria in light even if no extra carbon dioxide is provided in the medium. (Incidentally, fermentation could produce "narcotizing" acids also directly—and not via the reduction of the fermentation-produced carbon dioxide in light.) Removal of external carbon dioxide may have no effect on fluorescence in the absence of reductants, because enough CO_2 is produced by fermentation to prevent the photochemical process from becoming "[CO_2]-limited"; instead, it remains "[reductant]-limited" (*i. e.*, its rate and the concentration of the narcotic—and thus also the intensity of fluorescence—remain limited by the rate of the reaction between the photoperoxides and the reductants).

It will be noted that Franck's concept differs from the first picture (presented on p. 941) in 3 ways: (*1*) the ultimate *oxidant* itself, ACO_2, rather than an intermediate, X, is supposed to be coupled with chlorophyll; (*2*) no ultimate (or intermediate) *reductant*, ZH, is supposed to be associated with chlorophyll, in such a way that *its* depletion, too, can cause an increase in the yield of fluorescence; and (*3*) *all strong changes in the yield of fluorescence are ascribed to narcotization* by "half-oxidized" metabolites, rather than to the depletion of reactants. These three assumptions are independent of each other; the third one, in particular, which is perhaps the most important feature of Franck's interpretation, can be combined, if desired, also with the picture of the primary photochemical process as light-induced tautomerization of the complex $X \cdot Chl \cdot HZ$ ($\xrightarrow{h\nu}$ $HX.Chl.Z$).

C. Concentration of Reductants*

1. Effect on Rate of Carbon Dioxide Reduction in Bacteria

The ultimate reductant in ordinary photosynthesis of green plants is water. The activity of water in the cells can be changed by direct hydration and dehydration; or by immersion into solutions of different osmotic pressure. Both treatments have a considerable effect on photosynthesis. However, this effect cannot be treated as a kinetic phenomenon obeying

* Bibliography, page 963.

the mass action law, since it is related to changes in permeability and other colloidal properties of the protoplasm and the cell membranes, which affect, to a varying degree, all activities of the living cell. Dehydration effects were therefore discussed in chapter 13 (Volume I, page 333), where we dealt with various physical and chemical inhibitions and stimulations of photosynthesis.

It was mentioned there (page 334) that one of the ways in which dehydration may inhibit photosynthesis is by its influence on the stomata. Since the primary purpose of the stomata is to regulate transpiration, they

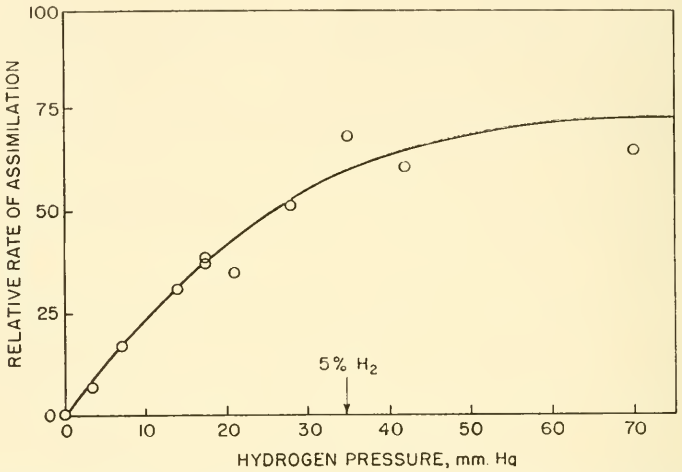

Fig. 27.10. Effect of hydrogen pressure on rate of hydrogen assimilation by *Streptococcus varians* (after French 1937). 114 mm.^{3} cells. Incandescent lamp below conical vessels; 25° C.; argon + 5% CO_2; rate in 95% H_2 = 100.

naturally close upon dehydration (by an increase in osmotic pressure in the guard cells; *cf.* page 912). In this way, the reduction in the activity of one of the reactants in photosynthesis (water) may result in the interruption of the supply of the other reactant (carbon dioxide).

A more favorable opportunity for quantitative kinetic study arises when *hydrogen, hydrogen sulfide, thiosulfate* or other inorganic or organic substances replace water in the role of the reductant reacting with carbon dioxide in purple bacteria or so-called "adapted" algae (*cf.* Vol. I, chapters 5 and 6).

Figure 27.10 (after French 1937) shows the effect of changes in the concentration of *hydrogen* on the rate of photoreduction of carbon dioxide by *Streptococcus varians.* The over-all reaction in this case is, according to Volume I (page 104), $2\ H_2 + CO_2 \rightarrow \{CH_2O\} + H_2O$. The "hydrogen

curve" has the same typical "saturation shape" as the curves representing the rate of photosynthesis as function of carbon dioxide concentration; half saturation is reached at *ca.* 20 mm. (2.5%) H_2, full saturation above 75 mm. (10%). Wassink *et al.* (1942) found, with *Chromatium* in nitrogen containing 5% CO_2, no signs of hydrogen saturation up to about 2% (*cf.* fig. 27.11A); single experiments at higher pressures indicated that saturation was reached probably at about 10%, certainly below 15%. (The rates at 15% and 30% H_2 were identical.) These hydrogen concentrations are approximately 100 times higher than those required for saturation of photosynthesis with carbon dioxide (about 0.03% CO_2 is needed for half saturation). In analogy with the interpretation of carbon dioxide saturation,

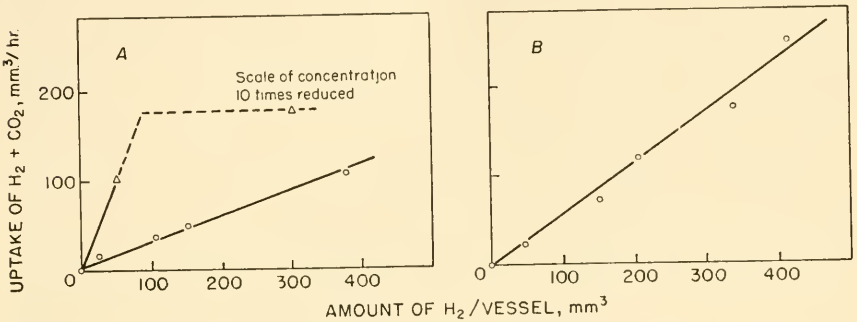

Fig. 27.11. Rate of carbon dioxide reduction by *Chromatium* (after Wassink 1942): (*A*) effect of [H_2]; (*B*) effect of [H_2S]. 5% CO_2 in N_2; *p*H 6.3; 29° C.; strong light.

given in the first part of this chapter, the simplest explanation of hydrogen saturation is to assume reversible formation of a hydrogen acceptor compound, with a dissociation constant of the order of 0.02 atmosphere (as compared with 3×10^{-4} atmosphere for the carbon dioxide acceptor compound). Thermodynamically, reversible hydrogenation (*i. e.*, hydrogenation with energy close to zero) presents no difficulties, since the free energies of hydrogenation of organic compounds can be either positive or negative, depending on the degree of resonance stabilization of the individual compound in the hydrogenated and the dehydrogenated form (*cf.* Vol. I, page 217). Kinetically, however, the problem is less trivial, since *in vitro*, no example is known of organic compounds behaving like metallic palladium, *i. e.*, taking up hydrogen under high pressure and releasing it when the pressure is reduced.

Quantitatively, the results of French with *Streptococcus varians* and of Wassink with *Chromatium* are similar enough to justify the suggestion that the hydrogen acceptor is the same in both species. This common acceptor may be either the enzyme hydrogenase, or the compound, desig-

nated in chapter 6 by A_H (*i. e.*, the compound assumed there to be hydrogenated to A_HH_2 by the mediation of the hydrogenase).

However, in interpreting the hydrogen saturation of photoreduction, one has to keep in mind that hydrogenation *equilibrium* is only one of the two possible explanations. As in the case of the carbon dioxide curves, the hydrogen curves may also be affected—or completely determined—by *kinetic* influences, such as slow rate of hydrogen supply and slow hydrogenation (in the linearly ascending part), and limitations of light supply, of oxidant supply, or of the availability of a catalyst, in the horizontal part of the curves, which follows saturation.

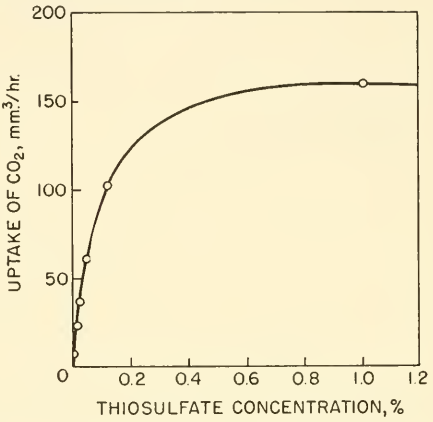

Fig. 27.12. Thiosulfate concentration and rate of photoreduction of carbon dioxide in *Chromatium* (after Wassink, Katz and Dorrestein 1942): 5% CO_2; *p*H 6.3; 29° C.; strong light.

Wassink (1942) made reaction rate studies on the same organism, *Chromatium*, also with varying concentrations of gaseous *hydrogen sulfide* as reductant. As shown in figure 27.11B, the (initial) rate was found to be proportional to $[H_2S]$ up to about 2% (in nitrogen); no signs of saturation were noticeable in the investigated range of concentrations. Higher doses of hydrogen sulfide could not be used because of rapid poisoning.

The influence of the concentration of *thiosulfate* as reductant was also studied on *Chromatium*. Eymers and Wassink (1938) first found the rate to be constant between 0.16 and 2% thiosulfate, indicating that the saturating concentration was $<0.16\%$. More detailed measurements were made by Wassink, Katz and Dorrestein (1942), who determined a complete "thiosulfate curve," $P = f$ [thiosulfate], reproduced in figure 27.12. It shows full saturation near 0.5% and half saturation a little below 0.1% thiosulfate (corresponding to 0.06 mole $Na_2S_2O_3$/l.).

As with variable [CO_2], the effect of variations in the concentration of the reductants disappears in weak light when the supply of light quanta becomes the rate-determining factor (*cf.* fig. 28.5B).

Photoreduction with *mixed reductants* offers an interesting kinetic problem. Wassink, Katz and Dorrestein (1942) conducted some experiments in which *Chromatium* cells were supplied with both hydrogen and thiosulfate (at *p*H 6.8). They used each reductant "in excess" (meaning that the quantity of each alone would have sufficed to produce saturation) and calculated (indirectly, from manometric measurements) the relative amounts in which the two reductants were consumed. The average was about two molecules of carbon dioxide reduced at the cost of thiosulfate for one molecule of carbon dioxide reduced at the cost of hydrogen. If the two acceptor systems (for H_2 and $H_2S_2O_3$) are separate, this result may mean either that the cells contain twice as much of the thiosulfate acceptor system as of the hydrogen acceptor system, or that the first acceptor reacts (in the hydrogenated state) twice as rapidly with the activated photocomplex as the second one. On the other hand, if only one hydrogen acceptor is present, and the two donors compete in supplying it with hydrogen, then the result, as reported, is not very significant, since, in this case, the relative utilization of hydrogen and thiosulfate would depend on relative concentrations (even if both reductants are present "in excess"—the only information provided).

In chapter 22, we mentioned the spectroscopic experiments of the same investigators that made them think that the composition of the photocomplex X·Bchl·HZ (Bchl = bacteriochlorophyll) may itself be specific for each reductant, *i. e.*, that bacteria contain a multiplicity of complexes, X·Bchl′·HZ′, X·Bchl″·HZ″ ···, adapted to the utilization of the several reductants R′H, R″H, ···· (This hypothesis was suggested to explain the multiplicity and varying relative intensities of the absorption bands of bacteriochlorophyll *in vivo*.) Wassink and co-workers now argued that, if this were true, the photoreduction of carbon dioxide by a mixture of reductants would be *additive*, rather than competitive. Since rate measurements indicated competition (the rate of total gas consumption in the presence of both reductants always was lower than in pure hydrogen and larger than in pure thiosulfate), the Dutch investigators concluded that their earlier explanation of the spectroscopic phenomena was incompatible with the results of kinetic studies. However, the argument would only be fully conclusive if it were definitely known that, under the conditions of the experiment, the rate of photoreduction was limited by the amount of the reductant available for reaction with the photocomplex. It was mentioned above that saturation of the over-all rate with respect to the reductant can often be due to a limitation elsewhere in the photosynthetic apparatus, *e. g.*, to

the deficiency of a "finishing" catalyst such as E_B. Whenever this is the case, the rate of photoreduction cannot be increased by increasing the available quantity of the reductant or by adding a second reductant and thus putting to work the (otherwise idle) photocomplexes specifically adapted to it.

In kinetic work with purple bacteria, one has to keep in mind their capacity to utilize organic materials—including intercellular ones—as reductants. Competition rather than additivity seems to be the rule in this case too. The "sigmoid" shape of the light curves of hydrogen consumption, noted both by French, with *Streptococcus varians*, and by the Dutch observers, with *Chromatium* (cf. figs. 28.8), can perhaps be interpreted as a consequence of such competition: In weak light, intracellular reductants (perhaps sugars or their derivatives) supply all the hydrogen necessary for the slowly proceeding photoreduction of carbon dioxide, and, therefore, only very little external hydrogen is used. In stronger light, the diffusion supply of internal hydrogen donors proves insufficient, and the more rapidly diffusing molecular hydrogen takes over as the main reductant.

To minimize the role of internal reductants, and thus to obtain light curves without a sigmoid initial section, Wassink (1942) has attempted to starve the bacteria before the experiment; however, he found no significant change in results.

The experimental material on the "reductant curves" of photoreduction is as yet rather limited and no attempts have been made to represent these curves analytically. If one accepts the general scheme of photosynthesis given in scheme 7.I (Vol. I, page 153), the kinetic role of the reductants appears symmetrical to that of carbon dioxide. An analytical treatment of the effect of reductants on the over-all rate would thus have to deal with the same type of partial processes as were treated in the analysis of the carbon dioxide factor, namely, supply by diffusion, preparatory catalytic dark reactions (such as binding of hydrogen by an acceptor with the help of the hydrogenase), liberation of the acceptor from the primary oxidation product, and "finishing" dark reactions (such as stabilization of the primary oxidation product). According to Franck's concept, repeatedly mentioned before, the finishing dark reactions on the oxidation side (which consist in the elimination of the primary oxidation products—"photoperoxides"—either by their conversion to molecular oxygen or by their reduction with reductants such as hydrogen or hydrogen sulfide) have the peculiar property that their failure to keep pace with the primary photoprocess leads not merely to the loss of a large proportion of primary products by back reactions but also to a reaction between them and oxidizable metabolites. This side reaction produces, according to Franck, a "narcotic," capable of enveloping chlorophyll and stopping the primary photochemical process.

The question whether the participation of water as reductant in photosynthesis involves some preliminary transformations similar to those of carbon dioxide remains open. The abundance of water in all cells may be advanced in favor of the convenient assumption that, even if a transformation of this kind—*e. g.*, hydration of a "water acceptor"—is needed before water can act as a hydrogen donor, the rate of this reaction is high enough to prevent it from playing a limiting role in photosynthesis. However, if the binding of water, which we have symbolized by $H_2O \rightarrow \{H_2O\}$ in scheme 7.I, and which we may now describe by the equation:

(27.87) $$H_2O + A' \rightleftharpoons A'H_2O$$

in analogy to equation (27.2), is an enzymatic process, the limited amount of the enzyme (particularly in an appropriately inhibited state) may well become a rate-limiting factor, despite the overabundance of the reactant H_2O in the cell.

2. Effect on Yield of Fluorescence

Hydration and dehydration of plant cells have a strong influence on the intensity of chlorophyll fluorescence *in vivo;* but in this case, as in that of gas exchange, it is difficult if not impossible to distinguish between the (undoubtedly possible) direct kinetic effects, and the indirect disturbances caused by changes in the colloidal structure of the pigment–protein–lipide complex. We must therefore refer here to the description of the relevant phenomena in chapter 24.

The study again becomes much more fruitful when purple bacteria are used. The supply of reductants, such as H_2, H_2S or $H_2S_2O_3$, has been found to strongly affect the yield of fluorescence of bacteriochlorophyll in these organisms. The light curves of fluorescence given in figs. 28.31, 28.32 and 28.33 (taken from the work of Wassink, Katz and Dorrestein 1942, on *Chromatium*) illustrate this phenomenon. The plots represent the fluorescence *intensity*, F, (not *yield*, φ) as a function of the incident light intensity I, with the concentration of the reductant, [HR], as parameter. Figure 28.31 shows the comparative effect of three different reductants; figure 28.32, a set of measurements with different concentrations of thiosulfate; and figure 28.33 a similar set with different hydrogen pressures. In both cases, increased concentration of the reductant causes an extension of the initial, linear part of the fluorescence curve (which corresponds to the "low light" yield of fluorescence, φ_1). Figure 28.34 shows the critical intensity," I_c (defined in fig. 28.27) as a function of the thiosulfate concentration; the curve has a great similarity with the corresponding gas exchange curve (fig. 27.12). It shows that deprivation of the reductant can

lower the beginning of the transitional range from 11 to 2 kerg/cm.^{2} sec.

In discussing the yield of photosynthesis in relation to the factor $[CO_2]$, we repeatedly encountered effects attributable to the exhaustion of the molecular species CO_2 in the immediate neighborhood of the plants; this disturbance could be reduced (*a*) by buffering with bicarbonate, (*b*) by using unicellular algae, to increase the surface of gas exchange and (*c*) by stirring. With hydrogen, the solubility of the gas in water is much smaller than that of carbon dioxide, and no buffering is possible; therefore, exhaustion effects can be expected to occur even more easily, and could perhaps not be fully avoided even with unicellular organisms, such as purple bacteria. Intense stirring is the only help available; the comparatively rapid diffusion of hydrogen in water may help to maintain uniform distribution. Wassink and co-workers (1942) have observed that, in the presence of 15% H_2 in the gas phase, the fluorescence of *Chromatium* in strong light soon rapidly increased by 25–20% (about one minute) after the cessation of shaking (fig. 28.33 would make even a larger increase easily understandable).

According to the Dutch authors, the influence of $[CO_2]$ on the fluorescence of bacteria disappears if the supply of reductants is stopped (fig. 28.29). Inversely, however, the effect of reductants on fluorescence remains considerable even if carbon dioxide is withheld (*cf.* fig. 28.35). Wassink and co-workers interpreted this difference as confirmation of their general concept that the reductants participate directly as "energy acceptors" in the photochemical process, while carbon dioxide does not react with the primary photoproduct at all. Franck and Herzfeld, on the other hand, assumed direct participation of carbon dioxide (in the form of a complex, ACO_2) in the primary photoprocess and quoted the effect of carbon dioxide removal on fluorescence as evidence of this participation (*cf.* above, page 941), while ascribing the (much stronger) effect of the absence of reductants to the indirect mechanism of "self-narcotization" in consequence of accumulation of unreduced "photoperoxides." Later (*cf.* page 942), Franck suggested that the effect of CO_2 deficiency also is due mainly to narcotization (caused by the products of photoxidation).

Without using the concept of narcotization, we can explain the effects of reductants on the basis of the picture of a "photocomplex," X·Chl·HZ, which undergoes primary photochemical conversion to HX·Chl·Z, followed by dark reactions that transfer H from X to CO_2, and supply H to Z from H_2O (or from one of the "substitute" reductants, such as H_2 or $H_2S_2O_3$). While CO_2 starvation may lead to the accumulation of the photocomplex in the "reduced" form, such as HX·Chl·HZ, the absence of reductants may lead to the accumulation of the "oxidized" form, X·Chl·Z; when both CO_2 *and* the reductants are deficient, the tautomeric form, HX·-

Chl·Z, may be accumulated in strong light. Each of the four forms should have its own characteristic yield of fluorescence, limited by the rate of internal energy dissipation and, in the case of the normal form X·Chl·HZ, also by the competition of fluorescence with the primary photoprocess ($X \cdot Chl \cdot HZ + h\nu \rightarrow HX \cdot Chl \cdot Z$). The conditions under which the three forms can be expected to predominate in strong light, are summarized in the following table.

Form	Formula	Conditions of accumulation	
		Oxidant (CO_2)	Reductant
Tautomeric	HX·Chl·Z	−	−
Reduced	HX·Chl·HZ	−	+
Oxidized	X·Chl·Z	+	−

Which form will be accumulated in the presence of very strong light when both the oxidant and the reductant are in good supply (case ++) cannot be predicted *a priori*, since this will depend on which of the supply processes first fails to keep pace with the primary photoprocess.

If the influence of carbon dioxide deprivation on fluorescence is due to the accumulation of the form HX·Chl·HZ, while the effect of "RH starvation" is caused by accumulation of the form X·Chl·Z, the absence of an effect of carbon dioxide in RH-starved cells can be interpreted as evidence of a similarity in the fluorescent capacity of the forms HX·Chl·Z and X·Chl·Z (or, perhaps as indication that in the absence of both carbon dioxide and reductant, the complex accumulates in the same form X·Chl·Z, as in the absence of reductants alone).

As mentioned on page 943, Franck's hypothesis, that all strong increases of fluorescence are evidence of narcotization of the chlorophyll complex, is not incompatible with the interpretation of the primary photochemical process as hydrogen transfer in the complex, X.Chl.HZ; it only implies that the differences in the capacity for fluorescence of the various forms of this complex (X·Chl·HZ, HX·Chl·Z, X·Chl·Z, and HX·Chl·HZ) are less significant than that between all of them and the "narcotized" form.

D. Concentration of Inhibitors*

1. Inorganic Ions

The effect of the hydrogen ion concentration on photosynthesis was discussed in chapter 8 (Vol. I), and also in the first part of the present chapter, from the point of view of the correlated shifts of the carbonate–bicarbonate–

* Bibliography, page 963.

carbon dioxide equilibrium. What we wanted to know—and did not quite succeed in finding out—was whether the rate of photosynthesis is determined uniquely by the concentration of free carbon dioxide *molecules*, $[CO_2]$, in the immediate surroundings of the cell, or whether the carbonic acid *ions* also contribute to photosynthesis directly (and not merely because they serve as a reserve for rapid replacement of neutral carbon dioxide molecules). One of the complicating factors was that a change in $[HCO_3^-]$ at constant $[CO_2]$ could not, and cannot be achieved without a change in $[OH^-]$, *i. e.*, a variation of the *p*H; and while some algae, such as *Chlorella*,

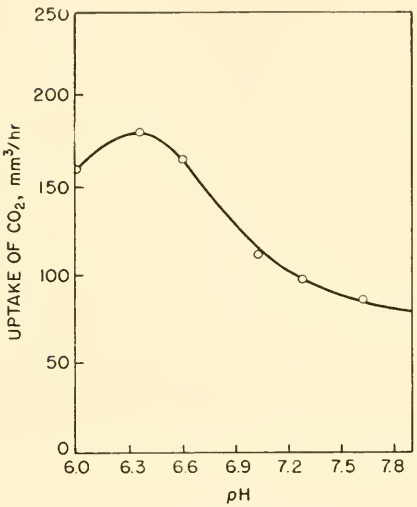

Fig. 27.13. Effect of *p*H on carbon dioxide reduction with thiosulfate by *Chromatium* (after Wassink, Katz and Dorrestein 1942): 5% CO_2; 1% thiosulfate; 29° C.; strong light.

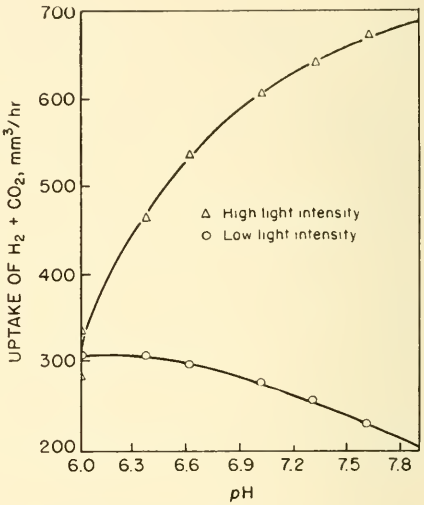

Fig. 27.14. Influence of *p*H on rate of carbon dioxide reduction by *Chromatium* with hydrogen (5% CO_2, 15% H_2) (after Wassink, Katz and Dorrestein 1942).

are capable of efficient photosynthesis over a wide range of acidities (say from *p*H 4.5 to 10), this does not prove that the observed changes in rate can be attributed entirely to variations of $[CO_2]$ and not to variations of *p*H. Some unicellular algae (*e. g.*, *Hormidium*) are definitely injured by alkaline media.

The observations in this field, described in Volume I (pages 339–340) can now be supplemented by the results of Wassink, Katz and Dorrestein (1942) with purple bacteria. When reductants such as thiosulfate or hydrogen sulfide (or inhibitors such as cyanide) are used, the *p*H effect is com-

plicated by changes in the ionic dissociation of these weak acids (in addition to the complications caused by the dissociation of carbonic acid).

Figure 27.13 shows the effect of *p*H on carbon dioxide reduction by *Chromatium* in 1% thiosulfate. The curve, obtained in phosphate buffers, shows a slight maximum of *P* at *p*H 6.3, followed by a sharp *decrease*. The effect disappears at low light intensities (as do all effects not connected with the primary photoprocess). With hydrogen as reductant, the effect was quite different (fig. 27.14); the rate, *P* (in strong light), *increased* steadily with increasing alkalinity.

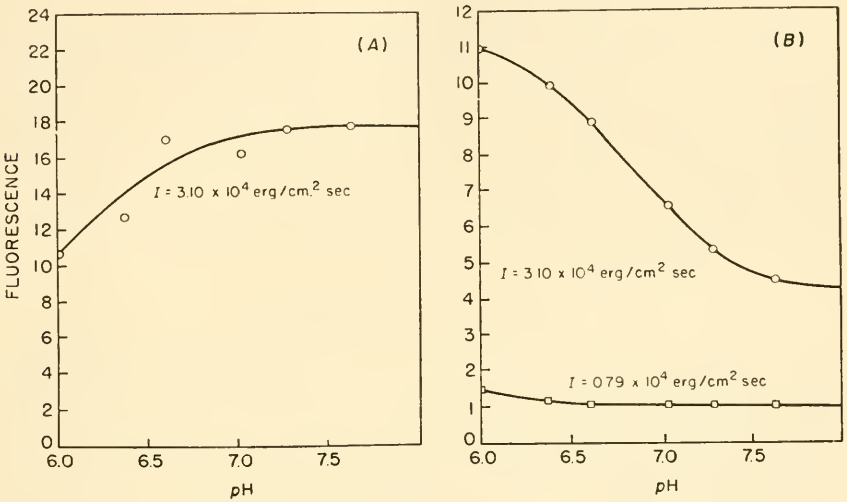

Fig. 27.15. Effect of *p*H on fluorescence of *Chromatium* (after Wassink, Katz and Dorrestein 1942): (*A*) 5% CO_2, 1% thiosulfate, 29° C., strong light; (*B*) 5% CO_2, 15% H_2, 25° C., strong and weak light.

One factor that may contribute to the *decline* of *P* with increased *p*H in thiosulfate is the decrease in concentration of undissociated $H_2S_2O_3$ molecules; this may be the main species that penetrates the cells and is used there as the reductant. According to figure 27.12, at 1% thiosulfate, the concentration of the reductant is not a "limiting" factor at *p*H 6.3. Whether it could become such a factor when alkalinity is higher, remains to be investigated.

The *increase* in *P* with *p*H, as observed with hydrogen as reductant, could perhaps be looked upon as new evidence of the direct participation of HCO_3^- ions in photosynthesis—if one were inclined to give weight to such evidence. Alternatively and more plausibly, both the decline of *P*

with *p*H in the case of thiosulfate, and its increase in the case of hydrogen, could be interpreted as evidence of different *p*H dependence of the enzymatic processes by which the two reductants are supplied to the photocomplex.

The influence of *p*H on the *fluorescence* of bacteriochlorophyll also was studied by Wassink and co-workers. With thiosulfate, the fluorescence was about 30% more intense at *p*H 7.6 than at *p*H 6.0 (fig. 27.15A), a result that may be attributed to a lower supply of effective reductant (undissociated $H_2S_2O_3$). As expected, the trend is reversed with hydrogen (fig. 27.15B), where the enzymatic supply of the reductant appears to be better in the more alkaline solution.

The effects on the rate of photosynthesis of various other *inorganic ions* also were described in chapter 13. The only systematic data, showing the rate in relation to the concentration of the inhibiting ion, were obtained by Greenfield (1941, 1942); some of his curves were reproduced in figure 34 (Vol. I, page 341). Among new results pertinent to this field we may quote the observations of Warburg and Lüttgens (1946) that the reduction of quinones by broken chloroplasts *in vitro* requires the presence of *chloride* ions (*cf.* Chapter 35).

2. Poisons and Narcotics

In chapter 12 (Volume I) we discussed the influence of various inhibitors on the rate of photosynthesis in a qualitative way, although some quantitative data also were given, such as a curve showing the rate of oxygen liberation by *Chlorella* as a function of the concentration of phenylurethan (fig. 30, page 323). Systematic kinetic study should include also rate measurements with varying amounts of poisons, and some such measurements have recently been reported, particularly by Wassink, Katz and Dorrestein (1942). These investigators found that the photoreduction of carbon dioxide by purple bacteria (*Chromatium* D) is as sensitive to cyanide as is the photosynthesis of *Chlorella*. With thiosulfate as reductant, some inhibition was observed even in weak light, such as 2 kerg/(sec. cm.^{2}), while in reduction with molecular hydrogen (as in normal photosynthesis), the cyanide effect disappeared completely in weak light, $I < 4$ kerg/(sec. cm.^{2}). Wassink and Kersten (1945) found a considerable effect of hydrogen cyanide also on the photosynthesis of diatoms in weak light ($\sim$2 kerg/sec. cm.^{2}), a result which differs from many observations on green algae and higher plants.

Figure 27.16 shows the relation between P and [KCN], in *Chromatium* (with hydrogen or thiosulfate as reductant) and in *Nitzschia*. Photosynthesis of *Chromatium* is half-inhibited by about 2.5×10^{-3} per cent KCN (with either hydrogen or thiosulfate). The *p*H (6.3 to 7.6) has an effect on inhibition in thiosulfate, but none in hydrogen, which makes the at-

tribution of its effect to a difference in the activity of HCN molecules and CN^- ions (*cf.* Vol. I, page 301) doubtful. Figure 27.16B shows that photosynthesis of *Nitzschia* is half-inhibited by about $1.5 \times 10^{-3}\%$ or about 2×10^{-5} mole/l. KCN. For similar figures for *Chlorella*, *Hormidium* and other algae, see Table 12.V (Vol. I, page 305).

The effect of cyanide on the steady *fluorescence* of *Chromatium* is shown in figure 28.45. It is somewhat complex, but, in general, amounts to an

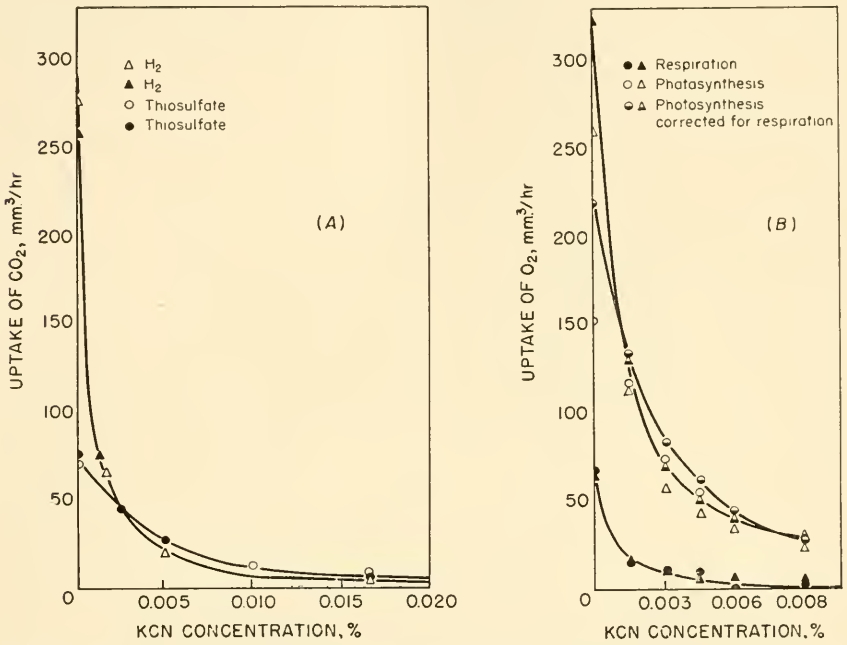

Fig. 27.16. (*A*) Effect of cyanide on carbon dioxide reduction by *Chromatium* in thiosulfate (1% solution) and hydrogen (15%) (after Wassink, Katz and Dorrestein 1942). (*B*) Effect of cyanide on carbon dioxide reduction and respiration of *Nitzchia* (after Wassink and Kersten 1944).

increase of F at the lower light intensities, and a decrease at the higher light intensities, and is thus reminiscent of the effect of carbon dioxide starvation (*cf.* fig. 28.30A and B). The analogy is supported by the observation that [KCN], like $[CO_2]$, has no effect on F in the absence of reductants (except for concentrations $\gg 0.01\%$ KCN, where the fluorescence becomes strongly depressed; complete suppression of carbon dioxide reduction occurs much earlier, at about 0.015% KCN.

Hydroxylamine. In chapter 12 (Vol. I, page 311) we found that hydroxylamine is a strong poison, the mode of action of which is rather complex. While it affects particularly strongly the oxygen-liberating process

in ordinary photosynthesis, hydroxylamine also produces, albeit only higher concentrations, two other inhibiting effects—on the reduction of carbon dioxide by "hydrogen-adapted" algae (in which no oxygen is liberated), and on the "de-adaptation" of such algae. Both effects could be explained by the assumption that hydroxylamine has an affinity either

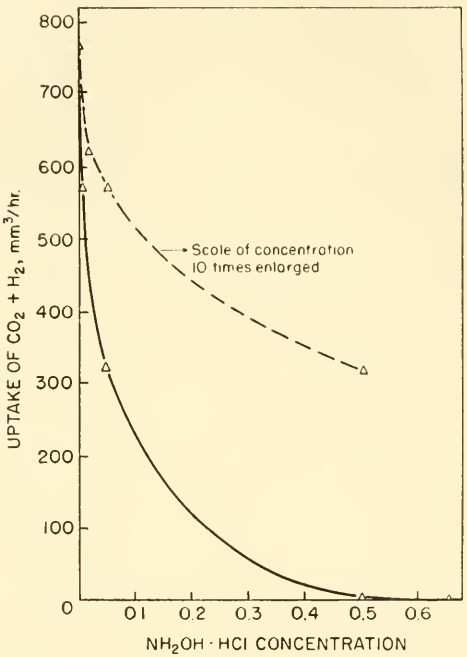

Fig. 27.17. Influence of hydroxylamine chloride on carbon dioxide reduction by *Chromatium* (after Wassink, Katz and Dorrestein 1942): 5% CO_2; 15% H_2; *p*H 7.16; 29° C.; strong light. $NH_2OH \cdot HCl$ concentration in per cent.

directly to the primary oxidation product Z (in photocomplex $X \cdot Chl \cdot HZ$), or to an enzyme with which Z must react to prevent this primary product from being lost by back reactions.

New experiments of Wassink and co-workers (1942) showed that the influence of hydroxylamine hydrochloride on the photoreduction of carbon dioxide by purple bacteria (*Chromatium*) is somewhat stronger than the above mentioned effect on the similar reaction in hydrogen-adapted *Scenedesmus*. (The following figures show this relation: Normal photosynthesis of *Scenedesmus*, 50% inhibition at 5×10^{-4} mole/l.; photo-

reduction by adapted *Scenedesmus*, no drop below 50% at *any* concentration up to 3×10^{-2} mole/l.; photoreduction by *Chromatium*, 50% inhibition at 3×10^{-3} mole/l., (*cf.* fig. 27.17). In experiments with bacteria (in contrast to those of Weller and Franck, 1941, on *Chlorella; cf.* Vol. I, page 312) no inhibition by hydroxylamine was found in weak light (*cf.* fig. 28.11C and fig. 28 in Vol. I). The difference is understandable if the effect of hydroxylamine on photosynthesis in weak light is due to its action on the oxygen-liberating enzyme (designated as E_0 or E_c in Vol. I); a possible "autocatalytic" mechanism of this effect was discussed on p. 312.

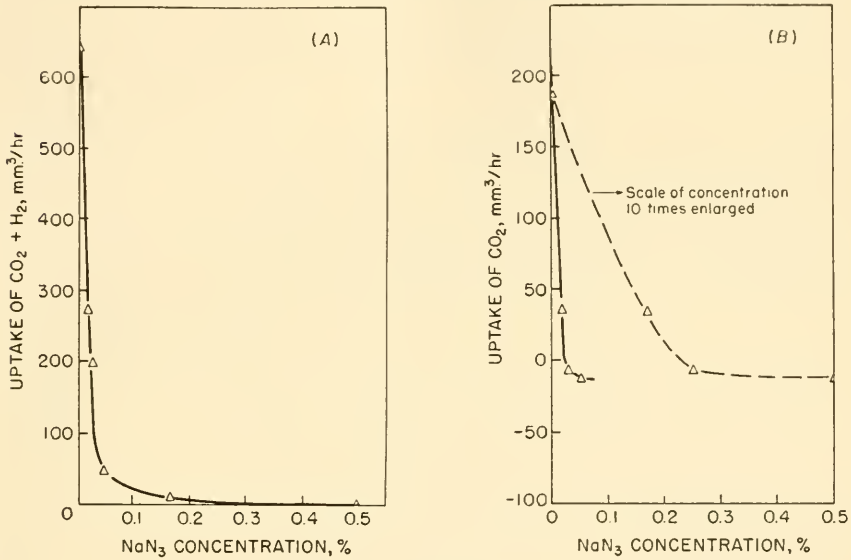

Fig. 27.18. Influence of sodium azide on rate of photoreduction of carbon dioxide by *Chromatium* (after Wassink, Katz and Dorrestein 1942): (*A*) 15% hydrogen; (*B*) 1% thiosulfate. 5% CO_2; *p*H 6.3; 29° C.; strong light.

In the absence of reductants, hydroxylamine hydrochloride was found to have no effect on the *fluorescence* of *Chromatium*, up to a concentration of 0.5%. In the presence of reductants, hydroxylamine causes, in general, an *increase* in the intensity of fluorescence; however, this effect becomes marked only above 0.05%, while the reduction of carbon dioxide is half inhibited by as little as 0.03% of the poison (*cf.* fig. 28.46). Thus, the inhibition of bacterial photosynthesis by hydroxylamine also seems to be a complex phenomenon, the first stage of which has no effect on fluorescence, while the second stage causes an increase in the fluorescence yield.

Sodium azide. Only one casual observation of the inhibition of photosynthesis by azide was mentioned in Volume I (page 318). Since then, the effect of this typical poison for heavy metal catalysts on the gas exchange and fluorescence of *Chromatium* has been studied quantitatively by Wassink, Katz and Dorrestein (1942). Figures 27.18A and 27.18 B show the rates of photoreduction, with molecular hydrogen and thiosulfate, respectively, as functions of azide concentration. The half-inhibiting concentration is about 0.02% sodium azide with hydrogen and about 0.01% with thiosulfate as reductant. Strangely enough, particularly strong inhibition was found in weak light (fig. 28.11D).

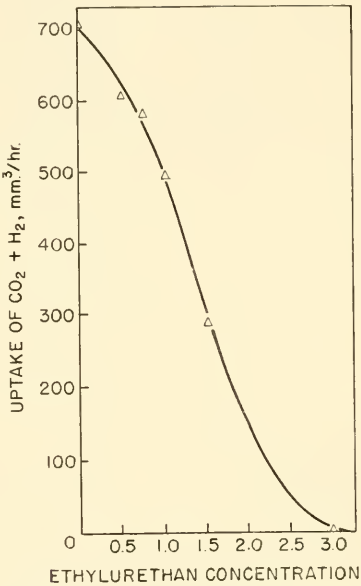

Fig. 27.19. Effect of ethylurethan on photoreduction of carbon dioxide in *Chromatium* (after Wassink, Katz and Dorrestein 1942): 5% CO_2; 15% H_2; *p*H 7.6; 29° C.; strong light.

The effect of azide on *fluorescence* also was different from that of the other enzyme poisons, such as cyanide and hydroxylamine. In the first place, fluorescence was strongly affected even in the absence of reductants (fig. 28.47A). In the second place, the typical effect was a considerable *lowering* of the yield of fluorescence, particularly at the higher light intensities (fig. 28.47B).

All three observations (inhibition of the gas exchange at low light intensities, effect on fluorescence in absence of reductants, lowering of fluores-

cence intensity) indicate that this poison does not, or not merely, interfere with the enzymatic supply to the photosynthetic apparatus of the oxidant (carbon dioxide), or of the reductant (such as hydrogen), but affects the primary photocomplex, X·Chl·HZ, *directly*, possibly by displacing the reductant HZ in this complex. Such a close association with the photocomplex could be expected to lead to the decomposition of the azide in light, an expectation which might, perhaps, be tested by experiments.

Urethans. In chapter 12 (Vol. I, page 321) the "narcotizing" effect of phenylurethan on photosynthesis of *Chlorella*, which appears to be equally strong at all light intensities, was illustrated by figure 30, taken from Warburg's early work on *Chlorella* (1920). This figure showed half inhibition at about 2×10^{-4} mole/l.; Table 12.VIII, taken from the same paper, indicated half inhibition at 5×10^{-4} mole/l. for phenylurethan and 0.22 mole/l. for ethylurethan.

An inhibition curve for *Chlorella*, given by Wassink and co-workers (1938) (fig. 31, page 323) indicated half inhibition by about 2.5 per cent, or about 0.02 mole/l. ethylurethan, and the measurements of Wassink, Katz and Dorrestein (1942) on *Chromatium* (fig. 27.19) gave about 1.3%, or 0.01 mole/l., as half-inhibiting concentration, at 29° C. Wassink and Kersten (1945) found about 2% as half-inhibiting concentration for the diatom *Nitzschia dissipata*.

One peculiar characteristic of the effect of urethan on *Chromatium*—similar to that of azide—is the enhanced inhibition at low light intensities, leading to a more pronounced sigmoid shape of the light curves of photoreduction in inhibited cells (fig. 28.11E).

Theoretically, a weaker inhibition at the higher light intensities (*i. e.*, light curve systems of "type 3"; *cf.* chapter 26) *could* be explained if one would assume that the narcotic partially covers the chlorophyll-bearing "photocomplex," but leaves free the enzyme that determines the limiting yield of photosynthesis in strong light. In this case, the decrease in inhibition would be brought about by continued increase, with increasing light intensity, of the rate of photosynthesis of narcotized cells—in the intensity range in which photosynthesis in noninhibited cells is light-saturated. Figure 28.11E shows, however, that light saturation occurs at the same intensity on both curves, so that this explanation appears inadequate.

In *Nitzschia*, according to Wassink and Kersten (1945), the inhibition by urethan is somewhat less strong in weaker than in stronger light.

The influence of ethylurethan on *fluorescence* of *Chromatium* is like that of azide. As with azide, addition of urethan causes the fluorescence to *decrease* in intensity in the absence of reductants (fig. 28.50A). When reductants, such as hydrogen or thiosulfate, are present, and the fluorescence of nonnarcotized cells is thus considerably weakened, addi-

tion of urethan causes an *increase* in F (*cf.* fig. 28.50B), so that the intensity of fluorescence finally becomes about the same with and without reductant.

It is noteworthy that in the completely narcotized state (*e. g.*, with 3% ethylurethan, when the gas exchange is completely inhibited; *cf.* fig. 27.19) the characteristic curvature of the fluorescence curves of bacteria disappears (fig. 28.50B).

These results can be explained by the assumption that urethan reacts directly with the primary photochemical complex X·Bchl·HZ, and that the product of this interaction is characterized by a yield of fluorescence about halfway between those of the forms X·Bchl·HZ and X·Bchl·Z.

Oxygen. In chapter 13 (Vol. I, page 328) we described the inhibiting effect of excess oxygen on photosynthesis. The light curves of photosynthesis and fluorescence in the presence and absence of oxygen will be discussed in chapter 28. The only systematic measurements with varying oxygen pressures permitting the plotting of an "oxygen curve," $P = f[O_2]$, were made by Warburg (1920); the results were reproduced in figure 32. This figure indicated the steepest rate decline between 0 and 20% oxygen; it was mentioned that this was contradicted by Wassink and co-workers (1938), who found no difference between the rates at 0 and 20% oxygen, but a considerable drop from 20 to 100% oxygen.

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Concentration Factors

A. Experimental Carbon Dioxide Curves

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Chapter 28

THE LIGHT FACTOR. I. INTENSITY *

A. Light Curves of Photosynthesis

The relation between photosynthesis and the *quantity* of light available to the plants was investigated for the first time in 1866, when the Russian botanist Volkov (Wolkoff) counted the oxygen bubbles evolved by submerged aquatic plants at different distances from a sun-illuminated, frosted glass window. He found that the rate of gas evolution was proportional to the intensity of illumination. In 1883, Reinke in Germany extended similar measurements to stronger illuminations and observed that, when the light intensity approached that of full sunlight, the "light curves" (*i. e.*, curves in which the rate of photosynthesis was plotted against the intensity of incident light) bent, and finally became horizontal. He had thus discovered the phenomenon of *light saturation.*

Ewart (1896, 1897, 1898) showed that, if the illumination is increased still further, far beyond the saturating value, the rate begins to *decrease* again, and photosynthesis may even be completely inhibited.

The initial increase of the rate with light intensity, the "light saturation" that follows and the ultimate "light inhibition" of photosynthesis were again observed by Pantanelli in 1903; at that time, it was natural to interpret these results in terms of the theory of "cardinal points" (*cf.* fig. 26.1). Blackman and Matthaei (1905) and Blackman and Smith (1911) pointed out, however, that photosynthesis requires no minimum light intensity (at least, if one considers the *true* rate, corrected for respiration, rather than the rate of *net* gas exchange). Furthermore, it shows a broad "saturation plateau" instead of a sharp optimum. Therefore, they argued, light curves can better be explained by means of Blackman's theory of "limiting factors" (*cf.* fig. 26.2) than by reference to the three "cardinal points."

Singh and Kumar (1935) and Lubimenko (1936) observed that the light curves of some land plants are *sigmoid* in shape, and Lubimenko saw in this the proof of the existence of a "light threshold" of photosynthesis; but this conclusion runs contrary to the results of all the other observers. On the other hand, sigmoid-shaped light curves appear to be the rule with *purple bacteria* (*cf.* French 1937; Wassink, Katz and Dorrestein 1942).

* Bibliography, page 1078.

Not satisfied with the qualitative similarity between the empirical light curves and the broken lines predicted by the theory of limiting factors, Blackman insisted on a *quantitative* agreement, and thus precipitated the controversy to which we have referred in chapter 26. He insisted that no decline in rate occurs at high light intensities, unless injuries are brought about by *overheating*, and denied the gradual character of the transition from the linearly ascending part to the horizontal part of the light curves.

Blackman probably was right in suggesting that the inhibition of photosynthesis by excessive light be attributed to destructive processes alien to the intrinsic kinetic mechanism of photosynthesis. (However, we believe these processes to be *photoxidations*, rather than thermal reactions caused by overheating; this theory of light inhibition was discussed in chapter 19, when we described the photoxidation phenomena in living plants.) It is, on the other hand, impossible to accept the second contention of Blackman—that the linearly ascending part of the light curves goes over abruptly into the horizontal part. All precise observations confirm that light saturation is reached *asymptotically*, sometime over an extended range of light intensities (*cf.* the early criticism of Blackman's interpretation by Brown and Heise 1917, 1918). It was shown in chapter 26 (*cf.* also fig. 28.20) that the inhomogeneity of light absorption, which is inevitable even in single chloroplasts, not to speak of multicellular systems, should in itself suffice to make practical observation of Blackman's angular light curves impossible—even if these curves correctly represented the relation between light intensity and rate of photosynthesis in a uniformly illuminated volume element. Application of the general laws of reaction kinetics shows, however, that even in the ideal case of completely uniform light absorption Blackman's broken lines could represent only a first approximation, which may be more or less satisfactory, depending on specific conditions.

1. General Review

Table 28.1 lists the most important determinations of the light curves of photosynthesis carried out since the time of Blackman.

A remark must be made on the *units* of light intensity used in these measurements. For *white light*, lux (meter candles), or foot candles, have been and still are widely used. A foot candle is equal to 10.8 meter candles; one meter candle corresponds (*cf.* chapter 25, page 838) to about 4.5 erg, or 1.4×10^{12} quanta, or 2.3×10^{-12} einstein of photosynthetically active light (400–700 mμ), falling each second on a square centimeter of the illuminated surface. For *colored light*, the intensity is usually given in ergs (or calories: 1 cal = 4.2×10^7 erg) per square centimeter per second, or in watts per square centimeter (1 watt = 10^7 erg/sec.). We will use the abbreviations klux for thousand lux, and kerg for thousand erg. In comparing the results obtained in light of different color, the most appropriate measure of intensity is the number of incident quanta ($N_{h\nu}$), or the number of einsteins (1 einstein = 6.1×10^{23} quanta) falling per second on one square

TABLE 28.I. DETERMINATION OF LIGHT CURVES. The values in parentheses are calculated from the approximate relation, 1 lux = 5 erg/(sec. × cm.²)

Observer	Year	Material	Species	Conditions			Half saturation		Saturation	
				CO_2 supply	Temp., ° C.	Illumination l. = lamp	Klux	Kerg per sec. per sq.cm.	Klux	Kerg per sec. per sq.cm.
				A. LAND PLANTS						
Willstätter, Stoll	1918	Excised leaves	*Ulmus, Sambucus*	Air, 4.5% CO_2	25	1500 W. lamp	~4	(20)	24	(120)
			Same, var. *aurea*	Air, 4.5% CO_2	25	1500 W. lamp	~12	(60)	~60	(300)
Lundegårdh	1921, 1922	Whole plants	*Oxalis acetosella*	0.03–0.24%	18	500 W. lamp	—	—	>15	(>75)
			Stellaria memorum	0.03–0.24%	—	Sun	—	—	15	(75)
Müller	1928	Arctic plants	*Salex glauca*	Air	10	Sun	—	—	7	(35)
			Salex glauca	Air	20	Sun	—	—	13	(65)
Boysen-Jensen	1929	Leaves	*Fagus silvatica*							
			Sun leaf	Air	20	Sun	~17	(85)	≫20	(>100)
			Shade leaf	Air	20	Sun	<1	(<5)	~3	(>15)
Boysen-Jensen, Müller	1929	Fern	*Marchantia*	Air	20	Sun	~0.5	(2.5)	1	(5)
		Lichen	*Peltigera*	Air	20	Sun	~5	(25)	>19	(>95)
Müller	1932	Plants	*Sinapis alba*	Air	20	Sun	~5.5	(27.5)	~20	(100)
Boysen-Jensen	1933	Leaves	*Sinapis alba*	0.07%	20	2 × 300 W. l.	6	(30)	~17	(85)
Hoover, Johnson, Brackett	1933	Plants	*Triticum* (*cf.* fig. 28.1)	0.013%	19	8 × 500 W. l.	~2	(10)	~16	(~30)
				0.026%	19	8 × 500 W. l.	~2.5	(12.5)	~10	(~50)
				0.032%	19	8 × 500 W. l.	~2.5	(12.5)	~10	(~50)
				0.050%	19	8 × 500 W. l.	~4	(20)	~15	(~75)
				0.178%	19	8 × 500 W. l.	>5	(>25)	>20	(>100)
Gabrielsen	1935, 1940	Leaves	*Sinapis alba*	Air	—	Sun, 0.6–0.75μ	(4.4)	22	(13)	66
						Sun, 0.48–0.64μ	4.4	22	(13)	66
Singh, Kumar	1935		*Raphanus sativus*	0.03%	—	Lamp	—	—	69	(345)
				0.3%	—	Lamp	—	—	72	(360)
Stålfelt	1937	Mosses	*Hylocomium squamosum, Proliferum parietinum, Sphagnum girgensohnii*	Air	—	Sun	~1.8	(9)	—	—

A. LAND PLANTS (*continued*)

Stålfelt	1937	Lichens	*Cetraria glauca* *Evernia prunastii* *Cetraria islandica* *Ramalina farinacea*	Air	Winter Summer	Sun Sun	4.7 5.9	(24) (30)	16–32 —	(80–160) —
Gabrielsen	1940	Leaves	*Fraxinus excelsior*							
			Sun leaf	Air	—	Vitalux lamp	~20	(100)	60	(300)
			Shade leaf	Air	—	Vitalux lamp	~8	(40)	18	(90)
Gabrielsen	1942	Leaves	*Triticum*	Air	—	Lamp	10	(50)	52	(260)
Kramer, Decker	1944	Trees	Pine (loblolly)	Air	30	Air	—	—	>100	(>500)
			Oak (red)	Air	30	Air	—	—	~30	(>150)
			Oak (white)	Air	30	Air	—	—	~15	(>75)
			Dogwood	Air	30	Air	—	—	~15	(>75)
Wassink	1946	(Leaves)	Strawberry	Buffer No. 9[a]	25, 17	Na lamp	—	20	—	50
			(*cf.* fig. 28.2)	9% CO_2	25	Na lamp	—	>30	—	>80
				9% CO_2	17	Na lamp	—	20	—	50
				1% CO_2	25	Na lamp	—	15	—	60
			Tomato	Buffer No. 9[a]	25, 17	Na lamp	—	15	—	30
				9% CO_2	25	Na lamp	—	>30	—	>80
				9% CO_2	17	Na lamp	—	15	—	50
			Chinese cabbage	Buffer No. 9[a]	25, 17	Na lamp	—	7	—	30
				9% CO_2	25	Na lamp	—	30	—	>80
				9% CO_2	17	Na lamp	—	22	—	>70
			Cucumber	Buffer No. 9[a]	25, 17	Na lamp	—	10	—	60
				9% CO_2	25	Na lamp	—	10	—	30
				9% CO_2	17	Na lamp	—	5	—	15
			Turnip cabbage	Buffer No. 9[a]	25, 17	Na lamp	—	8	—	30
			Asparagus	Buffer No. 9[a]	25, 17	Na lamp	—	3	—	15

B. AQUATIC HIGHER PLANTS

Harder	1921	*Fontinalis antipyretica*	$KHCO_3$ 0.01%	23–24	500 W.	~0.5	(2.5)	~2	(~10)
		(*cf.* fig. 28.3)	$KHCO_3$ 0.04%	23–24	500 W.	~4	(20)	>20	(>100)
			$KHCO_3$ 0.10%	23–24	500 W.	—	—	$\gg$20	($\gg$100)
			$KHCO_3$ 0.32%	23–24	500 W.	—	—	$\gg$20	($\gg$100)
Ashby, Oxley	1935	*Lemna minor*	Tap water	29	Lamp	—	—	~10	(~50)
Smith	1937	*Cabomba caroliniana*	Buffer No. 4[a]	25.3	500 W. lamp	3.3	(16.5)	~20	(~100)
			Buffer No. 9[a]	25.3	500 W. lamp	6	(30)	~30	(~150)
			Buffer No. 10[a]	25.3	500 W lamp	7	(35)	~50	(~250)
			Buffer No. 11[a]	25.3	500 W. lamp	7	(35)	~100	(~500)

Table continued

TABLE 28.I (*concluded*). DETERMINATION OF LIGHT CURVES

Observer	Year	Material	Species	Conditions			Half saturation		Saturation	
				CO_2 supply	Temp., ° C.	Illumination	Klux	Kerg per sec. per sq.cm.	Klux	Kerg per sec. per sq.cm.
			B. AQUATIC HIGHER PLANTS (*continued*)							
Smith	1938	*Cabomba caroliniana* (*cf.* fig. 28.4)		Buffer No. 1[a]	25.3	500 W. lamp	1.5	(7.5)	~5	(~25)
				Buffer No. 2[a]	25.3	500 W. lamp	1.5	(7.5)	~10	(~50)
				Buffer No. 4[a]	25.3	500 W. lamp	3	(15)	~30	(~150)
				Buffer No. 5[a]	25.3	500 W. lamp	4	(20)	~50	(~250)
				Buffer No. 9[a]	25.3	500 W. lamp	8	(40)	~100	(500)
				Buffer No. 11[a]	25.3	500 W. lamp	10	(50)	>123	(>615)
Gessner	1938	*Cerathophyllum demersum*		Hard tap water	ca. 20	200 W. lamp	~2	(10)	~30	(~150)
		Cabomba aquatica		Hard tap water	ca. 20	200 W. lamp	~2	(10)	~30	(~150)
		Elodea crispa (sun)		Hard tap water	ca. 20	200 W. lamp	>20	(>100)	>80	(>400)
		Elodea crispa (shade)		Hard tap water	ca. 20	200 W. lamp	~6	(30)	40	(200)
		Potomageton densus (sun)		Hard tap water	ca. 20	200 W. lamp	>10	(>50)	>80	(>400)
		Potomageton densus (shade)		Hard tap water	ca. 20	200 W. lamp	~3	(15)	40	(200)
		Ranunculus aquaticus		Hard tap water	ca. 20	200 W. lamp	~2	(10)	15	(65)
		Hottonia palustris		Hard tap water	ca. 20	200 W. lamp	8	(40)	60	(300)
			C. GREEN ALGAE							
Warburg	1919	*Chlorella pyrenoidosa*		Buffer No. 9[a]	25	300 W. lamp	3.5[b]		>16[b]	
Emerson	1929	*Chlorella vulgaris*, normal		Buffer No. 9[a]	20	100 W. lamp	50[b]		200[b]	
		Chlorella vulgaris, chlorotic		Buffer No. 9[a]	20	100 W. lamp	40[b]		140[b]	
Van der Paauw	1932	*Hormidium flaccidum*								
			Light green cells	Air, 1% CO_2	18	150 W. lamp	1.2	(6)	6	(30)
			Shade green cells	Air, 1% CO_2	18	150 W. lamp	0.2	(1)	2.4	(12)
Wassink, Vermeulen, Reman, Katz	1938	*Chlorella vulgaris* (*cf.* fig. 28.6)		Buffer No. 9[a]	10	Na lamp	—	1.2	—	5
				Buffer No. 9[a]	16.3	Na lamp	—	2.1	—	9
				Buffer No. 9[a]	22.5	Na lamp	—	3.6	—	18
				Buffer No. 9[a]	28.9	Na lamp	—	—	—	>18
Wassink, Katz	1939	*Chlorella vulgaris* (*cf.* fig. 28.12)								
			3 days old	Buffer No. 9[a]	29	Na lamp	45[b]		140[b]	
			23 days old	Buffer No. 9[a]	29	Na lamp	35[b]		120[b]	
			42 days old	Buffer No. 9[a]	29	Na lamp	30[b]		80[b]	

C. GREEN ALGAE (*continued*)								
Noddack, Eichhoff	1939	*Chlorella pyrenoidosa* (*cf.* fig. 28.7)	Buffer No. 9[a]	20	500 W. lamp			
					red light	—	—	—	6
					yellow light	—	—	—	6
					white light	5	(25)	>15	(>75)
Noddack, Kopp	1940	*Chlorella pyrenoidosa* (*cf.* fig. 28.7)	Buffer No. 9[a]	10	500 W. lamp				
					white light	~2	(10)	20	(120)
				10	red light, 0.65μ	—	7	—	24
				20	white light	~5	(25)	$\gg$20	($\gg$100)
				20	red light	—	~8	—	$\gg$25
Steemann-Nielsen	1942	*Rhizoclonium*	$[CO_2] + \frac{1}{2}[HCO_3^-]$ = 0.0014 mole/l.	19.5	—	~5	(25)	>15	(>65)
D. RED AND BROWN ALGAE								
Emerson, Green	1934	*Gigartina* (red)	10^{-4} mole/l. HCO_3^-	15	6 × 60 W. l.		22[b]		100
Barker	1935	*Nitzschia closterium*	5% CO_2, or buffer 9[a]	20	40–75 W. l.		15[b]		50[b]
		Nitzschia palea	5% CO_2, or buffer 9[a]	21	40–75 W. l.		—		>100[b]
Steemann-Nielsen	1942	*Fucus serratus*		5	—	4	(20)	25	(25)
				25	—	7	(35)	25	(125)
Wassink, Kersten	1945	*Nitzschia dissipata* (10 Trommsdorff units/ml.) (*cf.* fig. 28.5)	Buffer No. 9[a]	17	Na lamp	—	—	—	>30
			Richter's soln., 5% CO_2	17	Na lamp	—	~20	—	~60
E. PURPLE BACTERIA								
Katz, Wassink, Dorrestein	1942	*Chromatium* D							
		Conc. 3	5%	29	Na lamp	—	~30	—	$\gg$30
		Conc. 1	5%	29	Na lamp	—	~12	—	>30
		Conc. 1/3	5%	29	Na lamp	—	~8	—	~30
		Conc. 3, 1, 1/3, reduced to light intensity received by each cell (*cf.* fig. 28.13)	5%	29	Na lamp	—	~8	—	~30
Wassink, Katz, Dorrestein	1942	*Chromatium* D (*cf.* fig. 28.8)	5%, *p*H 6.3 (thiosulfate)	11.3	Na lamp	—	~2	—	6
				19.8	Na lamp	—	5	—	20
				28.5	Na lamp	—	10	—	30
			5%, *p*H 7.6 (hydrogen)	16.3	Na lamp	—	3	—	8
				22.4	Na lamp	—	6	—	20
				28.0	Na lamp	—	9	—	30

[a] $[CO_2]$ values for buffers (25°C.) (*cf.* table 8.V) in 10^{-6} mole/l.: No. 1, 0.48; No. 2, 0.90; No. 4, 2.29; No. 5, 4.48; No. 9, 78.7; No. 10, 131; No. 11, 290.

[b] Relative units.

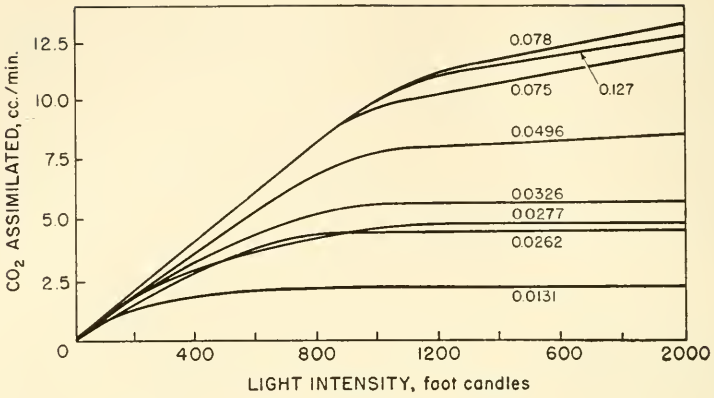


Fig. 28.1. Light curves of photosynthesis in whole young wheat plants at 19° C. (after Hoover, Johnston and Brackett, 1933). Parameters: Vol. % CO_2; one foot candle equals about 10 lux.

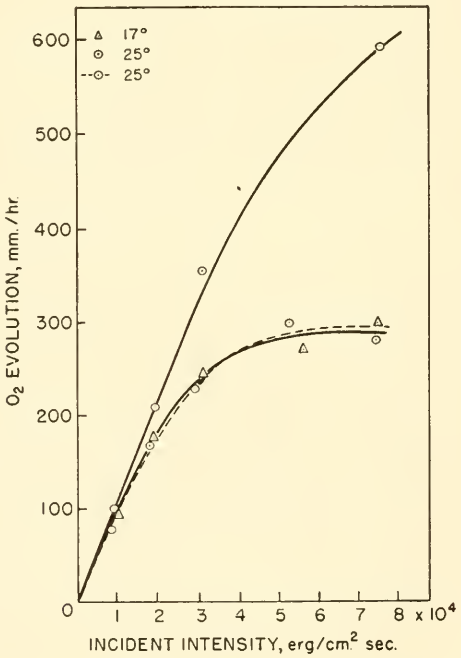


Fig. 28.2. Light curves of strawberry leaves (after Wassink 1946). Leaf disks floating on water in equilibrium with air containing 9% CO_2, at 17° and 25°. Broken line: same, in Warburg buffer No. 9, at 25°.

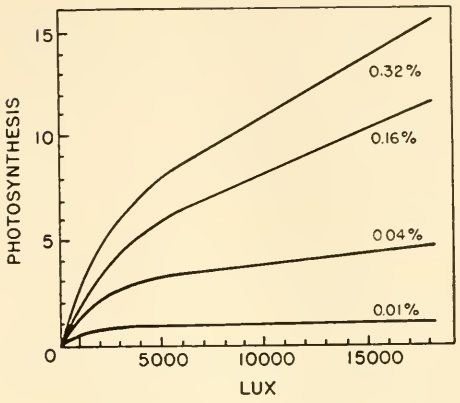


Fig. 28.3. Light curves of water moss *Fontinalis antipyretica* at 23–24° C. for different values of [$KHCO_3$], in white light (after Harder 1921). 1% $KHCO_3$ corresponds to [CO_2] = 1.1×10^{-3} mole/l.

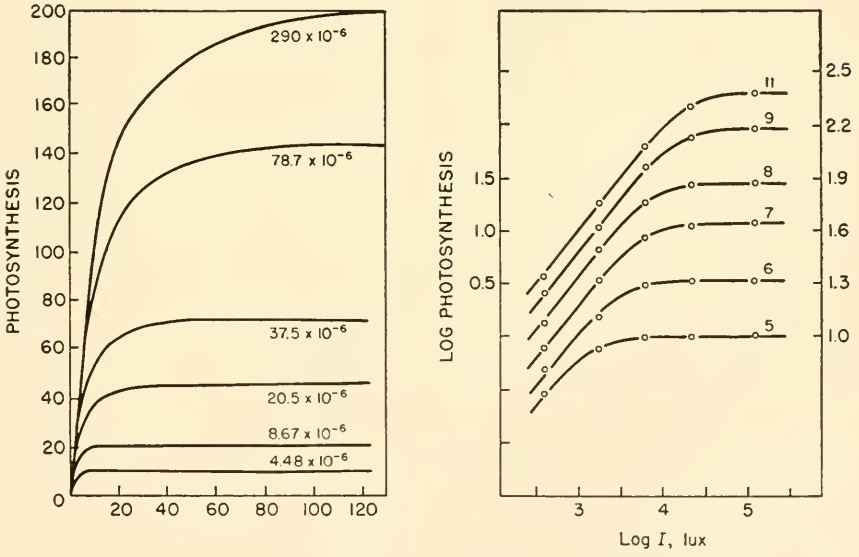


Fig. 28.4. Light curves of the water plant *Cabomba caroliniana* for various [CO_2] values (left) (after Smith 1938). Warburg buffers, white light, 25.3° C. In the right-hand figure log P is plotted against log I. Ordinates are correct only for the uppermost curve; others have been displaced downward in steps of 0.2 log unit each, with correct positions given at right of figure. Parameters are buffer numbers (Table 8.V and footnote *a*, p. 969). Left abscissa, klux.

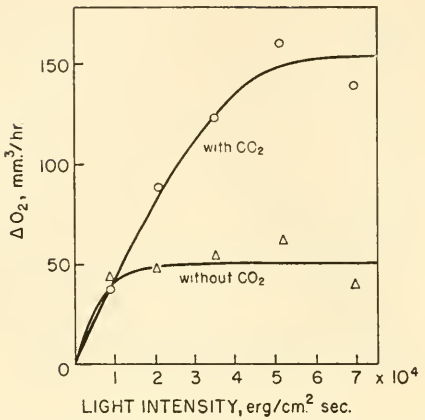


Fig. 28.5. Light curves of diatoms (*Nitzschia* sp.) in Richter solution (after Wassink and Kersten 1945). In 5% CO_2 and in CO_2-free air, 17° C.

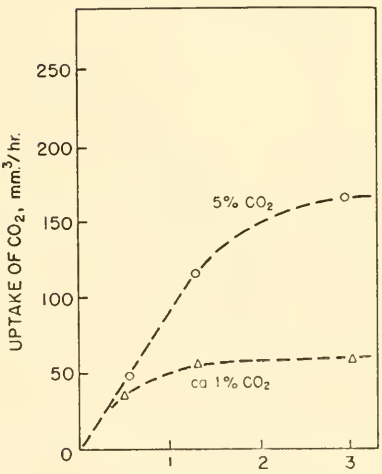


Fig. 28.5A. Light curves of CO_2 reduction by purple bacteria (*Chromatium* D) at two CO_2 concentrations (after Wassink, Katz and Dorrestein 1942). 1% thiosulfate, *p*H 6.3, 29° C.

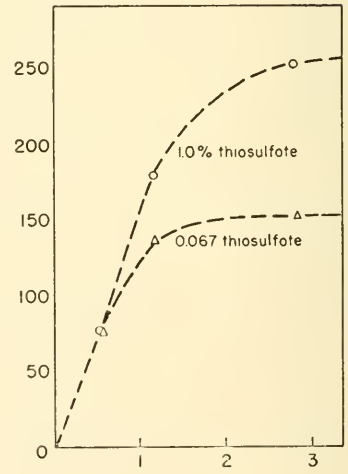


Fig. 28.5B. Light curves of CO_2 reduction by purple bacteria (*Chromatium* D) at two different thiosulfate concentrations (after Wassink, Katz and Dorrestein 1942). 5% CO_2, *p*H 6.3, 29° C.

Abscissa for both figures, incident intensity in (erg/cm.² sec.) $\times 10^{-4}$.

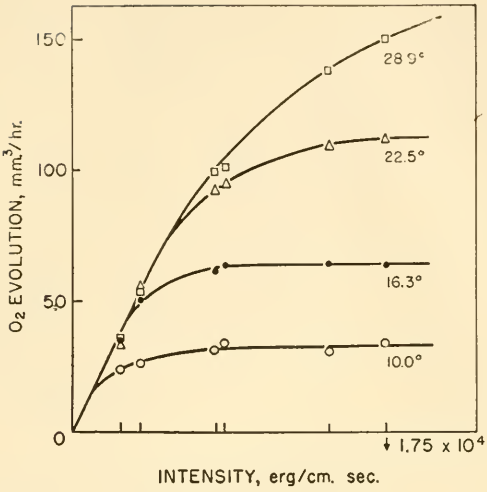


Fig. 28.6. Light curves of *Chlorella* at different temperatures (after Wassink, Vermeulen, Reman and Katz 1938).

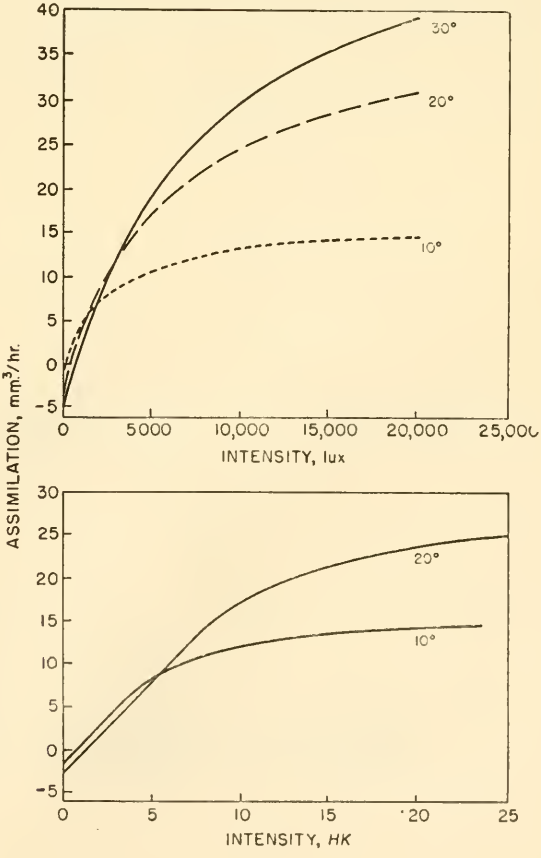


Fig. 28.7. Light curves of *Chlorella* at different temperatures (after Noddack and Kopp 1940). $[CO_2] = 7.87 \times 10^{-5}$ mole per liter. 1 *HK* = 940 erg/(cm.^{2} sec.). Upper figure, white light; lower figure, red light.

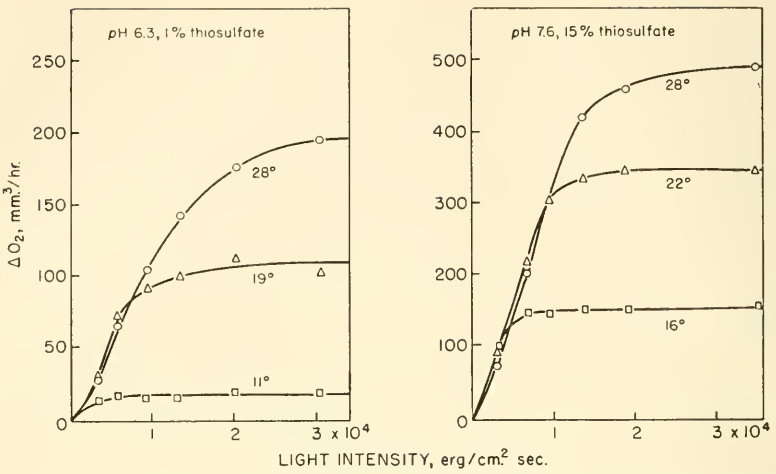


Fig. 28.8. Light curves of purple bacteria (*Chromatium* D) at different temperatures (after Wassink, Katz and Dorrestein 1942). (5% CO_2, concn. 1, corresponding to *ca.* 80% absorption of sodium light; *cf.* fig. 28.22.)

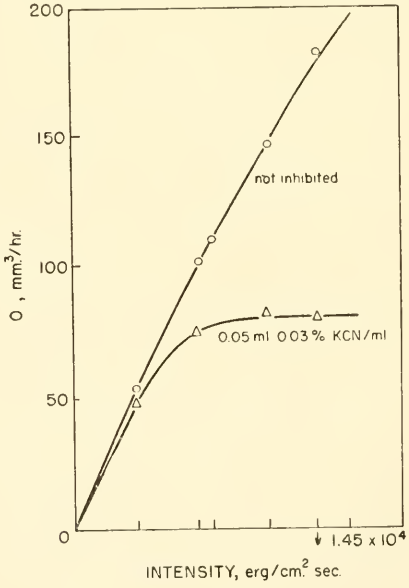


Fig. 28.9A. Light curves of HCN-inhibited *Chlorella* cells showing that HCN is ineffective in weak light (after Wassink, Vermeulen, Reman and Katz 1938).

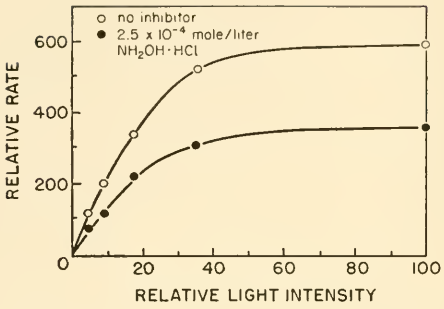


Fig. 28.9B. Light curves of inhibited *Chlorella* cells showing that $NH_2OH{\cdot}HCl$ is effective at all light intensities (after Weller and Franck 1941).

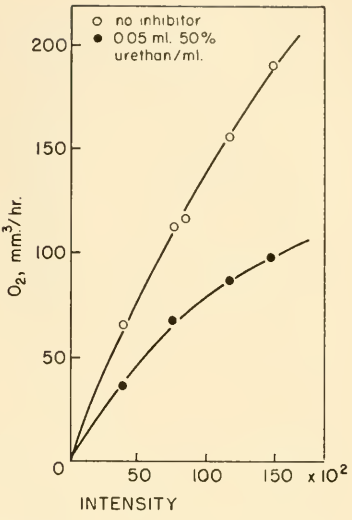


Fig. 28.9C. Light curves of inhibited *Chlorella* cells showing that ethylurethan is effective at all light intensities (after Wassink, Vermeulen, Reman and Katz 1938). Intensity is in erg/cm.^{2} sec.

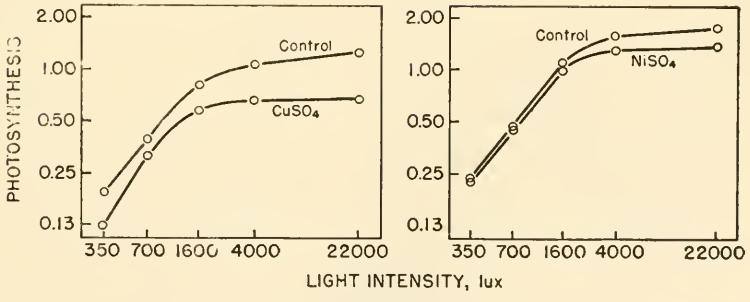


Fig. 28.9D. Light curves of inhibited *Chlorella* cells showing that $CuSO_4$ is effective, $NiSO_4$ ineffective at low light intensities (after Greenfield 1942).

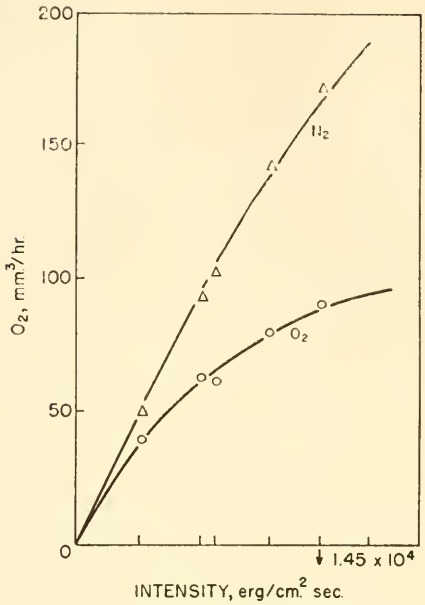


Fig. 28.9E. Light curves of *Chlorella* cells showing O_2 effect (after Wassink, Vermeulen, Reman and Katz 1938).

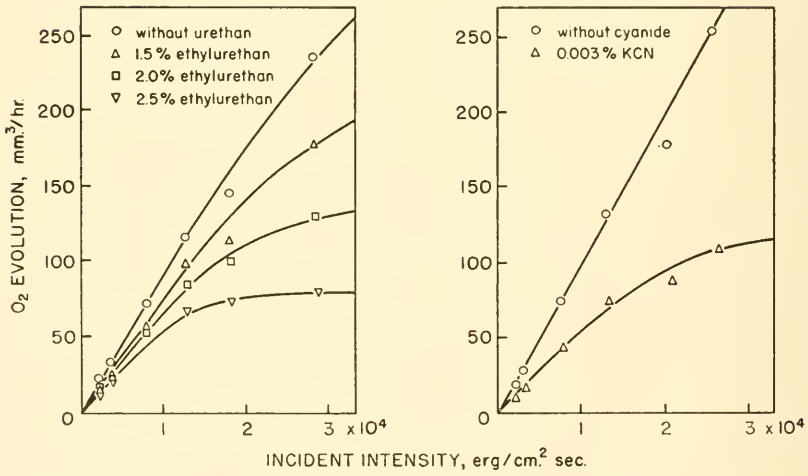


Fig. 28.10. Light curves of inhibited diatoms (*Nitzschia*) at 25° C. in Warburg buffer No. 9, showing both ethylurethan and cyanide (?) to be effective at low light intensities (after Wassink and Kersten 1945).

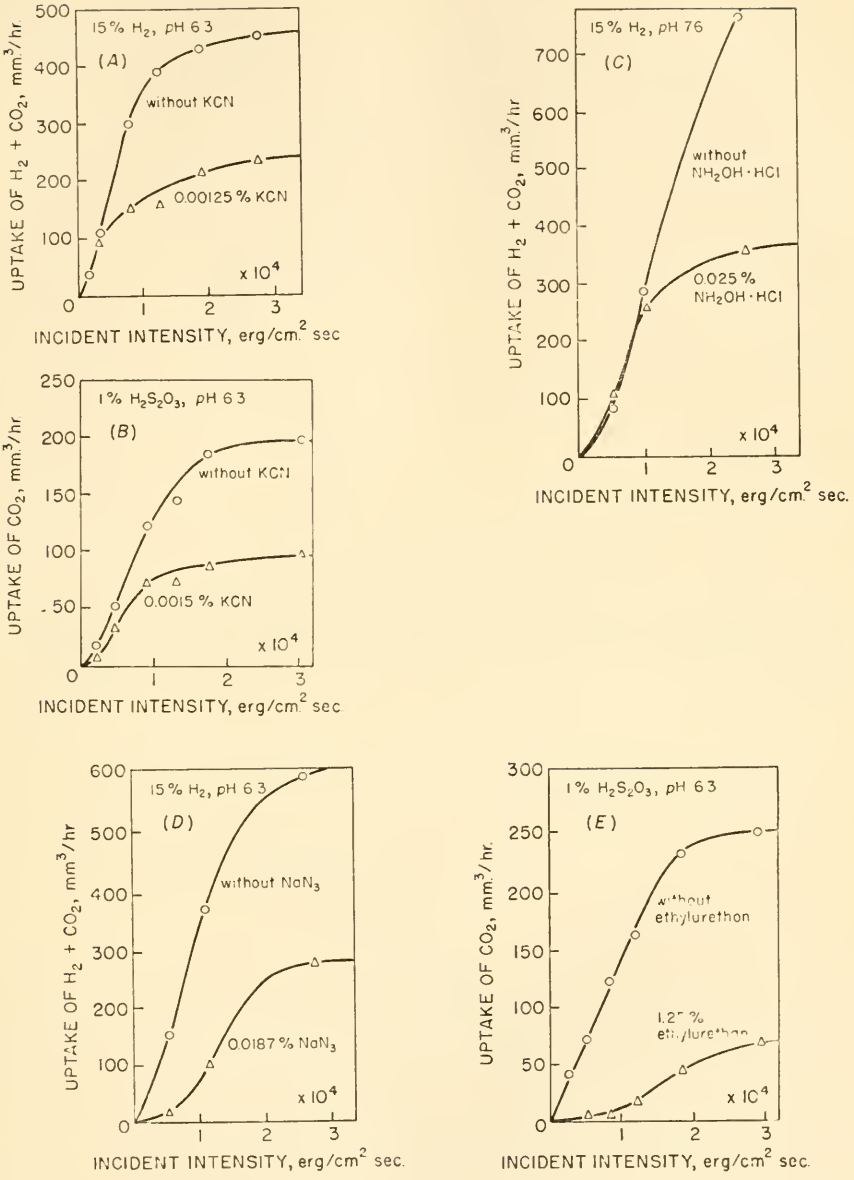


Fig. 28.11. Light curves of inhibited purple bacteria (*Chromatium*) (after Wassink, Katz and Dorrestein 1942). (H_2 or $H_2S_2O_3$ as reductant, 5% CO_2, 29° C.) HCN shows no effect in weak light with H_2. With $H_2S_2O_3$, effect is shown also in weak light. $NH_2OH \cdot HCl$ shows no effect in weak light (*cf.* fig. 28.9B). With ethylurethan, the effect is particularly strong in weak light.

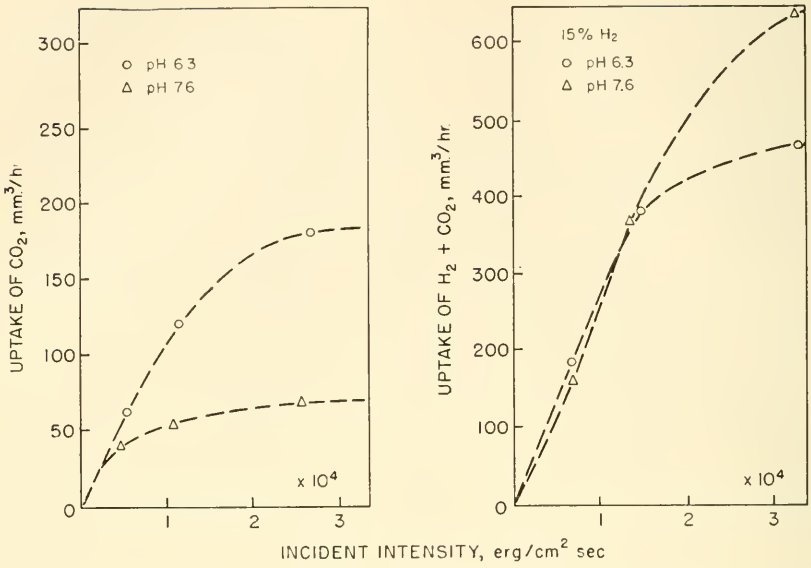


Fig. 28.12. Effect of *p*H on rate of CO_2 reduction by *Chromatium* (after Wassink, Katz and Dorrestein 1942). 15% H_2 (at right) or 1% $H_2S_2O_3$ (at left) as reductant, 5% CO_2, 29° C.

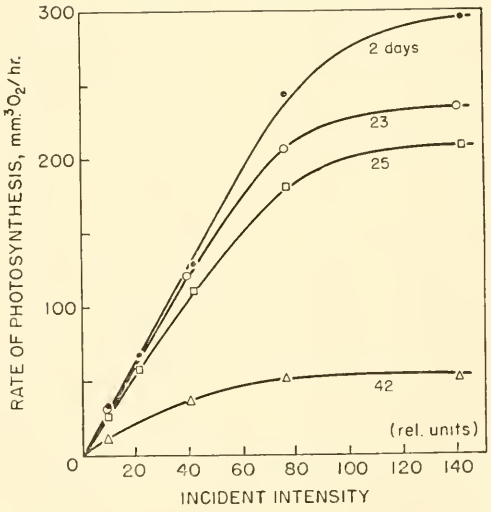


Fig. 28.13. Light curves in relation to age in *Chlorella* (after Wassink and Katz 1939). Gas phase air, 29° C.

centimeter. For relationships between $N_{h\nu}$ and intensity of illumination see chapter 25, page 838.

Table 28.I does not list the measurements of light curves in the presence of various poisons, such as potassium cyanide, hydroxylamine or azide, of narcotics, such as urethan, or of salts, such as copper sulfate. Several curves of this type are, however, reproduced in figures 28.9–11; for additional information, we refer to chapters 12 and 13 in volume I, and to chapter 37. In the latter, we will also describe the light curves of algae in the state of (almost complete) anaerobic inhibition, which Franck, Pringsheim and Lad (1945) were able to measure by the very sensitive phosphorescence method.

Figures 28.1–28.13 contain a selection of typical light curves. Attempt was made to include curves for all types of plants—higher land plants, aquatic higher plants, green and colored algae, diatoms and purple bacteria. Figures 28.1–28.5A represent families of curves in which the carbon dioxide concentration, $[CO_2]$, serves as parameter (strangely enough, no such set is available for *Chlorella*). Figure 28.5B shows light curves of purple bacteria for two concentrations of the *reductant* (thiosulfate); figures 28.6–28.8 represent curve sets with *temperature* as parameter. Figures 28.9–28.11 illustrate the effect of *inhibitors*. The effect of *p*H (in purple bacteria) is shown in figure 28.12, while figure 28.13 shows the influence of *age*. Later in this chapter, some additional sets of curves will be given to illustrate the influence of inherited or acquired *conditioning* to strong or weak light.

In chapter 26 we discussed three types of curve sets, $P = f[F_1]$ with a parameter F_2, which can be anticipated in photosynthesis. Examples of conditions under which each type can occur were given, for carbon dioxide curves, $F_1 = [CO_2]$, in chapter 27.

2. Linear Range

We will now consider some of the details of the light curves: the *linear range*, the *compensation point*, the *saturating light intensity* and the *maximum yield*. Perhaps the most important quantitative characteristic of the light curves is the *initial slope*, which determines the *maximum quantum yield*; it will be discussed separately in chapter 29.

Figures 28.1, 28.7, 28.9A, 28.10 and 28.14A,B show that many light curves exhibit a practically exact proportionality between rate and light intensity over a considerable range of intensities. This "linear range" is less clearly delimited in figures 28.2–28.6. In the light curves of purple bacteria, it is often obscured by an inflection (*cf.* figs. 28.8 and 28.11A–D). Data collected in Table 28.II indicate that (at room temperature and with an ample

TABLE 28.II

LIMITS OF LINEAR INCREASE IN PHOTOSYNTHESIS WITH LIGHT INTENSITY

Observer	Plant	Medium	Light quality	Limit of linearity	
				Klux	Kerg/sec. cm.^{2}
Hoover, Johnston, Brackett (1933) (*cf.* fig. 28.1)	*Triticum*	Air, 0.037 vol. % CO_2	White	1	(5)
		Air, 0.082 vol. % CO_2	White	4	(20)
		Air, 0.143 vol. % CO_2	White	10	(50)
Wassink (1946)	Nine horticultural plants	Floating on buffer No. 9	Yellow	—	6–25 (17 or 25° C.)
		9% CO_2	Yellow	—	20–70 (25° C.)
		9% CO_2	Yellow	—	15–125 (17° C.)
Warburg, Negelein (1922)	*Chlorella*	Water satd. with 5% CO_2	Yellow	(0.2)	1
Warburg, Negelein, (1923)	*Chlorella*	Water, satd. with 5% CO_2	Red	—	1
Manning, Juday, Wolf (1938)	*Chlorella*	Lake water	Sunlight under water	—	5
Petering, Duggar, Daniels (1939)	*Chlorella*	Water satd. with 5% CO_2	Red	—	6
Magee, DeWitt, Smith, Daniels (1939)	*Chlorella*	Water satd. with 5% CO_2	Red	—	8
Noddack, Eichhoff (1939)	*Chlorella* (Dense)	$KHCO_3 + K_2CO_3$[a]	White	2	(10)
	(Dense)	$KHCO_3 + K_2CO_3$[a]	Red	—	3
	(Dense)	$KHCO_3 + K_2CO_3$[a]	Green	—	3
Noddack, Eichhoff (1939)	*Chlorella* (Thin)	$KHCO_3 + K_2CO_3$[a]	Red	—	4
Noddack, Kopp (1940)	*Chlorella*	$KHCO_3 + K_2CO_3$[a]	White	1	5 (10° C.)
	Chlorella	$KHCO_3 + K_2CO_3$[a]	White	1	5 (20° C.)
	Chlorella	$KHCO_3 + K_2CO_3$[a]	Red	—	4 (10° C.)
	Chlorella	$KHCO_3 + K_2CO_3$[a]	Red	—	8 (20° C.)
Emerson, Lewis (1941) (*cf.* fig. 28.14A)	*Chlorella*	$KHCO_3 + K_2CO_3$[a]	White	1.3	(6.5)
Emerson, Lewis (1943)	*Chlorella*	$KHCO_3 + K_2CO_3$[a]	Red	—	5
French, Rabideau (1945)	*Chlorella*	$KHCO_3 + Na_2CO_3$[a]	Red	—	>80
Eymers, Wassink (1938) (*cf.* fig. 28.14B)	*Chromatium*	Phosphate buffer	Yellow	—	60
Wassink, Katz, Dorrestein (1942) (*cf.* fig. 28.8)	*Chromatium*	Phosphate buffer	Yellow	—	3 (16.3° C.)
			Yellow	—	9 (22.4° C.)
			Yellow	—	12 (28° C.)

[a] 0.085 mole/l. $KHCO_3$, 0.015 mole/l. K_2CO_3 or Na_2CO_3 (buffer No. 9).

supply of carbon dioxide) the linear range usually extends up to 5 or 10 kerg/cm.^{2} sec., corresponding to 1–2 klux of white light. In some cases, however, the first signs of curvature have been observed—despite ample supply of carbon dioxide—as early as at 1 kerg/cm.^{2} sec., or 200 lux; while in others, the linear increase continued up to 50 or even 100 kerg/cm.^{2} sec., *i. e.*, 10–20 klux (*cf.* figs. 28.1 and 28.14B).

Theoretically, no exact definition of the linear range can be given, since all light curves probably are hyperbolae (or curves of a higher order) and can only approach straight lines asymptotically. A formal definition of the upper limit of the linear range could thus be given only in terms of a definite deviation from linearity.

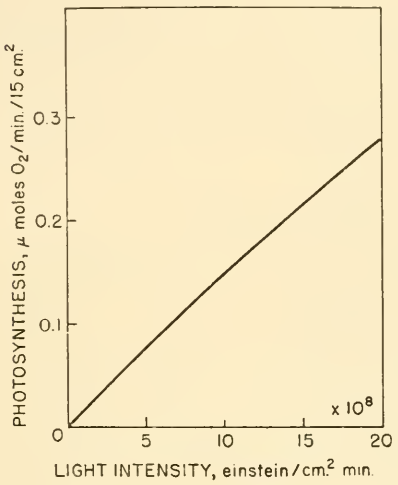


Fig. 28.14A. Approximate linearity of light curves of *Chlorella* in white light up to *ca.* 1300 lux (or 6.5 kerg/cm.^{2} sec.) (after Emerson and Lewis 1941).

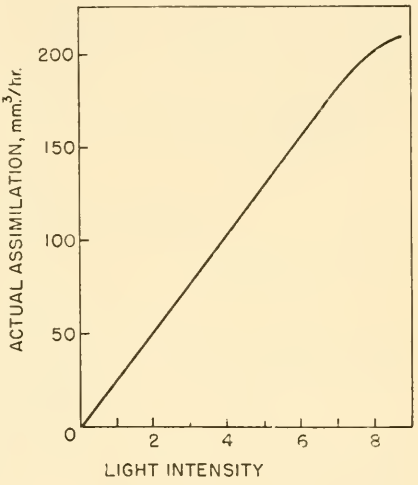


Fig. 28.14B. Light curves in purple bacteria in sodium light (after Eymers and Wassink 1938) (showing linearity up to 60 kerg/(cm.^{2} sec.)). Light intensity in (erg/cm.^{2} sec.) $\times$ 10^4.

Wassink (1946) gave incident intensities of monochromatic yellow light at which the yield of photosynthesis of nine horticultural plants showed 16% deviation from proportionality (*cf.* Table 28.II).

Kok (1948,1949) and van der Veen (1949) found that the linear range may consist of two segments, the lower one up to twice as steep as the upper one (*cf.* chapter 29, p. 1113).

3. Compensation Point

The compensation point is the light intensity I_c at which photosynthesis is balanced by respiration, so that the net gas exchange is zero.

One could also call "compensation point" the carbon dioxide concentration at which the gas exchange becomes zero at a given light intensity (*cf.* chapter 27); or the temperature at which the gas exchange becomes zero at a given combination of the parameters I and $[CO_2]$ (*cf.* chapter 31); but the word is seldom used in either of these two ways. Sometime, the designation "upper compensation point" is applied to the second crossing of the curves of photosynthesis and respiration, which may occur either at very high light intensities or at "superoptimal" temperatures (*cf.*, for example, fig. 31.1).

When the carbon dioxide supply is not too low, compensation occurs within the linear range of the light curve, where the slope of the latter is determined by the maximum quantum yield of photosynthesis and the rate of light absorption (*i. e.*, the optical density of the specimen). Probably (*cf.* chapter 29) the maximum quantum yield is approximately the same for all species (at least, when all cells are fully active—which is not always the case, *e. g.*, in "aged" cultures). Differences in the compensation points found under these conditions must therefore depend mainly or exclusively on two factors: *rate of respiration* and *optical density* of the specimen.

Respiration is proportional to the concentration of cells in a suspension;

TABLE 28.III

COMPENSATION POINT OF LEAVES AND THALLI

Authority	Plant	I_c, lux	Temp.
	HIGHER LAND PLANTS		
Boysen-Jensen, Müller (1929)	*Fraxinus excelsior* (shade leaves)	200	20° C.
	(sun leaves)	700	20° C.
	Fagus silvatica (shade leaves)	150	20° C.
	(sun leaves)	500	20° C.
	MOSSES		
Boysen-Jensen, Müller (1929)	*Marchantia polymorpha*	100	20° C.
Stålfelt (1939[2])	6 species (winter), average	390[a]	About 11° C.
	6 species (summer), average		
	LICHENS (SYMBIOTIC GROWTHS OF ALGAE AND FUNGI)		
Boysen-Jensen, Müller (1929)	*Peltigera canina*	4200[b]	20° C.
Stålfelt (1939[1])	12 species (winter), average	1020	13° C.
	12 species (summer), average	1160	
	AQUATIC HIGHER PLANTS AND MOSSES		
Plaetzer (1917)	*Elodea* (summer)	2	
	Elodea (winter)	18	
	Cabomba caroliniana	55	
	Miriophyllum spicatum	128	
	Fontinalis antipyretica	150	
	Cinclidotus aquaticus	140	

Table 28. III (*continued*)

Authority	Plant	I_c, lux	Temp.
AQUATIC HIGHER PLANTS AND MOSSES (*continued*)			
Harder (1923)	*Fontinalis* (shade plant)	95	
	After 12 days in dark	64	
	After 20 days in dark	27	
	Fontinalis (sun plant)	152	
	After 12 days in dark	84	
	After 20 days in dark	10	
Harder (1924)	*Fontinalis* grown at 4.6°	—1000	18° C.
	Grown at 20°	—580	18° C.
GREEN ALGAE			
Plaetzer (1917)	*Spirogyra* sp.	174	
	Cladophora sp.	253	
Ehrke (1929, 1931)	*Enteromorpha compressa*	457	16° C.
	Ulva lactuca	357	16° C.
	Cladophora rupestris	322	16° C.
Noddack, Eichhoff (1939)	*Chlorella pyrenoidosa* (thin suspension)	400	25° C.
COLORED ALGAE			
Ehrke (1929, 1931)	*Fucus serratus* (brown)	408	16° C.
	Laminaria saccharina (brown)	345	16° C.
	Plocamium coccineum (red)	299	16° C.
	Phyllophora brodiaei (red)	312	16° C.
	Delesseria sanguinea (red)	270	16° C.

[a] Values remarkably high for shade plants. [b] The high values of I_c are caused by the respiration of the (photosynthetically inactive) fungus.

while light absorption, in an optically dense system, increases more slowly than proportionally with the concentration (Beer's law). Therefore, if we compare a *dense suspension* with a *thin suspension* of identical cells, we can expect to find the compensation point of the second one at a lower light intensity. When, on the other hand, a decrease in optical density is brought about by a decline in the concentration of chlorophyll *within* the cells (without a change in the number of the cells per unit volume), the compensation point will be shifted in the opposite direction (*i. e.*, toward higher intensities), because in this case the decline in the total yield of photosynthesis will not be compensated by an even stronger decline in total respiration.

The respiration of chlorophyll-deficient cells is either the same as that of normal green cells (*cf.* Noddack and Kopp) or even stronger (chlorotic *Chlorella* cells grown by Emerson and Lewis; most sun-adapted, light-green leaves). Three examples of the latter behavior will be found in Tables 28.III and 28.IV (p. 989).

The relation between the kinetic properties of sun-adapted (heliophilic) and shade-adapted (umbrophilic) plants will be discussed on page 987; that between warmth-adapted (thermophilic) and cold-adapted (cryophilic) plants, in chapter 31. As shown by Harder's data in Table 28.III, the effects of adaptation to weak light and low temperature differ in sign—the first one reduces respiration and thus shifts I_c toward weaker illuminations, while the second one enhances repiration, and thus shifts I_c toward more intense light.

Algae that live deep under the sea, particularly red algae, are adapted both to weak light and to low temperature. The effect of umbrophilic adaptation predominates, however, and the compensation points of these algae generally are lower than those of the surface algae. Without low compensation points, these organisms could not develop 100–120 meters under the sea (the lowest level from which organisms have been recovered by dragnet) because the intensity of illumination at 120 meters depth is of the order of only 200 lux (see data of Seybold 1936, in chapter 22).

As discussed in more detail in chapter 15 (page 424), the deep-sea algae are adapted not only to low light intensity, but also to predominantly blue-green light. If the compensation points of the green surface algae and the colored deep-sea algae were compared in blue-green light, the lower compensation points of the latter probably would appear even more strikingly than in Table 28.III.

In general, the compensation points of different species shown in the table are comparable only if the experiments were carried out under closely similar conditions (same carbon dioxide concentration, temperature and previous history of the plants), since otherwise the intensity of respiration of a given species may vary widely. Plants allowed to photosynthesize efficiently for some time often accumulate assimilates and then respire many times stronger than similar plants "starved" for an extended period of time (*cf.* Harder's data in Table 28.III). Such special conditions may perhaps explain the very low I_c values found by Plaetzer for some aquatic plants.

The ratio between respiration and photosynthesis at low light intensities is generally changed in favor of respiration by an increase in *temperature* (*cf.* chapter 31); thus, higher temperature must cause an upward shift of the compensation point (*cf.* fig. 28.15 and Table 31.III). *Narcotics* have a similar influence, since they, too, reduce photosynthesis (at all light intensities) much more effectively than respiration (*cf.* chapter 12). Enzyme *poisons* (*e. g.*, cyanide) may have a lesser or even opposite effect, because their influence on photosynthesis in weak light usually is rather small (*cf.* figs. 28.9A, 28.11A), while most of them strongly inhibit respiration. In certain algae (*e. g.*, some *Scenedesmus* strains), the effect

of cyanide on respiration is stronger than on photosynthesis, even in strong light; in organisms of this type, addition of cyanide causes a strong downward shift of the compensation point (*cf.* chapter 12). Indications of a peculiar difference between cyanide effects of photosynthesis above and below the compensation point were mentioned in chapter 12 (Vol. I, p. 308).

Reduced *supply of carbon dioxide* decreases photosynthesis without affecting respiration. If, in consequence of carbon dioxide deficiency, the light curves begin to bend in very weak light, the compensation point may be shifted to high light intensities (*cf.* fig. 28.15), or never reached

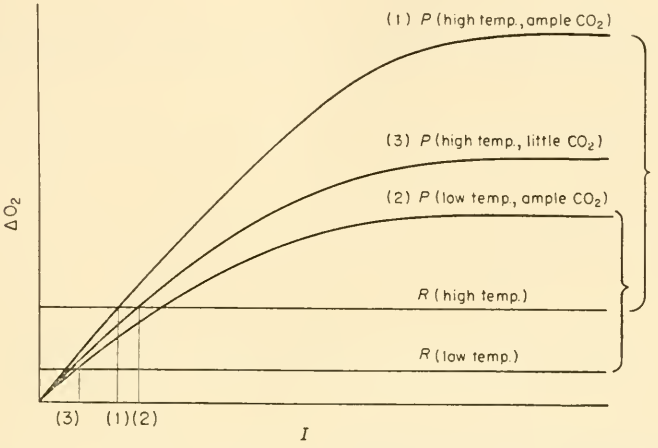


Fig. 28.15. Shift of compensation point with changing carbon dioxide concentration. (1) → (2) decreasing $[CO_2]$; (3) → (1) increasing temperature.

at all. This case was mentioned in chapter 26, when we spoke of the experiments of Chesnokov and Bazyrina (1932) and Miller and Burr (1935), in which gas balance was observed at light intensities of the order of 20 klux. Miller and Burr (1935) noticed that, in this "carbon dioxide-limited" range, the compensating light intensity was *independent of temperature.* This means that the temperature coefficient of the carbon dioxide supply process (diffusion or carboxylation?) was practically equal to that of respiration.

4. Saturating Light Intensity

When Reinke discovered the light saturation of photosynthesis, he found it to occur at an intensity close to that of sunlight at noon (S_0 = ap-

proximately 60 klux in moderate zones; *cf.* chapter 25). However, the saturating intensity varies widely from species to species and specimen to specimen. One reason for this is difference in optical density. Saturation begins when the most exposed chlorophyll molecules receive a certain light flux, and becomes complete when the most deeply shaded molecules obtain this saturating intensity. The intensity of incident light at which this complete saturation occurs obviously must depend on whether we use a thick or a thin leaf, a dense or a dilute cell suspension. This "density effect" already was described in chapter 25 and will be again discussed later in this chapter (page 1007).

Even with the density effect eliminated—either experimentally, by using optically very thin objects, or by calculation (*cf.* fig. 28.22)—the saturating light intensity still remains dependent, for a given species, on internal factors such as age and adaptation (to strong or weak light), and external variables, such as carbon dioxide supply and temperature. The effects of carbon dioxide concentration are illustrated by figures 28.1 to 28.5, those of temperature by figures 28.6 to 28.8. Using the notion of "ceilings" introduced in chapter 26 (page 869) we can say that everything that lowers the ceiling imposed on the over-all reaction of photosynthesis must shift the saturation toward lower light intensities. This may be a decrease in $[CO_2]$, a decrease in available reductants (in purple bacteria), or a decline in the amount of one of the catalysts. The temperature effect is complex, because changes in temperature affect all ceilings simultaneously—those imposed by diffusion, as well as those caused by enzymatic reactions of limited maximum yield.

Among the *internal* factors affecting the saturating light intensity, the most important is *adaptation* to strong or weak light. Shade-adapted plants often are darker green, *i. e.*, contain more chlorophyll (per unit area or unit volume) than the corresponding light-adapted species or individuals. This difference in optical density would in itself be sufficient to cause changes in the shape of the light curves: Darker, shade-adapted plants are more efficient light absorbers, and their light curves should therefore have a steeper initial slope. If the higher optical density is due to increased concentration of the pigment (with the concentration of all other constituents of the catalytic apparatus remaining the same), the saturation rate, *related to unit volume* of cells (or to *unit area* of leaves, assuming the leaf thickness is constant), should be the same for heliophilic and umbrophilic varieties; while the saturation rate related to *unit amount of chlorophyll* should be lower in the darker specimens. In practice, conditions are more complicated, because shade leaves often *are* thicker, and shade cells *do* grow larger than their heliophilic counterparts. These relationships will be discussed in more detail in chapter 32. The experimental result we

want to quote now is that the saturation rate of umbrophilic plants usually is much *lower* than that of the heliophilic plants, even if related to unit volume or unit area (not to speak of the rate per unit chlorophyll content). This indicates that adaptation to weak light involves, in addition to an *increase* in pigment concentration, a *decrease* in the amount of one or several catalysts that exercise a rate-limiting influence in photosynthesis. Coupled with steeper initial rise, this lower "ceiling" on the rate of photosynthesis in shade plants often leads to a very early light saturation. While the light curves of sun-adapted plants may continue to rise at or even beyond 100 klux (*cf.* data of Singh and Kumar, Smith, Boysen-Jensen, and Gabrielsen in Table 28.I), the light curves of shade plants may show saturation at light intensities as low as 1 klux (*cf.* figs. 28.16 and 28.18).

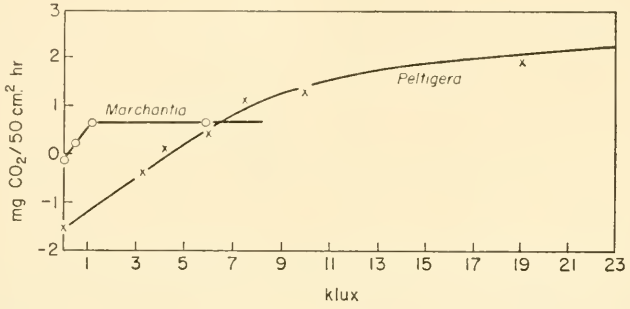


Fig. 28.16. Light curves of net gas exchange of an umbrophilic moss (*Marchantia*) and a heliophilic lichen (*Peltigera*) (after Boysen-Jensen and Müller 1929). The former is light-saturated at 1 klux; the latter at or above 20 klux.

The difference between the shapes of the light curves of heliophilic and umbrophilic land plants was first observed by Weis (1903), who compared the shade plant *Polypodium* with the sun plant *Oenothera*. This phenomenon was also investigated by Lubimenko (1905,1907,1908[1,2],1928,1929), Boysen-Jensen (1918, 1929), Boysen-Jensen and Müller (1929[2]) and Lundegårdh (1921, 1922), among others. Typical results are illustrated by figures 28.16, 28.17 and 28.18. The first of these figures refers to an umbrophilic moss (which is compared with a heliophilic lichen); the second compares shade-adapted specimens of two aquatic plants with sun-adapted individuals of the same species and the third contains a comparison of the light curves of a shade-adapted leaf and a sun-adapted leaf on the same plant (see also Table 28.IV). We see that the effect of phylogenetic adaptation (fig. 28.16) is similar to that of the individual adaptation of whole

plants (fig. 28.17) or single leaves (fig. 28.18). The figures in Table 28.IV further show that the respiration of shade-adapted plants is weaker than that of the sun-adapted specimens.

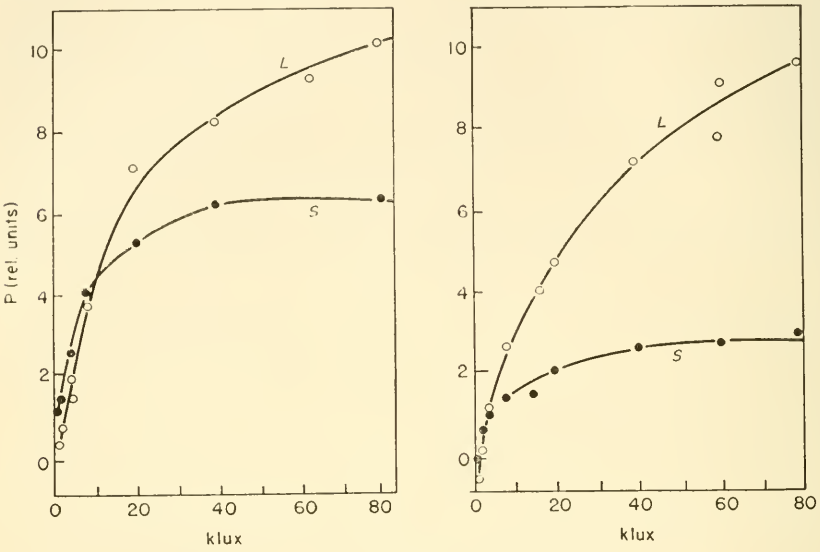


Fig. 28.17. Light curves of photosynthesis of shade plates (*S*) and sun plants (*L*) of the same species (after Gessner 1937). Former saturated at 40 klux; latter only far above 80 klux.

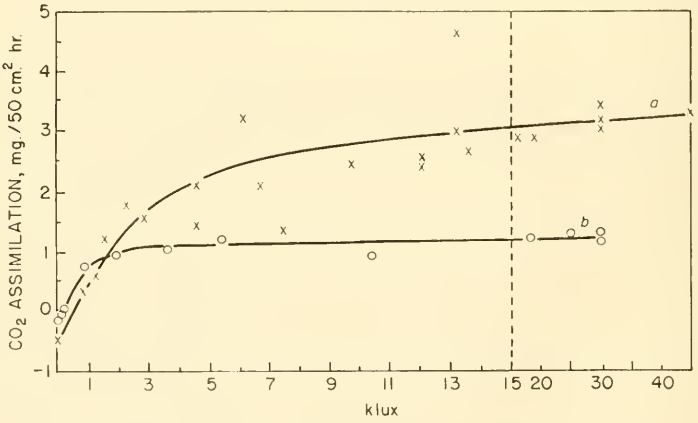


Fig. 28.18. Light curves of sun leaf (a) and shade leaf (b) of *Fagus silvatica* (after Boysen-Jensen and Müller 1929). Former saturated at or above 30 klux; latter at 3 klux.

Böhning (1949) noted that the rate of photosynthesis of shade-adapted leaves on trees of *Pyrus malus* declined in continuous illumination of 32 klux from an initial value of about 20 mg. to < 5 mg. CO_2/(hr. × 100 cm.^{2}) after 20 days. Sun-adapted trees, on the other hand, showed no decline during a similar period of continuous illumination, even in 50 klux. Kramer and Decker (1944) compared the light curves of white pine with those of three hardwood trees, and noted that the first one behaves as a heliophile and the deciduous trees as umbrophiles. This supports a previously suggested explanation of the fact that young deciduous trees "squeeze out" young pine trees on the floor of a forest.

TABLE 28.IV

PHOTOSYNTHESIS AND RESPIRATION OF SHADE LEAVES AND SUN LEAVES OF THE SAME PLANT (AFTER BOYSEN-JENSEN AND MÜLLER 1929)

Species	Specimen	R^a	$P_{1\,klux}$, net^a	$P_{max.}$, net	$P_{max.}/R$
Fraxinus excelsior	Sun leaf	1.2	1.4	9.8	8.2
	Shade leaf	0.4	2.2	4.2	10.5
Fagus silvatica	Sun leaf	1.0	2.2	6.6	6.6
	Shade leaf	0.2	1.8	2.4	12.0

[a] Respiration (R) and photosynthesis (P), in mg. CO_2/100 cm.^{2} hr.

Lubimenko (1928) and Montfort (1934) found that some species have "rigid" umbrophilic or heliophilic characteristics, *i. e.*, they are unable to adapt themselves to illuminations different from those to which the species as a whole has become adapted in its phylogenesis, whereas other species are capable of individual readjustment, as shown in figures 28.17 and 28.18 and in Table 28.IV.

Umbrophilic character is typical also of *algae* that have been adapted—phylogenetically or individually—to weak light. *Chlorella* cultures grown in dim light are richer in chlorophyll than those grown in strong light (*cf.* Table 25.I); the light curves of these "shade-adapted cells" rise more steeply, and reach saturation earlier than those of the "light-adapted cells." Similarly, van der Paauw (1932) found that *Hormidium* cells grown in dim light (2000 lux) become light-saturated in comparatively weak light (5000 lux), and show light inhibition in only slightly stronger light.

Algae that live deep under the sea, particularly the red ones, behave as extreme umbrophiles, and their photosynthesis, too, reaches saturation at light intensities of the order of a thousand lux.

5. Absolute Maximum Rate

It was mentioned before that simultaneous increase of carbon dioxide supply and light supply usually leads to saturation of photosynthesis long

TABLE 28.V

ABSOLUTE MAXIMUM RATES OF PHOTOSYNTHESIS OF DIFFERENT PLANTS

Material	$P^{max.}_{max.}$, g. CO_2/hr. Per 100 g. fresh wt.	Per 100 g. dry wt.	Per 100 cm.^{2} $\times 10^3$	Per g. chlorophyll (ν_A)	Assimilation time (T_A), sec.
Selected Data of Willstätter and Stoll (1918); 20° C., Air with 5% CO_2					
"Normal" leaves					
Aesculus hippocastanum	1.55	5.4	33	6.4	24
Acer negundo	1.92	8.6	40	7.7	20
Tilia cordata	1.88	5.8	28	6.6	24
Sambucus nigra	1.46	5.6	34	6.6	24
Leaves with high ν_A values					
Helianthus annuus	2.30	13.4	80	14.0	11
Populus pyramidalis	1.90	6.0	40	10.0	16
Pelargonium peltatum	1.19	19.8	—	14.5	10
Young and old leaves					
Acer pseudoplatanus, young	0.98	3.0	16	11.8	13
old	2.07	5.8	26	5.2	30
Tilia cordata, young	0.98	3.6	18	14.2	11
old	1.85	5.8	28	6.6	24
Green and yellow leaves					
Quercus robur, green	1.96	4.4	41	7.8	20
yellow	1.03	2.9	19	55	2.8
Sambucus nigra, green	1.96	5.3	34	6.6	24
yellow	0.88	4.7	18	120	1.3
Ulmus, green	1.11	3.8	21	6.9	23
Yellow	0.98	3.9	24	82	1.9

Data of Other Observers

Observer	Material	$[CO_2]$	° C.	$I_{max.}^{max.}$	ν_A	T_A
Boysen-Jensen (1933)	*Sinapis alba*	0.07%	20	22[a]	—	—
	Impatiens sultani	0.15%	18	24[a]	—	—
Stocker, Rehm, Paetzold (1938)	*Plectanthrus fruticosus*	0.15%	18	20[a]	—	
	Codineum hybridum	0.08%	18	8[a]	—	—
Wassink (1946)	Horticultural plants (strawberry, asparagus, cabbage, tomato, cucumber, etc.)	Water equilibrated with 9% CO_2	25	4–18[a]	—	—
Gessner (1938)	*Potomageton crispus*	Hard tap water	—	7[a]	—	—
Kniep (1914)	*Ulva lactuca*	—	23	1.3[a]	—	—
	Porphyra laciniata	—	23	1.8[a]	—	—
Emerson, Green (1934)	*Gigartina harveyana*	Sea water	15	14[a]	—	—
van der Honert (1930)	*Hormidium flaccidum*	—	20	—	6.8	23
Noddack, Kopp (1940)	*Chlorella pyrenoidosa* (shade cells)	Carbonate buffer 9	—	11.5[b]	4.1	36
	(light cells)	Carbonate buffer 9	—	13.4[b]	2.8	56

[a] (g. $CO_2 \times 10^3$/hr.)/100 cm.^{2}
[b] (g. CO_2/hr.)/100 g. dry weight.

before "carbon dioxide inhibition" or "light inhibition" becomes apparent. We have therefore assumed that, independently of any inhibition, certain intrinsic internal factors (such as limited availability of certain catalysts) impose an "absolute" ceiling (*i. e.*, a ceiling independent of both $[CO_2]$ and I) on the maximum rate of photosynthesis.

Determination of this maximum rate is of interest from the practical point of view (estimation of absolute and relative efficiency of different plants as producers of organic matter) as well as from the point of view of the kinetic mechanism of photosynthesis. However, the two aspects call for different methods of comparison. The practical problem can best be answered by using *unit surface* as the basis of rate determination (since what one wants to know is how much organic matter can be harvested from a unit area covered with plants of different species). From the point of view of a theorist, comparison should be based on *unit cell volume*, or *unit chlorophyll content*, rather than on unit area. Willstätter and Stoll (1918) designated the maximum quantity of carbon dioxide that can be reduced in unit time by unit quantity of chlorophyll in a cell or tissue as "assimilation number" (ν_A in Table 28.V), and the shortest time in which one molecule of chlorophyll can reduce one molecule of carbon dioxide (T_A in Table 28.V) as "assimilation time." (These constants will be analyzed in chapter 32.)

We designated, in chapter 27, the maximum rate of photosynthesis, at a given light intensity, reached with saturating concentrations of carbon dioxide, by $P_{\max.}$; we can use the symbol $P^{\max.}$ for the maximum rate reached, at a given carbon dioxide concentration, when light intensity becomes saturating; and the symbol $P^{\max.}_{\max.}$ for the "absolute" maximum rate, obtained when both carbon dioxide supply *and* light intensity are saturating.

Table 28.V shows that for the leaves of land plants the values of $P^{\max.}_{\max.}$ generally are of the order of 20 mg. CO_2/hr. 100 cm.^{2} of leaf surface, and sometimes reach 80–90 mg. Even *aurea* leaves, despite their very low content of chlorophyll, constitute no exception. Only some algae and aquatic plants investigated by Kniep (1914), Emerson and Green (1934) and Gessner (1938) fell far short of this production. A yield of 20–80 mg. CO_2/100 cm.^{2} hr.—assuming it is reached in light of 40 klux, 80% of which is absorbed by the leaf—corresponds to the conversion into chemical energy of 4 to 16% of absorbed light energy (in the photosynthetically active region, 400–700 mμ), and thus to a quantum yield between 0.018 ($\frac{1}{56}$) and 0.07 ($\frac{1}{14}$). (This estimate is based on factors given in chapter 25.) The relation of these yields, obtainable in *strong* light, to the maximum quantum yields observed in *weak* light will be discussed in chapter 29. In the case of *aurea* leaves, the quantum yield in the light-saturated state appears

to be higher, since roughly the same yield of carbon dioxide reduction is obtained here with a lower light absorption. However, the average absorption of white light by *aurea* leaves can vary, depending on their actual chlorophyll content, from as low as 30% or less, to as high as 75% of that of normal green leaves of the same species. An estimate of the quantum yield in the light-saturated state requires therefore that absorption determinations and yield measurements be performed on the same specimens.

The fact that *aurea* leaves may absorb only a slightly smaller proportion of incident light than normal green leaves, caused Seybold and Weissweiler (1942) to consider their higher "assimilation numbers" (table 28.V) as irrelevant (and not—as assumed by Willstätter and Stoll—as a sign of exceptionally high capacity for photosynthesis). However, the capacity for photosynthesis *in the light-saturated state*, $P_{max.}^{max.}$, is *not* a function of the efficiency of light absorption, but a measure of the amount of a limiting enzyme present in the cells. The values of $P_{max.}^{max.}$ for *aurea* leaves show that in these leaves an abnormally low chlorophyll content is *not* accompanied by a proportional reduction in the content of the rate-limiting enzyme.

Yields obtained by Noddack and Kopp (1940) with *Chlorella pyrenoidosa*, if related to dry weight, are higher than those given for most land plants in Table 28.V. However, because of the high concentration of chlorophyll in *Chlorella* (3–4%, instead of 0.5 to 1% in leaves), the assimilation numbers are not higher, but somewhat lower, and the assimilation times somewhat longer than those given by Willstätter and Stoll for the leaves of the higher plants.

Like the *maximum quantum yield* (at low light intensity), the *maximum rate* of photosynthesis (in strong light) is a constant of the plant, *i. e.*, it is independent of the optical density of the selected material. The only external factor that affects it (apart from the presence of poisons or inhibitors) is *temperature* (as illustrated by figs. 28.6–28.8). It is difficult, if not impossible, to define the absolute maximum rate of photosynthesis also as a function of temperature. In short experiments, the highest rates can be obtained, with plants adapted to moderate conditions, at about 35° C.; but, in prolonged experiments, "heat inhibition" is apt to occur even at temperatures as low as 22–25° C. (*cf.* chapter 31). We have used, in Table 28.V, mostly values obtained at 18–20° C., which are certainly smaller than the highest efficiencies of which most of the investigated plants were capable at higher temperatures, at least for short periods of time.

The maximum rate of photosynthesis of a species or individual plant depends on adaptation to strong or weak light. As described on p. 986, shade-adapted species or individuals generally have a lower "ceiling rate," indicating a decreased content of a rate-limiting catalyst. In addition, they often show an early onset of light inhibition (*cf.* fig. 28.19).

Since inhibition by excess light is a *time effect* (*cf.* chapter 19), the $P_{max.}^{max.}$ values of shade-adapted plants change with the duration of illumination. We recall in this connection the time curves that Harder (1933) found for *Fontinalis antipyretica* (fig. 26.8). The general impression made by these complex curves was that photosynthesis declined with time (*i. e.*, the plants suffered light injury) whenever the illuminating light was more intense than the light to which the specimens were accustomed during the growth period.

It was noted on page 987 that in the shade-adapted plants the apparent lower content of the enzyme responsible for the absolute saturation of photosynthesis is coupled with a *higher* content of chlorophyll. We will encounter, in chapter 32, other cases in which the content of the rate-limiting enzyme appears to be *independent* of that of chlorophyll (*Chlorella* cells grown in strong or weak light, *cf.* Tables 25.I and 28.V; green and *aurea* varieties of land plants which were mentioned above, *cf.* Table 28.V and fig. 32.2) as well as cases in which these two concentrations change in the same direction (*Chlorella* cells made chlorotic by iron deficiency, *cf.* figs. 32.3 and 32.4).

The shape of the light curves of shade-adapted plants has been much discussed in the ecological literature, particularly in relation to the photosynthetic production of aquatic plants at different levels under the surface. Even green algae, or submerged higher plants, found only a few meters under the surface, which should not have acquired extreme umbrophilic characteristics, were observed to produce a maximum of oxygen when placed at a certain depth, and to show light inhibition when exposed to direct sunlight. This, however, might have been, at least in part, a thermal effect. Much more pronounced optima on yield *vs.* depth curves were reported for the photosynthetic efficiency of colored (brown or red) algae at different levels under the sea.

Ruttner (1926) and Schomer (1934) observed that several aquatic higher plants (*Elodea*, *Myriophyllum*, *Cerathopyllum*) had a maximum efficiency 1–5 meters under the surface. Curtis and Juday (1937) found similar optima for the green algae *Anaboena* and *Gloethea* (in 9–10 meter depth). Van der Paauw (1932) found that *Hormidium* grown in a light of 2000 lux suffered light inhibition at 5000 lux. On the other hand, Gessner (1938) found no "optimum" in the light curves of shadow-grown or sun-grown *Elodea* plants in lamp light up to 30,000 lux. He tried ultraviolet light (360–400 mμ) to imitate sunlight, but this, too, produced no inhibition. He suggested that the reported depth optimum of *Elodea* may be caused by *chromatic* adaptation (to bluish-green light) rather than by intensity adaptation. However, this explanation is implausible since it implies that photosynthesis can be inhibited by the addition of red and blue-violet light to green light, which has never been observed. Perhaps, *carbon dioxide supply conditions* are more favorable at a certain depth than on the surface, and this causes the rate to increase with increasing depth, as long as illumination remains sufficient for light saturation.

Particular attention has been paid to the maximum efficiency and light inhibition of *colored algae* in relation to their vertical distribution in the sea. Engelmann suggested (see chapter 15, page 420) that the color of brown,

and especially of red, algae is the result of *chromatic adaptation* to the predominantly bluish-green light that prevails deep under the sea; Berthold (in 1882) and Oltmanns (in 1905), on the other hand, thought that colored algae are adapted not so much to the spectral composition of light in their natural habitats as to its *low intensity*. The ensuing controversy—which led to almost complete vindication of Engelmann's theory of chromatic adaptation—will be discussed in chapter 30. However, the fundamental importance of chromatic adaptation for the composition of the pigment system of deep-sea algae does not mean that these algae are not also adapted to low light intensity and do not use the same mechanism—shifts in relative concentrations of red, blue and green pigments—for chromatic as well as for intensity adaptation (*cf.* Harder 1923).

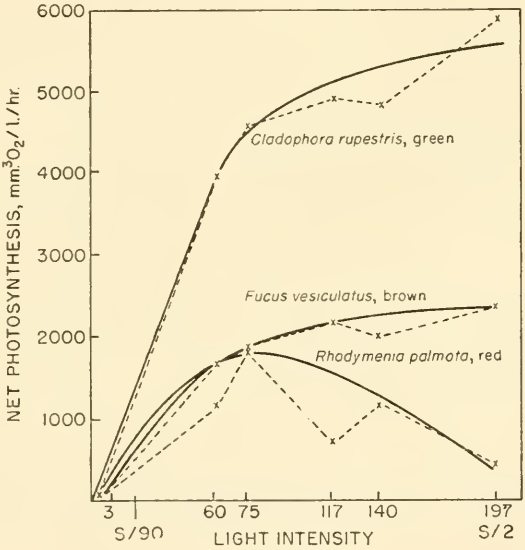


Fig. 28.19. Typical light curves of red, brown and green algae (after Montfort 1929). Light intensity in relative units and fractions of full sunlight. Equal fresh weights of algae used.

The response of colored algae from different depths to intense illumination has been studied, among others, by Maucha (1924, 1927), Marshall and Orr (1927, 1928), Ehrke (1931), Curtis and Juday (1937) and particularly by Montfort (1929, 1930, 1933,[1,2] 1934, 1936). Figure 28.19 shows a typical "optimum" curve, obtained by the last-named investigator. Montfort noticed that algae from one and the same level often show different resistance to strong light: Some red algae, containing much phycocyanin (such as *Rhodymenia palmata*), continued to synthesize effectively on the surface, while others, found in the same depth, but containing mainly phycoerythrin (such as *Delesseria alata*) suffered a "sunstroke" and died. The surface-living, almost pure-green form of

the blue alga *Gigartina* behaved like a typical shade plant, whereas the violet, deep-water form of the same species, rich in phycocyanin, maintained its photosynthesis at full efficiency even in direct sunlight.

6. Maximum Rate and Average Rate of Photosynthesis under Natural Conditions

The curve corresponding to $[CO_2] = 0.03\%$ is theoretically not more important than any other light curve of photosynthesis, but its saturation value has a considerable interest because it represents the *maximum rate of production of organic matter by land plants in the open air*. (In dense growth, or under otherwise abnormal conditions, the concentration of carbon dioxide may vary between 0.01 and 0.1%, and this must affect the maximum rate of photosynthesis in some natural habitats.)

It was stated in chapter 27 that with 0.03% carbon dioxide, and in intense light, the supply of carbon dioxide has a considerable rate-limiting influence, and the saturation value may therefore be below the "absolute" maximum, $P_{max.}^{max.}$ at the same temperature. Table 28.VI contains some relevant experimental data (for a more extensive table, see Stocker 1935). Most figures in this table represent the *net* consumption of carbon dioxide. For strongly photosynthesizing plants, the corresponding values of *true* photosynthesis are 10–15% higher; but for weakly photosynthesizing plants (*e. g.*, the arctic plants investigated by Müller) the difference may be much larger, as illustrated by the figures in parentheses. Table 28.VI contains some striking contradictions, which remain to be elucidated. There is a general contrast between the $P^{max.}$ values found by Boysen-Jensen and co-workers (usually 1–10 mg. CO_2/hr. 100 cm.^{2}, with the largest single values not exceeding 20–25 mg.), and the much larger values reported —often for the same species and under similar climatic conditions—by Kostychev and other Russian plant physiologists (usually 10–40 mg. CO_2/hr. 100 cm.^{2}, with the largest single values reaching 80 or 100 mg.).

Only in the case of *arctic* plants is there an approximate agreement between Boysen-Jensen's co-worker Müller, and Kostychev and his co-workers. In the case of sun-adapted plants from *moderate zones*, the average of Danish measurements (section B*a* of the table) is 13 mg., that of Swedish measurements (section B*b*), 16 mg., and that of English, Japanese and German measurements (with the exception of the early determinations of Sachs carried out by the half-leaf starch method), 10 mg. The average of the Russian analyses, listed in section B*c*, is as high as 24 mg. The results obtained by Kostychev, Bazyrina and Vasiliev (1927) by the determination of the synthesized assimilates did not differ significantly from those obtained by the same group by determination of absorbed carbon dioxide.

It was mentioned on page 908 that Kostychev and co-workers attributed the lower values of Boysen-Jensen to insufficiently rapid gas circulation.

This was denied by Boysen-Jensen and Müller (1929); but one notices in Table 28.VI that the newer measurements of the Danish school have given somewhat higher values than those of 1918–1929, and thus reduced the discrepancy between the averages in sections B*a* and B*c* to a factor of about 2.

In section C, containing plants from arid zones, we find a similar discrepancy between the result of Harder and co-workers in Algeria, and Wood in Australia (1–10 mg./hr. 100 cm.^{2}), and those of Kostychev and Kardo-Sysojeva in Central Asia (20–70 mg.).

In section D, practically all the listed values fall into the range 1–10 mg. (no Russian measurements are listed here, *cf.* however, the data of Kostychev and Kursanov for the subtropical vegetation of the Black Sea littoral in section B*c*).

In the group of alpine plants (section E), Mönch (1937) and the Russians agree in finding the highest yields ever recorded under natural conditions. Earlier, Henrici (1918) had reported, for the alpine plant *Bellis perennis*, a yield of 232 mg. CO_2/hr. 100 cm.^{2} This value appears so incredibly high that we did not include it in Table 28.VI; but even the results of Blagoveshchenskij (1935) and Mönch (1937) (90–100 mg./hr. 100 cm.^{2}) indicate remarkably high quantum yields (of the order of one CO_2 molecule reduced per twenty quanta, in light of 80 klux, and with not more than 0.03% CO_2 present).

It should not be assumed that the carbon dioxide concentration was exactly 0.03% in all measurements listed in Table 28.VI. In Blagoveshchenskij's experiments in the Pamir, for example, the [CO_2] assays varied between 0.01 and 0.02%, and the highest yields were obtained at the latter concentration (which is still considerably below the normal value of 0.03%). Stöcker found, in the undergrowth of the tropical forest, [CO_2] values up to 0.04%. (Compare also data given in Chapter 27, page 902.)

To sum up, it is certain that plants growing in moderate climates can reduce, in their natural habitats and under favorable conditions, 20 or 30 mg. CO_2 per hr. per 100 cm.^{2} of leaf surface; but it is much less certain whether *any* plants—desert and alpine plants not excluded—are capable of yields up to 100 mg./hr. 100 cm.^{2}, as is suggested by the measurements of Kostychev, Mönch and Blagoveshchenskij.

We will now discuss the relation between the *maximum* yield of photosynthesis of which leaves are capable under favorable natural conditions, and the *average* production of organic matter by whole plants or large plants assemblies.

Land plants in the open air, exposed to the sun, can be expected to maintain the above-estimated rate of photosynthesis (about 20 mg./hr. 100 cm.^{2}) for a considerable part of the day (barring such phenomena as the "midday rest"; *cf.* chapter 26). The intensity of illumination is sufficient,

TABLE 28.VI

MAXIMUM YIELD OF PHOTOSYNTHESIS BY LEAVES OF LAND PLANTS UNDER NATURAL CONDITIONS[a]

Species	Shade (Sh) or sun (S) plant	$P^{max.\,b}_{net}$ mg. CO_2 / hr. 100 cm.^{2}	$P^{max.}$ (estim.) mg. CO_2 / hr. 100 cm.^{2}	Observer
A. ARCTIC ZONE				
Salix glauca, 10° C.	S	4	(6)	Müller (1928) (Greenland)
Salix glauca, 20° C.	S	6	(9.5)	
Chamaenerium latifolium, 0° C.	S	10	(10.5)	
Rumex acetosella, 10° C.	S	12	(13)	
Rubus chamaemorus	S	8		Kostychev, *et al.* (1930) (Arctic Russia)
Average: *8*				
B. MODERATE ZONES				
(*a*) Denmark (Boysen-Jensen and others)				
Fagus silvatica, 20° C.	S	6.6	(7.6)	Boysen-Jensen (1918)
Fagus silvatica, 20° C.	Sh	2.4	(2.6)	
Fraxinus excelsior, 20° C.	S	9.8	(11.0)	
Fraxinus excelsior, 20° C.	Sh	4.2	(4.6)	
Alchemilla minor, 20° C.	S	7.2	(8.0)	Boysen-Jensen, Müller (1929)
Betula verrucosa, 20° C.	S	6.4	(7.6)	
Plantago major, 20° C.	S	11.0	(12.2)	
Spinacia oleracea, 20° C.	S	18.6	(20.4)	
Rumex acetosella, 20° C.	S	9.0		Boysen-Jensen (1932)
Sambucus nigra, 20° C.	S	4.6		
Sambucus nigra, 20° C.	Sh	1.7		
Oxalis acetosella, 20° C.	Sh	1.6		
Sinapis alba, 20°	S	16.0		
Avena, 20° C.	S	13.0		
Sinapis alba, 20° C.	S	26		Müller (1932)
N-deficient, 20° C.	S	14		
Sinapis alba, 20° C.	S	20		Gabrielsen (1935)
Sinapis alba, 20° C.	S	26		Kjär (1937)
Average: *13* (S)				

(b) Sweden (Lundegårdh and others)			
Atriplex latifolium, 18° C.	S	17.2	Lundegårdh (1921)
Oxalis acetosella, 18° C.	Sh	4.8	
Stellaria nemorum	Sh	2.9	
Anemone nemorosa	Sh	11.0	Lundegårdh (1930)
Eupteris aquilina	S	9.4	Johansson (1926)
Polypodium vulgare	Sh	5.6	
Solanum tuberosum	S	20.0	Lundegårdh (1924)
Solanum lycopersicum	S	16.8	
Beta vulgaris	S	20.1	
Phaseolus vulgaris	S	17.0	Yoshii (1928)
Average: *16* (S)			
(c) U.S.S.R. (Kostychev and others)			
Alchemilla sp., 14–22° C.	S	18–11, 18–26*	Kostychev, Bazyrina, Vasiliev (1927)
Plantago major, 11–17° C.	S	18–26, 12–18*	
Betula pubescens	S	15–22, 10–15*	
Phleum pratense, 17–20° C.	S	18–27, 9–16*	
Trifolium pratense, 20–29° C.	S	23–25, 16–24*	
Alchemilla	S	20.5	Kostychev, Bazyrina, Chesnokov (1928)
Betula pubescens	S	9.8	
Lappa major	S	17.0	
Rubus idaeus	S	6.5	
Alisma plantago	S	5.1	
Pinus silvestris, 23° C.	S	13.2[c], 5.1[d]	Ivanov, Kosovich (1929)
Pinus silvestris, 23° C.	Sh	6.4[c], 3.6[d]	
Fagopyrum esculentum	S	10.5	Maximov, Krasnoselskaja-Maximova (1928)
Hordeum sativum	S	29.5	
Glycine hispida	S	31.8	
Trifolium repens	S	36.0	
Epidemium colchicum	S	48.6	Kostychev, Berg (1930) (subtropics)
Tamus communis	S	49.8	
Bambusa auricoma	S	38.7	
Bambusa phyllostrachys	S	20	Kursanov (1933) (subtropics)
Eriobotyra japonica	S	29.1	
Pirus malus	S	34.9	
Average: *24* (S)			

[a] Figures in parentheses are estimated rates of *true* photosynthesis. All data were obtained by carbon dioxide determination, except those marked by an asterisk, which were obtained by the assay of assimilates.

[b] $P_{net}^{max.} = P^{max.} - R$; in mg. CO_2/100 cm.^{2} hr. [c] Maximum observed values. [d] Average values.

Table continued

TABLE 28.VI (*concluded*)

Species	Shade (Sh) or sun (S) plant	$P_{net}^{max.b}$ mg. CO_2 / hr. 100 cm.^{2}	$P^{max.}$ (estim.) mg. CO_2 / hr. 100 cm.^{2}	Observer
		(*d*) Other Countries		
Helianthus annuus	S	24*		Sachs (1884) (Germany)
Cucurbita pepo	S	20*		
Helianthus annuus	S	5.5		Brown, Escombe (1905) (England)
Tropaeolum majus	S	3.1		
Polygonum weyrichii	S	3.9		
Catalpa bignoinoides	S	4.7		
Patasitas albus	S	1.9		
Rhododendron brachycarpum	Sh	2.8		Hiramatsu (1932) (Japan)
Acer tschenoskii	Sh	4.1		
Pars tetraphylla	Sh	2.2		
Fagus silvatica	Sh	2.2		Daxer (1934) (Germany)
Anemone nemorosa	Sh	6.0		
Viola riviniana	Sh	2.5		
Lemna minor, 29° C.	S	10		Ashby, Oxley (1935) (England)
Pyrus malus	S	20		Böhning (1949) (Ohio)
Pyrus malus	Sh	20 → 3		
Average: *5* (S)				
		C. DESERTS		
Phoenix dactylifera	S	3.4		Harder, Filzer, Lorenz (1932) (Algerian desert)
Nerium oleander	S	10.3		
Zila macroptera	S	10.9		
Limoniastrum feei	S	1.3		
Cousinia pseudomollis	S	62.1		Kostychev, Kardo-Sysojeva (1930) (Central Asia)
Alhagi camelorum	S	56.3	Loam desert	
Capparis herbacea	S	68.5		
Heliotropium arguzioides	S	27.3	Sand desert	
Arthrophytum haboxylon	S	27.1		
Triticum sativum	S	36.5		
Andropogon halepensis	S	38.0	Irrigated desert	
Medicago sativa	S	22.4		
Vitis vinifera	S	16.1		
Atriplex vesicarium	S	10.0		Wood (1932) (Australian desert)
Average: *28* (S)				

D. TROPICAL ZONES				
Helianthus annuus	S	8.0		Giltay (1898) (Java)
Nicotiana rustica	S	9.0		
Cedrella serrulata	S	5.7		
Acalypha tricolor	S	4.8		
Cassia timorensis	S	19.2		
Sugar cane	S	5.0		McLean (1920) (Philippines)
Cocos nucifera	S	0.9		
Musa textilis	S	1.0		
Abutilon darwinii	S	7.5		Dastur (1925) (India)
Cassia fistula, 28° C.	S	10.9	(13.5)	Stöcker (1931) (Java)
Cassia fistula, 28° C.	Sh	8.6	(10.6)	
Stelechocarpus burahol, 28° C.	S	6.3	(8.3)	
Stelechocarpus burahol, 28° C.	Sh	2.6	(3.2)	
Calophyllum inophyllum, 28° C.	S	7.3	(10)	
Average: *6.9*				
E. MOUNTAINS				
Eurotia ceratoides, 24° C.	S	≥44		Blagoveshchenskij (1935) (Pamir)
Gentiana algicola, 20° C.	S	≥100		
Barley	S	≥30		
Crepis montana	S	60		Mönch (1937) (Alps)
Geum montanum	S	48		
Alchemilla alpina	S	90		

[a] Figures in parentheses are estimated rates of *true* photosynthesis. All data were obtained by carbon dioxide determination, except those marked by an asterisk, which were obtained by the assay of assimilates.

[b] $P_{net.}^{max.} = P^{max.} - R$; in mg. CO_2/100 cm.^{2} hr.

[c] Maximum observed values. [d] Average values.

or almost sufficient, for light saturation during most of the day, unless the sky is heavily overcast. On the other hand, the carbon dioxide supply, under natural conditions, may often be less constant than it is in laboratory experiments with a circulating gas containing 0.03% carbon dioxide and this may cause considerable variations in the rate of photosynthesis. In quiet open air, a carbon dioxide-deficient air layer will form around the plants, and cause a decline in rate, while the exhalations of the ground, which contain carbon dioxide produced by the decay of organic matter and by the respiration of the roots, can be caught in the foliage, and can provide an increased supply of carbon dioxide (*cf.* page 902). A certain amount of carbon dioxide, of the same origin, also may reach the leaves with the transpiration flow from the roots (*cf.* page 910).

Thus, variations in the supply of carbon dioxide, under natural conditions, may be considerable, and these variations, more than alterations in the intensity of illumination, may cause the rate of photosynthesis of *land plants* to vary from location to location, and to fluctuate with time in a given location. The same appears to be true of the multicellular *aquatic* plants. As mentioned on page 878, Gessner (1937) found that the decline in rate of photosynthesis of higher aquatic plants with time, reported, among others, by Arnold, was largely caused by insufficient circulation of water. Although the carbonates contained in natural waters (particularly hard waters) represent a considerable reserve of carbon dioxide, the diffusion of dissolved electrolytes is so slow that a carbon dioxide-deficient alkaline layer can easily be formed around a submerged plant.

Gessner (1938) found (*cf.* Table 28.V) a rather low value (7.0 mg./hr. 100 cm.^{2}) for the maximum rate of carbon dioxide reduction by *Potomageton crispus* in hard tap water; the value for *Potomageton perfoliatus* was even lower (4.8 mg. CO_2/hr. 100 cm.^{2}). These yields are less than one third of those of land leaves in ordinary air, and five to ten times smaller than the maximum yields produced by land leaves provided with an abundant supply of carbon dioxide. This indicates that Gessner's water plants might have been in a "carbon dioxide-limited" state, despite the relatively high carbonate content of the medium. (Gessner attributed the low yield of aquatic plants per unit area to the fact that these leaves consist of only a few layers of cells; however there is no indication that the light absorption in the leaves of aquatics could be three or five times smaller than in ordinary leaves.)

Unicellular algae, with their extremely favorable ratio of surface to volume, are unlikely to feel any deficiency of carbon dioxide supply, at least as long as the average carbonate concentration in the medium is high and stirring sufficiently intense. Since these algae form a vast majority of the organisms living in the sea, we can conclude that the total photosynthesis of the marine flora—in contrast to that of the continental flora—is not much affected by limitations of carbon dioxide supply.

In addition to vagaries of carbon dioxide supply, other external factors, such as variations in temperature and humidity, and internal changes responsible for permanent "aging" and temporary "resting" of plants, also affect the average rate of photosynthesis under natural conditions. And lastly, variations in the brightness of the daylight, even though less important than one is at first inclined to believe, certainly affect the yield of photosynthesis, particularly in the case of species adapted to strong light.

We estimated in chapter 1 the average conversion yield of incident solar energy as 1.5 to 6% (assuming 20 klux as the average intensity of illumination). We will now compare this estimate with the results of several investigations in which the determination of the yield of photosynthesis under natural conditions was combined with the measurement (or estimation) of the solar radiation that fell upon the plants during the same period. These investigations can be divided into two groups: experiments of short duration (several hours), and studies lasting several weeks or months. Table 28.VII contains the results of three short-time experiments. The figures of Brown and Escombe were obtained in the same investigation that gave the low absolute yields listed in Table 28.VI; the conversion yields were correspondingly low (of the order of 1% of *incident* energy, or an estimated 2.5% of the visible radiation *absorbed* by the plants). The figures of Purevich and Bose are considerably higher, and can be placed alongside the higher absolute reduction rates found by Willstätter and Stoll in 5% carbon dioxide (Table 28.V), and by many recent investigators in ordinary air (Table 28.VI).

TABLE 28.VII
ENERGY CONVERSION UNDER NATURAL CONDITIONS

Author	Plant	Incident energy, % converted	Absorbed energy, (400–700 mμ) % converted
Brown, Escombe (1905)	*Polygonum weyrichii*	0.5 to 1.7	2.5[a]
	Tropaeolum majus	0.8 to 1.4	
	Patasites albus	1.1 to 1.3	
	Helianthus annuus	0.3 to 0.7	
Purevich (1914)	*Acer platanoides*	0.6 to 2.7	7.5[a]
	Polygonum sacchalinensis	1.1 to 7.7	
	Helianthus annuus	4.5	
	Saxifraga cordifolia	5.0	
Bose (1924)	*Hydrilla*	—	6.7

[a] This average conversion of *absorbed* energy was obtained by multiplying the average conversion of *incident* energy by 2.5 (factor 2 to account for far red and infrared radiation, and factor 1.25 to account for reflection and transmission of visible light).

We decided above that the larger reduction rates are likely to be the correct ones. (We mean values of the order of 20–25 mg./hr. 100 cm.^{2}

in ordinary air, and 30–40 mg. in air enriched with carbon dioxide, and *not* the much higher yields found by Blagoveshchenskij, or Mönch.) We therefore suggest that in Table 28.VI, too, the results of Purevich and Bose should be given preference over those of Brown and Escombe. However, the whole problem is in need of renewed and more exact experimental analysis, which alone could link the rate of photosynthesis under natural conditions (strong illumination and limited carbon dioxide supply) to the much better known rates in weak light and in the presence of an ample amount of carbon dioxide.

We now turn to experiments of longer duration, in which the total yield of photosynthesis of an assemblage of plants was compared, over an extended period of vegetation, with the integral of insolation over the same period.

Noddack and Komor (1937) studied two plots of grass, one of 9 m.^{2} and another of 74 m.^{2} In two consecutive periods of 20 days each, they measured the total solar radiation falling on these two plots, $\int I\,dt$; after this, the grass was mowed, dried and combusted, and the heat of combustion, ΔH_c, was measured. Here are the results: *total irradiation* in 20 days, 2.6×10^3 cal./cm.^{2} (average irradiation 0.0015 cal./cm.^{2} sec., or approximately 6000 lux); *proportion of incident energy stored in the hay*, first plot, $\Delta H_c / \int I\,dt = 0.67\%$ (first period) and 0.80% (second period), *second plot*, 0.41% and 0.64%, respectively.

In these measurements, the growth of the root system was not taken into consideration. This correction is difficult to estimate; but it should bring the average value of $\Delta H_c / \int I\,dt$ up to almost 1%, and ϵ ($= \Delta H_c / \int A\,dt$) close to 2.5%.

In comparing these results with those of the short-time experiments listed in Table 28.VII, one has to consider that some obvious factors tend to decrease the long-time average value of energy conversion by a large assemblage of plants growing under natural conditions, compared with that of a few isolated plants or leaves, averaged over a few hours of full sunlight; but that other less obvious factors may act in the opposite direction. Such favorable factors are the lower average light intensity (which decreases $\int I\,dt$ without reducing strongly the rate of photosynthesis), and, possibly, partial retention of the respiratory gases in the dense foliage, permitting reutilization of exhaled carbon dioxide. On the unfavorable side we can anticipate that in a large assemblage of organisms a certain proportion will not be in a healthy state, and others will be "resting"; sometimes the temperature will be too low for maximum photosynthetic efficiency; sometime it will be so high as to cause inhibition (*cf.* chapter 31). Some leaves will be in the shade of others, at least part of the day. These unfavorable influences seem to predominate, judged by the fact

that the average yields of Noddack and Komor are two to three times smaller than the most reliable short-time averages in Table 28.VII.

The measurements of Noddack and Komor represent the only available parallel large-scale *measurements* of irradiation and production of organic matter by plants. There is, however, no dearth of *estimates*, often on a much larger scale, based on agricultural and meteorological statistics. They have been mentioned in chapter 1, where we used them for the estimation of the total yield or organic synthesis on earth. We will now consider these estimates somewhat closer. Pütter (1914) took the insolation data from observations of the brightness of daylight carried out by Weber in Kiel, Germany, over a period of several years, and used the relation 1 lux = 6.3 erg/cm.^{2} sec. to calculate the corresponding energy flux. He calculated, for the total irradiation over a year, 35.3 kcal./cm.^{2}, corresponding to an average illumination intensity of about 7000 lux.

For the calculation of the heat of combustion of the synthesized organic matter Pütter used estimates of "exceptionally high" crops from agricultural yearbooks; he then added the (estimated) heat of combustion of the roots and stubbles, and subtracted the heat of combustion of the seed. Thus he obtained values for ΔH_c; for the calculation of $\int I\,dt$, he integrated Weber's data over the periods of vegetation of the several crops, and subtracted the energy of infrared radiations (above 1 μ). (It would be more reasonable to subtract all radiations above 0.7 μ, since light between 700 and 1000 mμ probably is not used for photosynthesis at all, *cf.* chapter 30.) Table 28.VIII shows some of Pütter's results. In the last column, the conversion yields are corrected for losses by respiration, estimated at 15% of the weight increase due to photosynthesis during the vegetation period.

TABLE 28.VIII
ENERGY CONVERSION BY FIELD PLANTS (AFTER PÜTTER 1914)

Crop	Total irradiation $<1\mu$ during vegetation, $\int I\,dt$, kcal./cm.^{2}	ΔHc, kcal./cm.^{2}	$\Delta Hc/\int I\,dt$, %	$\Delta Hc/\int I\,dt$, corr. for respiration, %
Summer wheat	22.2	0.63	2.8	3.3
Summer rye	20.9	0.47	2.3	2.6
Summer barley	12.3	0.39	3.2	3.7
Oats	21.8	0.63	2.9	3.3
Potatoes	25.0	0.65	2.6	3.0
Beets	30.0	0.55	1.8	2.1
Clover	13.6	0.62	4.6	5.2

The average conversion yield in Table 28.VIII is 3.3%, referred to incident radiation below 1 μ. This corresponds to 2.3% referred to the *total* incident radiation, or about 6% referred to the absorbed radiation below 700 mμ. Thus, Pütter's average value is two to three times larger than the

experimental yields of Noddack and Komor, and about equal to the highest short-time averages of Purevich. Pütter attributed these comparatively high values to conditions that favor large-scale field experiments (particularly to the carbon dioxide supply from the ground). However, it seems more likely that his conversion yields were overestimated. An error by a factor of about 1.5 could have been caused by the use of too low a factor for the conversion of lux into energy units (in chapter 25 we stated that, in sunlight, one lux corresponds to about 10 erg/cm.^{2} sec., while Pütter used a factor of 6.3). Another error in the same direction may have been introduced by the comparison of *exceptional* crops with *average* insolation data.

Spoehr (1926), who made calculations similar to those of Pütter, but took into consideration only the grain in the field crops and the utilizable timber in the forests, obtained much lower values of energy conversion—*e. g.*, 0.13% of total incident radiation for a wheat field, and 0.35% for a forest of fast-growing eucalyptus trees. Similar figures were obtained, for forest trees, by Boysen-Jensen (1932). These calculations were intended to estimate the practical efficiency of plants as converters of solar energy—so that stalks, husks and roots of the wheat plants, and leaves and roots of the trees were neglected altogether; but the consideration of these terms could scarcely more than double the calculated conversion yields—which would thus become comparable to the results of Noddack and Komor, but could never approach the much higher figures of Pütter. To sum up, it seems that 1% of total incident solar energy, and about 2.5% of absorbed visible radiation, represent a fair estimate of the average utilization of light energy by field crops and forests, during the summer vegetation period, under moderate climatic conditions. The average quantum yield of photosynthesis under these conditions is of the order of 0.01 (1 molecule CO_2 reduced per 100 visible quanta absorbed).

Analysis of the data on plankton production in the sea (*cf.* Table 1.II) led Riley (1941) to the conclusion that the average utilization of light energy falling on the surface of the sea is between 0.6 and 0.8%,* *i. e.*, similar to average utilization of light by fields and forests. However, in the ocean, vegetation develops more or less uniformly throughout the year; and except for the part of the Arctic seas covered by ice, there are no large barren regions in the ocean comparable to the deserts or glaciers on the surface of the earth. These differences weigh heavily in favor of the oceans as the main producers of organic matter on earth (*cf.* Table 1.III).

In chapter 1 (page 9) we made one more step and calculated the *total production of organic matter on earth* by assuming that the average yield of conversion of the energy of visible radiations *absorbed* by the plants is 2% (corresponding to 0.8% of the total *incident* light energy). Now,

* Lanskaja and Sivkov (1950) gave much higher figures, 3–14%.

after having analyzed the foundations of this estimate, we feel certain that its *order of magnitude*, at least, is secure—even though the figure given may be in error by as much as a factor of two.

7. Interpretation of Light Curves

(*a*) *Influence of Inhomogeneity of Light Absorption*

The nonuniform illumination and supply of reactants in photosynthesis were discussed in general terms in chapter 26 (section 2). In chapter 27, while dealing with the carbon dioxide curves, we noted that these curves can be strongly affected by concentration gradients, which arise, particularly during intense photosynthesis, between the external medium and the immediate neighborhood of the chloroplasts. Similarly, the light curves may present a strongly distorted picture of the intrinsic relationship between the rate of light absorption and the yield of photosynthesis, because a considerable gradient of light intensity often must exist between the light-exposed and the shaded chlorophyll molecules. Even within a single chloroplast the rate of light absorption may decrease by a factor of five or ten from the light-exposed to the shaded side; or, in the case of diffuse illumination, from the surface to the center of the plastid. In suspensions containing millions of cells, as well as in leaves or thalli, the heterogeneous nature of light absorption is further enhanced by the mutual shading of the numerous chloroplasts (*cf.* fig. 26.5). Consequently, the light curves of different specimens of one organism, even if they all have the same content of all the relevant pigments and catalysts and are investigated under the same external conditions, may nevertheless differ in shape, depending on optical density (*i. e.*, the number of cells per square centimeter in an algal suspension, or the thickness of a leaf or thallus). The assumption of equal content of catalysts may itself be incorrect; for example, the cells of the spongy parenchyma may be adapted to weaker light (and thus contain less of certain catalysts) than the leaves of the palisade tissue.

Let us consider, as the simplest example, two suspensions of identical cells—one optically thin (*e. g.*, transmitting 80% of incident light), the other optically dense (*e. g.*, absorbing 80% of incident light). There is no reason (aside from the phenomena of "self-inhibition" by metabolic products mentioned in chapter 25) why these suspensions should differ in the maximum quantum yield at low light intensities, or in the maximum yield per chlorophyll molecule in strong light. However, the transition from the linearly ascending part to the horizontal part of the light curves will be sharper in the optically thin system (where saturation occurs more or less simultaneously in all cells), and more gradual in the optically dense system,

where saturation sets in at the surface of the vessel and spreads inward with increasing intensity of illumination.

Figure 28.20 shows, in a qualitative way, the expected differences in the plots of the absolute rate of photosynthesis, P, and the relative saturation, $P/P^{max.}$, against the incident energy flux, I, and the absorbed energy, I_a.

If the rate of photosynthesis, P, is plotted against the incident light intensity, I, as independent variable (curves *1*), the initial slopes of the

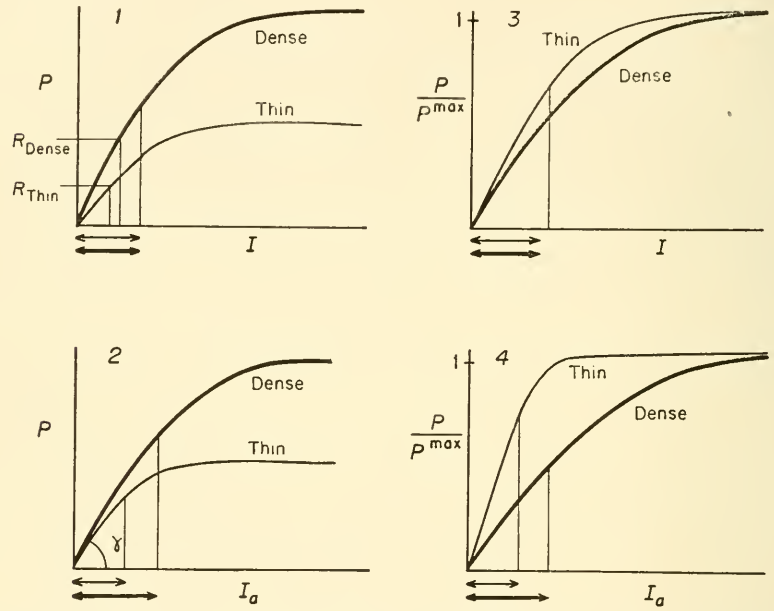


Fig. 28.20. Effect of optical density of a cell suspension on shape of light curves. Heavy and thin double arrows represent the "linear range" of dense and thin suspension, respectively; γ is the angle that determines the maximum quantum yield.

curves vary in proportion to optical density, but the extension of the linear range must be practically independent of optical density (since the curve of the dense suspension must bend as soon as saturation begins in the surface layer). But if P is plotted against the *absorbed* energy, I_a (curves *2*), the initial slopes must be the same, but the linear range of the thin suspension must be shorter than that of the dense one. On the other hand, if we plot the relative saturation, $P/P^{max.}$, against either I or I_a (curves *3* and *4*), the curves of the thin suspension will remain linear much closer to full saturation.

As an illustration, figure 28.21 shows the light saturation curves,

$P/P^{max.} = f(I)$, of two *Chlorella* suspensions of different density (*cf.* Table 25.1) given by Eichhoff (1939). Their relationship is in agreement with the prototype of figure 28.20 (curve *3*). Katz, Wassink and Dorrestein (1942) attempted to reduce analytically the light curves obtained with three suspensions of bacteria (*Chromatium*, D) of different concentration to a single curve showing the *average yield per cell* as function of *average illumination*. In a 2 cm. deep absorption vessel, the "dense" suspension,

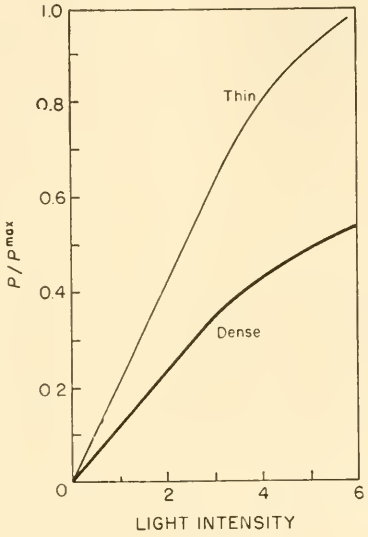


Fig. 28.21. Light curves of a thin and a dense *Chlorella* suspension, in red light, $\lambda = 6500$ Å (after Eichhoff 1939). Intensity in "energetic meter candles" (HK) (page 1098).

with a concentration of 30 "Trommsdorff units"/ml. (concentration 3) absorbed about 80% of incident light of a sodium lamp, the "medium" suspension (concentration 1; 10 Trommsdorff units), about 60%, and the "thin" suspension (concentration $\frac{1}{3}$; $3\frac{1}{3}$ units), about 30%. Figure 28.22A shows the empirical light curves of these three suspensions, $P = f(I)$. They have the relative positions anticipated in figure 28.20 (*1*) (except for the sigmoid initial shape, which is characteristic of the light curves of purple bacteria).

Near $I = 0$, the order of the three curves is reversed. The probable reason for this is that, at a given incident light intensity, the *average* illumination is lowest in the densest suspension; therefore, the deficiency of hydrogen consumption (which we think, is responsible for the sigmoid shape) is maintained, in the dense suspension, up to higher intensities than in the dilute one, and this influence apparently overcompensates that of stronger absorption.

Figure 28.22B shows the same three curves, replotted to represent yield per single cell. The densest suspension now shows the lowest yield. However, all three curves appear to approach (as expected) the same limiting yield at high light intensity; thus, except for the sigmoid shape, they

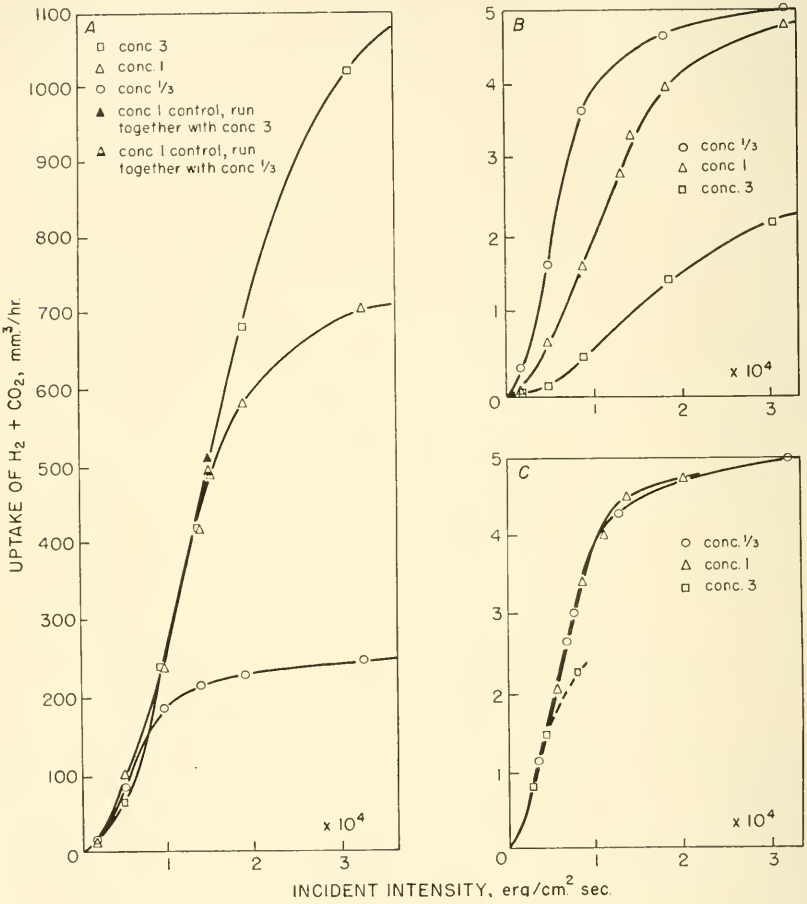


Fig. 28.22. Effect of cell concentration on light curves of photosynthesis (Katz, Wassink and Dorrestein 1942).

are of the type 3 in figure 28.20. (The latter refers to $P/P^{\max.}$, but, since $P^{\max.}$ is proportional to the number of cells, $P/P^{\max.}$ must be proportional to the yield per cell.) Figure 28.22C, finally, shows the yields per cell as function of *average* intensity of illumination throughout the vessel (in A and B, the abscissae were the *incident* light intensities, as measured at the

front wall of the vessel). The conversion is made by reducing the abscissae in the ratio a/c, where c is the concentration and a the per cent absorption, (*i. e.*, by $0.8/3 = 0.27$ for the dense suspension, $0.6/1 = 0.6$ for the medium suspension, and $0.3/0.3 = 1$ for the dilute one). This treatment causes the curves for $c = 0.3$ and $c = 1$ to coincide almost exactly; but the last point on the $c = 3$ curve still shows considerable deviation.

There are two obvious reasons why one cannot expect the reduction method used to be completely successful. In the first place, the averaging cannot be quite correct, because the cells are not actually exposed to the "average" light intensity, but some are illuminated with stronger, and some with weaker light. This would not matter if the yield were proportional to intensity; but, if the yield declines with increasing intensity (as it does in the saturation region), the yield that corresponds to a given average intensity will be lower when the spread of actual intensities is wider, *i. e.*, in the more concentrated suspension.

A second complication arises from the *stirring* of the reaction vessel, which causes the cells to come successively into light of different intensity. The effect of this variation is complex; it belongs to the group of phenomena (induction; photosynthesis in alternating light) which will be treated in chapters 33 and 34. Only if the illumination cycles are much shorter than the periods required for the completion of all dark processes of photosynthesis can one expect the cells to work, in alternating light, with the same efficiency as in steady light with the same average intensity. The known periods of dark reactions, associated with photosynthesis, include at least one with a period as short as $\tau = 0.01$ sec. at room temperature; stirring is not usually rapid enough to send each cell through the whole cycle of intensities within 0.01 sec. (*cf.* chapter 29, page 1106). Consequently, the cells in the stirred vessel are illuminated with an alternating light the average frequency of which is smaller than $1/\tau$. While the *frequency* of intensity variations is identical for all three suspensions, their *amplitude* is the larger the denser the suspension. Because of induction phenomena, the highest yield at a given average illumination is obtained in continuous light (*cf.* fig. 34.5); consequently, the efficiency losses caused by intermittency will be highest in the densest suspension. We have thus found two reasons, each of which may explain the deviation from the average of the last point in the $c = 3$ curve in figure 28.22C.

It may be useful to note that the changes in the illumination of individual cells, caused by stirring, may be discontinuous. The absorption by a single chloroplast (*i. e.*, in the case of *Chlorella*, a single cell) is so strong that the only significant intensities of illumination to which a cell is exposed may be those with no cells or with a very small number of cells (1 or 2) between it and the light source. The scattering of light in the suspensions tends to smooth over these discontinuities.

With respect to the inhomogeneity of light absorption, two cell suspensions with the same number of cells per square centimeter, but with a different concentration of chlorophyll within each cell, offer a case similar to that of two suspensions of identical cells, but different dilution. Whether the light curves of such two specimens will present a picture similar to that shown in figure 28.20, depends on their content of the catalyst that limit

the rate in strong light. In the case for which figure 28.20 was drawn, the catalyst content could be assumed to be proportional to the content of chlorophyll (in other words, the "assimilation numbers" could be taken as identical), since the two suspensions differed only in quantity and not in quality of the cells. When, however, changes in optical density are brought about by variations in the pigment concentration *within* the cells, the "assimilation number" does not necessarily remain constant. We will deal with these relationships in chapter 32; of the three cases discussed there (umbrophilic and heliophilic plants, *cf.* figs. 28.16–28.18; green and *aurea* varieties, *cf.* fig. 32.2; and normal and chlorotic plants, *cf.* fig. 32.4), only the last one is characterized by approximate constancy of the assimilation numbers, and thus leads to light curves such as those in figure 28.20. In other words, in this case only does the intracellular content of the rate-limiting catalyst change proportionally to the content of chlorophyll.

(*b*) *General Shape of Light Curves*

We now leave the effects of inhomogeneity of light absorption and inquire into the intrinsic shape of the light curves. All kinetic interpretations agree that the initial, almost linear segment of the light curves corresponds to a state in which the primary photochemical process is so slow that the catalysts which participate in the nonphotochemical steps can supply the substrates needed for, and transform the intermediates formed by, the primary process, without depletion of the former or accumulation of the latter. Only the light curves of purple bacteria generally show a sigmoid-shaped initial part; the probable reason for this was discussed on page 948.

In the linear section, the quantum yield of photosynthesis has its highest value along a given light curve. (In sigmoid light curves, the highest quantum yield is in the point where the tangent to the light curve passes through the origin of the coordinates.)

This yield may correspond to actual utilization of all absorbed light quanta for photosynthesis, or to a certain, not further reducible proportion of quanta wasted by complete or partial inactivity of a certain number of cells, or of a certain fraction of chlorophyll. A similar irreducible loss of energy can be caused by back reactions, if the proportion of photochemical products that they destroy is independent of the rate of formation of these intermediates (*cf.* page 1037, and chapter 29, page 1137).

The light curves bend toward the horizontal when the rate of the primary photochemical reaction ceases to be slow compared with the maximum possible rate of one or several of the nonphotochemical processes associated with photosynthesis. As demonstrated in chapter 26, the limit-

ing influence of a bottleneck reaction in a "catenary series" generally becomes felt long before the rate of the over-all process has reached the maximum speed of which this limiting reaction is capable. Consequently, the light curves must approach saturation asymptotically rather than suddenly "hit the ceiling" (even if we forget for the time being about the effects of inhomogeneity of light absorption, which further enhance the gradual character of saturation). For the same reason, the maximum rate reached in the light-saturated state will often be considerably lower than the "ceiling" imposed by the limiting process.

As to the nature of the processes that can cause light saturation, the general alternative is between "preparatory" and "finishing" reactions. These two types of dark processes have been first discussed by Warburg, and Willstätter and Stoll, respectively. Because all transformations that occur in photosynthesis must be cyclic as far as chlorophyll and other catalysts are concerned, the question whether a reaction takes place "before" or "after" the primary photoprocess is not always as easy to answer as one would at first imagine. We will assume that a dark reaction *precedes* the photochemical step, if its retardation prevents the occurrence of this step (and thus also all the succeeding ones), and that a dark reaction *follows* the primary photochemical process, if the latter takes place in any case, and the effect of the limited rate of the dark reaction is merely to cause an accumulation of the primary photoproducts. Since experience shows that no *large* accumulation of oxidation intermediates occurs in photosynthesis (this is evidenced by the abrupt stoppage of oxygen production after the cessation of illumination), we must assume that the primary oxidation products ("photoperoxides") are unstable; unless rapidly removed or chemically stabilized by a "finishing" process, they apparently disappear by back reactions.

The uptake of carbon dioxide may sometimes continue for about 20 sec. in the dark (*cf.* Vol. I, page 206, and Vol. II, chapter 36). This may mean that some intermediate reduction products survive for that length of time, or that the carbon dioxide acceptor, A, requires it to become recarboxylated. (It may also be that the CO_2-acceptor is itself a reduction intermediate of carbon dioxide *cf.* chapter 36.)

The two alternative mechanisms of light saturation can thus be described as *starvation,* which causes an "idling" of the primary photochemical mechanism, and *constipation,* which blocks the elimination of the primary products and compels most of them to return to their initial form.

As described before, the distinction between the effects of preparatory and finishing dark reactions becomes still more difficult, if we follow Franck in the assumption that one of the finishing reactions "backfires," so that its slowness, like that of the preparatory reactions, affects the composi-

tion of the photosensitive complex. According to Franck, this is the reaction that converts the intermediate oxidation products, formed by light, into free oxygen (or sulfur, or other final oxidation products formed in bacterial photosynthesis). When this reaction fails to keep pace with the primary photochemical process, the intermediate oxidation products ("photoperoxides") accumulate in amounts sufficient to oxidize certain metabolites, thus forming a product of narcotizing properties (perhaps an organic acid). The latter is adsorbed on the photosensitive complex, and this retards or stops altogether the primary process.

Each partial nonphotochemical process of limited maximum rate imposes its own "ceiling" on the over-all rate of photosynthesis; and, since the influence of such a ceiling is felt long before it has actually been reached, the saturation value of photosynthesis in strong light may be affected not by *one* limiting process, but by several such processes—particularly since the maximum capacities of different parts of the photosynthetic apparatus appear to be of the same order of magnitude (as one would expect of a well-adjusted catalytic system).

In the general discussion of the kinetic curves of photosynthesis in chapter 26, three types of curve sets, $P = f(F_1)$ with F_2 as parameter, were described and designated as the first (or "Blackman") type, the second (or "Bose") type and the third type, respectively (see figures 26.2, 26.3 and 26.4). We recall that curves of the *first type* must arise when the parameter F_2 determines the maximum rate of a partial process that does *not* depend on the independent variable, F_1. This process then imposes a horizontal ceiling on the curve $P = f(F_1)$, but does not affect its initial slope. In curve sets of the *third type* the parameter affects the initial slope of the light curve, but not its saturation level; this type results when F_2 codetermines the rate of a process that is also a function of the independent variable, F_1. In curve systems of the *second type*, the parameter F_2 affects both the initial slope *and* the saturation level. Carbon dioxide curves offered examples of all three types, depending on the nature of the parameter (*cf.* page 868). Since most parameters do not affect the rate of the primary photochemical process, and therefore do not change the initial slope of the light curves, the $P = f(I)$ curve systems usually are of the first type, *i. e.*, the various curves coincide at low light intensities, but diverge at saturation. Such are most of the curve systems observed with *carbon dioxide* concentration as parameter (figs. 28.1, 28.2, 28.4, 28.5 and 28.5A), the only exception being Harder's *Fontinalis* curves (fig. 28.3). The two light curves of *Chromatium* with *thiosulfate concentration* as parameter (fig. 28.5B) have the same general appearance, and the curve systems with *temperature* as parameter, illustrated by figures 28.6, 28.7 and 28.8, are of the same type.

The effect of inhibitors, however, is uneven and some results are contra-

dictory. It has been suggested, as a generalization of empirical results, that *catalyst poisons* affect only the saturation level, thus producing light curve systems of the first type, while *narcotics* depress also the initial slope, thus giving curve systems of the second type. However, not all experimental results conform to this rule.

In figures 28.9 A,B, the curves of *Chlorella* in the presence of cyanide are, as expected, of the Blackman type; but the hydroxylamine curves (as observed by Weller and Franck) are of the Bose type. With the diatom *Nitzschia* (fig. 28.10) the effect of cyanide was different: A distinct depression was observed at *all* light intensities between 2 and 30 kerg/cm.^{2} sec. (However, the per cent inhibition increased with increasing light intensity, for example, 0.003% KCN caused an inhibition by 35% at 2.4 kerg, by 45% at 13 kerg and by 57% at 27 kerg.) Other observations of cyanide inhibition of photosynthesis in weak light were quoted in Volume I (page 309). The light curves of cyanide-inhibited purple bacteria, as observed by Wassink and co-workers, show a similar "semi-Bose" behavior; reversing the results obtained by Weller and Franck with *Chlorella*, the bacteria exhibited a Blackman type behavior toward hydroxylamine!

The light curves of urethan-inhibited *Chlorella* (as given by Warburg, and by Wassink and co-workers, respectively) appear to be of the second type, according to rule; but the third type is not entirely excluded. Bose type curves were found also with urethan-inhibited *Nitzschia* (fig. 28.10), although in this case the per cent inhibition was not constant, but rose with increasing light intensity. Wassink's curves, showing the effect of urethan on *purple bacteria* (fig. 28.11E), exhibit the reverse change—the depression is more pronounced in weak light than in strong light.

The effect of *oxygen* on the photosynthesis of *Chlorella* (fig. 28.9E; *cf.* also fig. 33, page 329, Vol. I) is of the Bose type, and the same is true of the effect of *copper sulfate*, while that of *nickel sulfate* apparently is of the Blackman type (fig. 28.9D). The curves of *purple bacteria* with *p*H as parameter (fig. 28.12) are of the Bose type with thiosulfate, and of the Blackman type with hydrogen as reductant.

Theoretically, one can easily understand why specific *catalyst poisons* such as cyanide, should affect only the saturation level and not the initial slope of the light curves: The former is determined by the rate of a dark catalytic reaction, the latter by the rate of supply of light quanta. With the poisoning becoming more and more complete, the inhibition can be expected to spread to lower and lower light intensities. If one assumes (as suggested in Vol. I, page 307) that cyanide inhibits most strongly the carboxylating enzyme, E_A, the observed differences in the sensitivity of different species may be attributed to variations in the amount of this enzyme. Some plants may contain a considerable reserve of E_A, and there-

fore show poisoning effects only in strong light; in others, the concentration E_A^0 may be just sufficient to maintain photosynthesis in the absence of cyanide and very little inactivation is needed to cause marked retardation even in moderate or weak light. However, even in this case, the *per cent reduction* of photosynthesis should remain smaller in weak light than in light of saturating intensity (as was, in fact, observed with cyanide-poisoned *Nitzschia*, *cf.* above). This hypothesis cannot explain an apparently uniform per cent reduction of photosynthesis at all light intensities by a typical catalyst poison such as hydroxylamine (fig. 28.9B). An *ad hoc* interpretation, suggested by Franck and co-workers, was described in Volume I (page 312).

What was said about the effects of catalytic poisons should apply also to deficiencies of the reaction substrates (carbon dioxide in green plants, carbon dioxide and reductants in bacteria). Their effects, too, should gradually diminish, and finally disappear with decreasing light intensity.

The effect of *narcotics* on the initial slope of light curves can be understood if one assumes that they are adsorbed on chlorophyll (or "chloroplastin") in such a way as to prevent the access of reactants or catalysts. Consequently, in the partially poisoned state, only the chlorophyll molecules free of these adsorbents are capable of properly utilizing the absorbed light quanta. If it is true that light saturation is due to the limited amount of a catalyst, such as E_A or E_B, which is kinetically independent of chlorophyll, the fact that the light curves obtained in the presence of urethan appear to be of type 2 rather than 3 (*i. e.*, that narcotics affect also the saturation rate) requires special explanation. For example, it can be postulated that the narcotic becomes adsorbed on the molecules of E_A or E_B as well as on those of chlorophyll. Alternatively, it can be suggested that, if the light curves of narcotized plants were followed to still higher light intensities, they would finally approach the same saturation level as the light curves of normal plants (*i. e.*, they would actually prove to be of type 3 rather than 2).

A third explanation, based on the idea that definite catalyst molecules are "assigned" to definite chlorophyll molecules, and become useless when the latter are "narcotized," will be discussed in chapter 32.

Still another phenomenon needs to be taken into consideration. It will be shown later in this chapter that a saturation level of photosynthesis probably exists which is due to the distribution of the chlorophyll complex, during photosynthesis, between the normal photosensitive form and a changed (tautomeric, or reduced) form. The latter is formed as an intermediate in photosynthesis, and requires a certain time for reconversion to the original photosensitive form. While this saturation limit may not be generally apparent in non-narcotized plants, because another limit (im-

posed by the deficiency of a finishing catalyst, E_B) is lower, the two may be not too far apart. Therefore, in the narcotized state, when a large fraction of the chlorophyll complexes is blanketed by the narcotic and therefore inactive, the saturation level due to chlorophyll can become lower than that due to the catalyst E_B. This will cause photosynthesis to be inhibited by narcotics even in strong light; however, the per cent inhibition will be smaller than in weak light. (This prediction is in agreement with Wassink's findings on purple bacteria, but not with his observations on diatoms.)

(*c*) *Analytical Formulation: Effect of Preparatory Dark Reactions*

In chapter 27, a rather extensive effort was made to derive equations for the function $P = f[CO_2]$ under different assumptions concerning the preparatory dark reactions on the "reduction side" of the primary photochemical process. The influence of light intensity was expressed in these derivations (*cf.* equation 27.6) by assuming that the rate is proportional to the concentration of the reduction substrate, $[ACO_2]$, and that the proportionality factor, k_r^*, is a function of the light intensity, I. The resulting equations for P were then applied to the analysis of the carbon dioxide curves, by assuming constant values of the parameter I (*i. e.*, of k_r^*). The same equations can, however, equally well be considered as analytical expressions of the light curves, $P = f(I)$, with $[CO_2]$ as parameter. A specific assumption must be made in this case concerning the relation of k_r^* to I (*e. g.*, by postulating that k_r^* is proportional to I, $k_r^* = k^*I$, *cf.* eq. 28.13).

The simplest equation for P in chapter 27 was equation (27.8). It was based on the assumption of a dissociable carbon dioxide–acceptor complex with no limitations on the rate of its formation. The corresponding light curves are linear (at least, if $k_r^* = k^*I$), and show no saturation effects. (This is, of course, due to the fact that, in the derivation of equation 27.8, the equilibrium concentration of the compound $[ACO_2]$ was supposed to be undisturbed even by intense photosynthesis.) The equation of these straight lines is (using k_r^* as independent variable):

$$P = (nk_r^* K_a A_0[CO_2])/(1 + K_a[CO_2]) \tag{28.1}$$

Their slope is proportional to $[CO_2]$ at low carbon dioxide concentrations and approaches a maximum at high carbon dioxide concentrations:

$$(dP/dk_r^*)_{max.} = nA_0 \tag{28.2}$$

As soon as the assumption is made that the stationary concentration $[ACO_2]$ *is* affected by the rate of consumption of this complex by photosynthesis (*i. e.*, that the formation of ACO_2 is not infinitely fast), the light

curves cease to be straight lines and become hyperbolae. We can refer here to equation (27.16), which describes the combined effects of slow diffusion and slow carboxylation, or to equations (27.21) and (27.31), which express these two effects separately. In intense light, the hyperbolae $P = f(k_r^*)$ approach one of the following two saturation levels, either:

$$P^{\max.} = nk_d[CO_2] \tag{28.3}$$

if the rate of diffusion is the limiting factor; or:

$$P^{\max} = nk_a A_0[CO_2] \tag{28.4}$$

if the rate of carboxylation is limiting. It will be noted that both saturation values are proportional to $[CO_2]$; in other words, this approximation provides for no "absolute saturation" with respect to both *I and* $[CO_2]$.

Half-saturation is reached, in the case of limitation by diffusion, at:

$$_{1/2}k_r^* = (K_a k_d[CO_2] + 2\,k_d)/2\,K_a A_0 \tag{28.5}$$

and, in the case of limitation by carboxylation, at:

$$_{1/2}k_r^* = k_a' + k_a[CO_2] \tag{28.6}$$

In both cases, half-saturating light intensity increases linearly with carbon dioxide concentration. The initial slopes of the light curves are the same as those of the straight lines (28.1). This means that at low carbon dioxide concentrations, the light curve families are of the Bose type, the Blackman type being approached at the higher values of the parameter $[CO_2]$.

"Absolute" saturation follows, as usual, as soon as we postulate a rate-limiting step, the maximum velocity of which is independent of both $[CO_2]$ and light intensity, but is determined entirely by the available amount of a catalyst. Equations (27.40) or (27.51), derived for the case of limitation by carboxylase deficiency, contain in their denominators terms proportional to the product, $k_r^* \times [CO_2]$; when light intensity and carbon dioxide concentration are both very high, this term becomes predominant and the yield approaches the "absolute" saturation value:

$$P_{\max.}^{\max.} = nk_{ea}E_0A_0 \tag{28.7}$$

which is the maximum rate of the catalytic formation of ACO_2 by reaction (27.37).

For the reaction mechanism discussed in section *d* of chapter 27 (non-dissociable ACO_2 complex attached to chlorophyll for the duration of the eight photochemical steps, as in the Franck-Herzfeld theory), with the simplification (28.8) used in deriving equation (27.63), we have:

$$[E_A] \simeq E_A^\circ \tag{28.8}$$

$$P^{\max.} = nk_aKE^0_aA_0[CO_2]/(1 + K[CO_2]) \tag{28.9}$$

$$_1/_2k^*_T = (8\,k_aKE^0_A[CO_2])/(1 + K[CO_2]) \tag{28.10}$$

$$P^{\max.}_{\max.} = nk_aE^0_aA_0 \tag{28.11}$$

$$(dP/dk^*_T)_0 = nA_0/8 \tag{28.12}$$

with n probably equal to 1.

So far, we have considered the shape of light curves as determined exclusively by preparatory reactions on the "carbon dioxide end" of photosynthesis (the possible rate-limiting factors being the constant of carbon dioxide diffusion, the bimolecular rate constant of carboxylation and the available concentration of the enzyme E_A). Analogous derivations can be made for limiting influences on the "oxidation end," such as, the rate constant of diffusion of reductants, the rate constant of their preliminary transformations (*e. g.*, of the binding of hydrogen to an acceptor) and the deficiency of enzymes catalyzing these reactions (*e. g.*, the hydrogenase). In making these derivations, we could, for example, set the rate of photosynthesis proportional to the concentration of the primary *oxidation* substrate such as the hypothetical "bound water," $A'H_2O$ or, more generally, A'HR (instead of to the concentration of the primary reduction substrate, ACO_2, as we have done so far). However, we abstain from a detailed discussion of these possibilities, because, in the case of green plants, there is no positive proof that a dark hydration reaction actually is needed to make water available for the photochemical process. The abundance of water in cells may make this hydration, even if it were needed, practically instantaneous. In the photosynthesis of purple bacteria, preliminary transformations of reductants are known to occur, but no definite proof has as yet been given that these transformations must be considered as preparatory reactions (*i.e.*, reactions providing the oxidation substrate for photochemical process) rather than as finishing reactions removing the primary oxidation products, formed by the photochemical oxidation of water. (The second alternative is favored by van Niel, Gaffron and Franck; *cf.* Vol. I, p. 168.) It must, nevertheless, be borne in mind that the rather detailed consideration of the preparatory processes "on the reduction side," and the comparative neglect of the analogous processes "on the oxidation side" of the primary photochemical process, which is common to most discussions of the kinetics of photosynthesis, are not justified, being based only on our inability to study the fate of water before its oxidation in photosynthesis, and our present insufficient knowledge of the initial transformations of hydrogen and other reductants used by bacteria.

(d) Analytical Formulation: Effect of Processes in the Photosensitive Complex

Leaving aside the effects of hypothetical preparatory reactions "on the oxidation end," we return to equation (27.6), $P = nk_r^*[ACO_2]$, for closer consideration from the point of view of the likely mechanism of light participation in photosynthesis. As mentioned before, the assumption, more or less implicit in the derivations of chapter 27, was that k_r^* is proportional to I, the intensity of incident light:

$$k_r^* = k^*I \tag{28.13}$$

and consequently:

$$P = nk_r^*[ACO_2] = nk^*I[ACO_2] \tag{28.14}$$

One remark, limiting the practical applicability of the analytical expressions derived in this section, must be made immediately. Kinetic equations are based on the law of mass action; they presume homogeneity of the reacting system. The light intensity, I, is, however, not uniform throughout a leaf or cell suspension; it varies even within a single cell or a single chloroplast. This complication has been repeatedly mentioned before, and we shall return to it again on page 1044. In the meantime, we will proceed as if light absorption were uniform throughout the region under consideration. This means that our equations will be strictly valid only for optically thin layers. In the following equations, then, I must be taken as meaning the light flux *actually reaching* a chlorophyll layer, and *not* the light flux falling on the outer surface of the system. (These two fluxes are proportional to each other, but the proportionality factor varies with depth, as well as with the wave length of the incident light.) Practically, most if not all kinetic measurements have been made, not with optically thin pigment layers but with leaves, thalli or suspensions absorbing a large proportion (sometimes up to 100%) of incident light. We will consider on page 1044 to what extent kinetic relationships derived for optically thin layers are changed through integration over the path of the light in the system (and also over the differently absorbed components of non-monochromatic light). The treatment of this problem is further complicated by the structural effects discussed in chapter 22 (scattering and "sieve effect"). Still another complication arises in the treatment of cell suspensions rapidly agitated during the measurement, thus bringing the individual cells more or less periodically into light fields of different intensity. If stirring were so intense as to cause each cell to slip through all the various light fields in a time which is short compared to the "Emerson-Arnold period" (about 10^{-2} sec. at room temperature, *cf.* chapter 34), it would

have been permissible to take into account only the *average* illumination and to consider the latter as identical for all cells. In other words, the rate of absorption of light by each cell could be taken as equal to the rate of total absorption in the suspension divided by the number of the cells in it. No amount of stirring, however, can mix the contents of the chloroplasts, so that chlorophyll molecules situated deeper inside them always will receive less light than those situated on the illuminated surface. What is even more important, the rate of stirring usually is quite insufficient to make legitimate even the averaging of intensity for whole cells. Such fast stirring is difficult to achieve; it is unlikely that Warburg and Burk had the right to claim that in their experiments (*cf.* p. 1006) stirring was so effective that only the *average* intensity of illumination was important. Often, a danger exists that the periods spent by individual cells deep in the suspension, between two exposures to full light in the surface layer, could be long enough to cause induction losses during the subsequent exposure (*cf.* chapters 29, 33 and 34).

These qualitative considerations show that the way to obtain light curves of photosynthesis best suitable for kinetic interpretation is by using optically thin suspensions or tissues. A limit to this procedure is, however, set by the fact that even single chloroplasts may absorb up to 50% of incident light in the absorption peaks of chlorophyll (*cf.* fig. 22.35); so that diluting algal suspensions until they absorb much less than that amount (or using faintly green tissues such as green onion skins) may merely mean allowing a part of incident light to pass *between* the chloroplasts—without improving the uniformity of absorption within the plastids. This uniformity can only be improved by employing cells poor in pigment (chlorotic cells), or by employing light which is comparatively weekly absorbed by chlorophyll (*e. g.*, green light).

We are thus forewarned that the several equations of the light curves, which will be derived below from alternative kinetic models of photosynthesis, can be used for comparison with the experimental curves found in the literature, only with strong reservations. We nevertheless consider it worth while to continue with these derivations, as a step toward a more quantitative study of the problem in the future. The latter will require both improved theoretical treatment (including the effects of inhomogeneous structure and nonuniform light absorption), and, above all, precise kinetic experiments on optically thin objects.

In analyzing the validity of equations (28.13) and (28.14), two alternative pictures must be considered. According to one, favored by Franck and Herzfeld, the compound ACO_2 is part of the "photosensitive complex" proper and its reduction can therefore be considered the primary photochemical process, *e. g.;*

$$(28.15) \qquad ACO_2 \cdot Chl \cdot A'H_2O \overset{h\nu\dagger}{\rightleftharpoons} AHCO_2 \cdot Chl \cdot A'HO$$

In considering this alternative, it is not necessary also to adopt Franck and Herzfeld's complex mechanism, which involves eight consecutive photochemical steps; essentially the same conclusions can be reached also by considering a single photochemical step, such as reaction (28.15), and leaving the completion of the process to nonphotochemical reactions, such as dismutations and coupled oxido-reductions, as described in chapter 9, Volume I.

The second alternative—for which certain arguments were adduced in Volume I (page 166)—is that the compound ACO_2 (and perhaps $A'H_2O$ as well, although the two assumptions are separable) is kinetically independent of chlorophyll; its reduction is then a secondary process, a dark reaction brought about by the products of the primary photochemical reaction.

The analysis is simpler if the first alternative is chosen. If we assume that the acceptor, A, is part of the chlorophyll complex, and that it takes up or loses carbon dioxide without separating itself from this complex (and that consequently, $A_0 = Chl_0$, and $[ACO_2] \leq Chl_0$), then all quanta absorbed by the chlorophyll molecules carrying ACO_2 must be effective (as far as the primary photochemical process is concerned)—while all quanta absorbed by chlorophyll carrying "bare" A are lost. The rate of reduction of ACO_2 is then $k^*I[ACO_2]$, as required by equation (28.14); $k^*I[Chl]$ being the rate of absorption of quanta by chlorophyll in light of intensity I (assuming that the absorbing capacity is not affected by association of chlorophyll with either ACO_2 or A). This equation already was used in chapter 27, section 7d (*cf.* equations 27.58–27.66).

If ACO_2 is kinetically independent of the photosensitive complex, the concentration $[ACO_2]$ cannot be limited to Chl_0; equation (28.14) now appears to indicate that the quantum yield of photosynthesis, $\gamma = P/I_a$ (I_a = absorbed light energy), can increase indefinitely with increasing $[ACO_2]$. At least, this would be so if one would assume, as usual, that I_a is proportional to I, $I_a = aI$, so that:

$$(28.16) \qquad \gamma = P/I_a = ank^*[ACO_2]$$

Of course, a certain limit to the increase of γ is set by the fact that ACO_2 must be $\leq A_0$, the total number of available acceptor molecules. However, there is no reason why this limit could not be higher than the maximum yield possible under Einstein's equivalency law. Therefore, a limi-

† Or 2 $h\nu$, if it is assumed that one quantum is used to transfer one hydrogen atom from $A'H_2O$ to Chl, and another one to transfer the same atom from Chl to ACO_2. In (28.15), it is also assumed that the hydrogen donor is "bound water," $A'H_2O$; and that, like the hydrogen acceptor, ACO_2, this donor is stably associated with Chl.

tation on P/I_a must exist that is quite independent of the limited quantity of the carbon dioxide acceptor, A. One way in which this limitation can arise is for k^* in equation (28.14) to become a function of $[ACO_2]$, such that the product $k^* \times [ACO_2]$ never exceeds a certain maximum value; another is for I_a to cease to be proportional to I, *i. e.*, for k^* in equation (28.14) to become a function of P.

The first phenomenon is a common occurrence in photochemical processes *in vitro*, where the photochemical secondary reaction competes with the deactivation of the light-activated molecules. (The latter can occur by fluorescence, or by energy dissipation within the activated molecule, or by energy transfer to other molecules.) The competition between a bimolecular photochemical reaction (rate constant k_r) and one (or several) monomolecular deactivating reaction (combined constant k') leads to a yield equation (Stern-Volmer equation):

$$\gamma = k_r[S]/(k' + k_r[S]) \tag{28.17}$$

according to which the quantum yield, γ, does not increase indefinitely with the concentration [S] of the reactant, but approaches, at $k_r[S] \gg k'$, a maximum value (for the primary process!):

$$\gamma_{max.} = 1 \tag{28.18}$$

This maximum quantum yield is independent of the light intensity, I. Comparison of equation (28.17) with (28.16) indicates that in this case—assuming that ACO_2 is the reactant S:

$$ank^* = k_r/(k' + k_r[ACO_2]) \tag{28.19}$$

i. e., k^* is in fact a function of the concentration of the reactant, decreasing with increasing $[ACO_2]$ in such a way that the product $ak^*n[ACO_2]$ can approach, but never exceed, 1.

In our schemes of photosynthesis (*cf.*, for example, schemes 28.IA and 28.IB below), we do assume a competition of the secondary photochemical reaction (such as 28.20b or 28.21b) with monomolecular deactivation, (such as 28.20a'), but mean by the latter not the energy loss by fluorescence, or by immediate energy dissipation in the light-absorbing complex, but the slower back reactions that follow primary tautomerization. The photochemically altered forms of the chlorophyll complex, HX.Chl.Z (or HX.Chl.HZ), play the part of "long-lived activated states" discussed in Volume I, p. 484. This changes the order of magnitude of the deactivation constant, k', from $>10^7$ or 10^8 sec.$^{-1}$ (the inverse of the life-time of electronic excitation states in strongly light-absorbing molecules) to perhaps as little as 10^2 sec.$^{-1}$; but formally, relation (28.17) remains valid as long as the back reaction follows the monomolecular law.

The possibility that the "activated" complex can be deactivated before

encountering a molecule with which it has to react (be it ACO_2 or $A'H_2O$) creates a new source of dependence of the rate on the *concentration* $[ACO_2]$ (or $[A'H_2O]$).

We now consider the second anticipated phenomenon—the breakdown, in strong light, of proportionality between irradiation and absorption. This complication does not occur significantly in "ordinary" photochemistry, where the rate constant of the process by which the excited molecule returns to the normal state is at least a million times higher than the rate constant of light absorption, even in the strongest available light (order of magnitude of the maximum frequency of absorptions: 10 sec.$^{-1}$, *cf.* page 838; order of magnitude of the rate constant of deactivation, by fluorescence or energy dissipation: $\geq 10^7$ sec.$^{-1}$). In strongest available light, the photostationary concentration of activated molecules ceases to be negligible compared to that of the normal molecules if the life-time of the activated form exceeds 10^{-2} sec. (light absorption: once every 0.1 sec.; lifetime of the activated state: 0.01 sec.; therefore, stationary concentration of activated molecules: 10%). If we assume such a longevity for the activated state of the chlorophyll complex, a sizable proportion of this complex will be present in the changed state during strong photosynthesis, and this will lead to a lack of proportionality between the "useful" light absorption, I_a, and the incident light intensity, I ("useful" meaning the absorption of light by chlorophyll complexes in the unchanged form—assuming that, if the changed form does absorb visible light at all, this absorption is photochemically "useless"). A new reason is thus added for the dependence of the yield on *light intensity*.

We will analyze these two phenomena—(*1*) the competition of the "detautomerizing" back reaction in the photosensitive complex with the photochemical forward reaction; and (*2*) the depletion of the normal form of this complex during intense photosynthesis—by using two simple mechanisms in which the photochemical forward reaction is assumed to involve the tautomerized chlorophyll complex, HX.Chl.Z, and either the carbon dioxide acceptor compound, ACO_2 (mechanism 28.20), or the hydrogen donor, A'HR, where HR may stand for water, or for a "substitute" reductant (mechanism 28.21):

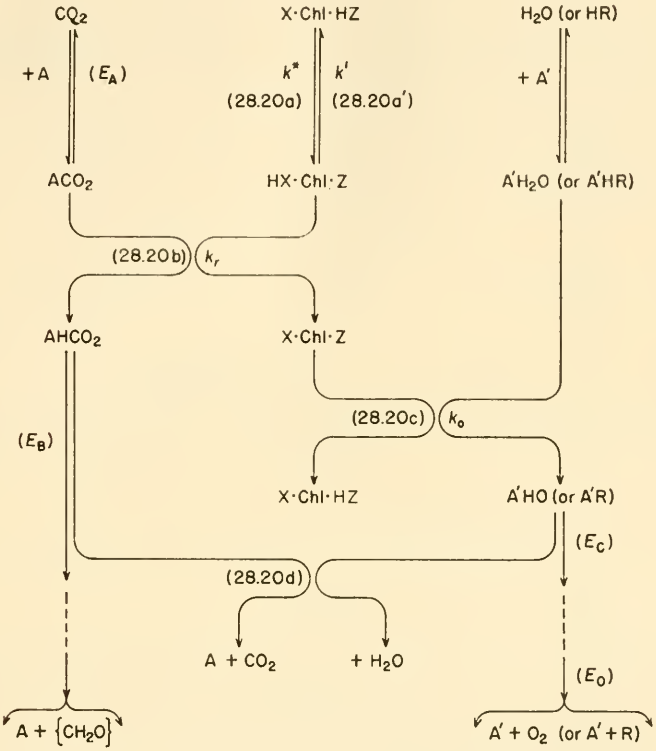
(28.20a and a') $$X\cdot Chl\cdot HZ \underset{k'}{\overset{k^*I}{\rightleftharpoons}} HX\cdot Chl\cdot Z$$

(28.20b) $$HX\cdot Chl\cdot Z + ACO_2 \xrightarrow{k_r} X\cdot Chl\cdot Z + AHCO_2$$

(28.20c) $$X\cdot Chl\cdot Z + A'HR \xrightarrow{k_0} X\cdot Chl\cdot HZ + A'R$$

(28.20d) $$AHCO_2 + A'R \xrightarrow{k'_{r_0}} ACO_2 + A'HR$$

This mechanism is represented in scheme 28.IA. The reason for assuming the occurrence of the *secondary* back reaction (28.20d) will be discussed later. If we assume, as suggested on page 1019, that $k_0[A'H_2O] \gg k_r[ACO_2]$, the concentration of the oxidized form, [X.Chl.Z], can be neglected in



Scheme 28.IA. Photosynthesis according to equations (28.20a–d).

green plants (though perhaps not in purple bacteria), compared to that of the tautomeric form, [HX.Chl.Z]; the latter is then the only one the accumulation of which may affect the photosynthesis of green plants in strong light.

It may be argued that, if the reaction of Z with water is so much more rapid than that of HX with carbon dioxide, the sequence of the secondary reactions (28.20b and c) should be reversed, resulting in the following mechanism (shown in scheme 28.IB):

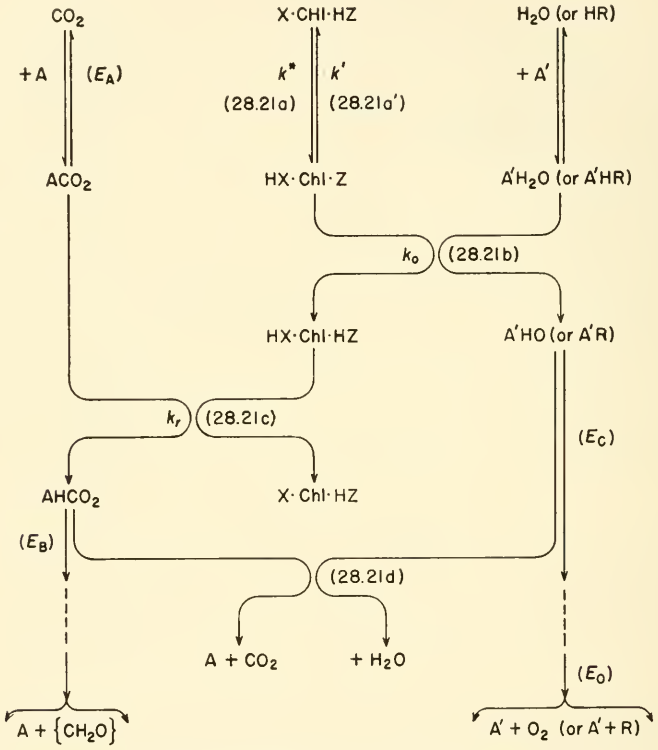
(28.21a and a') $$X\cdot Chl\cdot HZ \underset{k'}{\overset{k^*I}{\rightleftharpoons}} HX\cdot Chl\cdot Z$$

(28.21b) $$HX\cdot Chl\cdot Z + A'HR \xrightarrow{k_0} HX\cdot Chl\cdot HZ + A'R$$

(28.21c) $$HX\cdot Chl\cdot HZ + ACO_2 \xrightarrow{k_r} X\cdot Chl\cdot HZ + AHCO_2$$

(28.21d) $$AHCO_2 + A'R \xrightarrow{k'_{r_0}} ACO_2 + A'HR$$

In this case, the main form in which the chlorophyll complex accumulates during intense photosynthesis is the *reduced* form, HX·Chl·HZ.



Scheme 28.IB. Photosynthesis according to equations (28.21a–d).

We chose above equation (27.6) as starting point of our considerations (and not a similar equation for the reaction of the light-activated complex with the oxidant $A'H_2O$), and are now interested, first of all, in the possible interdependence of k_r^* and $[ACO_2]$, caused by the primary back reaction. Therefore, mechanism (28.20) is more convenient for our purpose than mechanism (28.21), (because in 28.20 the primary back reaction, 28.20a′, competes directly with the reduction of $[ACO_2]$).

In discussing the consequences of the primary back reaction, on the basis of mechanism (28.20), we will neglect the second anticipated phenomenon—the accumulation of tautomerized chlorophyll complexes in strong light. (We thus introduce a new cause for carbon dioxide saturation, but no new cause for light saturation.) Later, we will use mechanism (28.21) to consider the second phenomenon (accumulation of HX·Chl·HZ in strong light, and consequent light saturation), while in turn neglecting the primary back reaction.

By assuming $[HX \cdot Chl \cdot Z] \ll [X . Chl . HZ]$, conditions become formally analogous to those prevailing in "ordinary" photochemistry *in vitro*, as discussed above. From the reaction sequence (28.20a–c) we obtain, for the photostationary concentration $[HX \cdot Chl \cdot Z]$, the Stern-Volmer type equation:

$$(28.22) \quad [HX \cdot Chl \cdot Z] = k^* I [X \cdot Chl \cdot HZ]/(k' + k_r[ACO_2]) \simeq k^* I \, Chl_0/(k' + k_r[ACO_2])$$

and hence:

$$(28.23) \quad P = nk_r k^* I [ACO_2] Chl_0/(k' + k_r[ACO_2])$$

(in expected formal analogy to equation 28.1). Comparison with equation (27.6) shows that k_r^*, while proportional to I, is now in fact also a function of $[ACO_2]$:

$$(28.24) \quad k_r^* = k_r k^* I \, Chl_0/(k' + k_r[ACO_2])$$

If $[ACO_2]$ increases indefinitely, P approaches the maximum rate:

$$(28.25) \quad P_{max.} = nk^* I Chl_0 = nI_a Chl_0$$

which corresponds to the maximum quantum yield, n. Similarly to the yield (28.18), n is independent of light intensity.

Equation (28.23) shows that back reactions in the photosensitive complex can explain the increase in yield with increasing $[CO_2]$ and the final $[CO_2]$ saturation, even if the acceptor A is available in unlimited quantities (*e. g.*, if $[ACO_2]$ stands for dissolved carbon dioxide, the quantity of which can be increased practically indefinitely by raising the partial pressure of carbon dioxide over the system). In other words, equation (28.23) indicates how a limited supply of light quanta can account for hyperbolic carbon dioxide curves, without the assumption of a limited amount of a $[CO_2]$ acceptor, or slow diffusion, or slow carboxylation.

The effects of carbon dioxide supply can, of course, be superimposed upon those of limited light supply, by introducing the corresponding expressions for $[ACO_2]$ into (28.23). Using for this purpose "static" equation (27.3), *i. e.*, taking into consideration only the limited *amount* of the $[CO_2]$ acceptor, will not lead to light saturation; the latter will be intro-

duced if one uses for $[ACO_2]$ one of the "kinetic" expressions, taking into account the limited *rates* of supply processes (carbon dioxide diffusion and carboxylation). *Absolute* saturation, $P_{\text{max.}}^{\text{max.}}$ will result if it is assumed that a maximum rate of supply exists which is independent of $[CO_2]$, *e. g.*, as a consequence of a limited amount of the carboxylating catalyst, E_A.

We will now consider the second phenomenon, which is without parallel in "ordinary" photochemistry, and arises from the assumed longevity of the "activated state" of the photosensitive complex: the accumulation of these complexes in a changed form, and the consequent lack of proportionality between the incident light intensity, I, and the photochemically significant light absorption, I_a. For this purpose, we use the alternative mechanism (28.21), since the postulated practical instantaneousness of reaction (28.21b) gives us some right to neglect in this case the primary back reaction, (28.21a) (*i. e.*, to assume $k_0[A'H_2O] \gg k'$) The steady state concentration of the reduced form, [HX.Chl.HZ], is, under these conditions:

$$[HX \cdot Chl \cdot HZ] = k^* I \, Chl_0/(k^* I + k_r[ACO_2]) \tag{28.26}$$

(implying that, in the absence of ACO_2, *all* chlorophyll complexes would go over, in light, into the reduced form, $HX \cdot Chl \cdot HZ$). The rate of photosynthesis is:

$$P = nk_r[HX \cdot Chl \cdot HZ][ACO_2] = nk_r Chl_0 k^* I \, [ACO_2]/(k^* I + k_r[ACO_2]) \tag{28.27}$$

Assuming further that the complex ACO_2 is in equilibrium with free carbon dioxide, and no diffusion gradient exists, so that $[CO_2] = [CO_2]_a$ we obtain:

$$P = nk_r Chl_0 k^* I A_0 K_a[CO_2]/(k^* I + K_a[CO_2]k^* I + k_r A_0 K_a[CO_2]) \tag{28.28}$$

an equation for the rate of photosynthesis when neither the preparatory nor the finishing dark reactions, neither on the "reduction side" nor on the "oxidation side," have a rate-limiting influence. Light saturation is in this case due entirely to the limited amount of the acceptor, A_0; and carbon dioxide saturation, to the limited amount of chlorophyll, Chl_0.

The light curves (28.28) are hyperbolae:

$$P/(P^{\text{max.}} - P) = k^* I(1 + K_a[CO_2])/k_r A_0 K_a[CO_2] \tag{28.29}$$

The half-saturating light intensity is:

$$_{1/2}I = k_r A_0 K_a[CO_2]/(k^* + k^* K_a[CO_2]) \tag{28.30}$$

Extrapolating to $[CO_2] = \infty$, we obtain:

$$_{1/2}I_\infty = k_r A_0/k^* \tag{28.31}$$

If the carboxylation equilibrium is *not* maintained in light, we have, more generally, instead of (28.30):

$$_{1/2}I = k_r[ACO_2]/k^* \tag{28.31a}$$

Comparing equation (28.31a) with equation (28.26), we note that, in the particular picture we are considering now, "half saturation" with light corresponds to the equal distribution of chlorophyll between the forms X·Chl·HZ and HX·Chl·HZ:

(28.32) $$[HX\cdot Chl\cdot HZ] = Chl_0/2 = [X\cdot Chl\cdot HZ]$$

If we assume that reaction (28.21a) has a quantum yield of unity, the constant k^* must be equal to the frequency of light absorptions by a single chlorophyll molecule. We recall that I is the intensity of light actually reaching the volume under consideration, and that light absorption within this volume is supposed to be so small that the rate of absorption is practically the same everywhere within it. Under these conditions:

(28.33) $$k^* = \bar{a} \ln 10 \times 10^3 = 2.3 \times 10^3 \bar{a} \text{ (mole/cm.}^2\text{)}$$

where $\bar{a}$ is the average molar absorption coefficient for the incident light. This equation implies that I is measured in number of einsteins of light falling per second on a square centimeter and Chl_0 is expressed in moles per liter. According to page 838, for white light, 1 lux $\simeq 1.4 \times 10^{12}$ quanta/sec. cm.^{2} $= 2.3 \times 10^{-12}$ einstein/sec. cm.^{2} We thus have:

(28.34) $$k^* = 5.3 \times 10^{-9} \bar{a} \text{ (mole/cm.}^2\text{)}$$

applicable when I is expressed in lux (meter candles). Average absorption coefficient of chlorophyll for visible light is of the order of 10^4; this means $k^* \simeq 5 \times 10^{-5}$, or one photochemical act per second per chlorophyll molecule in light of 20,000 lux.

Experiments indicate that ${}_{1/2}I_\infty$ is of the order of 5×10^3 lux (*cf.* Table 28.I); assuming $k^* \simeq 5 \times 10^{-5}$ and $A_0 \simeq 5 \times 10^{-2}$ m./l. (roughly the concentration of chlorophyll in the plastids; *cf.* Vol. I, page 411), we obtain for k_r a value of 5. When both $[CO_2]$ and I are very large, the rate according to equation (28.28) approaches the "absolute ceiling":

(28.35) $$P^{max.}_{max.} = nk_r Chl_0 A_0 \; (= nk^* Chl_0 \; {}_{1/2}I_\infty)$$

which is the maximum possible rate of reaction (28.21c) reached when *all* chlorophyll is in the state HX·Chl·HZ, and *all* acceptor is occupied by carbon dioxide.

For low light intensities and ample supply of carbon dioxide, the light curves defined by equation (28.28) approach asymptotically the limiting straight line:

(28.35a) $$P^{lim.}_{I=0} = nk^* Chl_0 I$$

The simplifying assumption that the carbon dioxide acceptor is, even during intense photosynthesis, in equilibrium with external carbon dioxide could be dropped, and the more general expression (27.15) for $[A\cdot CO_2]$

could be introduced into equation (28.27) instead of the equilibrium expression (27.3). However, the resulting equations, embodying the effects of carbon dioxide supply limitations together with the limitation due to light supply, would become too unwieldy for practical use.

One may ask whether equation (28.35) provides a sufficient explanation of the "absolute saturation" of photosynthesis, *i. e.*, of the maximum yields discussed in section 5 of this chapter. We can obtain from equation (28.35) the following estimate of the maximum possible yield of photosynthesis per chlorophyll molecule per second:

$$P^{\text{max.}}_{\text{max.}}/\text{Chl}_0 = nk_r A_0 \; (= nk^*\,{}_{1/2}I_\infty) \simeq 0.025 \tag{28.36}$$

or, for "assimilation time," T_A:

$$T_A = \text{Chl}_0/P^{\text{max.}}_{\text{max.}} \simeq 40 \text{ sec.} \tag{28.36a}$$

The experimental values of "assimilation time" (*cf.* Table 28.V) actually range from 10 to 100 sec. (with the exception of the remarkably smaller values found for *aurea* varieties). It thus appears as if chlorophyll *could* be the component of the photosynthetic apparatus the slow rate of restoration of which imposes an "absolute ceiling" on the rate of photosynthesis. However, this is not the *only* adequate answer to the problem of absolute saturation, since flashing light experiments reveal the existence of a "finishing" catalyst (Franck's "catalyst B"), which appears to be present in an amount equivalent to about one two-thousandth of that of chlorophyll, and to have a "working time" of the order of 0.02 sec. at room temperature; the "ceiling" imposed by this catalyst is of the same order of magnitude as the one derived in (28.36), since $2 \times 10^3 \times 2 \times 10^{-2}$ is the same as 1×40.

It is useful to show why the approximate agreement of the value (28.36) with the experimental results is not a significant confirmation of the model used in the derivation of this equation.

We will see below (page 1043) that in a *rectangular* hyperbola, the three parameters which in general specify a hyperbola—such as (*a*) the initial slope, (*b*) the abscissa corresponding to half-saturation and (*c*) the saturation value of the ordinate—are *not independent of each other*, but related by equation (28.48C). What we did above was to insert the approximate experimental value of *two* of these parameters ($n \simeq 0.1$ and ${}_{1/2}I \simeq 10^4$ lux) into equation (28.28), which represents a rectangular hyperbola (*cf.* equation 28.29; the equation in parentheses in 28.36 is a special case of 28.48C); and to derive in this way, an approximately correct value of the third parameter, $P^{\text{max.}}$. This result merely shows that the light curves (or, at least, the limiting light curve at high $[CO_2]$) do approximate rectangular hyperbolae. *Any* kinetic mechanism which leads to light curves

approximating rectangular hyperbolae will permit a correct estimation of the third parameter from the experimental values of the two other parameters.

We will find later (*cf.* chapter 32) evidence that the principal rate-limiting reaction in photosynthesis is a dark reaction catalyzed by a catalyst (E_B) which is present in the cells in a concentration only 0.05% of that of chlorophyll. We will also see (page 1038) that this type of limitation leads to light curves which are hyperbolae, but *not* rectangular hyperbolae. It remains to be seen whether light curves can be measured precisely enough to exclude one of the two mechanisms.

Is it possible for *two* or more bottlenecks to exist in photosynthesis, each allowing the passage of about the same amount of reactants—the maximum rate of passage through one bottleneck being determined by the product of the concentration Chl_0, the (approximately equal) concentration, A_0, the (bimolecular) constant, k_r, of reaction (28.21c) and the quantum yield n ($0.05^2 \times 5 \times 0.1 = 1.25 \times 10^{-3}$); and the other, by the product of the enzyme concentration E_B^0 and its (monomolecular) rate constant ($2.5 \times 10^{-5} \times 50 = 1.25 \times 10^{-3}$). Such a coincidence seems not implausible; it could even be considered as admirable economy in the allotment of catalysts to the cell. (Why have more of a certain catalyst than can be utilized because there is not enough of another one?) However, certain experimental results are not consistent with the assumption that restoration of chlorophyll is the bottleneck which limits (or "co-limits") the maximum rate of photosynthesis; these data indicate that a chlorophyll molecule which has taken part in the primary photochemical process needs much less than $40n$ ($\simeq 4$) sec. to return to the photosensitive form. We mean here the observations of Willstätter and Stoll (*cf.* Table 28.V; see also chapter 32, fig. 32.2), that *aurea* leaves have $P^{\max.}$ values only slightly lower than those of ordinary green leaves, although they contain only one third (or less) of the normal amount of chlorophyll. This is obviously inconsistent with equation (28.35), and indicates that $P^{\max.}$ is determined *not* by the rate of restoration of the photochemically tautomerized chlorophyll complex (rate constant k_r in equation 28.35), but by the rate of transformation of a substrate by a catalyst (such as E_B) that is kinetically independent of chlorophyll. The above-estimated value of k_r ($\simeq 5$ (sec. mole)$^{-1}$) is therefore merely a *lower limit*; in fact, quantitative observations which *aurea* leaves indicate that the true value of this constant (which determines how often a given chlorophyll molecule is available for the primary photochemical reaction) is *at least* ten times higher. This means $k_r > 50$ sec.$^{-1}$ (for $[ACO_2] = 0.05$ mole/liter), assuming that the primary photochemical reaction is (28.21c). Because of fundamental significance of these conclusions, a reinvestigation of the kinetics of photosynthesis in *aurea* leaves seems desirable.

It was noted that, according to equation (28.32), half saturation of photosynthesis with light should take place, in the presence of excess carbon dioxide, when chlorophyll is distributed equally between the forms X·Chl·HZ and HX·Chl·HZ. This state should also correspond to the halfway point in the transition of fluorescence from the "low light" yield, φ_1 to the "high light" yield, φ_2 (*cf.* page 1049). It has been argued that accumulation of one half the total quantity of chlorophyll in a changed state during intense photosynthesis is unlikely, since no reversible change in the spectrum of strongly illuminated green plants has ever been noticed. An exact experimental re-examination of this statement remains desirable; but even if it were confirmed, this might only mean that the spectrum of the chlorophyll complex in the form which accumulates in strong light is practically identical with that of the form present in darkness. This is quite possible, since the difference in composition is supposed to lie not in the chlorophyll molecule itself, but in the associated hydrogen donors and acceptors.

One has to distinguish between this hypothesis and the assumption (favored, among others, by Franck and Herzfeld) that the conversion of X·Chl·HZ to HX·Chl·Z (or of ACO_2·Chl·A′H_2O to $AHCO_2$·Chl·A′HO to use Franck's picture) requires *two* quanta, with an intermediate, X·ChlH·Z, formed by the first quantum. According to this picture, when photosynthesis proceeds most efficiently, the rate of light absorption by ChlH must be equal to that by Chl (because only under these conditions can all quanta be utilized). If this assumption is made, we must postulate a close similarity between the spectra of the compounds Chl and ChlH. This, too, is not impossible, but more remarkable than a spectroscopic similarity between X·Chl·HZ and HX·Chl·HZ.

If the changed form of the chlorophyll complex is green, the question arises as to the photochemical effect of light absorbed by this form. One possibility is that the quanta absorbed by the changed form produce no photochemical effect at all; the other that they cause *photochemical back reactions,* such as:

$$\mathrm{HX{\cdot}Chl{\cdot}Z} \xrightarrow{h\nu} \mathrm{X{\cdot}Chl{\cdot}HZ} \qquad \text{or} \qquad \mathrm{HX{\cdot}Chl{\cdot}HZ + A'HO} \xrightarrow{h\nu} \mathrm{HX{\cdot}Chl{\cdot}Z + A'H_2O}$$

Kinetic considerations show that photochemical back reactions would not in themselves cause light saturation, but merely reduce the quantum yield by the same factor at all light intensities. Franck and Herzfeld, in one of their earlier speculations on the mechanism of photosynthesis (1937), suggested that light saturation may be caused by *photochemically induced chain back reactions:* but they abandoned this hypothesis later (1941) in favor of

the assumption that light saturation is usually caused by *nonphotochemical back reactions*, which compete with the forward dark reaction catalyzed by the catalyst "B."

(*e*) *Analytical Formulation: Effect of "Finishing" Dark Reactions*

The necessity of considering, in addition to the "preparatory" dark reactions and the reversible changes in the chlorophyll complex, the "finishing" dark reactions as possible sources of light saturation phenomena in photosynthesis arises from several observations. It was mentioned above that experiments in flashing light (to be described in chapter 34) demonstrated directly the existence of a "finishing" catalyst with a "working period" of the order of 10^{-2} sec. at room temperature. The maximum yield obtainable per single flash shows that this catalyst is present in a concentration equivalent to 0.05% of that of chlorophyll. Consequently, as indicated above (page 1031 it imposes a "ceiling" on the over-all rate of photosynthesis of about one molecule carbon dioxide reduced per chlorophyll molecule every 40 seconds—which is close to the actually observed maximum rate of photosynthesis at room temperature. A second relevant observation is made by comparing the light curves of photosynthesis of various plants with the light curves of their fluorescence (*cf.* part B of this chapter). If the light saturation of photosynthesis were due to a slow supply reaction (*i. e.*, to the depletion of one of the reactants, ACO_2 or $A'H_2O$, in intense light), or to slow regeneration of the photosensitive form of the chlorophyll complex, in both cases, the light saturation would be associated with accumulation of the photosensitive complex in a chemically changed form (such as $HX \cdot Chl \cdot Z$ or $HX \cdot Chl \cdot HZ$), and should therefore reveal itself by simultaneous changes in the fluorescence yield of the complex. This is actually the case sometimes, but not always. Figure 28.24, for example, shows light saturation of photosynthesis of *Chlorella* without any change in the yield of fluorescence; and even in figure 28.26, saturation is almost completed before fluorescence begins to change its yield. In all such cases, saturation must be due to the failure of a *finishing* dark reaction to keep pace with the primary photochemical process—a failure that produces no change in the composition of the photosensitive complex, but leads to the loss of a large part of primary photoproducts by back reactions.

It was stated before that the distinction between preparatory and finishing dark reactions is not so clear-cut in Franck's theory of "narcotic regulation" of photosynthesis. According to this theory, accumulation of primary oxidation products ("photoperoxides") leads not (or not only) to back reactions between these peroxides and the primary reduction products

(as was assumed above), but also to the oxidation of certain metabolites (sugars?) which produces a "narcotic" (a fatty acid?). The latter settles on the chlorophyll complex, prevents any further acceleration of the primary photochemical reaction, and causes a strong increase in fluorescence.

In the case of purple bacteria the following two alternative descriptions of the rate-limiting and fluorescence-enhancing effect of a limited supply of the reductants (H_2, $H_2S_2O_3$ . . .) are possible: Either one considers this supply as a *preparatory* reaction, whose slowness causes the photosensitive complex to accumulate in a changed (more strongly fluorescent) form, such as X·Chl·Z; alternatively, (using Franck's theory) one can treat this supply as part of a finishing reaction ("disposal of photoperoxides"), and explain its effect on fluorescence as a consequence of the production of the "narcotic" by accumulated peroxides. In both cases, the light curve of photosynthesis will approach, with increasing light intensity, a limit determined by the maximum rate of supply of the reductants (either by diffusion or by a preliminary enzymic transformation).

Another argument against general attribution of the assimilation numbers of green plants to the rate-limiting influence of preparatory reactions —specifically, those on the "carbon dioxide side"—was mentioned in Chapter 12 (Vol. I) in connection with the influence of cyanide on photosynthesis. It was stated there that the difference in the amounts of cyanide required to reduce by a certain factor the rate of photosynthesis in different plant species is most easily understood if one assumes that the cyanide-sensitive catalyst (which, in all probability, is the "carboxylase" E_A) is *not* rate-limiting in strong light in the absence of the poison, and becomes limiting only when a considerable part of it is inactivated. If a different ratio E_A/E_B prevails in various species and strains, the fraction of E_A that has to be inactivated in order to make this catalyst "limiting" also must change from case to case.

What kind of back reactions can compete with finishing catalytic reactions in photosynthesis? In section *d* of this chapter, we considered the "primary" back reaction, $HX\cdot Chl\cdot Z \rightarrow X\cdot Chl\cdot HZ$, which can be called "detautomerization" of the chlorophyll complex. In equations (28.20) and (28.21), this reaction competes with the secondary photochemical forward reaction, *e. g.*, (28.20b) or (28.21b). We noted on page 1024 that this competition can cause carbon dioxide saturation, but *not* light saturation, because the proportion of quanta lost by this kind of back reaction is independent of light intensity. If one would treat (28.20b) or (28.21b) as a catalytic reaction, assigning to it a catalyst with the concentration and working speed attributed above to "catalyst B," this would produce light saturation, but the latter will again be associated with the accumulation of the form X·Chl·Z or HX·Chl·HZ and thus with a change in the intensity

of fluorescence. To explain saturation *not* accompanied by changes in fluorescence intensity, the deficient catalyst must be placed, not between one of the two reactants (ACO_2 or $A'H_2O$) and the photosensitive complex, but between the primary and the finished products of photosynthesis. In other words, the back reactions caused by the catalytic deficiency must be "secondary" rather than "primary." A back reaction of this kind was therefore added as reaction d in mechanisms (28.20) and (28.21). We can postulate, *e. g.*, that reaction (28.20d) comes into play because the transformation of the first photoproduct, $AHCO_2$, into a more stable intermediate requires a catalyst, E_B (perhaps a "mutase"), which is present in limited quantity. A similar postulate could be made for the effect of the catalyst E_C, which is required for the first step in the conversion of A'HO into free oxygen. Because of the symmetry we have assumed between the right and left sides in schemes 28.IA and B, a limitation in the utilization of the oxidation products will have the same effect on the kinetics of the process as a whole as a limitation of the utilization of the reduction products. In the first case, the secondary back reaction will be accelerated by the accumulation of the primary oxidation product, A'HO; in the second case, by the accumulation of the primary reduction product, $AHCO_2$.

Using reactions (28.20) we can tentatively assume that E_B acts on the first reduction product, $AHCO_2$, according to the scheme:

$$AHCO_2 + E_B \underset{k'_{ae}}{\overset{k_{ae}}{\rightleftharpoons}} E_BAHCO_2 \tag{28.38a}$$

$$E_BAHCO_2 \xrightarrow{k_e} E_B + A + \{HCO_2\} \tag{28.38b}$$

where $\{HCO_2\}$ designates a stabilized reduction product.

(If reaction 28.38b is a *dismutation*, it may require the participation of *two* $AHCO_2$ radicals, but we will use here the simplest possible mechanism.)

The rate of photosynthesis is, according to this scheme:

$$P = nk_e[E_BAHCO_2] \tag{28.39}$$

and the "absolute maximum rate" is:

$$P^{\text{max.}}_{\text{max.}} = nk_eE^0_B \tag{28.40}$$

where E^0_B is the total available quantity of the "limiting" enzyme.

An equation for P as a function of I and $[CO_2]$ can be derived from this mechanism; but it is complicated, even if all the possible simplifying assumptions are made, and little could be achieved by writing it down here.

The situation is simplified if we again make use of Franck and Herzfeld's mechanism (scheme 7.VA, Vol. I), in which the oxidant, ACO_2, and the reductant, $A'H_2O$, belong to the photosensitive complex, and take part in the *primary* photochemical process, *e. g.*, in the way indicated in equation

(28.15). In this case, the primary back reaction (detautomerization) itself becomes a competitor to finishing dark reactions, and it can therefore be assumed that the role of the catalyst E_B is to prevent this reaction from destroying the photoproducts. This point of view was used in the elaboration of kinetic equations of photosynthesis by Franck and Herzfeld (1937). Their derivations are complicated by the assumption of four successive (different) photochemical steps "on the reduction side," alternating with four (identical) photochemical steps "on the oxidation side"; the product of each of these steps was supposed to require stabilization by the same "catalyst B," in order to prevent this step from being reversed by a dark reaction. Instead of trying to present here the derivations of Franck and Herzfeld we will use a simpler mechanism embodying the same basic concept of primary back reaction as cause of light saturation. This mechanism is similar to the one given in equation (7.13) in Volume I, but is further simplified by the substitution of a single primary photochemical step for the two steps (7.13a) and (7.13b). The reaction scheme is:

(28.41a, a′) $ACO_2 \cdot Chl \cdot A'H_2O \underset{k'}{\overset{k^*I}{\rightleftharpoons}} AHCO_2 \cdot Chl \cdot A'HO$ — Primary forward and back reaction

(28.41b) $AHCO_2 \cdot Chl \cdot A'HO + E_B \xrightarrow{k_e} E_BHCO_2 + A \cdot Chl \cdot A'HO$

(28.41c) $E_BHCO_2 \xrightarrow{k_e'} E_B + \{HCO_2\}$

(28.41b, c) — Catalytic stabilization of reduction product

(28.41d) $A \cdot Chl \cdot A'HO + H_2O \longrightarrow A \cdot Chl \cdot A'H_2O + \{HO\}$

(28.41e) $A \cdot Chl \cdot A'H_2O + CO_2 \longrightarrow ACO_2 \cdot Chl \cdot A'H_2O$

(28.41d, e) — "Reloading" of chlorophyll

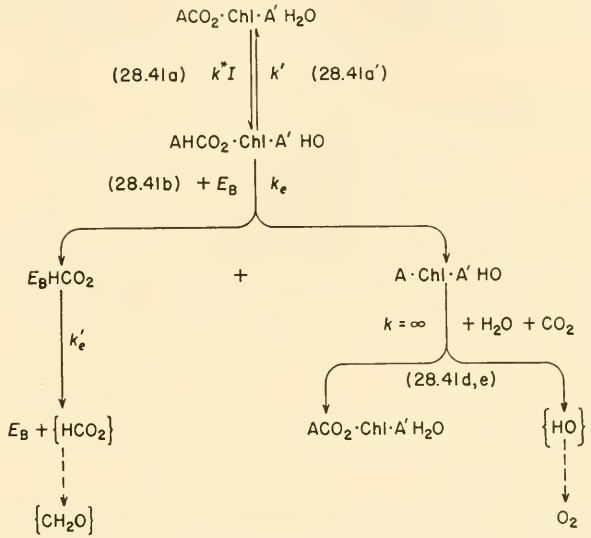
We assume that E_B is needed only for "stabilization" of the reduction product, $AHCO_2$, and that this stabilization is achieved, in reaction (28.41b), by taking the reduced group, HCO_2, away from the chlorophyll complex and thus preventing its back reaction with the oxidized group, HO. (It is not suggested that the radicals HCO_2 or OH occur in the free state; these symbols can stand for corresponding functional groups in larger molecules, as indicated by braces in 28.41c and d.)

In order to simplify the derivations still more and to elaborate only the effect due to the back reaction, we further assume that the reactions (28.41d and e), by which the photosensitive complex is supplied with fresh oxidant and fresh reductant, respectively, are practically instantaneous. Under these conditions, only two factors can cause light saturation: (*a*) accumulation of the photosensitive complex in the tautomeric form $AHCO_2 \cdot Chl \cdot A'HO$; and (*b*) accumulation of the catalyst E_B in the bound

form, E_BHCO_2. The equation for P which takes into account these two effects is quadratic; its one significant solution is:

$$(28.42)\quad P = 1/n \left\{ \frac{k_e k^* I \mathrm{Chl}_0 + k' k'_e + k'_e k^* I + k'_e k_e E^0_B}{2\, k_e} - \left[\left(\frac{k_e k^* I \mathrm{Chl}_0 + k' k'_e + k'_e k^* I + k'_e k_e E^0_B}{2\, k_e} \right)^2 - k'_e E^0_B k^* I \mathrm{Chl}_0 \right]^{1/2} \right\}$$

where n, as before, means the number of elementary photochemical steps (28.41a) required for the reduction of one molecule of carbon dioxide.



Scheme 28.II. Photosynthesis according to mechanism (28.41).

Equation (28.42) describes a hyperbolic light curve with an initial slope:

$$(28.43)\quad (dP/dI)_0 = nk_e E^0_B k^* \mathrm{Chl}_0/(k' + k_e E^0_B)$$

If $k' \ll k_e E^0_B$ (so that back reactions play no important part in weak light) we have:

$$(28.44)\quad (dP/dI)_0 = nk^* \mathrm{Chl}_0$$

In other words, in weak light, all light quanta absorbed are used for photosynthesis with the maximum possible quantum yield, n. If, however, k' is *not* $\ll k_e E^0_B$, the quantum yield will remain $< n$ even if extrapolated to zero illumination.

The maximum absolute yield obtainable in strong light, according to equation (28.42), is:

(28.45) $$P_{\text{max.}}^{\text{max.}} = nk_e\text{Chl}_0k_e'\text{E}_B^0/(k_e\text{Chl}_0 + k_e')$$

(the lower index being justified by the assumption that reaction with carbon dioxide is practically instantaneous). If $k_e' \ll k_e\text{Chl}_0$, equation (28.45) is reduced to:

(28.46) $$P_{\text{max.}}^{\text{max.}} = nk_e'\text{E}_B^0$$

which obviously is the maximum possible rate of restoration of the free catalyst E_B by reaction (28.41c). It will be noted that, if $k_e\text{Chl}_0$ is *not* $\ll k_e'$, this rate is not reached even in saturating light intensity; in other words, the catalyst E_B is never utilized to the maximum of its capacity. In the opposite extreme case, $k_e' \gg k_e\text{Chl}_0$, the saturation value becomes:

(28.47) $$P_{\text{max.}}^{\text{max.}} = nk_e\text{Chl}_0\text{E}_B^0$$

in other words, the limiting rate is the maximum rate of reaction (28.41b), reached when, in the photostationary state, practically all chlorophyll complexes are in the "tautomeric" form, $\text{AHCO}_2\cdot\text{Chl}\cdot\text{A}'\text{HO}$.

This last conclusion reminds us of the fact that the Franck-Herzfeld variant of the mechanism of E_B-limitation of photosynthesis does not strictly belong under the heading of "limitation by finishing dark reactions," since we have defined finishing reactions as those whose slowness does *not* affect the composition of the photosensitive complex (and thus the rate of the primary photochemical process). The reason why the reaction catalyzed by catalyst E_B in scheme (28.41) does not qualify as finishing reaction is obvious: it is the assumed stable association of the substrate of this reaction, $\{\text{AHCO}_2\}$, with chlorophyll. As long as this photochemical product has not reacted with E_B, it "blocks" the return of the chlorophyll complex into the photosensitive form, $\text{ACO}_2\cdot\text{Chl}\cdot\text{A}'\text{H}_2\text{O}$.

The half-saturating light-intensity according to equation (28.42) is:

(28.47A) $$_{1/2}I = \frac{k_e'\left(k' + k_e\text{E}_B^0\,\dfrac{k_e' + {}^1/_2\,k_e\text{Chl}_0}{k_e' + k_e\text{Chl}_0}\right)}{k^*(k_e' + k_e\text{Chl}_0)}$$

(It will be recalled that equation 28.42 was derived by assuming complete and instantaneous saturation of the acceptor with carbon dioxide; therefore no $[\text{CO}_2]$-proportional factor appears in equation 28.47A.)

The hyperbola represented by equation (28.42) is *not* rectangular; it therefore cannot be represented in the form $P/(P_{\text{max.}} - P) = \text{const.} \times I$. The degree of its deviation from the shape of a rectangular hyperbola, with the same initial slope and same saturation value, can be seen from a comparison of the half-saturating intensity (28.47A) with the half-saturating intensity derived from (28.43) and (28.45) by means of equation (28.48C):

(28.47B) $$_{1/2}I_{\text{rect.}} = \frac{k'_e(k' + k_e E_B^0)}{k^*(k'_e + k_e \text{Chl}_0)}$$

The two expressions become identical if $k'_e \gg k_e \text{Chl}_0$; in which case we write:

(28.47Ba) $$_{1/2}I = (k' + k_e E_B^0)/k^*$$

This was stated before to mean that the bottleneck reaction is (28.41b), while the catalyst, E_B, is present in excess. According to (28.47) it also means that the maximum yield $P_{\text{max.}}^{\text{max.}}$ is proportional to Chl_0; and the observations on *aurea* leaves, quoted above on page 1031, indicate that this condition is *not* realized in nature. The reverse relation, $k_e \text{Chl}_0 \gg k'_e$, which allows E_B to be fully utilized and the maximum rate (equation 28.46) to be *independent of chlorophyll concentration*, appears the more likely approximation to natural conditions. This inequality reduces (28.47A) to:

(28.47C) $$_{1/2}I = \frac{k'_e(k' + {}^1/_2 k_e E_B^0)}{k^* k_e \text{Chl}_0} \qquad (k'_e \ll k_e \text{Chl}_0)$$

Furthermore, we have seen above that, for the utilization of practically all the absorbed light quanta at low light intensities, k' must be $\ll k_e E_B^0$; we thus have approximately:

(28.47D) $$_{1/2}I \simeq \frac{k'_e + k_e E_B^0}{2k^* \text{Chl}_0} \qquad (k'_e \ll k_e \text{Chl}_0;\ \ k' \ll k_e E_B^0)$$

(nonrectangular hyperbola), and:

(28.47E) $$_{1/2}I \simeq \frac{k_e E_B^0}{k^*} \qquad (k'_e \gg k_e \text{Chl}_0;\ \ k' \ll k_e E_B^0)$$

(rectangular hyperbola). It thus appears that the hyperbola (28.42) ascends more steeply and reaches saturation more suddenly than a rectangular hyperbola with the same parameters n and $P_{\text{max.}}^{\text{max.}}$. This deviation from the shape of a rectangular hyperbola is in the direction actually noted in many experimental light curves (*cf.* the speculations of Brackett and Smith in the next section).

It was stated above that the empirical relation between the parameters n, $_{1/2}I$, and $P_{\text{max.}}^{\text{max.}}$ is approximately that required for a rectangular hyperbola. However, the calculation which led to this conclusion was one of *order of magnitude* only; more precise determinations of the light curves may show whether a deviation from the rectangular hyperbola by a factor of 1/2 in the $_{1/2}I$-value is compatible with the facts. Such a determination should not be beyond the precision with which kinetic measurements of photosynthesis can be carried out at the present time (although the persistent disagreement about a factor of about 1/2 in the value of n, to be described in the next chapter, indicates the difficulty of such measurements).

It is, of course, possible to combine the above derivations with the assumption of slow carbon dioxide supply (in consequence of slow diffusion, slow carboxylation or low content of the enzyme E_A), in other words, to drop the assumption that equation (28.41e) is an instantaneous reaction. In this case, one would have to take into consideration the accumulation of the chlorophyll complex also in the "carbon dioxide-denuded" form, $A \cdot Chl \cdot A'H_2O$. Such derivations were actually carried out by Franck and Herzfeld, using their eight-step mechanism.

It may be noted that in the case of mechanism (28.41), as in that of the previously considered mechanisms, the result would be formally the same if the limiting finishing catalyst E_B would be assigned to the stabilization of the oxidation product $\{HO\}$ instead of the reduction product, $\{HCO_2\}$, *i. e.*, if this catalyst would be needed to make possible reaction (28.41d), rather than reaction (28.41e).

The reason Franck and Herzfeld had to assume that the catalyst E_B works on all seven intermediate products (and does not merely create a single "bottleneck," *e. g.*, after the fourth, or seventh photochemical step) is as follows: If one assumes eight photochemical steps and only one bottleneck, then, in strong light, the intermediate just before the bottleneck will accumulate at the expense of all the others. If now the light intensity is suddenly reduced, a certain time must elapse until the distribution of intermediates can become uniform. Uniform distribution is, however, necessary to obtain the maximum quantum yield (since for this, all eight photochemical steps must occur with equal frequencies). It follows that sudden reduction of light intensity from the saturation range to the linear range should cause photosynthesis to drop "too low," and then gradually recover to the normal value. Franck and Herzfeld took it for granted that no such effect exists, and this caused them to assume equal proportions of back reactions (*i. e.*, equal role of the catalyst E_B) for all eight intermediates.

However, Steemann-Nielsen (1942, 1949) has described experiments in which "induction losses" following a transition from stronger to weaker light have in fact been observed in the algae, *Fucus serratus* (1942), and *Cladophora insignis* (1949). (These observations will be described in chapter 33, dealing with induction phenomena.) It is, however, not at all certain that the induction effects observed by Steemann-Nielsen are actually caused by unequal distribution of intermediates in strong light; the observed duration of the induction period (up to 30 min.) seems much too long for this origin. It seems more likely that these induction losses are related to "narcotization," photoxidation, or some other type of inhibition or partial destruction of chlorophyll in strong light. This loss of active chlorophyll is not noticeable when the over-all rate is limited by a chlorophyll-independent catalyst, such as E_B; consequently, in strong light, even if only a fraction of chlorophyll is active, its activity may suffice to keep all E_B occupied. In the light-limited state, on the other hand, absorption of light by "narcotized" (or otherwise inhibited) chlorophyll must reveal itself in a proportionally decreased rate of photosynthesis.

Obviously, questions about the distribution of intermediates become unnecessary if one postulates only one photochemical step followed by dark dismutations or coupled oxidoreductions.

Another consequence of the assumption of an eight times repeated action of the catalyst E_B is that the estimate of the ratio E_B^0/Chl_0, must be reduced from 1:2000 to 1:250 (*cf.* chapter 34).

(*f*) *Analytical Formulation: Narcotization*

Franck has evolved the concept—repeatedly referred to above—of internal regulation of photosynthesis by "self-narcotization." This self-narcotization occurs, according to Franck, whenever excess light, or lack of reactants, or presence of poisons, make the photochemical apparatus deficient in normal substrate, and creates the danger of this apparatus "running amok" and destroying valuable cell components (including itself). The specific form of this protective mechanism, envisaged by Franck, consists in the formation of a "half-oxidized" metabolite (a plant acid?), which is adsorbed on chlorophyll, blanketing all or part of it, preventing its photosensitizing activity and incidentally enhancing its fluorescence. We will derive kinetic equations for a simple "servomechanism" of this type, to see whether it can introduce fundamental changes in the shape of the light curves of photosynthesis (and fluorescence).

We consider the following mechanism:

$$\text{X}\cdot\text{Chl}\cdot\text{HZ} \xrightarrow{+k^*I} \text{XH}\cdot\text{Chl}\cdot\text{Z}$$

$$\text{XH}\cdot\text{Chl}\cdot\text{Z}(+\{\text{CO}_2\}) \xrightarrow{(k=\infty)} \{\text{HCO}_2\} + \text{X}\cdot\text{Chl}\cdot\text{Z}$$

$$\text{X}\cdot\text{Chl}\cdot\text{Z}\ (+\{\text{H}_2\text{O}\}) \xrightarrow{(k=\infty)} \text{X}\cdot\text{Chl}\cdot\text{HZ} + \{\text{OH}\}$$

(28·47Fa) $$\text{X}\cdot\text{Chl}\cdot\text{HZ}\ (+\{\text{CO}_2\} + \{\text{H}_2\text{O}\}) \xrightarrow{k^*I} \{\text{HCO}_2\} + \{\text{OH}\} + \text{X}\cdot\text{Chl}\cdot\text{HZ}$$

(28·47Fb) $$\{\text{OH}\} + \text{E} \xrightarrow{k_e} \text{EOH}$$

(28.47Fc) $$\text{EOH} \xrightarrow{k_e'} \text{E}(+\text{O}_2)$$

(28·47Fd) $$\{\text{OH}\} + \text{X}\cdot\text{Chl}\cdot\text{HZ}\ (+\text{H}_2\text{M}) \xrightarrow{k_n} \text{X}\cdot\text{Chl}\cdot\text{HZ}\cdot\text{HM} + \text{H}_2\text{O}\ (=\{\text{Chl}\} + \text{H}_2\text{O})$$

(28.47Fe) $$\text{X}\cdot\text{Chl}\cdot\text{HZ}\cdot\text{HM}\ (+\tfrac{1}{4}\text{O}_2) \xrightarrow{k_n'} \text{X}\cdot\text{Chl}\cdot\text{HZ}\ (+\text{M} + \tfrac{1}{2}\text{H}_2\text{O})$$

where {Chl} is an abbreviated expression for the "narcotized chlorophyll," X·Chl·HZ·HM.

The components in parentheses are supposed, for the sake of simplification, to be present in excess and to react practically instantaneously, so that their concentrations do not appear in kinetic equations. The summation of the first three equations gives equation (28.47Fa) for the primary photochemical process. It is further assumed that the *reduction* product.

$\{HCO_2\}$, is removed practically instantaneously, but that the *oxidation* product, $\{OH\}$, has to undergo an enzymatic transformation (28.47Fb, c), which includes a "bottleneck" reaction imposing an absolute ceiling (28.47H) on the over-all rate of photosynthesis.

If reaction (28.47Fa) runs too fast for the reactions (28.47Fb,c) to remove the oxidation products immediately, the "narcotization" mechanism (28.47Fd, e) comes into play; accumulated $\{OH\}$ reacts with a metabolite, H_2M (supposed to be present in excess in the cells), and oxidizes it to a "narcotic," HM, which is adsorbed on chlorophyll and inactivates it (supposedly without affecting the absorption spectrum, but increasing the yield of fluorescence). The narcotization is removed by reaction (28.47Fe), which completes the oxidation of the "half-oxidized" product. (At a constant concentration of oxygen, this reaction can be treated as monomolecular.)

The rate of photosynthesis is, in this scheme, equal to the rate of the limiting reaction (28.47Fc):

$$P = nk_e[\mathrm{EOH}] \tag{28.47G}$$

the maximum possible rate is (the subscript *max.* being justified by the above-made assumption of practically instantaneous supply of carbon dioxide)

$$P_{\text{max.}}^{\text{max.}} = nk_e\mathrm{E}_0 \tag{28.47H}$$

The factor n designates, as before, the number of elementary photochemical steps of type (28.47Fa) required for the production of *one* molecule of oxygen.

We introduce the abbreviation:

$$k = \frac{k_c k_n'}{k_e k_n} \tag{28.47Ha}$$

An equation for the rate of photosynthesis can be obtained by determining the photostationary concentration [EOH] and then applying equation (28.47G). The result is:

$$\frac{P}{n} = \frac{k^*Ik\mathrm{Chl}_0 + k_e k\mathrm{E}_0 + k_n'\mathrm{Chl}_0}{2(k-1)} - \left[\left(\frac{k^*Ik\mathrm{Chl}_0 + k_e k\mathrm{E}_0 + k_n'\mathrm{Chl}_0}{2(k-1)}\right)^2 - \frac{k_e k^*Ik\mathrm{Chl}_0\mathrm{E}_0}{k-1}\right]^{1/2} \tag{28.47I}$$

(One can easily ascertain that this equation gives correctly, $P = 0$ for $I = 0$, and $P^{\text{max.}} = nk_e\mathrm{E}_0$ for $I = \infty$.)

The light curves (28.47I) are *nonrectangular hyperbolae* with the initial slope:

$$\left(\frac{dP}{dI}\right)_{I=0} = \frac{nk_e k^*}{k_e k\mathrm{E}_0 + k_n'\mathrm{Chl}_0} = \frac{nk_e k_n k^*}{k_n'(k_c + k_n\mathrm{Chl}_0)} \tag{28.47J}$$

and the half-saturating intensity:

$$(28.48) \qquad {}_{1/2}I = \frac{k_e E_0(k+1) + 2k_n' \mathrm{Chl}_0}{2k^* k \mathrm{Chl}_0}$$

(g) Are Light Curves Hyperbolic?

It will be noted that all the kinetic models used in this chapter lead to *quadratic* equations for the rate of photosynthesis, P, as function of incident light intensity, I; in other words, to hyperbolic light curves, $P = f(I)$. The imposition of a rate "ceiling," caused by limited supply of a reactant or limited amount of an enzyme (in addition to a rate "roof," imposed by the minimum number of quanta required to bring about the reaction) changes the appearance of the light curves—for example, it can convert a rectangular into a nonrectangular hyperbola—but in the mechanisms discussed so far, general hyperbolical shape is preserved.

A hyperbola is defined by three parameters. In our presentation of light curves, the axis of abscissae, I, was parallel to one asymptote of the hyperbola (the equation of the latter being $P = P^{\max.}$), while the axis of ordinates, P, was chosen so as to make $P = 0$ at $I = 0$. One convenient form of writing the equation of a hyperbola in this system of co-ordinates is:

$$(28.48\mathrm{A}) \qquad \gamma_0 I = \frac{P}{P^{\max.} - P}\left(P^{\max.} + \frac{2\gamma_0 P_{1/2} I - 2PP^{\max.}}{P^{\max.}}\right)$$

The three parameters in this equation are γ_0, the initial slope of the curve, which is proportional to the maximum quantum yield of photosynthesis; ${}_{1/2}I$, the half-saturating light intensity; and $P^{\max.}$, the limiting rate in strong light. If the second term in parentheses vanishes, the equation becomes:

$$(28.48\mathrm{B}) \qquad \frac{P}{P^{\max.} - P} = \frac{\gamma_0}{P^{\max.}} I = \text{const.} \times I$$

which is the equation of a *rectangular* hyperbola. For the quadratic term in (28.48A) to vanish, the following relation between the three parameters must be fulfilled:

$$(28.48\mathrm{C}) \qquad \gamma_0 = \frac{P^{\max.}}{{}_{1/2}I}$$

Several equations derived in chapters 27 and 28 for the light curves of photosynthesis—such as (28.29)—actually had the simplified form (28.48B); however that was not always the case, as shown, *e. g.*, by equation (28.42), which cannot be represented in the form (28.48B).

Since γ_0 is not so easily measured as the other two parameters (in fact, determination of γ_0 may be one aim of analytical representation of the light

curves, *cf.* chapter 29, p. 1132 ff.), another parameter may be chosen instead, such as $_{1/4}I$, the light intensity at which photosynthesis reaches one-quarter of its saturation value. This choice leads to a very simple relation:

$$\gamma_0 = \frac{P^{\max.}}{6_{1/4}I - {}_{1/2}I} \tag{28.48D}$$

and gives, as equation of the light curve:

$$I = \frac{P}{P^{\max.} - P}\left[6_{1/4}I - {}_{1/2}I + \frac{4P}{P^{\max.}}({}_{1/2}I - 3_{1/4}I)\right] \tag{28.48E}$$

For the hyperbola (28.48E) to be rectangular, it must satisfy the very simple condition:

$${}_{1/2}I = 3_{1/4}I \tag{28.48F}$$

Its equation is then:

$$I = \frac{P}{P^{\max.} - P}(6\,{}_{1/4}I - {}_{1/2}I), \text{ or} \tag{28.48G}$$

$$P = \frac{IP^{\max.}}{I + 6\,{}_{1/4}I - {}_{1/2}I} \tag{28.48H}$$

More generally, a hyperbola could be represented in our chosen coordinates, using any three of its points (P_1,I_1; P_2,I_2; and P_3,I_3) as parameters; for a rectangular hyperbola, two points suffice.

These derivations should be kept in mind in evaluating papers, in which failure to represent empirical data by equations of type (28.48B) has been taken to mean that the light curves were "not hyperbolic" (*cf* Smith 1936, 1937, 1938).

It was stated on page 1020 that all our derivations of light curve equations were based on the assumption of uniform light absorption, and are therefore strictly applicable only to optically thin layers.

The question arises to what extent the considerable optical density of most actually studied plant objects distorts the shape of light curves—for example, whether the observed "integral" light curves will be nonhyperbolic if the "differential" light curves for each thin layer, with practically uniform light absorption, are hyperbolae. For monochromatic light, for which the absorption coefficient is α, the total light flux absorbed in a layer l (for the sake of simplicity, we assume uniform pigment distribution and absence of scattering) is:

$$I_a = I(1 - 10^{-\alpha[\mathrm{Chl}]l}) \tag{28.48I}$$

(assuming chlorophyll to be the absorbing pigment).

If the quantum yield of the over-all process is n, and if I_a is expressed in einsteins per cm.^{2} per sec., the total rate of photosynthesis is:

(28.48J) $$P = nI_a = nI(1 - 10^{-\alpha[\mathrm{Chl}]l})$$

(mole CO_2 reduced per second per cm.^{2} of illuminated area).

If the illuminating light is nonmonochromatic, the first part of this relation remains valid, but I_a is now expressed by an integral:

(28.48K) $$I_a = I - \int_{\lambda_1}^{\lambda_2} I_\lambda 10^{-\alpha_\lambda[\mathrm{Chl}]l}\, d\lambda$$

When the incident light intensity increases so that light saturation sets in, in the most exposed pigment layer, the light curve of the integral yield bends, and does not become horizontal until saturation has become complete in the deepest layer. Consequently (as discussed before, *cf.* p. 1007 and fig. 28.20), the qualitative effect of inhomogeneous light absorption must be to *broaden* the transitional region connecting the linearly ascending and the horizontal part of the light curves. What we want to know is how the *shape* of the light curves is changed by this integration. Let us assume for the sake of simplicity that the "differential" light curves are rectangular hyperbolae:

$$\frac{P_l}{P_l^{\max.} - P_l} = k^* I_l$$

(28.48L) $$P_l = \frac{k^* P_l^{\max.} I 10^{-\alpha[\mathrm{Chl}]l}}{1 + k^* I\, 10^{-\alpha[\mathrm{Chl}]l}}$$

The integrated rate (per unit area) is then:

(28.48M) $$P = \int_0^{l_0} P_l dl = \frac{P^{\max.}}{l_0 \alpha[\mathrm{Chl}] \ln 10} \ln \frac{1 + k^* T}{1 + k^* I 10^{-\alpha[\mathrm{Chl}]l}}$$

or:

(28.48N) $$\frac{P}{P^{\max.} - P} = \frac{\ln [(1 + k^* I)/(1 + k^* I 10^{-\alpha[\mathrm{Chl}]l_0})}{l_0 \alpha[\mathrm{Chl}] \ln 10 - \ln [(1 + k^* I)/(1 + k^* I 10^{-\alpha[\mathrm{Chl}]l_0})]}$$

an equation which does *not* represent a rectangular hyperbola. It can be developed into a series:

(28.48O) $$\frac{P}{P^{\max.} - P} =$$

$$k^* I \left(1 - \frac{\alpha[\mathrm{Chl}]l_0 \ln 10}{2!} + \frac{(\alpha[\mathrm{Chl}]l_0 \ln 10)^2}{3!2!} \frac{(2 + k^* I)}{(1 + k^* I)} - \frac{(\alpha[\mathrm{Chl}]l_0 \ln 10)^3}{4!3!} \frac{(3 + k^* I)(2 + k^* I)}{(1 + k^* I)^2} + \cdots\right)$$

This series shows that the integral light curve remains practically a rectangular hyperbola until the *third* term in the development ceases to be small compared to 1; then, it looses the hyperbolic shape (because of a third degree term, containing the product PI^2). Since the second factor in the third term decreases from 2 to 1 with increasing light intensity, the

maximum value of this term is $\alpha[\mathrm{Chl}]l_0 \ln 10/6$. This means that for the maximum deviation of the integral light curve from hyperbolical shape to exceed 5%, the integral absorption must exceed 50%:

$$(I_0/I = 2;\ \log I_0/I = \alpha[\mathrm{Chl}]l_0 \ln 10 = 0.30;\ \alpha[\mathrm{Chl}]l_0 \ln 10/6 = 0.05)$$

At 75% absorption, the maximum deviation will reach 10%. This indicates that to obtain experimental light curves which will permit one to judge the true shape of the function $P = f(I)$, objects with an optical density of up to $\log I_0/I = 0.5$ can be used.

A corresponding derivation for a general hyperbola is much more involved, but conclusions are likely to be not too different from those obtained here for a rectangular hyperbola.

In the literature one can find many attempts to derive analytical expressions for the light curves of photosynthesis, partly by using very simple kinetic models, and partly by empirical approximation.

Among the simplified kinetic equations, reference may be made to those of Ghosh (1928), Emerson and Green (1934), Baly (1935) and Burk and Lineweaver (1935); while among the *empirical* approximations, we may mention those of Brackett (1935) and Smith (1936, 1937, 1938).

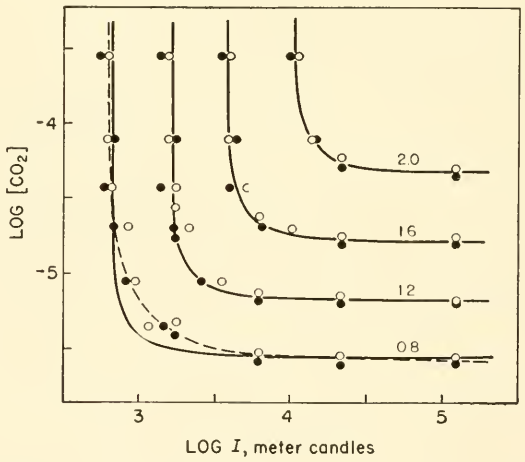


Fig. 28.23. Are photosynthesis curves hyperbolae? (after Smith 1938). Solid lines are derived from nonhyperbolic functions (27.77) and (28.48P); dashed line from rectangular hyperbolic function. See text page 1047.

Brackett sought to express the sudden approach of the light curves to saturation by an exponential curve, while Smith suggested an algebraic function of a higher order:

(28.48P) $$P/P^{\max.} = CI/\sqrt{1 + C^2I^2}$$

or:

(28.48Q) $$P/\sqrt{(P^{\max.})^2 - P^2} = CI$$

To compare the usefulness of functions (27.77) or (28.48P) with that of the rectangular hyperbola, Smith derived, for both types of functions, equations determining the combinations of the parameters $[CO_2]$ and I for which the yield P has a certain constant value. Figure 28.23 shows the results: For the lowest value of P used (log P = 0.8), the dashed curve derived from the hyperbolic function gave the better fit; but for three higher values of P (log P = 1.2, 1.6, and 2.0), a very good fit was obtained by means of equations (27.77) and (28.48P).

B. Light Curves of Fluorescence*

1. Relation between Light Curves of Photosynthesis and Fluorescence

In vitro the intensity of fluorescence usually is proportional to the intensity of illumination. This is so because both fluorescence and the "quenching" processes that compete with it (*i. e.*, energy dissipation, and photochemical reactions) usually are "first order" or "monomolecular" processes with respect to the concentration of excited pigment molecules. In other words, each excited molecule has a certain probability of fluorescing, a certain probability of being deactivated by energy dissipation and a certain probability of undergoing a photochemical reaction; all these probabilities are independent of the concentration of the excited molecules, and consequently do not depend on the rate of their production and thus also on light intensity. Under these conditions, the rate of photochemical reactions too must be proportional to light intensity. Thus, both the quantum yield of fluorescence, φ (= const. $\times$ F/I, where F is the intensity of fluorescent light), and the quantum yield of the photochemical change, γ (= const. $\times$ P/I, where P is the rate of photochemical change), usually are independent of the intensity of the incident light, I.

In photosynthesis, we know already that γ is approximately constant only within a limited range of low intensities (corresponding to the linear part of the light curves, *cf.* table 28.II), and then declines gradually. The question arises whether, in this case, φ, too, changes with light intensity. At first, experiments appeared to indicate that the intensity of fluorescence continues to increase proportionally with light intensity (*i. e.*, the yield, φ, remains constant) long after photosynthesis has begun to show light saturation (*i. e.*, γ has begun to decline). For example, Wassink, Vermeulen, Reman and Katz (1938), using a suspension of *Chlorella vulgaris*, found

* Bibliography, page 1081.

for F and P the two curves shown in figure 28.24: The fluorescence curve remained linear up to 16 kerg/cm.^{2} sec., while photosynthesis became light-saturated at about 10 kerg. Subsequent investigations have shown, however, that such an absence of correlation between φ and γ is by no means the general rule. More often, a definite relation—usually, antiparallelism—is found between the two yields (*i. e.*, the yield of fluorescence increases

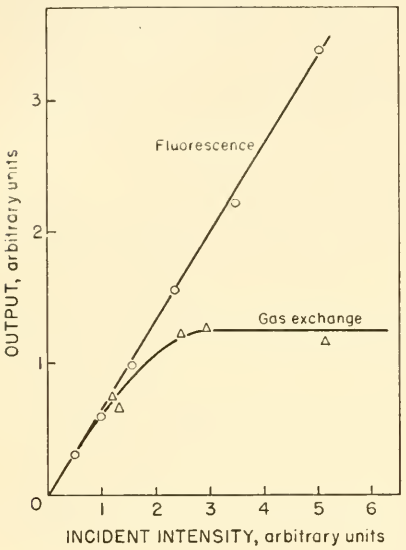


Fig. 28.24. Photosynthesis light saturated, fluorescence unchanged in *Chlorella* suspension (after Wassink, Vermeulen, Reman and Katz 1938).

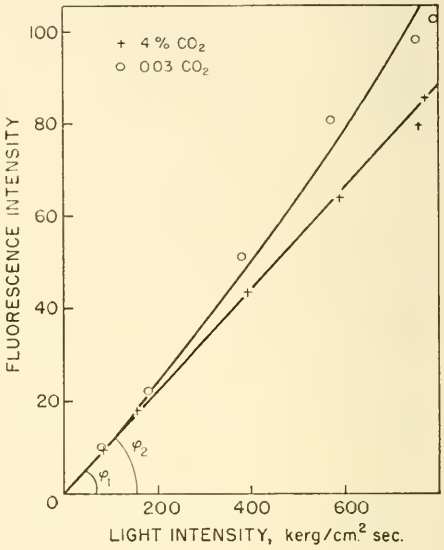


Fig. 28.25. Yield of fluorescence increases in wheat at high light intensity when CO_2 supply is low and remains constant when [CO_2] is high (after McAlister and Myers 1940).

when the yield of photosynthesis becomes smaller, and *vice versa*). An increase in the yield of fluorescence at high light intensities was first observed by McAlister and Myers (1940) in young wheat plants. It began at about 200 kerg/cm.^{2} sec. However, this increase occurred only when the carbon dioxide supply was low (0.03%); no change of φ was noticeable even up to 700 kerg/cm.^{2} sec., when this supply was ample (in 4% CO_2) (*cf.* fig. 28.25). It will be noted that McAlister and Myers used much higher light intensities than Wassink and co-workers; but in their experiments (*cf.* Table 28.I), saturation was not quite reached, in the presence of 4% CO_2, even at 600 kerg/cm.^{2} sec.

Franck, French and Puck (1941), working with *Hydrangea* leaves, found an increase in fluorescence yield above 20 kerg/cm.^{2} sec., even with ample

supply of carbon dioxide (1% CO_2). Measurements up to 130 kerg/cm.^{2} sec. indicated (*cf.* fig. 28.26) that, at very high light intensities, the yield of fluorescence again becomes stabilized. In other words the yield increases from an initial constant level, φ_1, to approach a final, also constant level, φ_2 ($\simeq$ 1.7 φ_1). The transition occurred, in *Hydrangea*, approximately in the same intensity region in which photosynthesis became light saturated (*cf.* lower curve in fig. 28.26).

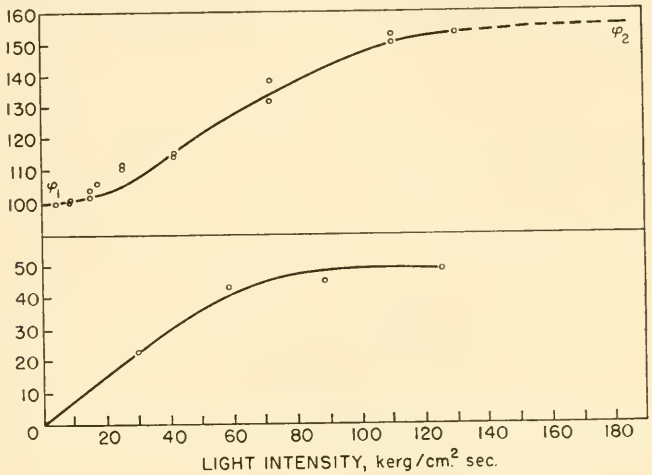


Fig. 28.26. Rate of photosynthesis (P, lower scale) and fluorescence (φ, upper scale) yield of *Hydrangea* leaves as function of light intensity (after Franck, *et al.* 1941). Note that upper curve represents *yield*, φ, while the two preceding figures show absolute rates, *i.e.*, *intensities*, of fluorescence, F.

New measurements with *Chlorella*, as well as with *Scenedesmus*, were carried out by Shiau and Franck (1947), and an increase in the yield of fluorescence at high light intensities was now found also in these unicellular algae—in *Chlorella* above 20 kerg/cm.^{2} sec. and in *Scenedesmus* above 15 kerg/cm.^{2} sec.

Extensive measurements of the fluorescence of *Chromatium* (strain D) were described by Katz, Wassink and Dorrestein (1942) and by Wassink, Katz and Dorrestein (1942), who found that the yield of fluoresence of these bacteria increased, at the higher light intensities, even more pronouncedly than that of green plants. This is illustrated by figures 28.30 to 28.33. These figures also show the influence that the supply of the reductants has on the shape of the light curves of fluorescence. In the presence of abundant reductant (either hydrogen or thiosulfate), the increase of φ in *Chromatium* first begins at 15 kerg/cm.^{2} sec., while without

the reductant the yield is high even at light intensities as low as 1 kerg/cm.^{2} sec.

Figure 28.27 shows the transition from the low intensity yield, φ_1, to the high intensity yield, φ_2. The yield in the upper figure becomes practically constant ($\varphi = \varphi_2$) when the difference between the angles φ and φ_2 in the lower figure ceases to be significant.

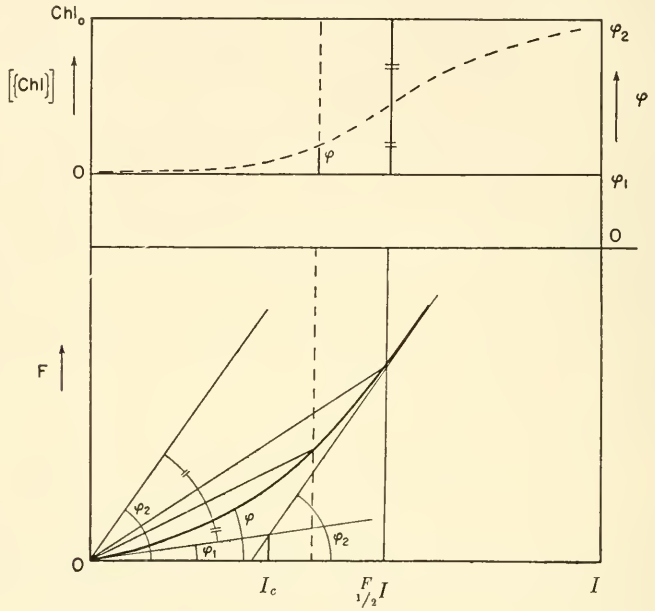


Fig. 28.27. Fluorescence intensity (F) and yield (φ) as function of incident light intensity (schematic).

Comparison of figure 28.32 with the light curves of photosynthesis of *Chromatium* in the presence of thiosulfate (*cf.* fig. 28.5B) indicates an approximate antiparallelism of φ and γ. The fluorescence yield, φ, begins to rise at about 10 kerg in 1% thiosulfate (somewhat earlier in 0.05%), while the first signs of saturation (*i. e.*, of a decline of γ) are noticeable a little above 10 kerg in 1% thiosulfate and at about 5 kerg in 0.067% thiosulfate.

The green plants and algae discussed so far all showed an *increase* in φ at high light intensities; different behavior was observed by Wassink and Kersten (1945) in a species of diatoms. There, the yield of fluorescence *declined*, in the presence of carbon dioxide, simultaneously with the saturation of photosynthesis (*i. e.*, φ_2 appeared to be *smaller* than φ_1); the yield φ_1 remained uniformly high if the carbon dioxide supply was low (*cf.* fig.

28.28) French and Koski (1951) reported that the chlorophyll fluorescence of a *red* alga declined when the exciting green light was raised to 4 kerg/cm.^{2} sec. The phycoerythrin fluorescence remained proportional to I.

2. Effect of Various Factors on Light Curves of Fluorescence

We have seen above that the yield of fluorescence, φ, is often affected by changes in the yield of photosynthesis, γ, when the latter are produced by variations of light intensity. We therefore expect φ to be affected also by other, external or internal factors that influence γ.

(a) Carbon Dioxide

The effect of the factor $[CO_2]$ on fluorescence was already mentioned before. First, we noted the results of McAlister and Myers (fig. 28.25), who found that, in wheat, φ rises at high light intensity in 0.03% CO_2, while no such change occurs in 4% CO_2. In qualitative agreement with McAlister's results, Franck, French and Puck (1941) found that at 17 kerg/cm.^{2} sec. the yield of fluorescence of *Hydrangea* was about 20% higher in the absence of carbon dioxide than in the presence of 5% CO_2.

As mentioned above, Wassink and Kersten (1945) found a decrease rather than increase in the yield of fluorescence of diatoms at high light intensities in the presence of carbon dioxide; in the carbon dioxide-free suspension φ remained constant up to 100 kerg/cm.^{2} sec. (*cf.* fig. 28.28). In other words, in this type of plant, as in *Hydrangea* or *Triticum*, the yield, φ_2, in strong light was higher without than with carbon dioxide; but this difference was caused by a decline of φ in the CO_2-supplied plant, rather than, as in the other species, by an increase of φ in the starved cells.

In evaluating figure 28.28, it must be borne in mind that according to figure 28.5 the photosynthesis of *Nitzschia* was not completely inhibited in CO_2-free air. This probably means that these cells produced, by dark metabolism, so much carbon dioxide (or intermediates which could be used directly for photosynthesis) that their photosynthesis could not be stopped by bubbling CO_2-free air through the suspension. Whether this is the correct explanation of the continued oxygen production by *Nitzschia* in "CO_2-free" air or not, the notation "no CO_2" in figure 28.28 certainly means "no external CO_2 supplied," and *not* "photosynthesis totally inhibited by absence of CO_2."

Comparison of figure 28.28 with the effects of low temperature (fig. 28.39) and cyanide inhibition (fig. 28.44) in the same species shows that the results would be more plausible and consistent if the designations of the two curves in figure 28.28 were reversed.

A still different effect of carbon dioxide on the light curves of fluores-

cence was observed by Wassink, Katz and Dorrestein (1942) in purple bacteria. In the first place, they found no effect of carbon dioxide deficiency at all when reductants were absent (fig. 28.29)—a condition that cannot be duplicated in green plants. In the presence of thiosulfate or hydrogen, the effect of carbon dioxide deprivation was small but noticeable. Figure 28.30A obtained in the presence of thiosulfate could be interpreted as analogous to the findings of Franck and McAlister; here, too,

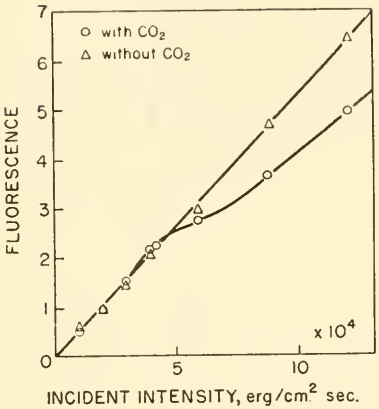


Fig. 28.28. Fluorescence and CO_2 supply in diatoms (after Wassink and Kersten 1945).

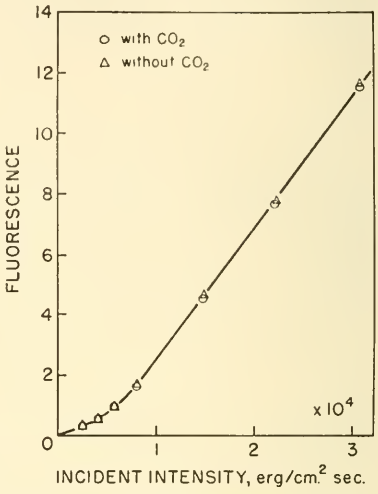


Fig. 28.29. No effect of $[CO_2]$ on fluorescence in purple bacteria when reductant is absent (after Wassink *et al.* 1942).

the region of transition from the lower yield, φ_1, to the higher yield, φ_2, appears to be shifted toward lower light intensities by the absence of carbon dioxide. Figure 28.30B obtained with hydrogen as reductant, on the other hand, shows that the yield is enhanced by the absence of carbon dioxide only in a limited intensity region below 10 kerg; unexpectedly, the relation is reversed at the higher light intensities, so that at 30 kerg the fluorescence is about one-third stronger in the presence of carbon dioxide than in its absence.

(*b*) *Reductants*

The influence of the concentration of reductants (thiosulfate, hydrogen, etc.) on the shape of the fluorescence curves of *Chromatium* is illustrated by figures 28.31–28.33. Comparison with figure 28.30 shows that the

influence of the reductant is much stronger than that of carbon dioxide. Figure 28.34 shows the "critical" intensity, I_c, (fig. 28.27) in relation to the thiosulfate concentration. It rises from 2 kerg without thiosulfate to 10 kerg in 0.5% thiosulfate, and then becomes more or less constant. Figure 27.13 indicated that thiosulfate "saturation" of photosynthesis occurs in about the same concentration region.

Wassink and co-workers noted, however, that, with hydrogen as reductant, at *p*H 7.6, the transition point of fluorescence was markedly higher than the saturation point of the gas exchange.

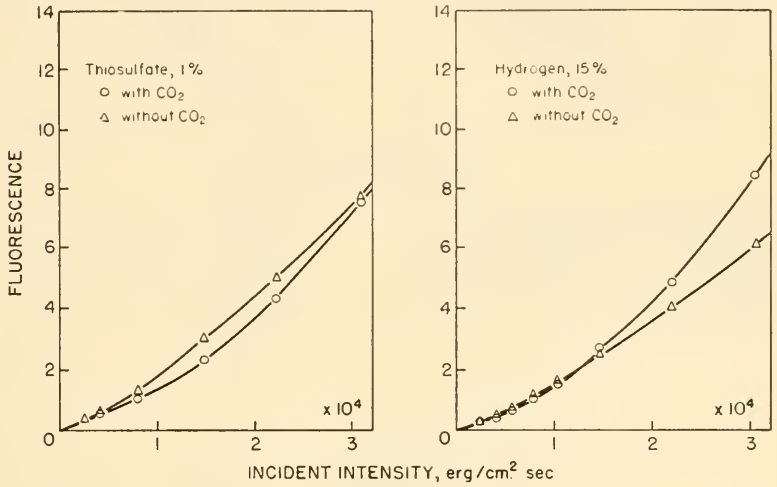


Fig. 28.30. Effect of CO_2 on fluorescence of purple bacteria in presence of reductants (after Wassink *et al.* 1942). *p*H 6.3, 29° C.

The characteristic initial bend upward of the fluorescence curves of *Chromatium* does not quite disappear even in complete absence of reductants. This can be attributed to the presence of "internal reductants," which in weak light suffice to prevent a complete conversion of the "photocomplex" into the strongly fluorescent form. The linearity can in fact be improved by preliminary starvation of the bacteria, depriving them of metabolites that could serve as internal reductants.

Figure 28.35 shows that the effect of the reductants on the fluorescence of *Chromatium* persists even in the absence of carbon dioxide. This fact must be compared with the above-mentioned observation (fig. 28.29) that the removal of carbon dioxide had no effect on fluorescence in the absence of a reductant. We will return to the discussion of this interesting difference on page 1077.

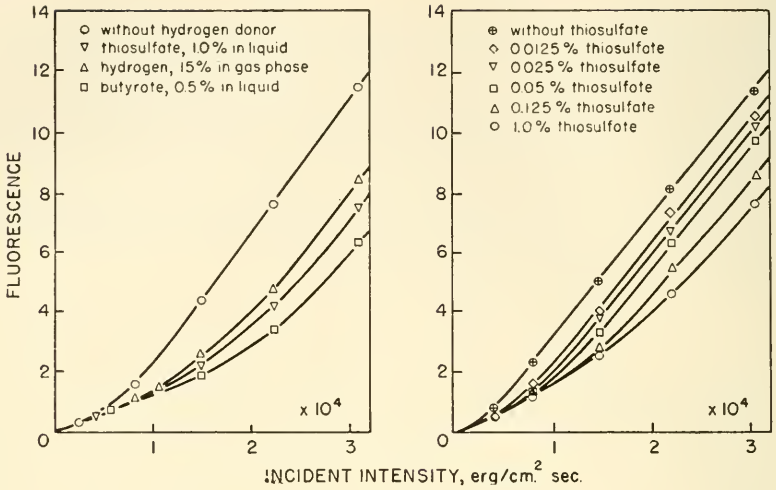


Fig. 28.31. Fluorescence of purple bacteria as function of the concentration of reductants (after Wassink *et al.* 1942). 5% CO_2, *p*H 6.3, 29° C.

Fig. 28.32. Influence of concentration of thiosulfate on intensity of fluorescence (Wassink *et al.* 1942). 5% CO_2, *p*H 6.3, 29° C.

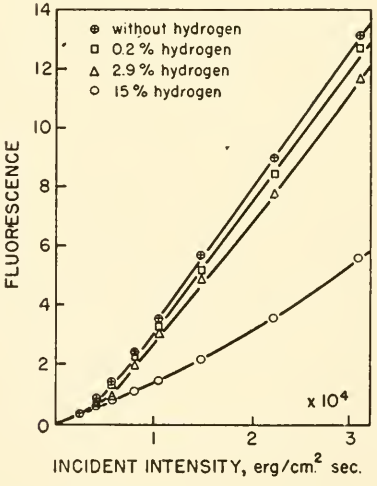


Fig. 28.33. Influence of concentration of H_2 on intensity of fluorescence (after Wassink *et al.* 1942). 5% CO_2, *p*H 7.6, 29° C.

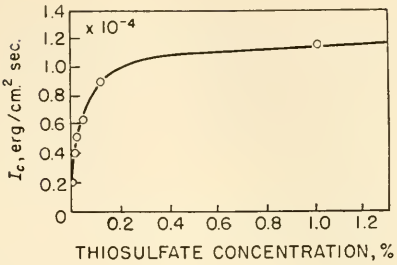


Fig. 28.34. Fluorescence transition point I_c as function of thiosulfate concentration (after Wassink *et al.* 1942) (*cf.* fig. 28.27 for meaning of I_c).

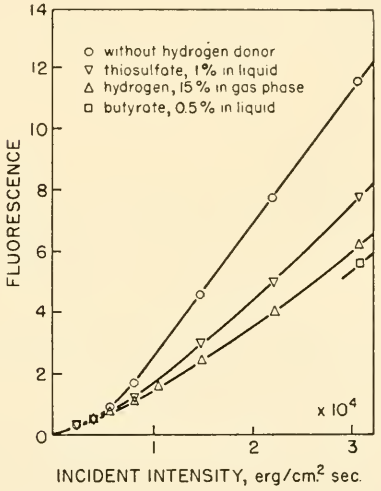


Fig. 28.35. Influence of excess reductants on intensity of fluorescence in absence of CO_2 (after Wassink *et al.* 1942). *p*H 6.3, 29° C.

(*c*) *Effect of Temperature*

Kautsky and Spohn (1934) noticed that the fluorescence of leaves was stronger at 0 than at 30° C.; but Wassink, Vermeulen, Reman and Katz (1938) found no such effect on the light curves of fluorescence in *Chlorella* suspensions (10–30°) (*cf.* fig. 28.36 on page 1056).

Franck, French and Puck (1941) found that a sudden cooling of *Hydrangea* leaves from 23 to 0° C. produced a "burst" of fluorescence, which subsided after 10 or 20 minutes (*cf.* fig. 28.37). We will return to this ob-

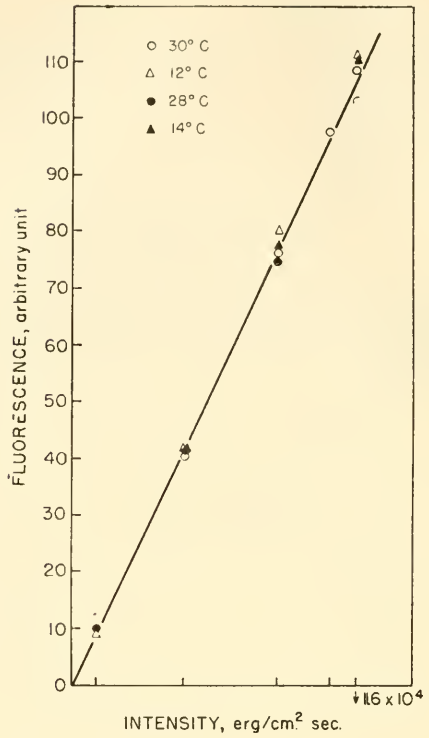


Fig. 28.36. Fluorescence of chlorophyll in suspensions of *Chlorella* at different temperatures (after Wassink, Vermeulen, Reman and Katz 1938).

servation in chapter 33, in dealing with "induction" phenomena; here we note that, even after the burst was over, the steady yield of fluorescence remained greater at 0° C. than at room temperature (fig. 28.37c, levels A and E).

This temperature effect could be observed only in a certain medium range of intensities (*e. g.*, at 5 and 27 kerg/cm.^{2} sec., *cf.* figs. 28.37b and c); but not in high light (*e. g.*, 220 kerg/cm.^{2} sec., *cf.* fig. 28.37a); or low light (*e. g.*, 1.8 kerg/cm.^{2} sec., *cf.* fig. 28.37c). In other words, a decline in temperature appeared to have no effect on the steady fluorescence levels, φ_1 and φ_2 (*cf.* fig. 28.27), but caused the transition from φ_1 to φ_2 to occur at lower light intensities (fig. 28.38). (Figs. 28.37 to 28.45 are on pages 1058–1061.)

The effect of temperature on the light curves of fluorescence was also observed by Wassink and Kersten (1945) with diatoms. Figure 28.39 shows that the peculiarity noted in the fluorescence curves of the same

species with and without external CO_2 (fig. 28.28) is repeated in the fluorescence curves at low and high temperature: The yield of fluorescence either remains constant, or declines at high light intensity. An additional peculiarity is that the curve obtained at low temperature resembles that found with carbon dioxide, and the curve found at high temperature resembles that found without external CO_2; usually, the reverse relation prevails, the effect of lowering the temperature being similar to that of removing carbon dioxide. In figure 28.39 the yield at 25° C. remains constant up to 100 kerg/cm.^{2} sec., while the saturation of photosynthesis begins at this temperature at about 20, and is complete at about 45 kerg/cm.^{2} sec.

A temperature effect similar to that observed in green plants was found in purple bacteria by Wassink, Katz and Dorrestein (1942). At 16° C. the transition point, I_c, was at about 10 kerg and, at 29°, at about 20 kerg/cm.^{2} sec. In more detail, observations by Wassink, Katz and Dorrestein are summarized in figure 28.40.

(*d*) *Cyanide*

A shift of the fluorescence transition point toward lower light intensities can be produced also by the addition of *cyanide*. A "stimulating" effect of cyanide on the yield of steady fluorescence was first observed by Kautsky and Hirsch (1935) in experiments with leaves. Wassink, Vermeulen, Reman and Katz (1938) found no such effect in *Chlorella* suspensions (fig. 28.41); but Wassink and Katz (1939) later proved that cyanide stimulation *does* occur in this organism as well, although only when the cyanide concentration is high enough to cause complete inhibition of photosynthesis. This concentration completely inhibits respiration also, which is an important factor from the point of view of the "narcotization" theory of Franck (since this theory assumes that the "narcotic", which protects the "idling" photosynthetic apparatus, undergoes rapid oxidation when respiration is strong, but can be preserved for a considerable length of time if respiration is weak). Figure 28.42 shows that the cyanide effect on steady fluorescence of *Chlorella* is caused by the disappearance of the decline of fluorescence otherwise observed after the first half-minute of illumination. This decline is entirely eliminated in 2.5×10^{-3} M potassium cyanide solution ($p = 0.33$ in fig. 28.42). The effect of cyanide on the stationary intensity of fluorescence increases at first with light intensity, as shown by figure 28.43; but Franck, French and Puck (1941) found that it disappears again if the light intensity is increased still further. For example, 2% gaseous HCN in the atmosphere increased the yield of fluorescence of *Hydrangea* leaves (in 1% CO_2) by 12% when the incident light intensity was 2 kerg,

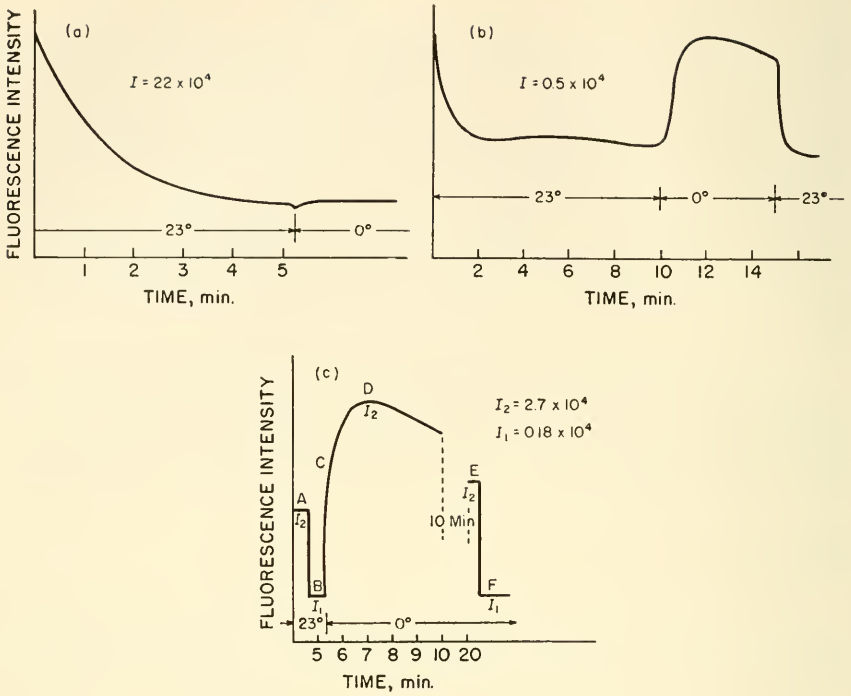


Fig. 28.37. Effect of low temperature on steady state fluorescence at various light intensities (after Franck, French and Puck 1941).

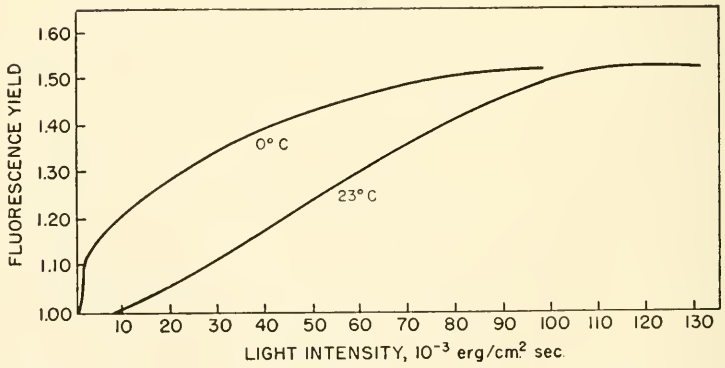


Fig. 28.38. Fluorescence yield as function of intensity of exciting light at room temperature, and at 0° C., where photosynthesis is inhibited (after Franck, French and Puck 1941).

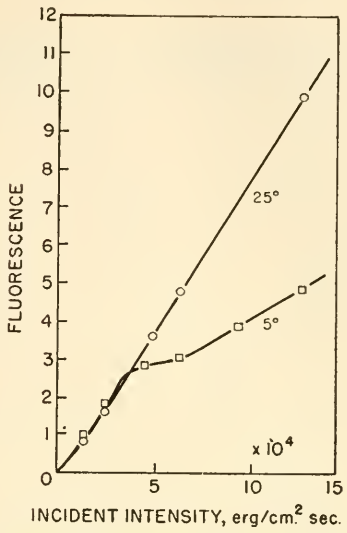


Fig. 28.39. Fluorescence intensity of diatoms as function of light intensity at two temperatures (Wassink and Kersten 1945).

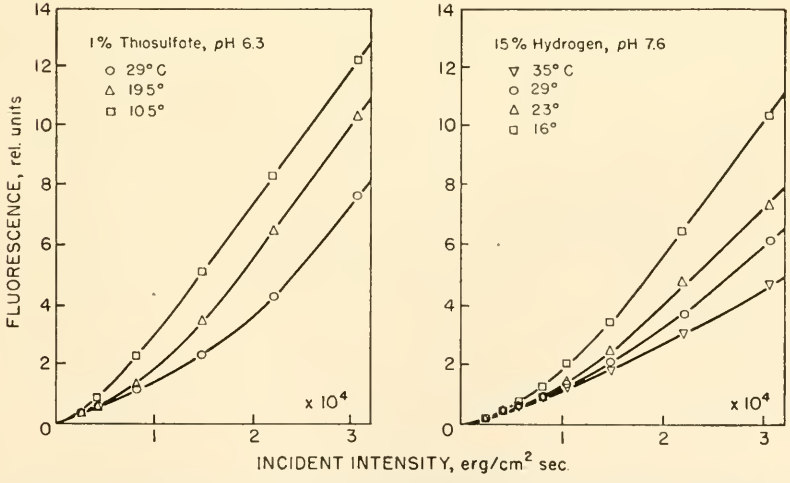


Fig. 28.40. Fluorescence curves of *Chromatium* at different temperatures (after Wassink, Katz and Dorrestein 1942). (5% CO_2.)

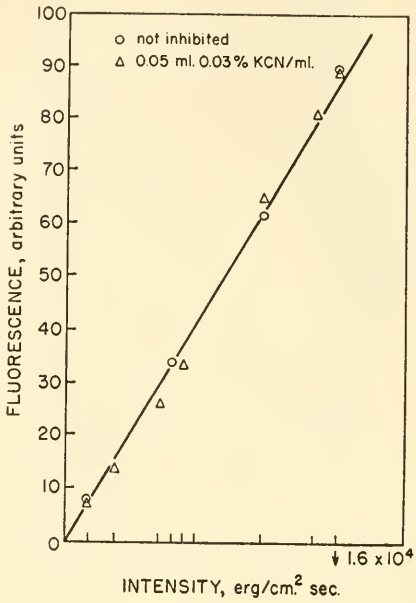


Fig. 28.41. Influence of cyanide on fluorescence of chlorophyll in *Chlorella* suspensions (after Wassink, Vermeulen, Reman and Katz 1938).

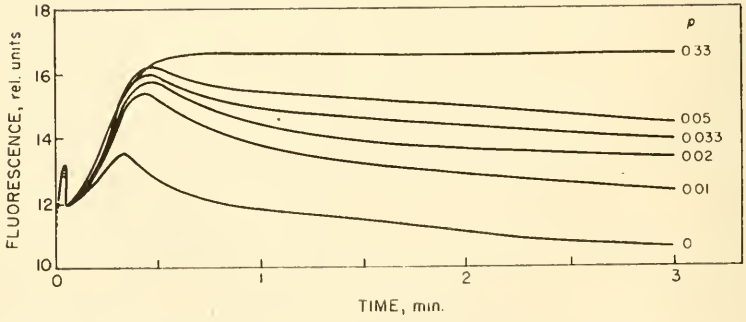


Fig. 28.42. Fluorescence–time relation in air as function of inhibition of photosynthesis by cyanide (after Wassink and Katz 1939). Parameters are percentage KCN in solution from which 0.1 ml. was added to 2 ml. of cell suspension (29° C.).

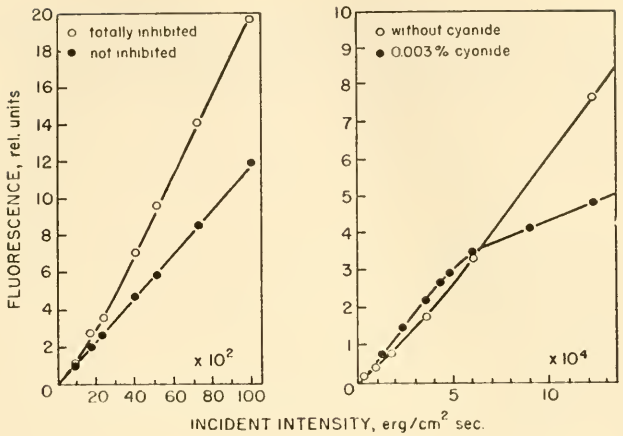


Fig. 28.43. Stationary values of fluorescence of *Chlorella* as function of light intensity with and without cyanide (after Wassink and Katz 1939). (Gas phase air, 29° C.)

Fig. 28.44. Cyanide effect on fluorescence of diatoms (25° C.) (after Wassink and Kersten 1945).

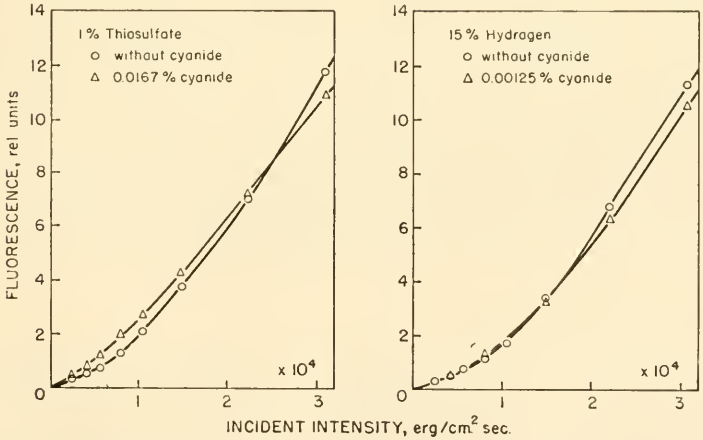


Fig. 28.45. Effect of cyanide on fluorescence of *Chromatium* (after Wassink, Katz and Dorrestein 1942). 5% CO_2, *p*H 6.3, 29° C.

and by 6% when it was 4.7 kerg/cm.^{2} sec., but left it unchanged at 70 kerg/cm.^{2} sec. In other words, the effect of cyanide was in this case similar to that of low temperature (or carbon dioxide deficiency); all three caused a shift of the critical intensity, I_c, toward lower light intensities, but did not affect the two "limiting" yields, φ_1 and φ_2. Working with diatoms, Wassink and Kersten (1945) found the cyanide effect similar to that of lowering of temperature (fig. 28.33) and of *addition* (not removal!) of carbon dioxide. Up to about 6 kerg/cm.^{2} sec., the yield of fluorescence (at 25° C.) was slightly increased by 0.003% KCN but, above this intensity, the yield became much smaller in the presence of the poison (fig. 28.44). Here again, we note the contradiction to other observations in the apparent similarity between the curve obtained with carbon dioxide and that observed with hydrogen cyanide (usually addition of hydrogen cyanide has been found to produce the same effect as deprivation of carbon dioxide). Furthermore, one notes that in figure 28.44 the noninhibited sample showed a marked *increase* in φ at high light intensities in contradiction to the perfectly straight line given for similar conditions in figure 28.39).

At 6° C., the yield, φ, remained constant in the presence of cyanide, up to 130 kerg; it looked as if in this case the low yield, φ_2, prevailed down to the lowest light intensities used.

Katz, Wassink and Dorrestein (1941) stated that in *purple bacteria,* as in *Chlorella,* the effect of cyanide on the transition intensity, I_c, is similar to that of a decrease in the concentration of carbon dioxide. Wassink, Katz and Dorrestein (1942) gave several fluorescence curves for potassium cyanide-poisoned bacteria. In the absence of reductants, the addition of up to 0.0167% KCN had no effect on fluorescence—a result analogous to that obtained in carbon dioxide starvation experiments, and thus in agreement with the assumption that hydrogen cyanide is primarily a poison for carbon dioxide fixation. Very high cyanide concentration, on the other hand, had a strong *depressing* effect on fluorescence of *Chromatium,* even in the absence of hydrogen donors (>50% depression in 0.05% KCN). In the presence of reductants, fluorescence curves with and without cyanide (figs. 28.45) showed small but distinct differences, again somewhat similar to those found with varying carbon dioxide supply (*cf.* fig. 28.30).

(*e*) *Hydroxylamine and Azide*

Observations of the effect of these two poisons on fluorescence were made by Wassink and co-workers (1942) in purple bacteria. They are illustrated by figs. 28.46-28.47 (p. 1064). The influence of hydroxylamine appears similar to that of potassium cyanide—no effect up to 0.05% (a concentration at which photosynthesis is about 50% inhibited), then (at

0.1%) a strong *stimulation* of fluorescence. The effect of sodium azide differs from that of either potassium cyanide or hydroxylamine in two respects. Fluorescence is affected (*cf.* fig. 28.47) even in the absence of reductants; and the typical effect is a *decline*, rather than a rise of the yield of fluorescence.

(*f*) *Ion Concentration*

In *Chromatium*, the yield of fluorescence can be affected by changes in *p*H. According to Wassink and co-workers (1942) the sign of this effect depends on whether molecular hydrogen or thiosulfate is used as hydrogen donor. (We saw on page 952 that the same is true of the influence of *p*H on the yield of carbon dioxide reduction by these two reductants.) This is illustrated by figure 28.48, page 1065.

No experiments are available on the effect of other cations or anions on the yield of fluorescence of green plants or purple bacteria.

(*g*) *Narcotics*

Narcotics were found by Kautsky and Hirsch (1935) to increase the steady fluorescence of aquatic plants; this result was confirmed by Wassink, Vermeulen, Reman and Katz (1938), *cf.* fig. 28.49. According to Franck, French and Puck (1941), very high concentrations of carbon dioxide (*e. g.*, 20%) produce a similar effect. (It was stated in chapter 13 that the effect of excessive concentrations of carbon dioxide on photosynthesis resembles narcotization.)

The phenomenon was also studied in purple bacteria, by Wassink, Katz and Dorrestein (1942). Figure 28.50 shows typical results obtained in the presence and in the absence of reductants. The picture is similar to that with sodium azide: In the absence of reductants, the addition of increasing amounts of ethylurethan causes a progressive *quenching* (rather than stimulation) of fluorescence (although the effect is reversed at $>1.5\%$ urethan, at least below 15 kerg/cm.^{2} sec). In the presence of reductants, moderate quantities of urethan have little if any effect on the fluorescence curve, while quantities $>2\%$ have a strong *enhancing* effect.

(*h*) *Oxygen*

Kautsky, whose theory of an exclusive transfer of excitation energy to oxygen was described in Volume I, (page 514), was naturally interested in the quenching of the fluorescence of leaves by oxygen. However, neither Kautsky, Hirsch and Davidshöfer (1932), Kautsky and Hirsch (1935) nor Wassink, Vermeulen, Katz and Reman (1938) could find any distinct influence of changes in the external oxygen concentration (between 1 and

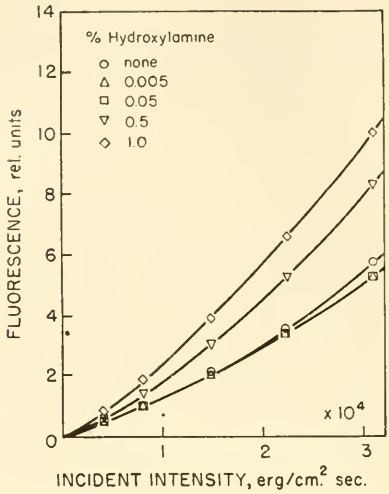


Fig. 28.46. Effect of $NH_4OH \cdot HCl$ on fluorescence of *Chromatium* (after Wassink, Katz and Dorrestein 1942). 5% CO_2, 15% H_2, *p*H 7.6, 29° C.

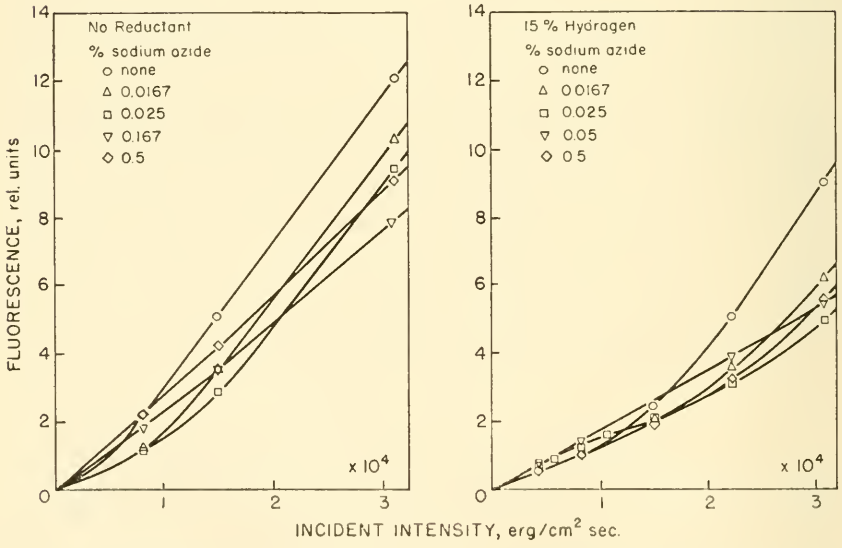


Fig. 28.47. Effect of NaN_3 on fluorescence of *Chromatium* (after Wassink, Katz and Dorrestein 1942). 5% CO_2, *p*H 6.3, 20° C.

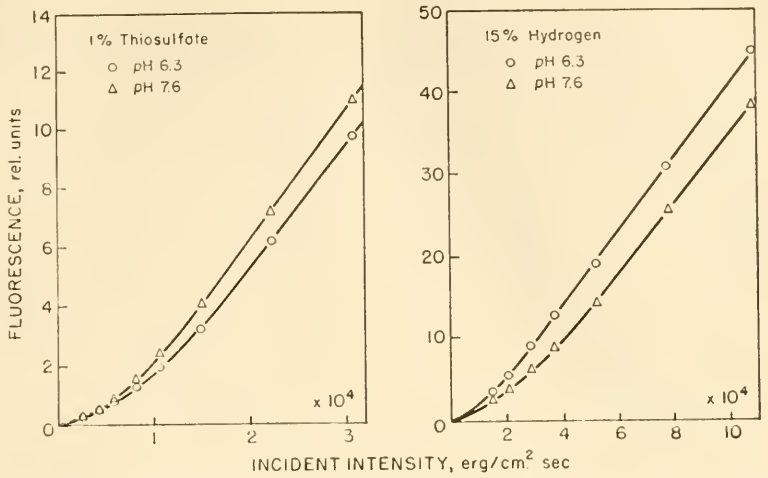


Fig. 28.48. Effect of *p*H on fluorescence of *Chromatium* (after Wassink, Katz and Dorrestein 1942). 5% CO_2, 29° C.

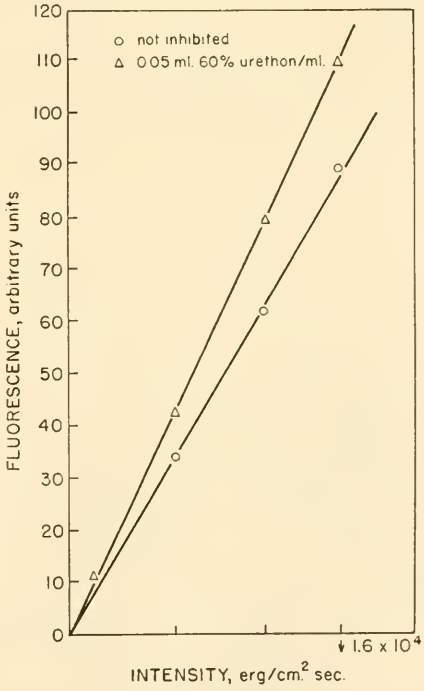


Fig. 28.49. Influence of ethylurethan on fluorescence of chlorophyll in suspension of *Chlorella* (after Wassink, Vermeulen, Reman and Katz 1938).

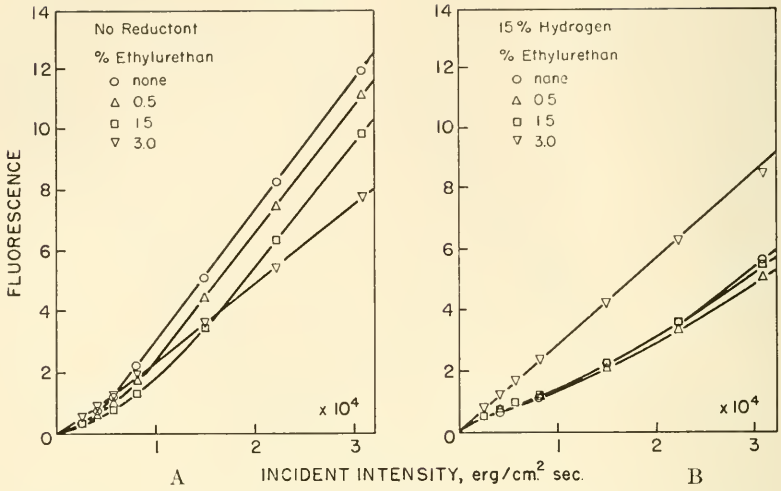


Fig. 28.50. Effect of ethylurethan on fluorescence of *Chromatium* in the absence and presence of reductants (after Wassink, Katz and Dorrestein 1942). 5% CO_2, *p*H 7.6, 29° C.

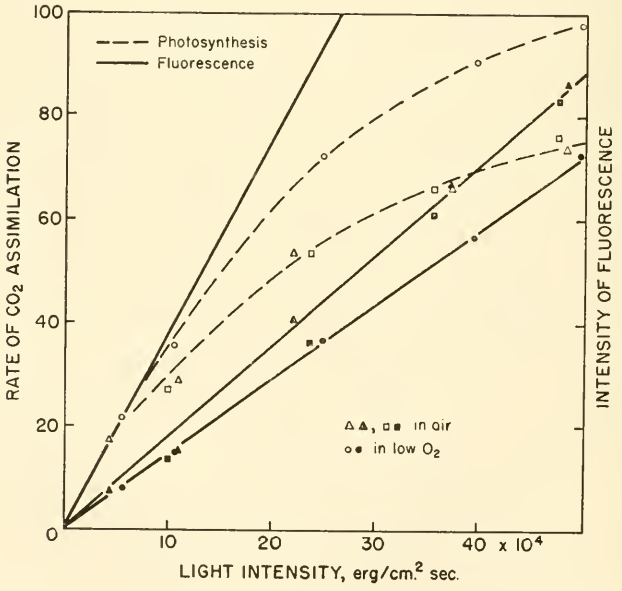


Fig. 28.51. CO_2 assimilation and fluorescence *vs.* incident light intensity (after McAlister and Myers 1940). Solid symbols, intensity of fluorescence, *F;* open symbols, rate of CO_2 uptake, *P*.

100%) on the yield of steady fluorescence of leaves and algae. McAlister and Myers (1940) found that an increase in oxygen concentration from 0.5 to 20% resulted in a marked *increase* in fluorescence (*cf.* fig. 28.51)—an effect opposite to quenching, and probably associated with the inhibiting influence of oxygen on photosynthesis, described in Volume I (chapter 13).

Shiau and Franck (1947) noted that, at low light intensities, fluorescence of green algae was stronger in nitrogen than in air, but that the increase of φ with increasing light intensity began earlier in air. In some cases the two curves even crossed each other, so that in strong light the aerated suspension fluoresced stronger than the nonaerated one.

We have spoken in this chapter only of changes in chlorophyll fluorescence caused by *internal* chemical transformations associated with photosynthesis—a relation that reveals itself *indirectly*, by comparison of the influences of light intensity, temperature and poisons on the yields of photosynthesis and fluorescence. *In vitro*, quenching of chlorophyll fluorescence can be produced *directly*, by the addition of certain substances undergoing autoxidation, as well as of many oxidants, including free oxygen (chapter 23, section A6). No observations have been made on the quenching of chlorophyll fluorescence *in vivo* by amines (or other possible substrates of sensitized photoxidation), while the effect of oxygen was described above as complex and probably mostly indirect.

Shiau and Franck (1947) found that quinone depresses fluorescence in *Chlorella*, if added in the dark or in light after long anaerobic incubation.

3. Interpretation of Light Curves of Fluorescence

In absence of positive information to the contrary, we have assumed, throughout the preceding sections, that all the observed changes of fluorescence were increases and decreases in the fluorescence yield, φ, without significant shifts in the position of the fluorescence bands. This point could, however, profit by exact investigation. Changes in the structure of the chlorophyll complex (*e. g.*, conversion of $X \cdot Chl \cdot HZ$ to $HX \cdot Chl \cdot Z$, not to speak of reversible hydrogenation, $Chl \rightleftharpoons rChl$) could well find expression in the variation of the position and shape of the fluorescence bands; and the selective spectral sensitivity of the photometric devices used could convert these changes into apparent variations in the *intensity* of fluorescence. While it is extremely unlikely that such spectral effects were responsible for all or even a large fraction of the described intensity changes, it might be unwise to ignore their possibility.

True changes in the intensity of fluorescence can be caused by two factors: alterations in the relative probabilities of fluorescence and energy dissipation in the light-absorbing complex, and changes in the probability

of the primary photochemical process. As described previously (*cf.* Vol. I, page 546, chapter 23.A8, and chapter 24, the three processes—energy dissipation (rate constant, k_i; quantum yield, δ), chemical transformation (rate constant, k_i; quantum yield, γ) and fluorescence (rate constant, k_f; quantum yield, φ)—compete for the absorbed light energy. If all three competing processes obey the law of monomolecular reactions, their quantum yields are determined by equations of the type:

$$\varphi = k_f/(k_f + k_t + k_i) \tag{28.49}$$

(*cf.* equation 19.8). If the primary photochemical process requires encounters with a kinetically independent reaction partner, A, the quantum yield equation becomes:

$$\varphi = k_f/(k_f + k_A^*[A] + k_i) \tag{28.50}$$

where k_A^* is a bimolecular rate constant (*cf.* equation 23.18, p. 797). In photosynthesis, we assumed the primary photochemical process to be a tautomerization (such as $X\cdot Chl\cdot HZ \xrightarrow{+h\nu} HX\cdot Chl\cdot Z$, or $ACO_2\cdot Chl\cdot A'H_2O \xrightarrow{+h\nu} AHCO_2\cdot Chl\cdot A'HO$, or $ACO_2\cdot Chl\cdot A'H_2O \xrightarrow{+h\nu} ACO_2\cdot ChlH\cdot A'HO \xrightarrow{+h\nu} AHCO_2\cdot Chl\cdot A'HO$). Since tautomerization is a monomolecular process, equation (28.49) can be used; changes in φ are thus indicative of variations in the composition or structure of the photosensitive complex, which affect the rates k_t and k_i. (The fluorescence rate constant, k_f, itself remains practically unchanged as long as the intensity of the absorption band is not changed significantly, since both are determined by the transition probability between the ground state and the excited state.) One could, of course, also consider the possibility of fluorescence quenching by collisions with alien molecules (*i. e.*, the addition of "bimolecular" terms in the denominator of eq. 28.49), since this is often observed in fluorescent gases and solutions; however, it seems plausible that changes in fluorescence associated with photosynthesis are due to changes within the chlorophyll complex, rather than to the formation or disappearance of new kinetically independent quenching substances. The "natural" life-time of the excited state of the chlorophyll molecule has been estimated (*cf.* page 534) as of the order of 8×10^{-8} sec.; and the low yield of fluorescence *in vivo* (order 0.1%) indicates that the actual life-time is about one hundred times shorter, or $\sim 8 \times 10^{-10}$ sec. To produce, under these conditions, a marked effect on the intensity of fluorescence by kinetic encounters the quenching molecules must occur in concentrations high enough for the encounter intervals to be not much longer than 10^{-10} sec.; and this requires concentrations of the order of at least 0.01 and more probably 0.1 mole per liter. It seems unlikely that such high concentrations of freely moving molecules of reaction products should actually arise and disappear during photosynthesis.

The relationship between the yield of photosynthesis and the yield of fluorescence has often been presented as a simple either-or; if this were correct, then whenever P increases, F should decrease and *vice versa*. In fact, however, φ is much too small ($\simeq 0.01$) to be an effective competitor of γ ($\simeq 1$ for the primary process, although as many as 4 or 8 primary processes may be needed to reduce one molecule of carbon dioxide). The actual competition is between φ and δ, between the primary photochemical reaction and the dissipation of energy. The yield of fluorescence can be considered an index of the value to which these two much more efficient processes together have reduced the life-time of the excited chlorophyll molecules. In other words, we can write, in good approximation, instead of (28.48):

(28.51) $\varphi \simeq k_f/(k_t + k_d)$ and also $\gamma \backsimeq k_t/(k_t + k_d)$; $\delta \simeq k_d/(k_t + k_d)$

Equation (28.51) shows that φ does not necessarily increase by the same amount by which γ decreases (or *vice versa*), since simultaneous changes in δ can change not only the absolute magnitude of the effect but even its sign. If k_t decreases—*e. g.*, if the primary photochemical reaction becomes altogether impossible, while k_d remains more or less unchanged, fluorescence must of course increase; but if simultaneously with the decline of k_t, k_d is strongly increased, in other words, the dissipation of light energy is strongly accelerated by the change in the structure of the pigment complex, the yield of fluorescence, φ, may decline parallel with the yield of the photochemical transformation, δ.

In discussing the theory of light curves of photosynthesis in section A7 of this chapter, we assumed that normally the photosensitive chlorophyll complex has the composition X·Chl·HZ (or ACO_2·Chl·A′HR, if we postulate direct association of chlorophyll with bound carbon dioxide and the reductant). In intense light or when one or both of the reactants, CO_2 and HR, are absent, or when the preparatory catalysts are poisoned, the chlorophyll complex may go over predominantly into a tautomerized or chemically changed state. If the normal state is X·Chl·HZ, the likely changed states are HX·Chl·Z (tautomeric), HX·Chl·HZ (reduced state) and X·Chl·Z (oxidized state); if the normal state is ACO_2·Chl·A′RH, the possibilities include, in addition to the tautomeric, oxidized and reduced states, also three "starved" states, namely A·Chl·A′R (carbon dioxide-starved), ACO_2·Chl·A′ (reductant-starved) and A·Chl·A′ (totally starved state).

Furthermore, one can envisage states in which CO_2 or RH are not merely missing, but are replaced by other molecules—either also suitable to serve as hydrogen acceptors or donors (such as oxygen, or the "substitute oxi-

dants" and "substitute reductants" considered in chapter 8; see, for example, page 543, Vol. I) or inert, such as various "narcotics." The latter may perhaps displace from the chlorophyll complex not only the reactants, CO_2 or RH, but even their "carriers," A and A′. Franck and co-workers (1941–1950) concluded, mainly from studying induction phenomena (chap. 33), that narcotizing substances are formed within the cell, *e. g.*, in the dark, by fermentation, particularly under anaerobic conditions, and in light, by photoxidation, or by the mechanism which we have repeatedly described previously—the reaction of the accumulated oxygen precursors, "photoperoxides," with oxidizable cellular constituents, a reaction that occurs whenever the removal of these peroxides is too slow, *e. g.*, in consequence of insufficient concentration of the enzyme E_c. This "self-narcotization" is considered by Franck the main cause of the most striking changes in fluorescence—when φ increases by a factor of two or more (*cf.* fig. 28.50). Many instances of an increase in the yield of fluorescence by adsorption of "protective" substances have been observed *in vitro*, although it is by no means a general rule that *all* adsorption increases fluorescence. In chapter 23 (page 776) we have seen that association with such substances as lecithin or oleic acid protects the fluorescence of chlorophyll, while adsorption on starch, alumina or proteins quenches it more or less completely. It seems likely that it is the nature and orientation of the forces between the pigment molecule and the adsorbent that determine whether the effect of these forces is to permit the excitation energy to spread over a larger number of degrees of freedom, including those of the associate molecules, and thus facilitate its conversion into heat, or whether their effect is to orient and stiffen some otherwise freely vibrating or rotating parts in the pigment molecule itself, thus making the dissipation of energy within it more difficult. Another way in which complexing or adsorption may delay internal dissipation of excitation energy, offers itself if dissipation occurs only after the excited molecule assumes a certain configuration (corresponding to the crossing point of two potential energy curves in the diatomic model). Temporary dissipation of the excitation energy over a larger number of degrees of freedom (which becomes possible when the molecule is complexed or adsorbed) can lengthen the average time needed by the molecule to reach the "critical" configuration.

With chlorophyll fluorescence *in vivo*, the effect of a typical narcotic ethylurethan was found to consist in an increase in yield of fluorescence in *Chlorella* by about 25% (fig. 28.49), in a quenching of fluorescence of *Chromatium* in the absence of reductants (fig. 28.50A), and a considerable enhancing effect (up to about +40%) on the same fluorescence in the presence of a reductant (fig. 28.50B). (The yield of fluorescence in the presence of 3% urethan is the same with and without the reductant—about halfway

between the two yields without the narcotic.) Franck suggested that "self-narcotization" may be a protective device of major importance for the preservation of plants from destructive photochemical reactions (such as photoxidations) that are likely to occur whenever the photosynthetic apparatus is for some reason or another prevented from working on its normal substrates.

It is obvious from this discussion that we should not be astonished to find rather complicated changes in the yield of fluorescence when we accelerate or retard photosynthesis by changing external factors such as light intensity or the concentrations $[CO_2]$ or [RH], or by adding various inhibitors.

As discussed previously on page 1013, one would expect, in general, that, whenever photosynthesis is limited or slowed down by "starvation" (*i. e.*, by the slowness of a preparatory dark reaction), the composition of the chlorophyll complex will change, the photosensitive form will be used up, k_t will therefore go down and φ will increase correspondingly—unless k_d increases simultaneously and so strongly that its increase overcompensates the decrease of k_t (*cf.* equation 28.51). On the other hand, if photosynthesis is limited or slowed down by "constipation" *i. e.*, by the slowness of a "finishing" dark reaction, the composition of the chlorophyll complex will remain unaffected and fluorescence yield will show no change, unless one is produced *indirectly* by the "self-narcotization" postulated by Franck.

It should be recalled here that, according to the criterion we have established to distinguish preparatory from finishing dark reactions (page 1013), slow return of the photochemically changed form of the chlorophyll complex into the normal photosensitive form must be considered a *preparatory* reaction (since its slowness reduces the rate of the primary photochemical process). This remains true whether this restoration occurs through a "forward" reaction with the oxidant $\{ACO_2\}$ or the reductant ($\{A'H_2O\}$, or H_2R in purple bacteria); or by reaction with an intermediary catalyst such as (28.41b), or by "primary back reaction" such as (28.41a′). Many of the mechanisms of light saturation which we have considered involved the transition of the chlorophyll complex in strong light into a changed (tautomerized, oxidized, reduced, denuded or narcotized) form. The accumulation of this form—which we assume to be photochemically inert ($\gamma = 0$)—is held responsible for changes in the fluorescence yield observed in strong light. We will designate this inactive form generally as {Chl}. If the quantum yield of fluorescence of the photosensitive form of the chlorophyll complex is φ_1, and that of the inactive form {Chl} is φ_2, and the absorption coefficients (and the spatial distribution) of the two forms are the same, then the observed yield of fluorescence ($\varphi = F/I$; number of quanta emitted per quantum absorbed) is:

$$\varphi = \varphi_1 + (\varphi_2 - \varphi_1)\frac{[\{\mathrm{Chl}\}]}{\mathrm{Chl}_0} \tag{28.51A}$$

The fluorescence *yield* light curves $\varphi = f(I_a)$ are thus linear derivatives of the function $[\{\mathrm{Chl}\}] = f(I_a)$. In practice, we use as independent variable, the incident light intensity I; since we assume uniform light absorption (*i. e.*, an optically thin system), we have (*cf.* equation 28.33) $I_a = k^*I\mathrm{Chl}_0$. The relation of the fluorescence *intensity* curve $F = f(I_a)$ to the fluorescence *yield* curve $\varphi = f(I_a)$ is:

$$F = \varphi I_a = \varphi_1 I_a + (\varphi_2 - \varphi_1)\frac{[\{\mathrm{Chl}\}]I_a}{\mathrm{Chl}_0} \tag{28.51B}$$

(I_a = absorbed flux; F = emitted flux; I is measured in number of einsteins per sec. per cm.^{2}; Chl_0 in moles/liter.)

It is easily possible to derive fluorescence yield curves for those of the kinetic models discussed in section A7 in which light saturation is associated with the slowness of a *preparatory* dark reaction (including in this definition the reactions which restore the chlorophyll complex after its photochemical change).

We begin with the case in which strong light causes accumulation of the chlorophyll complex in the carbon dioxide denuded form (such as $\mathrm{A\cdot Chl\cdot A'H_2O}$), or in the totally reduced form (such as $\mathrm{HX\cdot Chl\cdot HZ}$), because of slow rate of reaction of carbon dioxide (with the acceptor, A, or with the reduced intermediate, HX). We can use here the simplest mechanism of this type (rather than the more elaborate mechanisms discussed in chapter 27), namely:

$$\mathrm{ACO_2\cdot Chl\cdot A'H_2O} \xrightarrow[+\mathrm{H_2O}]{k^*I} \mathrm{A\cdot Chl\cdot A'H_2O} + \{\mathrm{OH}\} + \{\mathrm{HCO_2}\} \tag{28.51Ca}$$

$$\mathrm{A\cdot Chl\cdot A'H_2O} + \mathrm{CO_2} \xrightarrow{k'} \mathrm{ACO_2\cdot Chl\cdot A'H_2O} \tag{28.51Cb}$$

or:

$$\mathrm{X\cdot Chl\cdot HZ} \xrightarrow[+\mathrm{H_2O}]{k^*I} \mathrm{HX\cdot Chl\cdot HZ} + [\mathrm{OH}] \tag{28.51Da}$$

$$\mathrm{HX\cdot Chl\cdot HZ} + \mathrm{CO_2} \xrightarrow{k'} \{\mathrm{HCO_2}\} + \mathrm{X\cdot Chl\cdot HZ} \tag{28.51Db}$$

The stationary concentration $[\{\mathrm{Chl}\}]$ (= $[\mathrm{A\cdot Chl\cdot A'H_2O}]$ or $[\mathrm{HX\cdot Chl.HZ}]$) is:

$$[\{\mathrm{Chl}\}] = \frac{k^*I\mathrm{Chl}_0}{k'[\mathrm{CO_2}] + k^*I} \tag{28.51E}$$

For the light intensity at which the yield of fluorescence, φ, is equal to the arithmetic mean of φ_1 and φ_2 we obtain:

$$^{F}_{1/2}I = \frac{k'[\mathrm{CO_2}]}{k^*} \tag{28.51F}$$

In this special case, photosynthesis reaches half-saturation at the same intensity as fluorescence. The rate of photosynthesis according to schemes (28.51C or D) is:

$$P = k'k^*I\mathrm{Chl}_0/(k'[\mathrm{CO}_2] + k^*I)$$

and the saturation rate is $P^{\max.} = k'\mathrm{Chl}_0$, which gives for ${}^{P}_{1/2}I$ the value (28.51F).

We can next consider the case discussed on page 1028, in which light saturation is due to the accumulation of chlorophyll in the reduced form, HX·Chl·HZ, because of slow *primary back reaction.* (In other words, we assume mechanism 28.21, with the specific assumption that reaction 28.21b is practically instantaneous.) This leads us (for the simplest case when the carboxylation equilibrium is undisturbed by photosynthesis) to equations (28.28) for the rate P, and equations (28.30) and (28.31) for the half-saturating light intensity of photosynthesis. The concentration of chlorophyll complexes in the inactive form is given in this case by equation (28.26); the midpoint of fluorescence transition again coincides with the half-saturation of photosynthesis.

We can further consider reaction mechanism (28.41) (with the simplifying assumption of instantaneous "reloading" with CO_2 and H_2O), in which light saturation is ascribed to the combined effects of slow restoration of the catalyst E_B (assumed to "stabilize" the first reduction product, HCO_2), and of the slow primary back reaction (28.41a′). The equation for the concentration of "inactivated" chlorophyll complexes is in this case quadratic, and its one significant solution is:

(28.51G) $$[\{\mathrm{Chl}\}] (= [\mathrm{AHCO_2{\cdot}Chl{\cdot}A'HO}]) =$$

$$- \frac{k'k'_e + k_ek'_eE^0_B - k_ek^*I\mathrm{Chl}_0 + k'_ek^*I}{2k_e(k' + k^*I)} + \left[\left(\frac{k'k'_e + k_ek'_eE^0_B - k_ek^*I\mathrm{Chl}_0 + k'_e\,k^*I}{2k_e(k' + k^*I)}\right) + \frac{k'_ek^*I\mathrm{Chl}_0}{k_e(k' + k^*I)}\right]^{1/2}$$

The midpoint of the fluorescence transition is:

(28.51H) $$ {}^{F}_{1/2}I = \frac{k'k'_e + k_ek'_eE^0_B + k_ek'\mathrm{Chl}_0/2}{k^*(k'_e + k_e\mathrm{Chl}_0/2)}$$

As could be expected, this midpoint coincides with the half-saturation of photosynthesis (28.47A) only in the case $k'_e \gg k_e\mathrm{Chl}_0$, when (28.51H) reduces itself to (28.47Ba). (This extreme case is practically identical with the one derived above from mechanism 28.21; in both of them, half-saturation is reached when one-half of all chlorophyll complexes are in the changed state—catalyst E_B not being fully utilized even in the light-saturated state.) ${}^{F}_{1/2}I$ differs from ${}_{1/2}I$ when the E_B-limitation is significant (*i. e.*, when k'_e is *not* $\gg k_e\mathrm{Chl}_0$).

In the other extreme case, when $k'_e \ll k_e Chl_0$, we have:

$$(28.51I) \qquad {}^{F}_{1/2}I = \frac{k_e k'_e E^0_B + k' k_e Chl_0/2}{k^* k_e Chl_0/2}$$

Comparison with (28.47C) shows that in this case

$$(28.51J) \qquad {}^{F}_{1/2}I = {}^{P}_{1/2}I \left(1 + \frac{k'_e k_e E^0_B + k' k_e Chl_0}{k'_e k_e E^0_B + 2k' k'_e}\right)$$

in other words, half-saturation of fluorescence occurs *later* (in fact, *much later*, because $k_e Chl_0 \gg 2k'$) than the half-saturation of photosynthesis.

Finally, we can derive the equations for φ and ${}^{F}_{1/2}I$ for the "narcotization" mechanism (28.47A). The photostationary concentration of "narcotized" chlorophyll [{Chl}] is:

$$(28.51K) \qquad [\{Chl\}] = -\frac{kk_e E^0_B - (k-2)k^* I Chl_0 + k'_n Chl_0}{2(k-1)(k'_n + k^* I)} + \left[\left(\frac{kk_e E^0_B - (k-2)k^* I Chl_0 + k'_n Chl_0}{2(k-1)(k'_n + k^* I)}\right)^2 + \frac{k^* I Chl_0^2}{(k-1)(k'_n + k^* I)}\right]^{1/2}$$

where k has the meaning defined in eq. (28.47Ha).

This expression reduces itself, as required, to [{Chl}] = 0 at $I = 0$, and to [{Chl}] = Chl_0 at $I = \infty$.

The equation for the midpoint of fluorescence transition, [{Chl}] = ½ Chl_0, is:

$$(28.51L) \qquad {}^{F}_{1/2}I = \frac{Chl_0 k'_n (k+1) + 2kk_e E_0}{k^*(k+1)Chl_0}$$

Comparison with equation (28.48) shows that fluorescence can be half-saturated either earlier or later than photosynthesis, depending on whether:

$$(28.51M) \qquad k\left(=\frac{k_e}{k_c}\,\frac{k'_n}{k_n}\right) \text{ is } < 1 \text{ or } > 1$$

(fluorescence is half-saturated when [{Chl}] = ½ Chl_0, photosynthesis is half-saturated in this model when [E_BOH] = ½ E^0_B; the two conditions are satisfied at the same light intensity only when $k = 1$).

The curves representing fluorescence intensity, F, as function of light intensity can be derived from the various expressions we have obtained for [{Chl}] by inserting them into equation (28.51B). The resulting equations are either second or third degree, indicating that the curves $F = f(I)$ are either hyperbolae or third order curves. They begin at $I = 0$ with the slope φ, and approach at high I-values the slope φ_2 (lower part of fig. 28.27).

A number of experimental fluorescence curves of this type are reproduced earlier in this chapter (*e.g.*, figs. 28.29, 43–48, 50). We are more interested, however, in fluorescence yield curves, $\varphi = f(I)$; a plot of this kind is shown in the upper part of figure 28.27. This figure indicates that the "critical" intensity, I_c, as defined by Wassink and co-workers (fig.

28.27) is quite different from the "midpoint" ${}^{F}_{1/2}I$—the latter is situated at much higher light intensities. The φ-curves too, often are hyperbolae; this is, for example true for the kinetic models leading to equations (28.51E) (slow reaction with CO_2), and (28.22) (slow primary back reaction). On the other hand, equations (28.51G) (E_B-limitation) and (28.51K) ("narcotization") indicate *cubic* equations for $\varphi = f(I)$. This is of interest in connection with Franck's idea of "self-regulation" of photosynthesis: Franck envisages a narcotization mechanism which would not affect photosynthesis (and fluorescence) at low light intensities, but would become operative rather suddenly when a finishing dark reaction ceases to be able to cope with the photoperoxides produced by the primary photochemical process, and would shut off a part of the chlorophyll apparatus sufficient to reduce the formation of photoperoxides to the amount which the limiting reaction can handle. The assumption of a self-regulating mechanism of this kind is a tempting hypothesis, because of the general importance which "feedbacks" and "servomechanisms" have acquired in mechanical interpretations and imitations of life processes. If such a mechanism were operative, the curves $[\{\mathrm{Chl}\}] = f(I)$ (and with this, also the curves, $\varphi = f(I)$) would have to have *sigmoid* shapes as indicated by dotted line in figure 28.27.

It was shown above that half-saturation of fluorescence will occur simultaneously with the half-saturation of photosynthesis whenever the latter corresponds to one-half of all chlorophyll complexes being in the inactive state. This will be the case, *e. g.*, when saturation is caused by insufficient supply of carbon dioxide (or other reactants). On the other hand, if saturation is caused by slow removal of photoproducts from chlorophyll (*e. g.*, by E_B-limitation in mechanism 28.41), we can expect half-saturation of fluorescence to require *stronger* light than half-saturation of photosynthesis. This seems to be the case in figure 28.26 (*Hydrangea* leaf) where half-saturation of φ occurs at about 60 kerg, and that of P at about 30 kerg.

We will now review briefly the experimental results described in sections 1 and 2 (*cf.* figures 28.24–28.51) in the light of these theoretical concepts. The occasionally observed light saturation of photosynthesis unaccompanied by changes in the yield of fluorescence (illustrated most strikingly by fig. 28.24) must indicate that saturation was caused by a *finishing* dark reaction that did not affect the composition of the chlorophyll complex, either directly or indirectly (through the formation of a "narcotic"). This may be an example of pure "catalyst B" limitation, and saturation by *secondary* back reactions. It can be asked whether, under these conditions, a change in φ will be observed at some higher intensity in the saturation region, when the primary process becomes too fast for some preparatory reaction to keep pace with it. Whether this is the case might depend on whether the back reactions give products suitable for direct use in the primary photochemical process, or products that have to undergo again the slow preparatory catalytic reactions. For example, if the back reaction is:

(28.52) $$AHCO_2 + A'HO \longrightarrow ACO_2 + A'H_2O$$

or:

(28.53) $$AHCO_2 \cdot Chl \cdot A'HO \longrightarrow ACO_2 \cdot Chl \cdot A'H_2O$$

and if no exhaustion of the photosensitive form has occurred at the time light saturation has been produced by E_B deficiency, there is no reason why such exhaustion should occur if the light intensity is stepped up still further, since the products of the back reaction are ready to participate again in the primary photochemical reaction. Experimentally, an increase of fluorescence at "supersaturating" light intensities has been noted in several cases discussed above. This could be explained by assuming, with Franck, that the back reaction liberates so much energy as to cause the reversal not only of the first oxidation-reduction step, but also of the carboxylation reaction:

(28.54) $$AHCO_2 + A'HO \longrightarrow A + CO_2 + A'H_2O \text{ (or } A + CO_2 + A' + H_2O)$$

or:

(28.55) $$AHCO_2 \cdot Chl \cdot A'HO \longrightarrow A \cdot Chl \cdot A'H_2O + CO_2 \text{ (or } A \cdot Chl \cdot A' + CO_2 + H_2O)$$

In this way, the products of the back reaction are added to the pool of free carbon dioxide and water rather than to the immediately available substrates of the primary photochemical process, ACO_2 and $A'H_2O$.

We recall that this hypothesis was first suggested by Franck to explain an entirely different observation—the "carbon dioxide burst" sometimes observed in the first minutes of illumination (*cf.* Vol. I, page 207, and chapter 29, page 1093).

When the yield of fluorescence goes up with increasing light intensity, as in figure 28.25, and reaches a new steady value, φ_2, in the region of the light saturation of photosynthesis (fig. 28.26), this can be taken as a sign that saturation is due to a preparatory dark reaction; it is thus understandable why, in McAlister's experiment represented in figure 28.25, this change was observed in a CO_2-deficient medium and not in 5% CO_2.

The results obtained by Wassink and Kersten with *Nitzschia* (fig. 28.28) are puzzling. The fact that above 50 kerg/cm.^{2} sec. φ *decreases* rather than increases with light intensity could be formally explained by assuming that, in this organism, the form of the chlorophyll complex that accumulates during intense photosynthesis has a higher value of k_i (*i. e.*, dissipates energy more rapidly), so that the sum $k_t + k_i$ increases in strong light even if k_t declines to zero. What is more difficult to explain is that in the absence of carbon dioxide the diatoms retain the high yield of fluorescence (φ_1) in strong light, while one would offhand expect that, in this case, the *lower* value (φ_2) would prevail from the very beginning. (It was mentioned

on page 1051 that the curves would be easier to understand if the designations "with carbon dioxide" and "without carbon dioxide" were exchanged!)

In the case of purple bacteria, several states seem to be needed to explain the light curves of fluorescence. First of all, the low value of φ in weak light (the sigmoid shape) needs interpretation. It is probably associated with the substitution of intercellular hydrogen donors for the external reductants, which occurs while photosynthesis is slow. The coincidence of the two curves in figure 28.29 seems to indicate that, when photosynthesis is prevented by the absence of reductants, either chlorophyll accumulates in one and the same form in the presence and in the absence of carbon dioxide, or the two forms (*e. g.*, HX·BChl·Z and X·BChl·Z) accumulated under these conditions have a practically identical rate of energy dissipation, k_i. In the presence of reductants, the two forms accumulated with and without carbon dioxide (perhaps X·BChl·HZ and HX·BChl··HZ) possess, to the contrary, a very different fluorescence capacity. However, as the light intensity is increased, a further change in the composition of the complex occurs, leading to the crossing of the curves with and without carbon dioxide. Figures 28.31–28.35 confirm that the absence of reductants causes (in the presence as well as in the absence of carbon dioxide), the accumulation of a form with considerably increased capacity for fluorescence (which may be X·BChl·Z, or ACO_2·BChl·A′).

An alternative explanation of the effect of reductants on fluorescence can be given on the basis of Franck's concept of "self-narcotization." Franck assumes that reductants such as hydrogen or thiosulfate intervene in bacterial photosynthesis by reducing the "photoperoxides" formed by the primary photochemical process. If the reductants are deficient, the peroxides accumulate and produce the "narcotic," that blankets the chlorophyll and causes fluorescence to become stronger. The absence of CO_2 has less effect in bacteria because they are studied under anaerobic conditions, permitting no photoxidation. Wassink, Katz, *et al.* (1938, 1942, 1949) explained the effect of reductants by assuming that an "energy acceptor," capable of taking light energy over from bacteriochlorophyll, thus quenching its fluorescence, can be formed exclusively by enzymatic transformation of the reductants. They followed that CO_2 must have no effect on fluorescence at all—which is not true.

The effects of *cyanide* and of *low temperature* on fluorescence (and photosynthesis) can often be explained by assuming that the primary effect of both is the retardation of the carbon dioxide supply processes. However, we have seen, in part A, that not all the experimental results on cyanide

inhibition agree with this simple explanation; the same is true of the fluorescence measurements. It is, for example, not clear why the concentrations of cyanide needed to markedly affect fluorescence are so much higher than those needed to inhibit photosynthesis. These and the results obtained with several other poisons and narcotics require so many *ad hoc* explanations that we do not want to attempt them here. Parallel measurement of gas exchange and fluorescence in the presence of various inhibitors seems to be a very promising approach to the unravelling of the complex happenings in the photosensitive chlorophyll complex; but the presently available results are hardly sufficient to warrant a detailed attempt.

Franck—to whom we owe both the fundamental concepts of fluorescence and the demonstration of how these concepts can be usefully applied to the study of photosynthesis—has written two reviews of this subject (1949, 1951), which contain many observations and interpretations that could not be covered in the above presentation.

Bibliography to Chapter 28

Light Factor I. Light Intensity

A. Light Curves of Photosynthesis

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Chapter 29

THE LIGHT FACTOR. II. MAXIMUM QUANTUM YIELD OF PHOTOSYNTHESIS*

In analyzing the light curves of photosynthesis in chapter 28, we did not discuss the *slope* of the initial linear part. This is a particularly important quantity, since it determines the *maximum quantum yield* of photosynthesis and the *maximum conversion of light into chemical energy* achieved in this process. The present chapter will deal with these two subjects.

The definition of maximum quantum yield as the limiting slope of the light curve at low light intensities implies that this curve has no inflection. An inflection has often been observed in the light curves of purple bacteria, but it has usually been assumed that the light curves of algae and higher plants show no such complication. Certain recent observations (Kok, van der Veen) lead, however, to new doubts concerning the shape of the light curves below the compensation point; we will discuss these observations and their possible significance for the determination of the maximum quantum yield later in this chapter (page 1113).

If the yield of photosynthesis is expressed in moles of reduced carbon dioxide (or liberated oxygen), P, and the light absorption, I_a, is given in einsteins of absorbed photons, the ratio $\gamma = P/I_a$ is the *quantum yield.* (In many papers on photosynthesis, and photochemistry in general, the quantum yield is designated by the letter φ, which we reserved—(*cf.* Vol. I, page 546)—for the quantum yield of *fluorescence.*

If the yield is measured by the energy content (heat of combustion) of the produced carbohydrates, $-\Delta H_c$, and I_a is given in calories, the ratio $\epsilon = -\Delta H_c/I_a$ can be called the *energy conversion factor.* The relation between γ and ϵ is:

$$\epsilon = -\Delta H_m \gamma / N_A h\nu = 3.96 \times 10^{-3}\, \lambda_{m\mu} \gamma \backsimeq 4 \times 10^{-3}\, \lambda_{m\mu} \gamma \tag{29.1}$$

where ΔH_m is the molar heat of combustion of one $\{CH_2O\}$ group in carbohydrates (approximately 112 kcal, or 4.69×10^{12} erg); N_A, Avogadro's number (6.02×10^{23}); h, Planck's constant (6.55×10^{-27}); and ν, the frequency of light ($3.00 \times 10^{17}/\lambda_{m\mu}$).

The concept of the "quantum yield" of a photochemical process arose from Einstein's application of quantum theory to photochemistry in 1913. Einstein suggested, in elaboration of Planck's concept of vibrational energy quanta of electrons in atoms and molecules, that light energy, too, consists

* Bibliography, page 1139.

of finite quanta (photons); from this, he deduced that the number of molecules, N, changed photochemically by the absorption of a certain amount of light, must be equal to the number of absorbed photons, $N_{h\nu}$ (Einstein's law of photochemical equivalency). In the following years, rapidly accumulating rate measurements of photochemical reactions made it clear that Einstein's principle applies only to the *primary* photochemical process, while the observed over-all rates of photochemical reactions usually depend on the efficiency of secondary reactions, which follow the primary photochemical step. Over-all rate measurements therefore only seldom lead to straightforward confirmation of the equivalency law (in other words, the empirical quantum yields usually are smaller—or larger—than unity).

Photosynthesis, as the most important photochemical process in nature, naturally came under scrutiny from the point of view of its quantum yield. The problem appeared particularly intriguing because of the strongly endothermal character of the photosynthetic reaction. It could easily be calculated that one quantum of visible light (energy available: 40–60 kcal/einstein) is insufficient to convert one molecule of carbon dioxide (and one molecule of water) into a link in the carbohydrate chain and a molecule of oxygen (energy needed: about 112 kcal/mole). It was obvious that several quanta must cooperate in the reduction of one molecule of carbon dioxide. The question was: how many (or rather: how few—since, from the point of view of reaction mechanism, we are above all interested in the *maximum* quantum yield obtainable under the most favorable conditions).

According to equation (29.1), the answer to this question meant also the determination of the maximum efficiency of plants as converters of light energy into chemical energy. Over a century ago, in 1845, Robert Mayer recognized that storage of light energy by conversion into chemical energy is a most important aspect of plant activity on earth (*cf.* Vol. I, chapter 2). We have described in the preceding chapter several investigations in which the yield of this conversion was measured over considerable periods of time, and concluded that under natural conditions it is rather low—of the order of 2–5%.

It was known, however, since Reinke's investigation in 1883 (*cf.* page 964) that the light curves of photosynthesis are convex; the curvature sometimes becomes apparent even at very low light intensities (*cf.* chapter 28, section A2). This means that the energy conversion efficiency and the quantum yield increase as light intensity decreases. Warburg and Negelein (1922) set out to determine the maximum quantum yield by measuring the yield in very low light. Their work marked the beginning of a new stage in the quantitative study of photosynthesis.

The inverse of the quantum *yield* (or quantum *efficiency*, which is the same thing), is called the *quantum requirement*. Regrettably, the first term is often used when the second one would be appropriate—for example, it is said that "the quantum yield of photosynthesis is 4" (or 8, or some other number, where $n > 1$), instead of saying that it is $^1/_4$ (or $^1/_8$, or, generally, $1/n$).

1. Quantum Yield Measurements by the Manometric Method

Most quantum yield determinations of photosynthesis were carried out by the manometric method described in chapter 25 (see fig. 25.3A). In this method, the change of gas pressure is measured first above a darkened, and then above an illuminated cell suspension. The net effects observed are the result of pressure changes due to the production and consumption of both carbon dioxide and oxygen. If both the respiratory quotient and the photosynthetic quotient are unity, the net pressure changes are different from zero only because of the greater solubility of carbon dioxide in water (or, still more, in alkaline buffers), as compared with that of oxygen. To obtain a check on the two quotients, the measurement can be repeated, in darkness and in light, with a different ratio of gas-filled and liquid-filled volumes (*cf.* fig. 25.3B).

(*a*) *Investigations of Warburg and Negelein*

Warburg and Negelein (1922, 1923) were the first to apply the manometric method. They worked with suspensions of the unicellular green alga *Chlorella*. (The species was described by them as *Chlorella vulgaris*, but subsequent experience makes it uncertain whether it was this species, or *C. pyrenoidosa*.) To avoid the difficulties of the measurement of light absorption in plants caused by scattering (*cf.* chapter 22), they used dense suspensions, absorbing practically all the incident light. Consequently, at any given moment, most of the cells were shaded, and their contribution to photosynthesis was small; on the other hand, all cells contributed equally to respiration. For this reason and because of the low light intensities used (of the order of 1000 erg/cm.^{2} sec.), the total volume of respiration was larger than that of photosynthesis. (In other words, Warburg and Negelein worked below the compensation point.) They noted that the respiration of *Chlorella* was markedly stimulated by prolonged exposure to light (*cf.* chapter 20, page 564). In order to avoid such changes in respiration during the experiment, Warburg used illumination periods of not more than 10 minutes, separated by equal or longer periods of darkness. Because of the sluggish response of the manometer to changes of gas concentration in the liquid, the determination of the gas exchange

generally requires an interpolation, illustrated by figure 29.1. The uncertainty caused by this interpolation is unimportant for extended illumination periods, but can markedly affect the results obtained in short experiments, for example, experiments of 5 or 10 minutes. Furthermore, short illumination periods increase the importance of *induction* phenomena. Warburg knew from his earlier work (*cf.* chapter 33) that an "induction loss" (*i. e.*, delayed onset of photosynthetic activity) can occur after dark periods of the order of several minutes, but he also knew that this effect disappears if the light intensity is reduced considerably below the saturation region (*cf.* fig. 33.8). Since in the quantum yield work very low

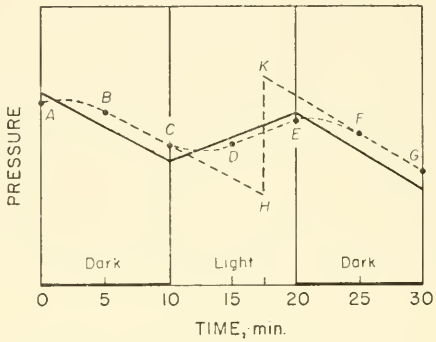

Fig. 29.1. Manometric determination of quantum yield (after Rieke 1939). Solid lines represent assumed course of photosynthesis and respiration; dotted curve, the pressure changes read from manometer. *KH* is interpolated yield of photosynthesis in 10 min.

light intensities were used, Warburg assumed that induction can be neglected. Subsequently, Emerson and Lewis (1939, 1941) found evidence of an induction phenomenon of a different kind, consisting in photochemical liberation of carbon dioxide during the first few minutes of illumination. This carbon dioxide "gush" or "burst" does *not* disappear with decreasing light intensity, and could be of particular importance in measurements in very low light.

An explanation of the carbon dioxide burst, suggested by Franck (1942), already was described in Volume I (page 167). This theory suggests that the "burst" is caused by the decomposition of the carbon dioxide–acceptor complex, ACO_2, accumulated in the dark. This decomposition follows the photochemical reduction of ACO_2 to $AHCO_2$, and the reversal of this reduction by back reactions, *e. g.*, in Franck's notation:

$$(29.2)\quad \{ACO_2 + HChl\} \xrightarrow[\text{light}]{\text{forward reaction}} \{AHCO_2 + Chl\} \xrightarrow{\text{back reaction}}$$

$$\{HChl + ACO_2^*\} \xrightarrow{CO_2\ \text{liberation}} HChl + CO_2$$

or, in the notation used in chapter 28 (pp. 1032, 1036, etc.):

$$(29.2a)\quad ACO_2\cdot Chl\cdot A'H_2O \xrightarrow[light]{\text{forward reaction}} AHCO_2\cdot Chl\cdot A'OH \xrightarrow{\text{back reaction}}$$
$$A'H_2O^{*}Chl\cdot ACO_2^{*} \xrightarrow{CO_2\ \text{liberation}} A\cdot Chl\cdot A' + H_2O + A + CO_2$$

Here, asterisks indicate that the compounds formed by back reactions contain considerable excess energy and therefore tend to decompose into their constituents. These back reactions normally occur only in saturating light (they are, in fact, supposed to be responsible for saturation); but in the first moment of illumination, practically *all* $AHCO_2$ formed (even the small amounts produced in weak light) undergoes back reaction, because during this "induction period," certain catalysts have not yet been "reactivated," and are unable to take care of the products of the first photochemical reaction.

A difficulty of this hypothesis is that, even with a 100% yield of the back reaction, the rate of production of ACO_2 in weak light must be small compared with the same rate in strongly oversaturating light. In the latter case, all intermediates formed in excess of the saturating rate are supposed to undergo back reactions; and yet, under appropriate supply conditions, no carbon dioxide limitation is observed, indicating that either the ACO_2 complexes formed by back reactions do not dissociate, or the recombination of A and CO_2 is so fast as to prevent any exhaustion of ACO_2 (in other words, the rate ceiling imposed by the formation of ACO_2 must be high compared with the full rate of the primary photochemical process and not only compared with the rate of the finishing dark reaction).

The total volume of the gush—which is about equivalent to the quantity of chlorophyll present in the cells—is in agreement with Franck's hypothesis; but the slow reabsorption of carbon dioxide in the dark (*cf.* fig. 29.3B, p. 1092) requires an explanation, since the time course of the "pick-up" (*cf.* Vol. I, fig. 22) indicates that the carboxylation equilibrium $CO_2 + A \rightarrow ACO_2$ usually is established in a few seconds. It may be noted that a similar difficulty was encountered in the attempt to attribute the uptake of radioactive carbon dioxide in the dark (fig. 21, Vol. I) to the same carboxylation process. Another problem is presented by the necessity of a high carbon dioxide concentration ($\geq 5\%$) for the "saturation" of the gush, since the shape of the carbon dioxide curves of photosynthesis indicates that the acceptor must be saturated with carbon dioxide even below 0.1% CO_2.

These discrepancies suggest that perhaps the gush and its reversal in the dark are manifestations of a carbon dioxide metabolism related to respiration and fermentation rather than to the first step of photosynthesis; but this hypothesis, in turn, fails to explain the apparent close relation of the carbon dioxide liberated in the gush to the chlorophyll complex (without such a relationship, a photochemical liberation of carbon dioxide with a high quantum yield would be difficult to understand).

The "cross-linking" of respiration and photosynthesis at an intermediate reduction level, between CO_2 ($L = 0$) and carbohydrate ($L = 1$), *e.g.*, on the level of oxalacetic or malic acids ($L = 0.625$ and 0.75, respectively), hypothesized by Calvin and co-workers (chapter 36), if confirmed, could explain how respiratory decarboxylations might be affected by reactions in the photochemical reaction sequence.

A carbon dioxide burst of the volume observed by Emerson and Lewis would be largely absorbed in carbonate buffers. As it was, Warburg and Negelein had decided that acid solutions (*e. g.*, water equilibrated with an

atmosphere containing 5% CO_2) offer a better promise of full photosynthetic efficiency, and chose to use them instead of the "unphysiological" alkaline buffers. Since only a small part of the carbon dioxide liberated in the burst can be caught in pure water, the larger part must escape into the gas space, and the consequent increase of pressure will be interpreted as increased photosynthesis, unless a check on the photosynthetic quotient, Q_P, reveals that the liberated gas is mostly carbon dioxide and not oxygen. It will be noted (*cf.* Vol. I, page 31) that we designate the photosynthetic quotient as Q_P and define it as the (positive) ratio $-\Delta O_2/\Delta CO_2$, while Warburg (and many others) designate the photosynthetic quotient as γ (using the symbol φ for the quantum yield), and define it as the (negative) ratio $+\Delta CO_2/\Delta O_2$.

The discovery of a carbon dioxide gush by Emerson and Lewis has made the interpretation of the results of Warburg and Negelein uncertain. The latter's quantum yield values, calculated from net pressure changes in 10 minute exposures, with the assumption $Q_P = 1$ (or more exactly, 1.09), turned out to be close to 0.25 or $1/4$. Offhand, this result seemed eminently satisfactory in consideration of the fact that the reduction of carbon dioxide by water involves the transfer of *four* hydrogen atoms (*cf.* chapters 3 and 7, Vol. I).

If the value $1/4$ had not been so plausible chemically, the fact that this high yield could be obtained only by following a specific schedule of experiments, combined with special methods of cultivation of the algae, would perhaps have attracted more attention. At first, using *Chlorella* cells grown in full light, Warburg and Negelein obtained only quantum yields of ≤ 0.06. Later they found much higher yields are obtainable with suspensions adapted to weak light. These experiments were carried out in yellow + orange light; high values of γ (up to 0.3) were obtained by extrapolation to $I = 0$, since the light curves bent markedly even below 1000 erg/cm.^{2} sec. In a second paper (1923), in which monochromatic light was used, the curvature was less pronounced and Warburg and Negelein calculated, without recourse to extrapolation, quantum yields ranging from 0.20 in blue, to 0.23 in red light, corresponding to energy conversion factors from 0.34 to 0.59. The least number of quanta of red light containing sufficient energy to cover the energy expenditure of the reaction $CO_2 + H_2O \rightarrow O_2 + \{H_2CO\}$ is three; the lowest plausible number of elementary reaction steps is four (corresponding to the transfer of four hydrogen atoms from water to carbon dioxide). From Warburg and Negelein's results, it appeared that plants are able to achieve photosynthesis with not more than four photochemical steps, leaving only a small margin to cover losses of energy by dissipation into heat, which appear inevitable in a complicated chemical process.

When theorists tried to devise a detailed mechanism of photosynthesis, plausible not only from the point of view of chemistry, but also from that of energetics, they found themselves badly hampered by the straight-jacket into which the limitation to four quanta had put them.

Franck and Herzfeld (1941), in particular, pointed out that, if the reduction of carbon dioxide has to be brought about by several consecutive photochemical steps (as in scheme 7.VA), the intermediates must have a certain degree of stability in order to avoid reoxidation while waiting for the supply of another quantum of light energy. Consequently, the "stabilization energy" of several intermediate products must be added to the energy requirements of photosynthesis; and a further allowance must be made for the heat of formation of the $\{CO_2\}$ complex and the heat of decomposition of a peroxide (which is the probable precursor of free oxygen evolved in photosynthesis).

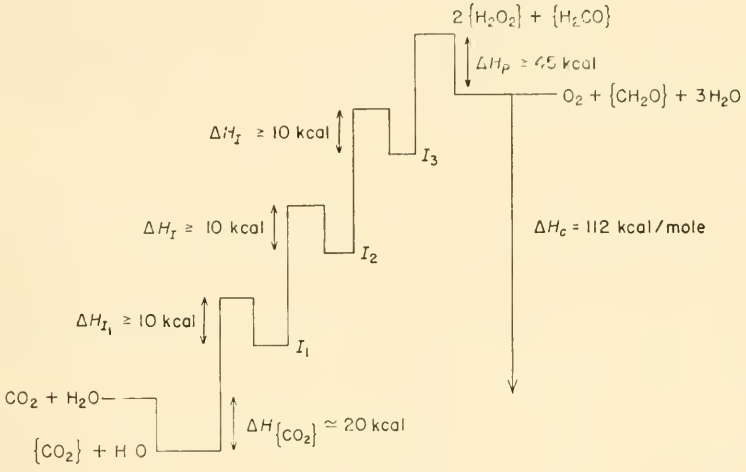

Fig. 29.2. Energy requirements of a linear four-stage mechanism of photosynthesis (after Franck 1941). Total energy required $\Delta H + \Delta H_c + \Delta H_{\{CO_2\}} + \Delta H_{I_1} + \Delta H_{I_2} + \Delta H_{I_3} + \Delta H_P = 210$ kcal./mole.

The energy relations in photosynthesis, according to Franck and Herzfeld, are illustrated by figure 29.2, in which $\Delta H_{\{CO_2\}}$ (the energy of formation of the carbon dioxide–acceptor complex), ΔH_{I_1}, ΔH_{I_2}, ΔH_{I_3} (the "stabilization energies" of three intermediates) and ΔH_P, the energy of stabilization of the end products (which includes the decomposition energy of the peroxide, $\{H_2O_2\}$) are shown as additional energy terms, which together with the accumulated chemical energy, ΔH_C, must be supplied by light.

The stabilization energy required for the prevention of "backsliding" of intermediates in periods of several minutes (which must pass, in weak light, in a dense suspension of green cells, between the absorption of two light quanta by one and the same chlorophyll molecule) must be of the order of 10 kcal/mole; the heat of formation of the $\{CO_2\}$ complex was estimated in chapter 27 as $\geq$20 kcal/mole. Altogether, three intermediates, the $\{CO_2\}$ complex and the primary peroxide, must increase the energy requirements of photosynthesis from 112 kcal/mole to probably as much as 210 kcal/mole—whereas 4 einsteins of red light (λ = 660 mμ) provide only 170 kcal.

The energy requirements are somewhat different if a "pyramidal" mechanism is postulated instead of the "linear" reaction sequence represented in figure 29.2 (*i.e.*, if it is assumed that four light quanta produce four *identical* pairs of intermediates, which then undergo dismutation by dark reactions, finally giving one pair of finished products, $[CH_2O] + O_2$; *cf.* Vol. I, pages 156, 158 and 164). In this case, the four quanta required to reduce one molecule of carbon dioxide can be absorbed by four different chlorophyll molecules. (The same result can be achieved by other physical or chemical mechanisms permitting a "collection of quanta" absorbed by several pigment molecules in one "reaction center." These "photosynthetic unit" theories will be presented in chapter 32.) In this case, the total energy requirement is obtained by adding to the accumulated energy (112 kcal/mole) approximately 20 kcal liberated in the formation of the $[CO_2]$ complex, and the energy amounts liberated in the several dismutations (or other "quanta-collecting" processes). It may be noted that one dismutation reaction (dismutation of a peroxide, yielding an oxide and free oxygen) was included also in the "linear" scheme. The dismutation of hydrogen peroxide liberates as much as 46 kcal per mole O_2 (Table 11.I, Vol. I); but dismutations of organic compounds, such as the Cannizzaro reaction, are less exothermal (about 10 kcal/mole; *cf.* Table 9.III, Vol. I). Even so, three such dismutations, together with one dismutation of a peroxide, will bring the total energy requirement of the "pyramidal" reaction scheme up to the same 210 kcal, which were estimated above for the "linear" reaction sequence.

For 16 years, the "4 quanta mechanism" of photosynthesis was the object of admiration and the source of headaches for those who approached the problem of photosynthesis from the point of view of energy conversion. During this time, no serious attempts were made to check the experimental foundations of this mechanism, and the results of Warburg and Negelein were considered final.

We will see in the next section that even during this time some measurements were made with the higher plants that gave considerably lower quantum yields; but because of less suitable objects and less precise methods, they were not considered to throw doubt on the validity of Warburg and Negelein's results. Beginning in 1938, however, a series of investigations appeared, in which the photosynthesis of the same algae as used by Warburg was studied by several methods (gas analysis, polarography and calorimetry) in the laboratories of the University of Wisconsin; these measurements gave rather widely scattered results, but the yields were invariably much lower than 0.25. The maximum quantum yields observed in this work (to be described in some detail in section 2) were of the order of 0.1. These publications induced several investigators to repeat Warburg and Negelein's determinations, adhering as closely as possible to the original technique.

Rieke (1949) used monochromatic light (mercury lines 546 and 578 $m\mu$) and an "integrating box" for the determination of light absorption. The pretreatment of the algae (adaptation to weak light), the light intensity (about 1000 erg/cm.^{2} sec.) and the illumination periods (10 minutes)

were the same as in Warburg's work. The quantum yields calculated from ten experiments at 578 mμ ranged from 0.18 to 0.24, and those calculated from six measurements at 546 mμ, from 0.17 to 0.20.

Thus, Rieke found Warburg and Negelein's results reproducible, but only by strict adherence, not merely to the original method of culturing of the algae, but also to Warburg's schedule of illumination. Wassink, Vermeulen, Reman and Katz (1938) noted another peculiarity—the quantum yields determined according to the procedure of Warburg and Negelein, were affected by temperature—they increased from 0.11 to 0.20 when the temperature was changed from 0° to 29° C.

Another peculiar observation was made by Rieke, who found that the *kind of water* used in the preparation of the algal culture had an effect on the quantum yield. Emerson and Lewis (1938, 1939) confirmed this. They used water from seven different sources, and obtained, under otherwise identical conditions, variations in the quantum yield from 0.16 to 0.27.

The lowest values were obtained in glass-distilled water; addition of a stock mixture of "microelements" (B, Zn, Co, Mn, Mo, Cr, Ni, Co, W, Ti, V) increased it markedly; a similar result was achieved by an increase in the amount of ferrous sulfate in the nutrient mixture (apparently, this compound contained all the micronutrients as impurities).

Emerson and Lewis found that, for securing the highest yields, the cells had to be grown for 5 or 10 days at 15–20° C., 20 cm. from four grouped 60 watt lamps followed by 3 days 30 cm. from a single 100 watt lamp. In agreement with Wassink and co-workers, they found a temperature effect—the highest quantum yield was observed at 10° C. At least 5% CO_2 had to be present during the quantum yield measurement, and the light intensity could not exceed 350 erg/cm.^{2} sec.

These experiments could have been interpreted as indicating possible reasons for the low yields observed at Wisconsin, and thus supporting the validity of the results of Warburg and Negelein, if a new difficulty had not appeared. Emerson and Lewis found that, by combining all the favorable factors, γ values could be obtained that were considerably above Warburg and Negelein's value of 0.25. With 10 min. illumination periods, γ values up to 0.31 were obtained; and these were further increased by making the illumination periods even shorter. Since even a quantum yield of $\frac{1}{4}$ presented grave difficulties from the point of view of thermochemistry, yields of one third or higher were clearly incompatible with the accepted over-all reaction of photosynthesis.

Emerson and Lewis suspected (1938, 1939) that the oxygen production in the first minutes of illumination may occur by reduction of accumulated

intermediates of dark metabolism, rather than of carbon dioxide, and thus require less energy. This caused them to inquire into the value of the photosynthetic quotient in the first minutes of illumination. It was in this

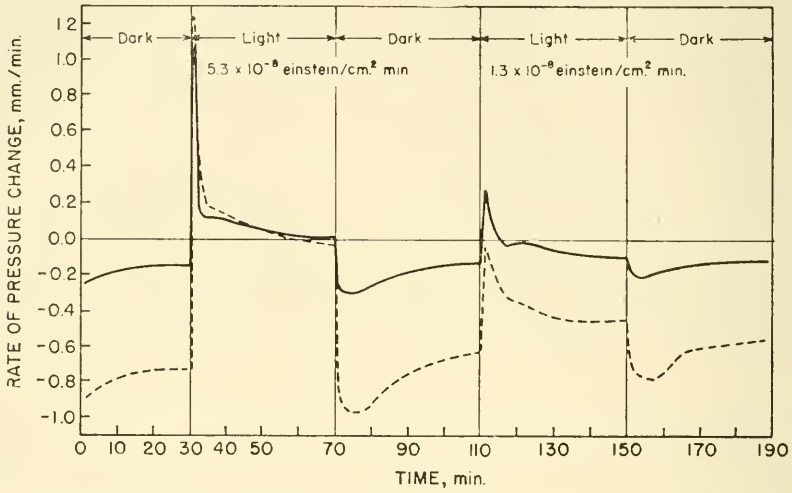

Fig. 29.3A. Rates of pressure change during alternating periods of light and darkness, for the same quantity of cells, in two vessels (after Emerson and Lewis 1941). Vessels differ in gas volume, thus permitting separate calculation of $[O_2]$ and $[CO_2]$ (cf. fig. 25.3B and 29.4A).

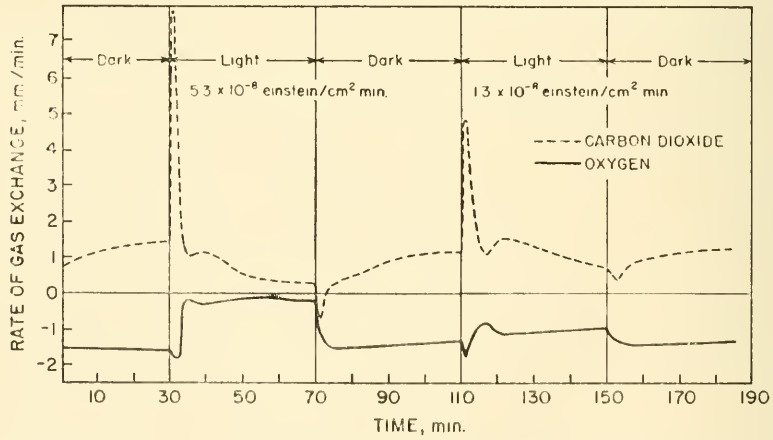

Fig. 29.3B. Rate of exchange of O_2 (solid curve) and CO_2 (broken curve) calculated from the rates of pressure change shown in fig. 29.3A for two different vessels (after Emerson and Lewis 1941).

inquiry that they noticed the carbon dioxide "burst" (1940, 1941), and suggested that failure to recognize it could have been responsible for the large pressure changes observed, and the high quantum yields calculated by Warburg and Negelein.

Figure 29.3, taken from Emerson and Lewis (1941), shows the rate of pressure changes as observed in minute-to-minute measurements in two vessels with different liquid : gas ratio. It indicates that upon illumination, an initial gush occurs, which lasts, in the light of the particular intensity used, for about three minutes, and then gives place to a more or less steady rate of pressure change. In dark, too, the steady rate of gas consumption is not established at once; after rapid, irregular variations, a rather extended, slow decrease in the rate is observed; subsequent experiments have shown that it takes about one hour for the rate of gas uptake to become quite steady.

The quantum yields of Warburg and Negelein were obtained by averaging the pressure changes over ten minute periods, *which included the time during which the gas burst* could have occurred, according to figure 29.3A. Obviously, values of the quantum yield calculated in this way could be deceptive, and higher averages could result from the use of periods shorter than ten minutes.

By comparing the curves obtained with two vessels, Emerson and Lewis (1940, 1941) sought information as to the relative role of oxygen and carbon dioxide in the gas burst, and in the extra gas consumption in darkness. They found (fig. 29.3B) that both were due to *carbon dioxide* and not to oxygen; the absorption and liberation of the latter (solid line in fig. 29.3B) showed only minor disturbances, which could perhaps be attributed to uncertainties in the evaluation of the measurements.

The factors that Warburg and Negelein, Rieke and Emerson and Lewis have described previously as indispensable for the realization of the highest quantum yield were found by Emerson and Lewis to affect mainly or exclusively the carbon dioxide gush.

The *carbon dioxide concentration* in the medium affected the quantity of carbon dioxide taken up in the dark, and therefore also the amount of the gas released in the light. This offered an explanation of the observation that large concentrations of carbon dioxide (such as 5%) were needed to obtain high quantum yields. (The light curves for different values of the parameter $[CO_2]$—*cf.* figures 28.1 to 28.5—indicate that the latter should be without influence at such low light intensities.)

A similar consideration applies to the role of *temperature*. It is known (*cf.* Figs. 28.6–28.8, and chapter 31) that at low light intensities, when the photochemical process proper limits the over-all rate of photosynthesis, changes in temperature have no influence on the maximum quantum yield. The observed effect of temperature contradicted this experience. Now, it became likely that the effect of temperature was due to its influence on the carbon dioxide uptake in the dark and its subsequent disengagement in the light, and not on photosynthesis itself.

The *pretreatment* of the algae also seems to be much more important for the carbon dioxide gush than for the steady rate of photosynthesis. Cells having a high rate of respiration produced a greater carbon dioxide gush than cells with a low rate of respiration; by culturing *Chlorella* cells in dim light throughout, cells with low respiration, showing almost no gush, could be obtained.

When the carbon dioxide gush was neutralized by substituting carbonate buffers for carbonic acid solution, Emerson and Lewis obtained quantum yields of the order of 0.1, independently of the previous illumination of the cells, the age of the culture and the kind of water used in its preparation. (However, the presence of certain microelements—particularly manganese—still appeared to be important.)

The conclusions of Emerson and Lewis agree with the findings of Daniels and coworkers (1939), who grew *Chlorella* with the addition of soil extracts, of a nutrient solution of 28 elements, or of sea water, in lake water, well water and distilled water, without appreciable changes in the quantum yield.

Emerson and Lewis did not calculate quantum yields from measurements of the type illustrated by figure 29.3B, but merely drew from the evaluation of these experiments the conclusion that with *Chlorella* the quantum yield of oxygen liberation (and of carbon dioxide uptake, once the burst is completely over) does not differ significantly in acid phosphate buffer and in alkaline carbonate media. If this is so, then the absolute determination of the quantum yield is better carried out in carbonate buffer, where all effects caused by carbon dioxide exchange are eliminated and therefore no need arises for the use of the two-vessel method. The latter depends on comparatively small differences, and has a correspondingly low precision.

The quantum yield measurements made by Emerson and Lewis with *Chlorella in alkaline buffers* consistently gave values between $\frac{1}{8}$ and $\frac{1}{11}$; and we will see below that Warburg, Burk and co-workers have since confirmed this result (leaving Eichhoff as the only investigator to have claimed that quantum yields of the order of $\frac{1}{4}$ can be obtained with *Chlorella* in carbonate buffer).

A set of manometric quantum yield determinations with *Chlorella pyrenoidosa* was made by French and Rabideau (1945) as a check on their measurements with isolated chloroplasts (*cf.* section 4). Using carbonate buffer No. 9 as medium they found in red light (660–720 mμ) γ values between 0.063 and 0.113, or from 9–16 quanta per reduced carbon dioxide molecule, with no significant variations between 1.4 and 8 kerg/cm^2 sec.

According to Emerson and Lewis, the true maximum yield of photosynthesis does not change much from species to species. This is indicated by the similarity between the above-mentioned results with *Chlorella* and Gabrielsen's observations on the leaves of the higher plants (page 1118), and confirmed by Emerson and Lewis' measurements listed in table 29.I. These measurements were made in carbonate buffers—after preliminary tests had shown that in none of the organisms used was the yield in phosphate medium significantly higher than in carbonate buffer.

TABLE 29.I

MAXIMUM QUANTUM YIELDS OF DIFFERENT SPECIES (AFTER EMERSON AND LEWIS 1941)

Species	γ (at *ca.* 1200 erg/cm.^{2} sec.) (Na light)	
	γ	$1/\gamma$
Green algae:		
Chlorella pyrenoidosa	0.101	9.9
Chlorella vulgaris	0.092	10.9
Chlorococcus	0.104	9.6
Eudorina	0.095	10.5
Sticchococcus bacillaris	0.107	9.3
Scenedesmus D[1]	0.094	10.6
Scenedesmus D[3]	0.100	10.0
Gyorffiana humicola	0.090	11.0
Oocystis naegeli	0.096	10.4
Blue-green algae:		
Chroococcus	0.086	11.6
Aquatic flowering plant		
Wolffiella lingulata	0.060	16.6

Rieke, working in Franck's laboratory at Chicago, carried out in 1941 a series of quantum yield determinations which were not published until much later (Rieke, 1949). He used relatively dilute and therefore only partially absorbing *Chlorella* suspensions, and determined the absorbed light energy by means of an integrating sphere. Because of the comparatively low cell density, the respiration correction was relatively small.

In experiments of this type, Rieke found quantum yields of about 0.08 for *Chlorella* (in acid solutions as well as in alkaline buffers), and from 0.09 to 0.11 for *Scenedesmus* (in 0.025 *M* bicarbonate, saturated with 4% CO_2, *p*H 8.4). Somewhat lower yields ($\gamma = 0.074$) were obtained with *Scenedesmus* in the more alkaline carbonate–bicarbonate buffers. The quantum yields (in buffers) were the same at 10° and 20° C.; they were not significantly affected by variations in light intensity during the last days of the culture period (from "very low" up to 10,000 lux), by doubling the salt concentration in solution, by culturing in 4% CO_2 or in normal air, and by adding a mixture of micronutrients. It was noted, however, that growing the cells to a culture density of over 1 ml. wet cells/l. impaired their photosynthetic efficiency.

The quantum yields decreased slightly with increasing light intensity, between 1100 and 4400 erg/cm.^{2} sec., *e. g.*, from 0.077 to 0.072 in one experiment with *Scenedesmus*, and from an average of 0.084 at 1900 erg to an average of 0.079 at 4400 erg in *Chlorella*.

Wassink (1946) applied Warburg's manometric technique to the measurement of photosynthesis in the leaves of several horticultural plants. (Small discs punched out of these leaves were allowed to float in buffer solutions in a Warburg apparatus.) At low or moderate light intensities,

in yellow sodium light, the yields were approximately independent of the initial carbon dioxide concentration (1–9%), and of temperature (17–25° C.). The light curves remained fairly straight up to 10 or 20 kerg/cm.^{2} sec. The quantum yields were calculated from the rates observed at 10 kerg/cm.^{2} sec. The resulting γ values are given in Table 29.II. The smallest $1/\gamma$ values measured were of the order of 11, corresponding to $\gamma \leq 0.09$.

Table 29.II
Quantum Yields of Oxygen Production by Horticultural Plants[a]
(after Wassink 1946)

Plant	$1/\gamma$	Number of measurements
Strawberry	12.1–17.8	23
Kohlrabi	13.5–15.4	5
Chinese cabbage	10.8–14.7	8
White succory	13.6–16.8	4
Tomato	16.0–43.5	17
Cucumber	13.8–24.6	5
Endive	21.8	2
Asparagus	14.6–18.2	4

[a] At 10 kerg/cm.^{2} sec.

The significance of the variations of the quantum yield of *Chlorella* with *wave length* will be discussed in chapter 30, primarily from the point of view of the function of carotenoids in photosynthesis. Most experiments on the quantum yield of photosynthesis of "colored" algae (brown, red or blue-green) were carried out with a similar aim in mind—elucidation of the role of phycobilins and carotenoids. As far as the *absolute value* of the maximum quantum yield is concerned, there seems to be no fundamental difference between these algae and the green plants, as is shown by the following observations:

Emerson and Lewis (1941, 1942) measured manometrically the quantum yield of photosynthesis by the blue-green unicellular alga *Chroococcus*, and found a maximum γ value of about 0.08 in red light. Working with a suspension of diatoms (*Nitzschia closterium*), Dutton and Manning (1941) found an average quantum yield of 0.063, with single γ values up to 0.1. Wassink and Kersten (1944) used another diatom, *Nitzschia dissipata*, and calculated the quantum yields from manometric measurements, with illumination periods of 30–60 min., in sodium light of about 10 kerg/cm.^{2} sec. (The light curves obtained by these authors showed only small deviations from linearity at these comparatively high intensities; *cf.* fig. 28.5). From one third to one half of the incident light was absorbed in the vessel. In five experiments at 25° C., and one at 6° C., $1/\gamma$ values from 11.4 to 13.9 were obtained, with an average of 12.9, or $\gamma = 0.078$, and $\gamma_{max.} = 0.09$.

Tanada (1951) made a systematic study of the quantum yield of the diatom *Navicula minima* in dependence on wave length. This work will be described in chapter 30 (p. 1173); what is significant for the purpose of the present discussion is that the maximum yield obtained in carbonate buffer No. 9 was $\gamma_0 = 0.11(0.05)$, corresponding to a quantum requirement of $1/\gamma_0 = 9$; and that the yield in acid phosphate buffer appeared to be, at all light intensities, 5–10% *lower* (and not 10–20% *higher*, as in *Chlorella*) than that in alkaline carbonate buffer. In this case, Warburg's attribution of a quantum requirement of $\simeq 10$ to a "nonphysiological" *p*H does not seem plausible.

Similarly to Kok (p. 1113), Tanada found the quantum yield to depend on the age of the culture. Both the quantum yield in weak light and the maximum rate in strong light dropped sharply from their original values ($\gamma_0 = 0.115$, $P^{max} = 3.0\ mm^3\ O_2/mm^3$ cells hr), between the sixth and the eighth day of cultivation ($\gamma_0 = 0.07$; $P^{max} = 1.0$ on the eighth day); the decline continued steadily but more slowly for the next 12 days until, on the 20th day, γ_0 was down to 0.06 and P^{max} was down to 0.75.

We thus see that a whole series of manometric determinations of the maximum quantum yield, applied to green higher plants, green algae, blue-green algae and diatoms, and made in acid solutions as well as in carbonate buffers, gave γ_{max} values of 0.1 ± 0.02. With the exception of Emerson and Lewis' measurements, which have revealed the carbon dioxide burst, they were all carried out under conditions when the burst, if it did occur at all, was either absorbed by the medium or minimized by the averaging of results over an extended period of time. Many of the experiments were performed in somewhat stronger light (up to ten times that used by Warburg), and this, too, is likely to minimize the effects of the burst. The experiments of Emerson and Lewis, and of Rieke, carried out under exact adherence to the Warburg-Negelein procedure, showed that Warburg's results can be duplicated, if the carbon dioxide burst is treated as part of normal gas liberation by photosynthesis.

We now turn to the other side in the controversy—investigations in which Warburg and Negelein's results were confirmed under conditions which did not seem to admit of the interpretation suggested by Emerson and Lewis.

First, we have to mention the investigation by Eichhoff (1939), carried out in Noddack's laboratory. Eichhoff suspended *Chlorella* cells in carbonate–bicarbonate buffers and measured the quantum yield after a preliminary illumination for 15 or 30 min. (obviously these two precautions should have prevented the carbon dioxide burst from affecting the results). Eichhoff worked either with a "dense" suspension of *Chlorella pyrenoidosa*,

absorbing 67% of red light (a band 10 mμ wide at 650 mμ, isolated by a Christiansen dispersion filter) and 39% of green light (567.5 mμ), or with a "thin" suspension absorbing 22% of red light; the absorption was determined by means of the "ellipsoid photometer" (*cf.* chapter 25, page 844). Each run included (*a*) a "dark adaptation" period, (*b*) a period in which respiration was measured, (*c*) a "light adaptation" period, (*d*) an illumination period, (*e*) a second "dark adaptation" period and (*f*) a final period of respiration measurement—each period lasting from 15 to 30 min. The illumination intensity was high enough for photosynthesis to exceed respiration (from 500 to 5000 erg/cm.^{2} sec.). The quantum yields obtained varied between 0.25 and 0.19, for both dense and thin suspension, with the lower value (0.19 to 0.22) observed only at the higher light intensities and interpreted as indications of an incipient light saturation. Similar γ values were found at 567.5 mμ. The quantum yield was found to be constant over a wide spectral region, including the near infrared (where other observers found no photosynthesis at all; *cf.* chapter 30, page 1155).

According to a review by Franck and Gaffron (1941), Emerson and Lewis, as well as Rieke, have tried to imitate Eichhoff's experiments (particularly with respect to the method of cultivation of the algae), but were unable to obtain the high yields claimed by him.

One peculiar feature of Eichhoff's light curves (*cf.* fig. 30.7) is the early saturation in red light. 70% of maximum photosynthesis is reached, according to these curves, with an incident intensity of only 3 kerg/cm.^{2} sec. Eichhoff's figures suggest that as little as 5 "energetic meter candles" of red light (he calls a monochromatic energy flux equal to the total "white" flux from a Heffner candle an "energetic meter candle") are equivalent, as far as photosynthesis is concerned, to 15 klux of white light from a 500 watt incandescent lamp! The latter, according to page 838, corresponds to a flux of at least 60 kerg/cm.^{2} sec., counting only the photosynthetically active region (400-700 mμ), while 5 "energetic meter candles" are equivalent (using Gerlach's value for the radiation of a Heffner candle) to only 4.7 kerg/cm.^{2} sec. Even though the suspension may absorb red light three or four times more efficiently than the (infrared-free) white light, the difference between the amounts of red light and white light required to bring about the same rate of photosynthesis remains striking. It suggests that the absolute intensity of the red light might have been underestimated by Eichhoff by as much as a factor of three or five. If this was the case, all quantum yields calculated by Eichhoff must have been in error by the same factor (since no quantum yield determinations were made in white light).

More recently, Warburg (1946, 1948) undertook, with Kubowitz, to repeat the original experiments of Warburg and Negelein, taking into consideration Emerson's criticism. Warburg calculated that, in order to account for his 1923 results in the way suggested by Emerson, the average photosynthetic quotient during the 10 min. exposure must have been $Q_P = \Delta O_2/-\Delta CO_2 = -0.26$. (In other words, four volumes of carbon dioxide must have been produced in light for each volume of oxygen liberated.)

Fig. 29.4. Quantum yield measurements with *Chlorella* (after Warburg 1948). Yellow light, 10°C. Shaded areas represent "induction losses" attributed to sluggishness of manometer. (Curve *A* shows a slight excess instead of deficiency of gas evolution at the beginning of illumination.) Quantum yield is determined from difference between slope in light (*AB* or *CD*, depending on the method of calculation) and slope in darkness.

The value used by Warburg and Negelein, $Q_P = +1.09$, was determined by gas analysis, in much stronger light than was used for quantum yield determinations. Warburg now redetermined the Q_P value in less intense light, manometrically, by means of two sets of measurements in one vessel filled with two different volumes of liquid (5 and 8 ml.). He found a Q_P value of +1.07, practically identical with the quotient used by Warburg and Negelein.

Furthermore, Warburg and Kubowitz could find no evidence of a "gas burst" in the first few minutes of illumination, except for a comparatively small effect, observed at the higher light intensities, especially when foaming occurred in the reaction vessel (*cf.* fig. 29.4A).

With the belief in the validity of the original experimental procedure thus strengthened, Warburg and Kubowitz proceeded to make new determinations of the quantum yield by the one-vessel method. The vessel was not silvered to better observe bubble formation (which Warburg considered the most serious source of error); readings were made without interruption of shaking. The light used was mostly the yellow mercury lines (578 mμ) with an intensity of 325–2920 erg/cm.^{2} sec.

To minimize effects caused by sluggish gas exchange, a smaller fluid volume was used than in 1923 and two glass beads were put into each vessel to act as stirrers. Curves such as those in figure 29.4B and C were considered by Warburg as confirmation of the interpretation of pressure disturbances at the beginning of the light and dark periods, as consequences of this sluggishness. He described these disturbances as "symmetric," meaning that the two disturbances cancelled each other and the γ values therefore were the same, whether they were determined from the steady rates, omitting the measurements in the first few minutes of light (*i. e.*, from slope AB), or by interpolation, as in figure 29.1 (*i. e.*, from slope CD). This procedure is equivalent to integration of the gas exchange over the whole period of the experiment, including the two transition intervals.

This obviously does not apply to the curve in figure 29.4A, where the pressure increase in the first minute is *faster* than afterward. Calculation from the steady state (slope AB) gives in this case a $1/\gamma$ value 25% higher than that obtained by integration (slope CD).

Table 29.III summarizes the results. Warburg concluded from these experiments that the limiting quantum yield in weak light is 0.25 and that it declines to about 0.20 at 1500 erg/cm.^{2} sec. He suggested, as general explanation of the smaller values found by other observers, failure to culture algae of the highest efficiency; but his description of the methods of culture revealed no significant difference from those used by Emerson or Rieke.

TABLE 29.III
QUANTUM YIELDS AFTER WARBURG (1946, 1948)[a]

λ, mμ	I: 10^{-6} einstein min. 17 cm.²	I: erg/cm². sec.	$1/\gamma$, quanta per molecule O_2	γ, molecules O_2 per quanta	See figure
578	0.158	324	3.96[b]; 3.60[c]	0.25	29.4D
578	0.161	330	4.35[b]	0.23	
578	0.177	363	4.50[b]	0.22	
578	0.196	403	3.60[b]	0.28	
436	0.201	—	(5.0)	(0.20)	29.4C
578	0.330	677	4.5[b]; 4.6[c]	0.22	
436	0.390	—	(6.0)	(0.17)	
578	0.400	824	4.25[b]	0.24	
578	0.750	1540	5.03[b]; 4.79[c]	0.20	29.4B
578	1.42	2920	5.56[b]; 4.45[c]	0.18	29.4A

[a] Chlorella suspension, 10° C., complete absorption. 450 mm.³ cells in 5 or 8 ml. liquid; bottom area 17 cm.² The two values in parentheses probably are affected by the light absorption by carotenoids.
[b] From the steady state.
[c] By integration.

In discussing Warburg's results, Emerson and his co-workers (1949, 1950) pointed out that the pressure changes in the first minutes of illumination are affected by two factors: the sluggishness of the manometer (emphasized by Warburg) and the carbon dioxide burst. In very weak light, the burst may be spread almost uniformly over a 10–15 min. illumination period, and the sluggish transition is then clearly revealed by the initial measurements, as in fig 29.4B and C. In stronger light, the burst is much more sudden, and its rapid decay overcompensates the effect of sluggish gas exchange, leading to curves such as that in figure 29.4A. The spread of the burst in low light over the whole illumination period, accentuated by the sluggishness of the manometer, may answer one of Warburg's objections—the absence of a visible pressure burst on the low light curves (figs. 29.4B–D).

Warburg's other (and main) objection against Emerson's criticism was that a check "under the conditions of the quantum yields measurements" confirmed the validity of the Q_P value of approximately +1, and that an extreme deviation of Q_P from unity would have been needed to calculate from Warburg's experimental data (obtained with the one-vessel method) a quantum yield of about 0.1 for the liberation of oxygen in light. To this, Emerson and co-workers answered that Warburg's determination of Q_P was based on subtraction of the rate of pressure change in darkness from the rate of pressure change in light (this difference was called the "light effect"). From the comparison of "light effects" in experiments with different amounts of liquid in the same vessel, Warburg derived the ratio $\Delta O_2/\Delta CO_2$ for *the light effect*; and finding it close to 1, concluded that no

significant carbon dioxide burst could have occurred during the illumination period. However, this procedure would only be permissible if the gas exchange in light were the result of the superposition of a photochemical process (photosynthesis, with possible addition of a carbon dioxide burst) upon a dark process *whose rate is the same in darkness and in light.* This is usually assumed to be true of respiration (although some doubts exist even here); but it is *not* true of the *reabsorption of the carbon dioxide burst* (since this process occurs *only* in the dark). By neglecting this component of the gas exchange in the dark after a period of illumination, one automatically eliminates from the calculated "light effect" a part, if not practically all, of the carbon dioxide burst—in the same way in which the effects of the sluggishness of the manometer are eliminated in the procedure illustrated by figure 29.1; no wonder that the ratios Q_P for the calculated "light effect" prove to be close to unity. (Whether the elimination of the burst is practically complete or only partial, depends on what fraction of the burst is reabsorbed during the dark period utilized in the calculation of the "light effect.")

To decide whether a significant carbon dioxide burst does occur in light (and is reabsorbed in darkness), the ratios $\Delta O/\Delta CO_2$ should be calculated for the illumination and the dark period *separately* instead of calculating them directly for the "light effect." The ratios Q_{dark} and Q_{light} might each be quite different from 1—and yet, the ratio "Q_P" for the "light effect" might show no significant deviation from unity. Thus, the method of calculating Q_P used by Warburg (1948) to prove the absence (or at least, practical insignificance) of the carbon dioxide burst is inappropriate for this purpose—even if the experimental data used had been adequate.

Emerson and co-workers argued, however, that the experiment itself was open to criticism. They pointed out that the value $Q_P = 1.07$ was derived from measurements lasting for about 40 minutes. The plot given by Warburg shows that if only the first 10 minutes of these measurements, *i.e.*, the period of quantum yield measurements, were taken into consideration, much smaller values of Q_P would have been obtained. Furthermore, the Q_P measurements were made in light of 3780 erg/cm.^{2} sec. (ten times as strong as that at which quantum yields close to 0.25 were obtained) and in red light, while the γ measurements were made in yellow light, which is considerably less strongly absorbed.

The conditions of Q_P measurement differed from those of γ measurement also in temperature (20° C. instead of 10° C.), carbon dioxide concentration (8% CO_2 in O_2, as compared with 5% CO_2 in air) and volume of respiration (twice as high in quantum yield measurements as in Q_P measurements, indicating different culture conditions). It was mentioned before

that the volume of the carbon dioxide burst depends on all these conditions; the statement that the Q_P measurements were made "under the conditions of quantum yield determinations" was therefore not justified.

Rabinowitch (1947) pointed out that experiments show the integrated volume of the "burst" to change only little with light intensity, the main effect of the latter being on the *suddenness* of the burst. This relation is to be expected for photochemical emptying, with a high quantum yield (perhaps as high as $\gamma = 1$) of a "carbon dioxide reservoir," containing a finite volume of carbon dioxide (the exact volume being dependent on conditions that prevailed prior to illumination). If the volume of the reservoir corresponds to about one molecule carbon dioxide per molecule chlorophyll, the time required for complete emptying must be of the order of the time required for each chlorophyll molecule in the suspension to absorb a quantum of light; in the dense suspensions and in the low light used for the quantum yield determinations, this time is of the order of ten minutes (*cf.* chap. 32); this is then the expected duration of the burst. In stronger light, the burst will be proportionally shorter.

If the volume of the burst increases only little or not at all with light intensity, its importance at 3780 erg/cm.^{2} sec. would be much smaller than at 320 erg./cm.^{2} sec. (where the value $\gamma = 0.25$ was found).

Emerson and Nishimura (1949) criticized also other experimental aspects of Warburg's work. They pointed out that the use of equal liquid volumes and different gas volumes in the two-vessel method (Emerson and Lewis, *cf.* fig. 25.3B) assured better comparability of the gas exchange than Warburg's use of a single vessel filled with different amounts of liquid (since the efficiency of gas exchange between the two phases depends on the volume of the liquid). Objection was raised also to Warburg's time schedule, which involved consecutive runs first with the more concentrated and then with the more dilute suspension. Because of continuous change in the rate of respiration of cell suspensions, only simultaneous exposure and darkening of two aliquots of the same cell material could vouchsafe the required high degree of their physiological comparability. Emerson and Lewis themselves did not quite meet this requirement: they, too, worked first with one, and then with the other vessel, but in contrast to Warburg, they used a fresh aliquot of the stock suspension for each experiment (considering this a less objectionable compromise than the use of a single sample) first for a series of measurements in a smaller volume of liquid and, after dilution, for a second series of measurements in a larger volume.

The discussion of these apparently minor details points to the great practical difficulty of the (theoretically so simple) two-vessel method: it rests on the assumption that the observed difference between the two

pressure changes is due *entirely* to different relative volumes of liquid and gas. Even a very small difference in the actual amounts of gas produced (or consumed) in the two vessels, or in the speed with which this gas is transferred into the manometer, can lead to large errors in calculation.

Several reasons for such variations can be anticipated. Small physiological differences may exist between samples taken at different times from the same stock solution or may arise in the course of the experiment. Considerable discrepancies of light absorption can be caused by different position of the two vessels in the light beam or by differences in their shape and wall material. Shaking thins out the liquid layer in the middle of the vessel, and the consequent incompleteness of absorption depends on the total amount of the liquid present and on the shape of the vessel.

In 1948–1949, an unsuccessful attempt was made to settle the quantum yield controversy by a combined effort of Warburg and Emerson in the latter's laboratory. Subsequently, in the summer of 1949, Warburg, Burk and co-workers (1949[1,2], 1950[1,2]) carried out quantum yield measurements by the two vessel method (this time with equal liquid volumes) at the National Cancer Institute in Bethesda and at the Woods Hole Marine Biological Laboratory. In these experiments, single yields as high as $1/\gamma_0 = 0.44$ ($\gamma_0 = 2.3$) were observed; even the average yield was markedly above 0.25. The conditions under which these high yields have been obtained were quite different from and, in some respects, opposite to those which had been recommended by Warburg and Negelein in 1923.

Cell Culture. The reduction of light intensity in the last day of cultivation, recommended by Warburg and Negelein to adapt *Chlorella* cells to weak light, was discarded by Warburg and Burk. Another precaution, called unimportant by Warburg and Negelein, was now found to be essential: fast bubbling of the carbon dioxide-bearing gas through the culture bottle, preventing the cells from settling out, and thus assuring adequate supply of oxygen and carbon dioxide to all of them.

Very concentrated suspensions were used: 0.3 cc. cells in 7 cc. culture medium (phosphate buffer, *p*H 4.9, saturated with 5% CO_2 in air). (In 1948, Warburg used only 0.1 cc. cells in 5 or 9 cc. solution.) This was done to ensure complete light absorption, despite an increased rate of shaking, which created a more pronounced "hole" in the center of the reaction vessel; but the respiration correction—the main source of uncertainty in measurements of this type—was thus made even larger than before.

Response of the Manometer. Compared to the 1923 experiments, the efficiency of shaking was increased (horizontal, back-and-forth motion of a rectangular vessel with an amplitude of 2 cm., 150 times per min.). It was asserted that stirring was thus made so effective that the response of the manometer to gas production (or absorption) in the liquid was

practically instantaneous. No correction (of the type illustrated in fig. 29.1) was therefore used to account for diffusion through the liquid and the exchange between the two phases. Instead, the yields were now calculated from manometer readings made at the very moment of changing from darkness to light, or from light to darkness. Errors caused by "physical lag" had been considered of prime importance in 1923 and 1948; in some examples given in these earlier papers the calculated quantum yields would have been quite different without correction for this lag.

Time Schedule. The two vessels were filled simultaneously with aliquots of the same culture, and exposed alternatively to the same beam of light (*e.g.*, 10 min. light on vessel I, then 10 min. light on vessel II, then again 10 min. light on vessel I, and so on). Both vessels (total volumes 14 and 18 cc., respectively) contained the same amount of liquid (7 cc.). It was argued that whatever physiological differences may have existed between the cells in the two vessels during the first exposure (because of a "phase difference" of 10 min.), must have disappeared after several light-dark cycles. This is plausible; however, Emerson and coworkers found that at least five or six (10 min. light + 10 min. dark) cycles may be needed to eliminate the initial difference, while in many of Warburg and Burk's published experiments (*cf.* table 29.IV) only 2 or 3 cycles were used. The alternate exposure schedule was altogether abandoned in almost one half of all experiments—namely those in which "background" illumination was used to compensate respiration.

Light Measurement. No physical determination of light intensity was made by Warburg and Burk. (Bolometers had been used in earlier experiments, both by Warburg and by Emerson.) Instead, light intensity was determined by means of the ethyl chlorophyllide – thiourea actinometer, for which a quantum yield of 1.0 was previously found (bolometrically) by Warburg and Schocken in Emerson's laboratory (*cf.* chap. 35). The quantum yield of the actinometer is known to decline with increasing light flux, particularly >0.1 μeinstein/min. Many runs of Warburg and Burk were carried out in stronger light; the intensity of the beam was reduced in these experiments to about 0.1 μeinstein/min. by means of calibrated wire screens before it was directed on the actinometer.

Light Intensity. In Warburg's 1923 and 1940 measurements, the use of very weak incident light was considered important, since the quantum yield was found to decline significantly (*cf.* fig. 29.8) with increasing light intensity, beginning as early as at 1000 erg/cm.^{2} sec. (about 0.03 μeinstein/cm.^{2} min.). In the Warburg-Burk work, much higher incident light intensities were used: instead of uniform illumination of almost the whole bottom area, as used in earlier experiments (1923, 1948),

a sharp beam was now thrown on the bottom of the vessel (cross section of the beam, about 3 cm.^{2}; bottom area, 8.3 cm.^{2}). The total light flux (red light, 630–650 mμ) was 0.2–0.6 μeinstein/min., *i.e.* 0.07–0.2 μeinstein per cm.^{2} min.—*ca.* ten times higher than the intensity at which quantum yields of 0.25 had been obtained in 1948. Because of the extremely high density of the suspension, practically all this light was absorbed within a 1 mm. thick bottom layer (0.3 cc.) of the suspension; thus, at any given time, >95% of the cells were in darkness, while <5% were exposed to light, the incident intensity of which was close to the saturating value (the photosynthesis of light-adapted *Chlorella* is saturated, in red light, in a flux of about 0.5 μeinstein/cm.^{2} min.).

Intermittency Effect. The finding of the highest quantum yields ever observed when the incident light was of almost saturating intensity appears startling. Warburg, Burk and co-workers explained this paradox by the *intermittency of illumination*: because of fast shaking, individual cells remain only for a very short while in the illuminated zone, and then plunge into darkness. (Assuming uniform stirring, each cell must spend >95% of the total "illumination time" in darkness, and less than 5% in light). Warburg and Burk proclaimed as a "new principle" that this type of intermittency of illumination permits maximum light utilization. This assertion is not easily reconciled with the results of experiments in flashing light, to be discussed in chapter 34:

According to these experiments, intermittent illumination cannot increase light utilization *above* the maximum value possible in steady low light. All that intermittency can do is to bring the quantum yield in partially or even completely saturating light close to—but never quite up to—the quantum yield in low steady light.

For the quantum yield increase caused by intermittency to be at all significant, the light periods must not be longer than the "Emerson-Arnold period" (0.01 sec. at 20° C., *cf.* chapter 34), allowing the limiting catalyst to work in the dark, after the flash is over, for a period of time which is significant compared to the duration of the flash itself. It is doubtful, however, whether shaking at the rate of 2.5 swings per second could lead to illumination flashes of 0.01 sec., or shorter.

Certainly, the "dark periods" in Warburg and Burk's experiments must have been $\gg$0.01 sec.; therefore, the assumption of Warburg and Burk, that under the conditions of their experiments all cells are engaged uniformly in photosynthesis throughout the "illumination period," requires revision of the major conclusions derived from experiments in flashing light. However, this assumption is not necessary for the validity of their argument (while the duration of the light period, mentioned in the preceding paragraph, is of crucial importance).

Background Light. Because only 5% of all cells were illuminated at any given moment, even the very high incident intensity of the red light used in the Warburg-Burk measurements did not prevent the respiration correction from being equal to or larger than the photochemical gas exchange. It has been suggested (this hypothesis will be discussed later in this chapter), that the effect of light below the compensation point could consist in reversing respiration *midway* (and not after it has led to the ultimate products, CO_2 and H_2O). To check this hypothesis, Warburg, Burk and co-workers made experiments in which the net gas exchange in light was made positive by substituting for dark periods, periods of diffuse illumination of the reaction vessels by white "background light" of such intensity that photosynthesis equalled or exceeded respiration. This background illumination was maintained also during the "light" period (when a measured beam of red light was added to it), so that the "light effect," from which the quantum yield was calculated, was the *increment* of gas exchange caused by an *increment* of illumination. Warburg and Burk argued that the quantum yields obtained in this way must be those of true photosynthesis, with the storage of 112 kcal. chemical energy per mole of liberated oxygen, and could not be those of a partial reversal of respiration (with an unknown, and possibly small, conversion of light energy into chemical energy), since this reversal, if at all possible, should be accomplished already by the background illumination.

It will be noted that this argument is tied up with the assumption of uniform photosynthetic activity of all cells—those that are momentarily illuminated by the flash as well as those that are momentarily in darkness. If only the actually illuminated cells (or cells <0.01 sec. out of the illumination zone) can contribute significantly to photosynthesis, then only the part of the background light that falls on these particular cells is of importance. This part is insignificant if the background light falls from above and is absorbed in the *top* layer of the suspension—while measured red light enters the vessel from below and is absorbed in a thin *bottom* layer of the suspension. Warburg and Burk (1950) described a single experiment in which the background light, similarly to the measured light, was thrown on the vessel from below. This light was so strong as to overcompensate respiration about fivefold; nevertheless, the addition of the measured light produced an increment of oxygen production equivalent to a quantum requirement as low as 2.8. It is unfortunate that this particularly important experiment was carried out with a particularly unsatisfactory time schedule —three 5-min. light-dark cycles in one vessel, followed by two 10-min. light-dark cycles in the other vessel.

Quantum Yield in Carbonate Buffers. The same cells which gave, in Warburg and Burk's experiment, high quantum yields at *p*H 5 (culture

medium) gave 2–3 times lower yields ($\gamma = 0.10$–0.09) in carbonate buffers (pH $\simeq 9$), both with and without compensating white light (last section of table 29.IV). Yields of 0.12 or less were obtained also in bicarbonate solutions equilibrated with 5% carbon dioxide in air (pH 7–8). It thus appears that for Warburg and Burk's cells (grown in acid medium) even neutral solutions were "unphysiological."

Respiration in Light. The question whether respiration is affected by light is crucial for the measurement of the rate of photosynthesis in weak light. Different indirect methods (Vol. I, Chapter 20, and Chapter 36) have been used to answer it, and have given contradictory answers (including all three alternatives: "no change," "stimulation," and "inhibition"). The simplest approach to this problem is to remove (*e.g.*, by absorption in alkali) all carbon dioxide (including that produced by respiration) and to measure the oxygen consumption in light unobscured by photosynthesis. This procedure was attempted repeatedly, but without success, because immediate photosynthetic reutilization of respiratory carbon dioxide competed too effectively with its absorption by the comparatively remote external absorber. In fact, it proved difficult to reduce photosynthesis in this way much below the compensation point. Warburg *et.al.* (1949[2]) reported, however, that with the increased frequency of shaking, they were now able to absorb respiratory carbon dioxide in an alkali-filled side arm of the reaction vessel so effectively that the rate of oxygen uptake by a *Chlorella* suspension in light was exactly the same as in the dark. They saw in this experiment the proof that respiration as such is quite unaffected by (red) light, and refutation of all hypotheses which postulate an exchange of intermediates between photosynthesis and respiration.

Complete prevention of photosynthetic reutilization of respiratory carbon dioxide by absorption of the latter in an external absorber (although reutilization must be possible even before the carbon dioxide had escaped from the cell into the medium), is a remarkable achievement. A possible reason why Warburg and co-workers were successful where others have failed is intermittent illumination. For 95% of the "light period" each individual cell is practically in darkness. Respiration goes on during all this time; all, or at least a large part of the carbon dioxide produced while the cell is in the shade may be able to escape into the medium before the cell had moved into the illuminated zone. Once a carbon dioxide molecule is in the medium, it may have a much greater chance to diffuse into the gas space than to diffuse into the small illuminated volume. In this way, 80 or 90% of respiratory carbon dioxide produced during the "light period" could perhaps escape re-utilization by the cells and reach the external absorber.

It will be noted that this explanation could not be used if Warburg and Burk's concept of all cells being uniformly engaged in photosynthesis throughout the "light period" were correct. More specifically, this explanation requires that not only the photocatalytic mechanism responsible for the liberation of oxygen, but also the enzymatic mechanism responsible for the uptake of carbon dioxide, should cease operating within <0.1 sec. after the cells are darkened. This seems to contradict the assumptions which we used on p. 207 in the explanation of the "pick up" of carbon dioxide after intense illumination in CO_2-deficient medium, on p. 308 in the explanation of the effect of cyanide on yield of photosynthesis in flashing light, and will use in chapter 36 in accounting for C^*O_2 uptake by preilluminated cells. In all these cases, we have assumed that the capacity to take up carbon dioxide survives, in preilluminated cells, for several seconds (or even minutes) after the cells had been darkened. However, as in many such cases, apparent contradictions may arise from the use of a qualitative, "yes or no" approach, where a quantitative, "more or less" analysis is required.

Summary of Warburg and Burk's Quantum Yield Measurements. Table 29.IV gives a summary of the quantum efficiencies reported by Warburg and Burk (1950); several of the experiments in this table have already been discussed above.

Whittingham, Nishimura and Emerson (1951) were able to reproduce Warburg and Burk's results by strict adherence to the same experimental arrangement and schedule of operations. However, they concluded that these results were affected by a systematic error. Following are the major points of their criticism.

1. The two-vessel method is very sensitive to slight errors in manometric determinations. Thus, a difference of 0.3 mm. in the pressure change registered in one of the two vessels over a 10-min. period may change the calculated oxygen yield by a factor of two. Such a difference is well within the limits of experimental error of the method of Warburg and Burk (as contrasted to the much more precise measurements with the differential manometer, employed by Emerson and Lewis.)

2. This low precision of the method leads to random scattering of results, (for example, in experiment No. 7, the $1/\gamma$ values derived from individual cycles scattered from 2.3 to 14). This can be corrected by averaging over a sufficiently large number of cycles. However, only in a few experiments of Warburg and Burk, as many as five or six 10 min cycles were used; in most others, only two or three. This explains why even the averaged $1/\gamma$ values scattered from 2.3 to 4.9.

3. Random errors can explain the scattering of the results, but only a *systematic* error can explain the consistent finding of $1/\gamma$ values considerably

TABLE 29.IV

QUANTUM REQUIREMENTS OF OXYGEN PRODUCTION ($1/\gamma$) AND OF CARBON DIOXIDE CONSUMPTION (Q_P/γ) CALCULATED BY WARBURG AND BURK (1950) FOR *Chlorella pyrenoidosa*

Expt. No.	Description	Number, duration and order of cycles l = light d = dark (or background light)	$1/\gamma$	Q_P[1]
	A. Experiments at *p*H 5 (two vessels)			
1	No background illumination	6 10′l/10′d. cycles alternating in two vessels	4.6[2]	1.25
2	"	2 10′l/10′d cycles, alternating	3.6	0.84
3	"	3 10′l/10′d cycles, alternating	4.2	1.03
4	"	1 10′l/10′d; 1 20′l/20′d; 1 30′l/30′d	2.8[3]	0.82
5	"	5 10′l/10′d, alternating	2.5[4]	0.80
7	" $-KNO_3$	4 10′l/10′d alternating	4.2	0.96
	" $+KNO_3$	3 10′l/10′d alternating	3.2	0.76
9	"	1 20′l/10′d	4.8	1.22
1	Same material as in exp. 1 above, slightly overcompensating white background light	5 5′l/5′d cycles in first vessel, then 3 5′l/5′d cycles in second vessel	2.9	0.98
	Same material, 2X overcompensating background light	5 5′l/5′d cycles in first vessel, then 6 5′l/5′d cycles in second vessel	4.5	1.11
2	Same material as in exp. 2 above & below 2X overcompensating background light	2 15′l/15′d cycles in first vessel, then 2 15′l/15′d cycles in second vessel	3.9	0.96
6	Continuous background illumination by white light from above. Red measuring light from below added at various times	1 10′l/10′d and 2 30′l/10′d cycles in one, then same in other vessel, at the beginning of experiment	4.2	0.90
		2 10′l/10′d cycles first in one, then in other vessel, 19 hr. later	4.8	1.11
		3 10′l/10′d cycles first in one, then in other vessel, 14 hr. later[5]	3.4	0.78
	3X overcompensating white light from above	2 10′l/10′d cycles in one then same in another vessel	3.0	0.90
	Same material, 4X overcompensating orange red light "mostly from below"	3 5′l/5′d cycles in one, then 2 10′l/10′d cycles in another vessel	3.5	0.98
9	Same material as in exp. 9 above, 3X overcompensating background light from above	1 20′l/20′d cycle in each vessel	4.4	1.00
		Average	3.8	1.04

Table continued on page 1111

	B. Experiments at pH 9.2 (Single vessel)			
2	Same material as in exp. 2 above; respiration overcompensated by white light from above	5′d/15′l/15′d/15′l/10′d	10.5	...
	Same, no background light	10′d/10′l/10′d/10′l/10′d/-20′d	9.8	...
		Average	10.15	...

[1] ($\Delta O_2/\Delta CO_2$) for "light effect" (p. 1101).
[2] Average of six cycles, single cycles give $1/\gamma$-values from 2.3 to 14.
[3] No significant difference between 10′, 20′ and 30′ cycles.
[4] Four cycles gave very closely similar results; one value off.
[5] Cells washed and re-suspended in fresh medium before this measurement because of apparent drop in yield.

below those which Emerson and co-workers consider correct on the strength of their own manometric experiments, and of the nonmanometric measurements performed in various other laboratories. Emerson sees a possible source of such an error in the decision of Warburg and Burk to reverse Warburg's earlier practice, and to make no allowance for the "physical lag" between the time of gas exchange in the chloroplasts and the time when pressure changes are registered in the manometer (*cf.* below).

4. Warburg and Burk's claim that this lag was negligible because of fast shaking could not be confirmed by Emerson and co-workers. The fact that in Warburg and Burk's experiments the pressure change often was the same (within the limits of experimental error) in the first, and in the second 5 minutes of a 10-min. light period, can be explained, according to Emerson, by compensation of the lag by the carbon dioxide burst. Emerson and co-workers, too, could obtain time curves in phosphate buffer without an apparent induction period; and yet, experiments made in the same two vessels, and with the same rate of shaking, but in carbonate buffer (eliminating the CO_2 burst), showed a lag of considerable duration.

5. In each vessel, taken separately, the physical lag tends to *decrease* the manometric effect of the transition from darkness to light (and *vice versa*) and thus to make the calculated "light effect" *smaller*. This does not mean, however, that the calculated γ values also must be too small. The quantum yield and the ratio Q_P are calculated from the light effects in the two vessels; and whether the calculated γ value is too small or too large depends on the ratio of the two lags. With the two vessels used by Warburg and Burk, the lag is larger in the vessel with the larger gas space, and this makes the calculated quantum yields *too high*. The systematic error caused by this unequal lag needs to be only very small (of the order of 0.3 mm. per 10 min.) for the calculated γ to be increased by a factor of two.

6. While Emerson and co-workers were able to closely reproduce the measurements of Warburg and Burk ($1/\gamma = 3$–4), the results became quite different if light and dark periods were lengthened to 30 minutes or if the vessel shape h_2 (fig. 29.4A) was substituted for h_1 in the two-vessel combination. Either change led to $\gamma^{-1} \geq 9$, with the same cells which gave values of 3–4 by following Warburg and Burk's specifications. These changes should diminish errors due to different physical lag, though perhaps not eliminate them, nor overcome all the disadvantages in the technique of Warburg and Burk. However, it is significant that the yield was found to be dependent on both timing and vessel shape.

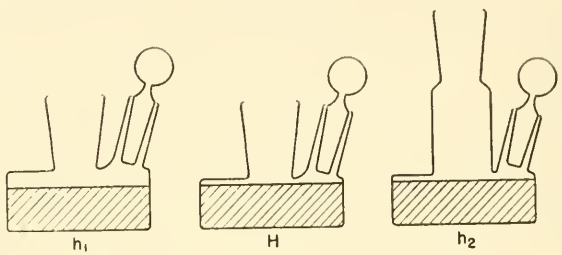

Fig. 29.4A. Manometric vessels for two-vessel method of quantum yield measurements. *H*, vessel with small gas space; h_1 h_2, vessels with large gas volume.

7. Warburg and Burk have calculated $-\Delta O_2/\Delta CO_2$ values of the order of $1(\pm 0.2)$ for the "light effect" in the two-vessel experiments. However, this does not prove that no significant carbon dioxide burst and gulp had occurred in their experiments, but—as already was explained on page 1101—merely that the "gulp" in the dark period compensated more or less completely for the burst in the light period. This compensation is inevitable in a series of light-dark cycles in which approximately stationary conditions are established after a few cycles. Separate calculation of $-\Delta O_2/\Delta CO_2$ in light and in darkness (suggested on p. 1102) gives—in the few cases where the necessary data are provided by Warburg and Burk—values quite different from 1, with deviations in the direction required by the burst-and-gulp hypothesis.

8. Warburg and Burk's experiments with white (or red) background light have additional uncertainty because they were carried out by consecutive, and not alternate, measurements in the two vessels. Earlier experiments of Emerson and Lewis had indicated that the burst occurs not only upon change from light to dark, but also upon change from one light intensity to a higher one; thus, the interpretation of experiments with light-compensated (or overcompensated respiration as "base line" can be the same as suggested above for the light-dark experiments.

9. To sum up the conclusions of Emerson and co-workers, the experi-

ments of Warburg and Burk can be duplicated by strict adherence to their specifications. However, the results obtained in this way are not only of low precision (as revealed by wide scattering), but, what is more important, contain a systematic error.

The differential manometer experiments of Emerson and Lewis (1941) had been far more precise than either the experiments of Warburg, Burk *et al.* (1948–1950), or the experiments which Emerson and co-workers made in 1949–1950 under conditions closely imitating those of Warburg and Burk. It seems that the most reliable of the presently available data on the quantum yield remain those derived from these older measurements.*

Manometric quantum yield measurements have also been reported by Kok (1948, 1949). He considered the loss of light by scattering in thin suspensions as a lesser experimental difficulty than the large respiration, the wide variation of local light intensity, and the intermittency of illumination inevitable in strongly agitated, dense suspensions. He therefore worked with *Chlorella* suspensions that absorbed only 30–40% of the incident light (yellow sodium light), and used an Ulbricht sphere for the measurement of absorption. He found practically linear light curves up to remarkably high incident intensities—sometimes as high as 20 times the respiration-compensating light! (Compare chapter 28, section A2). Quantum yield determinations were made by Kok in four different ways: (*1*) by measuring the carbon dioxide exchange only, oxygen being absorbed by chromous chloride in the side arm of the Warburg vessel; (*2*) by measuring the oxygen exchange only, carbon dioxide being absorbed, in the usual way, in carbonate buffer; (*3*) by measuring both the carbon dioxide and the oxygen exchange by the two vessel method, and (*4*) by measuring the net exchange in a single vessel, and assuming $Q_P = 1.09$.

The quantum requirements, $1/\gamma$, were calculated from the *slope of the straight, ascending section of the light curves*, thus avoiding explicit use of a respiration correction. [The underlying assumption is, of course, that the respiration, R, is the same at all light intensities at which a straight line is obtained for the function $P - R = f(I)$]. Kok found the so-calculated efficiencies to depend on the age of the suspension (*cf.* fig. 28.13). (This probably means, primarily, dependence on the freshness of the culture medium.) The yields were almost independent of the temperature and the light intensity used in the cultivation of the algae. They were about 20% higher in acid media (water, culture liquid, or phosphate buffer) than in alkaline carbonate buffers. Lowering the oxygen pressure to 0.25% had no effect on the quantum yield, and the same seemed to be true of changing the temperature from 10 to 20 or 30°C.

The $1/\gamma$-values obtained by the four methods ranged (apart from a

* New results by Warburg and Burk (1951[1,2]) pertain not so much to the question of the quantum yield of photosynthesis, as to that of the mechanism of utilization of the quanta. They will be described in chapters 36 and 37.

few exceptionally high figures) from 6.9 to 12.9; the average for non-alkaline media (methods 1, 3, and 4) was $1/\gamma = 7.85$; for alkaline buffers (method 2) about 10. Kok estimated $1/\gamma = 6.75$ as the most probable lowest value of the quantum requirement.

The most interesting (and controversial) finding of Kok was that linear extrapolation of the light curves to $I = 0$ consistently lead to considerably smaller values of the gas exchange than would have corresponded to the respiration of the same cells in the dark. Upon closer study, he concluded that the light curve underwent a sudden change of slope by a factor of about 2, somewhere near the compensation point (fig. 29.4B). He took this to mean that the quantum efficiency was constant from near the saturation point down to the compensation region, and then doubled suddenly. This shape of the light curve—according to Kok it consists of three practically linear segments—has not been found by any of the previous observers; however, Kok claimed a confirmation of the sharp break in the $P = f(I)$ curve by new analysis of the data of Kopp and of Gabrielsen.

If the slope of the light curve changes by a factor of two at the compensation point, the rate of respiration in strong light, determined by linear extrapolation of the light curve from above the compensation point to $I = 0$, must indicate a rate of respiration in light equal to one half of the rate of respiration in darkness.

Later (1949) using a more precise manometric device (a "differential volumeter") Kok found, as an average of 50 experiments with *Chlorella* cells grown in Knop's medium, $R_{\text{light}} = 0.5\ R_{\text{dark}}$. (To increase R_{dark},

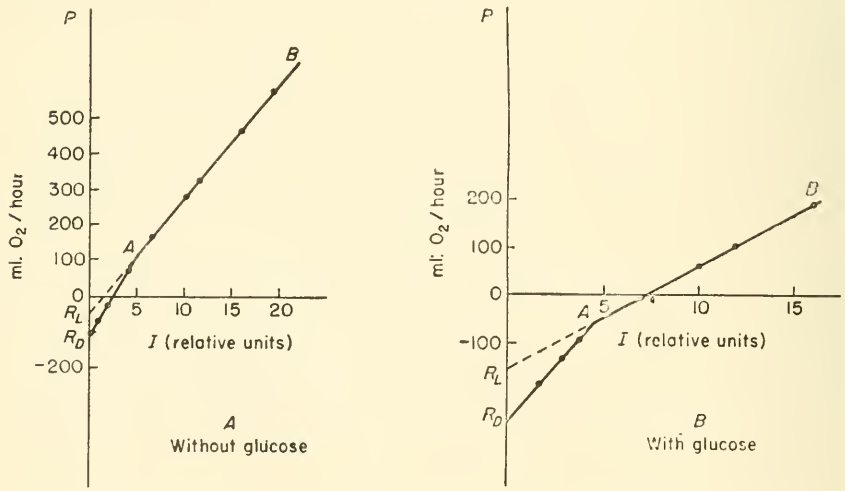

Fig. 29.4B. Light curves of photosynthesis at low light intensity after Kok (1949), showing knick near compensation point.

these measurements were made at 30°C.) However, the break in the $P = f(I)$ curve was now found not exactly at the compensating intensity, but at about twice this intensity (fig. 29.4B(A)). Since R_{light} was equal to 0.5 R_{dark}, the ratio of the slopes below and above the break is, for these algae, 1.33 rather than 2.0.

With *Chlorella* cells grown in glucose solution, the break was *below* the compensation point (fig. 29.4B(B)); the slope below the break was, in this case, exactly one half of that above it, which meant that R_{light} was greater than 0.5 R_{dark}—perhaps reflecting enhanced respiration in the cytoplasm. A break in the same region (*i.e.*, below the compensation point) was found also in the *Haematococcus pluvialis* grown in inorganic medium; but in this case, the slope below the break was less than twice that above it. Light curves obtained with *Cabomba* leaves (floated on carbonate buffer) indicated that the break was present there too, and that $R_{\text{light}} \simeq 0.5\ R_{\text{dark}}$.

Kok suggested that these experiments indicate the existence of two light processes, with the quantum requirement of the "low-light process" (which he called "light respiration," *cf.* below) exactly one half that of the "high light process" (true photosynthesis). When the slope in the low-light region was less than twice that above it, he interpreted this as indication that the two light processes were occurring simultaneously. When the slope in low light was exactly ½ of that in high light, Kok assumed that the high light process did not begin until the low-light process was saturated.

Kok considered these experiments (which had indicated a probable lowest $1/\gamma$-value of 6.75 above the break), as making plausible a quantum requirement of 6 for the high light process (true photosynthesis), and 3 for the low light process ("light respiration"). However, according to Franck (1949), Rieke found in Kok's method of light measurement an error which might have reduced the calculated quantum requirements by 20%; with this correction, the results become consistent with the assumption of quantum requirements of 8 and 4, respectively.

The sharp breaks in the light curves, found by Kok, are very improbable (*cf.* the discussion in chap. 26 of the impossibility of a sharp break between the ascending and the horizontal part of the light curve, postulated by Blackman). However, even if the light curves are smoothly curved rather than broken lines the possibility remains that they may decline in the low light region more steeply than would be expected from their shape in the region of higher light intensities. Once before—in the explanation of the alleged incapacity of cyanide to reduce photosynthesis below the compensation point (Vol. 1, page 308)—we have been led to the hypothesis that compensation of respiration in light may not requrie complete photosynthesis. We will confront the same situation in the description of the study, by Calvin and co-workers of respiration in light with the help of tracer

carbon (Chapter 36). From the latter experiments, Calvin drew the same conclusion as Kok—that the rate of respiration in strong light is only about one half of that in darkness. (The remaining one half may represent the proportion of the total cell respiration taking place outside the chloroplasts and therefore not affected by light.)

Kok suggested that photosynthesis *substitutes* for respiration by producing energy carriers (such as high energy phosphates or "HEP" molecules) which the organism requires for its metabolic activity, and which it ordinarily derives from respiration. More specifically, Kok postulated that the primary light reaction in photosynthesis has a twofold function: (*1*) to produce reducing and oxidizing agents (HX and Z, *cf.* Vol. I, scheme 7.IV) capable, respectively, of reducing CO_2 to CH_2O and of oxidizing H_2O to O_2; and (*2*) to produce HEP-molecules by transphosphorylations coupled with back reactions between these primary products:

$$HX + Z + \text{phosphate} \longrightarrow HZ + X + HEP$$

Until respiration is fully compensated—or, rather, suspended as unnecessary (at least, in the chloroplasts)—the absorbed light is used only or mainly to produce HEP molecules. Respiration of one $\{CH_2O\}$ group has been reported to produce six HEP molecules (Ochoa, Lippman); Kok suggested that the same number can also be obtained by recombination of six (HX + Z) pairs, and that these six pairs can themselves be produced by *three* quanta. Thus, two HEP molecules, containing about 20 cal./mole disposable energy, are formed by one quantum of red light (about 40 cal./einstein).

Above the compensation point, Kok assumed a quantum requirement of 6; he postulated that here, too, each quantum produces *two* (HX + Z) pairs, and that out of twelve such pairs (produced by six quanta), four (produced by two quanta) react further to reduce $\{CO_2\}$ to $\{CH_2O\}$ and to oxidize H_2O to O_2, and eight (produced by four quanta) react back, converting eight low energy phosphates into eight HEP molecules (which, in turn, are utilized as "boosters" in the reduction process). The quantum requirement would then be 3 for the reversal of respiration and 6 for true photosynthesis. (No explanation was given by Kok why eight HEP molecules are needed in the latter case, as against only 6 in the first one.)

This, obviously highly arbitrary scheme made to fit the (supposedly) experimentally indicated $1/\gamma$-values of 3 and 6, is closely related to the "energy dismutation" schemes (such as scheme 9.III) proposed (among other possible reaction schemes of photosynthesis and chemosynthesis) in chapter 9. The assumption that *one* third of all quanta are used in photosynthesis to provide oxidation and reduction agents, and *two* thirds for the formation of energy boosters (HEP molecules), imitates the mechanism of

chemosynthesis postulated for hydrogen bacteria (schemes 9.IV) in which *two* hydrogen molecules reduce carbon dioxide, utilizing the energy liberated by the oxidation of *four* hydrogen molecules by oxygen.

According to Franck (1949) (*cf.* page 1115), it is permissible to interpret Kok's results as indicating quantum requirements of 8 (rather than 6) for true photosynthesis and 4 (rather than 3) for the "low light process." If one wants to retain Kok's picture, one can, for example, suggest that four quanta produce four oxidation and reduction agents (4HX + 4Z), while the other four produce—by back reaction of another four (HX + Z) pairs—eight HEP-molecules. (The numerical analogy with the hydrogen bacteria would be lost in this way; but it is more plausible that one quantum produces a single HX + Z pair than that it produces two such pairs, as was suggested by Kok).

Van der Veen (1949), in the course of a study of induction phenomena by the thermal conductivity method (chapter 33), found, for tobacco leaves, a light curve of photosynthesis similar to that recorded by Kok—a straight line up to 450 lux, and another straight line, with about half of the slope of the first one, from 450 to 3200 lux. He combined Kok's concept of the reaction mechanism of photosynthesis with scheme 9.IV, interpreting the "energy dismutation" postulated in this scheme (*cf.* pages 164 and 239, Vol. I), as production of HEP molecules by recombination of a part of the primary photochemical oxidation and reduction products, and "boosting" by these HEP molecules of the reductive power of the remaining reduction products. The specific numbers used in his scheme (eight recombinations to four oxidation-reductions) taken from Kok, could equally well be replaced by others—*e.g.*, by four recombinations and four oxidation-reductions, as in scheme 9.III; and the same is true of the number of quanta required (six, or eight, or even twelve).

A somewhat different—and perhaps more plausible—interpretation of a comparatively low quantum requirement of "anti-respiration" in weak light has been suggested (Franck, 1949): This is the (repeatedly mentioned) possibility that intermediates of respiration can be drawn into the photosynthetic cycle and reduced back to the carbohydrate level, and that a smaller number of quanta is required for this process than for complete photosynthesis. It is important to note that such a half-way interception of respiration would not cause a deviation of the $\Delta O_2/\Delta CO_2$ ratio from its normal value of (approximately) 1—since the only gas exchange measured in low light will be that due to residual normal respiration (*e.g.*, respiration outside the chloroplasts). Calvin suggested, on the basis of certain C(14) tracer experiments, that a cross-link between respiration and photosynthesis exists on the level of malic and oxalacetic acid; however, these observations are still controversial (*cf.* chapter 36).

Franck (1949), in offering an explanation of Warburg and Burk's results in terms of photochemical half-way reversal of respiration, suggested that the extent to which this process occurs depends on the capacity of respiration intermediates (which probably are organic acids) to penetrate from the protoplasm into the chloroplasts, and that this capacity is affected by the physiological state of the cells. It remains to be seen whether this explanation can suffice to explain why many careful experiments have failed to show the existence of the phenomenon. Thus, Emerson and co-workers never had observed any curvature of the light curves in the region of the compensation point, which would indicate a lower quantum requirement in very low light. Brown and co-workers (1950) found no evidence that light interferes with respiration in mass-spectrographic experiments: The uptake of O(16)O(16) from the air continued in light, while O(16)O(18) was evolved simultaneously by photosynthesis from algae suspended in O(18)-enriched water. It was mentioned before (page 1108) that Warburg and co-workers (1949) arrived at a similar conclusion by observations of the rate of oxygen consumption in darkness and light under conditions assuring rapid removal of respiratory carbon dioxide from the medium; it was, however, suggested that these findings might have been contingent on the intermittency of illumination, which prevented the utilization for photosynthesis of a large proportion of respiration products.

To sum up, the possibility of photochemical utilization of respiratory intermediates remains controversial, and the effect of this re-utilization on the quantum requirement in weak light, an open question. Suggestive experimental evidence is available for both a negative and a positive answer. (On the positive side: Warburg's cyanide experiments, Kok's and van der Veen's broken light curves, Calvin's carbon tracer experiments. On the negative side: Emerson and Lewis' smooth light curves, Warburg's experiments in CO_2-free medium, Brown's respiration study with oxygen isotopes.) Whether Franck's suggestion, that the chloroplasts sometimes are and sometimes are not permeable to respiration intermediates formed in the cytoplasm, can explain these contradictions is uncertain. A knowledge of the relative contribution of chloroplasts and cytoplasm to total cell respiration in the dark would be useful in this connection; but no estimate of this relation has as yet been made.

2. Nonmanometric Measurements of Quantum Yield

The results of nonmanometric measurements of the quantum yield on the whole agree with the lower figures ($1/\gamma = 10 \pm 2$) found by Emerson and Lewis, Rieke, and others by manometric studies rather than with the higher figures ($\gamma = \frac{1}{3}$ to $\frac{1}{4}$) claimed by Warburg and Burk.

(*a*) *Chemical Methods*

Wurmser (1923, 1925, 1926) made a few quantum yield determinations with the green alga *Ulva lactuca*. Each experiment lasted for several hours, and consisted in the measurement of *change in oxygen concentration in solution* by Winkler's method. The rate of absorption of light was calculated from comparison of transmission by green and discolored thalli (*cf.* chapter 22, page 675), using a theoretical equation to take into account scattering (*cf.* page 713). Wurmser found, in some of these experiments, energy conversion factors up to 50%, corresponding to quantum yields up to ¼. However, the calculated yield in the (weakly absorbed) green light turned out to be so much higher than in the (strongly absorbed) red light, that it indicated probable grave errors in the calculation of absorption. Warburg (1925) therefore did not consider these experiments of Wurmser as significant confirmation of his own results.

Briggs (1929) obtained, with leaves of *Phaseolus vulgaris*, at light intensities 5–10 times stronger than those used by Warburg and Negelein, yields from 7–17 cc. O_2/500 cal absorbed energy; with yellow elm leaves, from 5.3 to 8.9 ml.; with green elm leaves, from 12 to 20 ml.; and with leaves of *Sambucus nigra*, from 9 to 19 ml. These values correspond to quantum yields <0.1.

In 1935, Gabrielsen, working with plants of *Sinapis alba*, calculated, also from gas-analytical measurements, by extrapolating the light curves (fig. 30.8A,B) to zero illumination, ϵ values from 0.13 in blue, to 0.36 in red light, corresponding to maximum quantum yields of 0.1 ± 0.02. Gabrielsen did not question at that time the correctness of Warburg's results, and thought that his lower yields must have been due to the use of a less efficient species.

Later (1947), Gabrielsen repeated these experiments with *Sinapis*, *Corylus* and *Fraxinus* leaves, and found γ_0-values between 0.082 and 0.078.

In the first of a series of investigations emanating from the University of Wisconsin, Manning, Stauffer, Duggar and Daniels (1938) determined the quantum yield by gas analysis, comparing the composition of a gas (containing approximately 5% CO_2 and 5% O_2) conveyed through a *Chlorella* suspension in the light and in the dark. The suspensions were less dense than in Warburg and Negelein's work, absorbing only 10–50% of the incident light; the intensity of the latter (green line from a mercury lamp) was somewhat higher than in Warburg and Negelein's experiments (1000–1750 erg/cm.^{2} sec.); 60 minute periods of illumination were used. The γ values derived from the absorption of carbon dioxide were not very different from those calculated from the increase in the concentration of oxygen, thus indicating that the quotient Q_P was close to unity.

The quantum yields obtained in these experiments scattered considerably—from 0.01 to 0.1—but never exceeded the latter limit. Still lower quantum yields (0.002 to 0.027) were obtained in experiments in *white* light; in this case, however, about ten times higher intensities of incident light were used, so that saturation effects appeared possible. Experiments with a different technique (closed reaction bottles, no stirring, analytical determination of the change in $[O_2]$ in solution by Winkler's method) yielded γ values between 0.02 and 0.065.

In another paper from the same laboratory, Manning, Juday and Wolf (1938) described experiments in which bottles containing *Chlorella* suspensions were deposited at different depths in a lake, and thus exposed to different intensities of illumination, ranging from full sunlight (600 kerg./cm.^{2} sec., not counting the infrared) down to 6 kerg/cm.^{2} sec. The change in color of the light with depth (*cf.* Table 22.XI) complicated the calculation of the number of absorbed quanta; the results were therefore less exact than those of the first paper. However, the approximate magnitude of γ values was the same as in other experiments—about 0.05 at the lowest light intensities (at 10 meter depth); *cf.* figure 29.5.

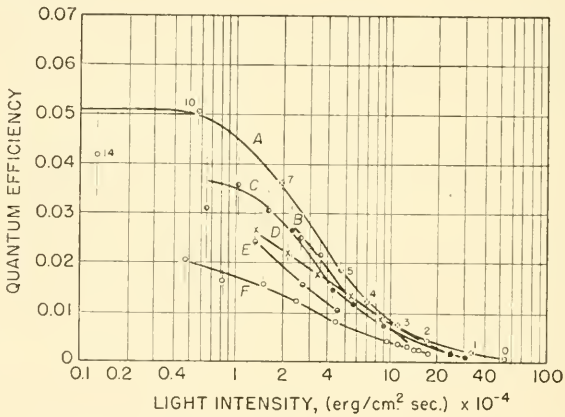

Fig. 29.5. Quantum efficiencies for *Chlorella* (after Manning, Juday, and Wolf, 1938). Curve *A*, 3.17 hr., cell concn. 3,250,000/ml.; *B*, 1.03 hr., cell concn. 718,000/ml.; *C*, 4.00 hr., cell concn. 331,000/ml.; *D*, 4.00 hr. cell concn. 718,000/ml.; *E*, 4.05 hr., cell concn. 1,900,000/ml.; *F*, 3.30 hr., cell concn. 1,210,000/ml.

(*b*) *Polarographic Method*

In a third investigation from the Wisconsin laboratories, Petering, Duggar and Daniels (1939) applied the *polarographic method* (*cf.* page 850) because it permitted the determination of respiration *immediately* before and after a period of photosynthesis, without the delays (illustrated by fig. 29.1) inherent in the manometric method. Figure 29.6 shows the polaro-

graph to respond almost immediately to transitions from respiration (in darkness) to photosynthesis (in light) and *vice versa*. In the top and bottom curves, the illumination is below the compensation point, and photosynthesis manifests itself in a reduced rate of consumption of oxygen; in four other curves, the oxygen concentration increases during photosynthesis. The curvatures of the respiration curves show the uncertainty involved in calculation of the respiration correction. The rate of oxygen consumption in the five minutes immediately following the cessation of illumination was used by the authors in the calculation of this correction. (This method gives the highest respiration correction and consequently the highest quantum yield values.)

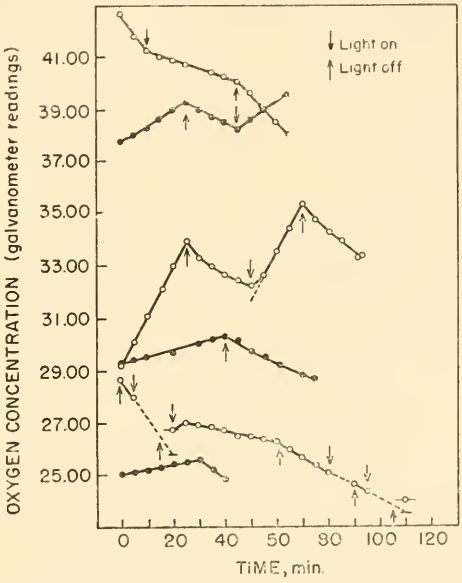

Fig. 29.6. Photosynthesis and respiration of *Chlorella* measured by a polarograph (after Petering, Duggar and Daniels 1939).

The experiments were made with white light; from 27 to 48% of the incident light was absorbed by the suspension. The calculated quantum yields ranged from 0.045 to 0.100, clustering around 0.07, and showing no trend with light intensity in the range from 1000 to 6000 erg/cm.^{2} sec.

New experiments with the polarograph were conducted by Moore and Duggar (1949). In these *Chlorella* cells were first illuminated with light of one color (intensity, 800–2500 erg/cm.^{2} sec.) and then light of another color was added, and the additional yield determined. The idea behind this procedure was that the uncertainty concerning the amount of respira-

tion in light can be eliminated by subtracting from the total gas exchange in the two combined beams the gas exchange in one beam alone. (There seems to be no difference between this method and calculation of γ from the difference in yield at two light intensities in light of the same color.) The results of these measurements are shown in Table 29.V. The γ values

TABLE 29.V

POLAROGRAPHIC DETERMINATION OF QUANTUM YIELDS OF *Chlorella* IN RED AND RED PLUS BLUE LIGHT
(AFTER MOORE, AND DUGGAR 1949)

Initial beam				Added beam				
λ	I_a	Abs., %	γ	λ	I_a	Abs., %	γ	$1/\gamma$
6500	1958	76	0.074	4358	1575	100	0.11	9.1
6500	783	46	0.12	—	—	—	—	—
4350	1288	91	0.09	6500	1180	50	0.10	10.0
6500	1610	49	0.10	5461	2532	40	0.08	12.5
6500	1392	74	0.10	5461	2437	64	0.09	11.0
6500	1188	52	0.11	4047	460	77	0.10	10.0

[a] Intensities in erg/cm.^{2} sec.

for the superimposed beam (0.08 to 0.11) are not significantly different from those for the single beam (0.074 to 0.12), a result that can be interpreted as indicating two things: approximate identity of quantum yields in blue and in red light (despite the absorption of light by carotenoids in the first-named region; *cf.* chapter 30), and approximate linearity of the light curve up to the total intensity of the two combined beams.

Several objections can be made (and have been made by Warburg) against the polarographic quantum yield determinations:

1. The determination of the number of absorbed photons was not satisfactory. A thermopile was placed immediately behind the reaction cell; the energy flux falling onto the thermopile with and without the algae in the reaction vessel (illuminated by parallel light) was multiplied by the ratio vessel area:thermopile area and the difference between the two products was assumed to be the absorbed flux. This assumption implies that scattering *out* of the beam intercepted by the thermopile is compensated by scattering *into* this beam, and thus neglects large angle scattering. The error caused by this could lead to too high a value for absorbed light energy, and hence to too low a value for the quantum yield.

2. The suspension was not stirred. (Stirring *during* measurements is impossible by the nature of the method; stirring *between* measurements was attempted, but found not to influence the results and was therefore abandoned). The algae did not settle during a run; and stirring is obviously of less importance when oxygen determination is made in the body of the liquid than when it is carried out in the gas phase above it. On the other hand, carbon dioxide exhaustion conceivably could occur in the immediate neighborhood of the cells, and cause a diminution of the quantum yield. However, this danger should not be serious when measurements are made at or below the compensation point.

3. Although the medium (nutrient solution, *p*H 5.5) satisfied Warburg's requirements of "physiological" conditions, presence of mercury drops introduced a danger of poisoning. In fact, such poisoning has been observed, but deemed too slow to affect the measurements.

(*c*) *Calorimetric Method*

The basis of the calorimetric determination of the yield of photosynthesis—which is a direct measurement of the energy conversion yield, ϵ, rather than of the quantum yield, γ—was described in chapter 25 (page 854). The first to carry out such measurements was Arnold in 1936–1937; however, so strong was the belief at that time in the correctness of Warburg's value, $\gamma = \frac{1}{4}$, that Arnold took his inability to obtain this yield as indication of a failure of the method, and did not publish his results until 1949 (reference to them was made by Franck and Gaffron 1941). Arnold used a modified Callender's radiobalance, originally designed to measure heat production by radioactive materials. Its period was so small that complete measurements could be made in from 1 to 10 minutes. Between 0.05 and 4 mm.^{3} of cells, in Knop's solution or carbonate buffer, were used. One run was made with healthy *Chlorella* cells, and one run with the same cells inhibited by ultraviolet irradiation. Respiration was assumed to be unaffected by ultraviolet light (*cf.* Vol. I, page 344). The results are shown in Table 29.VI.

TABLE 29.VI
CALORIMETRIC DETERMINATION OF QUANTUM YIELD (AFTER ARNOLD 1949)

Cells	Extra heat evolved in light[a]: With inhibited cells, I_a	With healthy cells, $I_a - \Delta H_c$	ΔH_c	$\frac{100\,\Delta H_c}{I_a}$, %	$1/\gamma$
HEAT PRODUCTION IN MICROWATTS					
C. pyrenoidosa	5.08	3.70	1.38	27.2	9.5
	4.60	3.80	0.80	17.4	14.8
	4.46	3.90	0.56	12.5	20.6
	2.24	1.90	0.34	15.2	16.9
Avocado leaf	0.786	0.656	0.130	16.5	15.6
C. vulgaris	0.912	0.700	0.212	23.2	11.1
C. pyrenoidosa	1.86	1.34	0.52	27.9	9.2
	16.2	12.9	3.3	20.4	12.6
HEAT PRODUCTION IN ARBITRARY UNITS					
Scenedesmus	2.63	2.04	0.59	22.4	11.5
	3.24	2.40	0.84	25.9	10.3
C. pyrenoidosa	2.08	1.65	0.43	20.7	12.4
	1.75	1.36	0.39	22.3	11.5

[a] The illuminating light was from a neon arc (ten to fifteen red lines isolated by a red filter), and had a very low intensity—from 61 to 126 erg/sec. cm.^{2}

Measurements with a photocalorimeter (*cf.* page 854) were carried out also at Wisconsin, by Magee, DeWitt, Smith and Daniels (1939), and gave γ values from 0.049 to 0.110 (the average of 17 experiments was $\bar{\gamma} = 0.077$ or $\overline{1/\gamma} = 13$). No pronounced change with light intensity was noted between 1200 and 8000 erg/cm.^{2} sec.

More recently, Tonnelat (1944, 1946), who worked in Wurmser's laboratory in Paris, published similar results of an investigation initiated in 1939. Tonnelat measured the heat developed in an adiabatic microcalorimeter under three conditions: (*1*) when the calorimeter contained an illuminated black-bottomed vessel with pure water (heat evolution, E_0); (*2*) when it contained the same vessel with an equal volume of an algal suspension in the dark (heat evolution, E_R, due to respiration); and (*3*) when it contained the same suspension and was illuminated (heat evolution, $E = E_0 + E_R - \Delta H_c$). The energy yield, ϵ, was then:

$$\epsilon = \Delta H_c/E = (E_0 + E_R - E)/E \tag{29.3}$$

and the quantum yield, according to equation (29.1), assuming $\lambda = 530$ mμ (green light isolated by Wratten filter No. 62):

$$\gamma = \epsilon/(4 \times 10^{-3} \times 530) = \epsilon/2.12 \tag{29.4}$$

Illumination lasted for 16 hours without noticeable deviation from linearity (*i. e.*, presumably, from the constancy of both R and P); even in the very low light used (about 1.5×10^{-8} einstein of green light/cm.^{2} min., or 550 erg/cm.^{2} sec.), such long duration of the experiments seems dangerous. The results of Tonnelat's determinations are shown in Table 29.VII.

TABLE 29.VII

EFFICIENCY OF PHOTOSYNTHESIS (AFTER TONNELAT 1944)
MEASURED WITH PHOTOCALORIMETER

Experiment	Chlorella concn., cells/0.5 cm.^{3}	Energy conversion factor
(1) Reflecting bottom.....	32×10^6	0.26 (±0.06)
(2) Reflecting bottom.....	33	0.34 (±0.05)
(3) Reflecting bottom.....	37	0.26 (±0.06)
(4) Reflecting bottom.....	56	0.31 (±0.05)
(5) Reflecting bottom.....	194	0.08 (±0.08)
(6) Black bottom.....	36	0.12 (±0.07)
(7) Black bottom.....	51	0.31 (±0.05)
(8) Black bottom[a].....	48	0.18

[a] With 0.1% agar.

Experiment 5 shows that the yield became low in very concentrated suspensions (a result Tonnelat attributed to inhibited gas exchange in the dense layer of cells on the bottom of the vessel, but which also could be due

to "self-inhibition" effects, described on page 880). Experiment 8 shows decreased efficiency in the presence of agar—an observation that Tonnelat suggested may explain some of the low values found by McGee, DeWitt *et al.* (who used agar to prevent the suspension from settling).

Experiments were performed in vessels with black or reflecting bottom. In the first case, γ values could be too low (if absorption of light by the blackened bottom was counted as absorption by the cells); in the second case, they could be too high (because of possible escape of reflected radiation). Figures in Table 29.VII indicate that the first effect is real (experiment No. 6 shows a lower value of γ at low cell concentration), but reveal no effects due to reflection.

Tonnelat concluded from these experiments that the energy conversion factor, ϵ, is about 0.30, and calculated from this a quantum yield of $\frac{1}{8}$. Equation (29.1) gives, however, $\gamma = 0.030/2.12 = 0.145$, or approximately $\frac{1}{7}$. Tonnelat's error was probably due to the use of an incorrect value, 8.9×10^{-12} instead of 7.9×10^{-12} erg/mole, for the heat effect of photosynthesis.

3. Quantum Yield of Bacterial and Algal Photoreduction

French (1937[1]), who worked in Warburg's laboratory, used the bacterial species *Streptococcus varians* to study the quantum yield of the reduction of carbon dioxide by molecular hydrogen (*cf.* chapter 5, page 104). The rate of hydrogen disappearance was determined manometrically. The lines 852 and 894 mμ were isolated by filters from the light of a cesium lamp; from 17 to 58% of this light was absorbed by the suspension. The quantum yields calculated by French ranged from 0.07 to 0.23 molecule of carbon dioxide (*i. e.*, from 0.14 to 0.46 molecule of hydrogen) transformed per quantum, depending on the pretreatment of the bacteria. French considered these experiments proof that carbon dioxide reduction by *Athiorhodaceae* requires four quanta per molecule of carbon dioxide, similarly to the assimilation of green plants according to Warburg and Negelein. As mentioned before, the light curves obtained by French in this work were *sigmoid;* the $\gamma_{max.}$ values were derived from the *maximum slope* of these curves (reached in the inflection point). This procedure requires justification. To divide the *increase in yield* in a certain region of the light curve by the corresponding *increase in light absorption*, and to call the quotient "quantum yield" presupposes that the increase in light intensity produces a certain additional amount of photosynthesis, characterized by its own quantum yield. Wassink, Katz and Dorrestein (1942) suggested that this may, in fact, be the case, if the bacteria use, in weak light, mainly intracellular organic compounds, instead of the externally supplied reactants. As light intensity increases, this photochemical process is soon light

saturated (because of supply limitations, or because of the limited amount of a necessary enzyme); the normal reduction of carbon dioxide with external reductants then comes into its own. The manometrically determined quantum yield of the photochemical process using intercellular substrates can be smaller than that of normal photoreduction, for two reasons:

(*a*) When *hydrogen* is used as external reductant, any utilization of nonvolatile, intracellular reductants will diminish the rate of gas consumption, even if the quantum yield of carbon dioxide reduction is the same with both types of reductants. In French's experiments (1937) with *Streptococcus varians*, the light curve had total gas consumption, $\Delta H_2 + \Delta CO_2$ as ordinate; its sigmoid shape may have been due entirely to an initial deficiency in the consumption of hydrogen alone. In the experiments of the same author with *Spirillum rubrum* (1937[2]) a nonvolatile reductant was used, and the curve showing ΔCO_2, as function of light intensity, showed no inflection. However, in the more recent experiments of Wassink, Katz and Dorrestein (1942) with *Chromatium*, sigmoid curves were obtained not only with hydrogen but often also with thiosulfate as reductant (*cf.* fig. 28.11). A different explanation is needed in this case.

(*b*) It was described in chapter 5 (Vol. I, page 106) how, when externally supplied organic compounds are utilized by photosynthesizing purple bacteria, the proportion of "coassimulated" carbon dioxide can vary widely (or carbon dioxide may even be liberated), depending on whether the organic compound is utilized mainly or exclusively as hydrogen donor (as in Foster's experiments with secondary alcohols), or serves also as the source of carbon. Wassink, Katz and Dorrestein suggested that the same applies to photochemical utilization of intracellular organic materials; here, too, the consumption of external carbon dioxide may be more or less completely suppressed by the utilization of the carbon (in the form of freshly formed carbon dioxide, or of oxidation intermediates) produced by dehydrogenation of the organic reductant.

These two considerations provide a plausible explanation of sigmoid gas exchange curves, but do not fully justify calculation of the maximum quantum yield of photoreduction from the slope of the steepest section of the light curve. In the first place, the utilization of internal reductants is at present merely a hypothesis. In the second place, assuming this hypothesis is correct, it is still possible—and, indeed, likely—that, as light intensity increases, photoreduction *replaces* (and not merely *supplements*) the photochemical transformation of intercellular substrates. This can occur either because of exhaustion of the intracellular material, or because of changes in the enzymatic system (as in the "de-adaptation" of hydrogen-adapted green algae; *cf.* Vol. I, chapter 6). More precise measurements of the photosynthetic ratio $\Delta[CO_2]/\Delta[\text{reductant}]$, at different light intensi-

ties, and investigations of the effect of intensity and duration of illumination on the shape of the light curves, could help to elucidate the situation. Until there is proof that the sigmoid shape of the light curves actually *is* due to an internal photochemical process resulting in no (or only little) gas consumption; and until it has been proved that this process, having become saturated in very low light, continues at a constant rate as the light grows stronger, the legitimate way to interpret the light curves is the conservative one: to consider the sigmoid shape as evidence that the average quantum yield of photoreduction, $\bar{\gamma}$, first increases with light intensity and then decreases again. The measure of $\bar{\gamma}$ in each point of the curve then is the slope of the straight line drawn from this point to the origin of the coordinates, and not the slope of the tangent. (Similarly, we do not attribute the convex part of the light curves to a superposition of low-yield photosynthesis upon persisting high-yield photosynthesis, but to a decrease in the *average* yield.)

In this way, we can deduce from the sigmoid light curves only a *lower limit* of the maximum quantum yield (this limit being given by the slope of the tangent to the curve that passes through the origin of the coordinates). This limit, derived from French's light curve of *Streptococcus varians*, is about $\gamma_{\text{lim.}} = 0.11$.

In French's study of *Spirillum rubrum* (1937[2]), the yield was measured by the uptake of carbon dioxide. As mentioned above, the light curves showed, in this case, no initial curvature; their slope corresponded to a quantum yield of the order of 0.06 to 0.07. These values were termed "unreliable" by French because of inexact determinations of light absorption. Subsequently, however, yields of similar magnitude were found in several investigations by the Dutch group (Wassink, Katz and co-workers). In their measurements, the initial concavity of the light curves often was only slight, and did not affect essentially the calculated quantum yield.

Eymers and Wassink (1938) measured the quantum yield of photosynthesis by *Thiorhodaceae*, with thiosulfate serving as a reductant and a cesium or sodium lamp as light source. The results are shown in Table 29.VIII. One notices that these organisms have a very strong dark meta-

Table 29.VIII

Quantum Yields of Carbon Dioxide Reduction by Purple Bacteria with Thiosulfate as Reductant (after Eymers and Wassink 1938)

Light source	Intensity, $10^3 \times$ erg/cm.^{2} sec.	ΔCO_2 in light	ΔCO_2 in dark	$1/\gamma$[a]	γ
Cesium (850–890 mμ).....	5.2 to 83	+30 to −121	+47 to −1	9.2 to 35	0.03 to 0.109
Sodium (590 mμ).	12 to 105	+12 to −302	+55 to −4	8.8 to 33[b]	0.03 to 0.114

[a] Quanta/molecule CO_2.
[b] In old cultures, $1/\gamma$ values up to 175 were observed.

bolism, which makes the exact evaluation of the quantum yield difficult. The largest γ values ever observed by Eymers and Wassink were about 0.11.

In a subsequent investigation from the same laboratory (Wassink, Katz and Dorrestein 1942), a summary of additional γ determinations for the same species (*Chromatium* D) was given, which included values obtained at two different *p*H values and with hydrogen as well as with thiosulfate as reductant. They are shown in Table 29.IX. The figures are described

Table 29.IX

Quantum Yields of *Chromatium* under Different Conditions
(after Wassink, Katz and Dorrestein 1942)

Reductant	*p*H	Temp., ° C.	Quantum yields, $1/\gamma$			
			Determinations	Limits	$1/\bar{\gamma}$	$\bar{\gamma}$
Thiosulfate...	6.3	29°	12	8.5–13.8	10.9	0.092
Thiosulfate...	6.3	19°	1	—	12.8	0.079
Hydrogen....	6.3	29°	7	8.5–16.0	11.4	0.088
Hydrogen....	7.6	29°	6	10.6–15.4	12.6	0.080
Hydrogen....	7.6	22°	1	—	12.1	0.083

as having been calculated from ΔCO_2, and ($\Delta CO_2 + \Delta H_2$) values of the order of 50 or 100 mm.^{3}/hr. According to the figures in text of the paper, this means light intensities of the order of 1000–3000 erg/cm.^{2} sec.

Sapozhnikov (1937) concluded, from his own not further described measurements, that the quantum yield of CO_2 reduction by *Thiorhodaceae* is 1.0. The scepticism one is bound to feel about an experimental finding so in variance with all other observations in the field is not reduced by the thermodynamic treatment the author uses to make his results plausible. He argues that the light energy required to reduce carbon dioxide depends on the oxidation-reduction potential of the medium in which the reduction takes place. Experimentally, he found this potential (in the medium in which bacteria have lived for a while) to be positive enough for the reduction of carbon dioxide to be possible with only 40 kcal/mole extra free energy. He suggested that, for the same reason, the photosynthesis of green plants could also require only one quantum per molecule of carbon dioxide. Sapozhnikov's argument ignores two facts: (*a*) that free energy is also needed to establish and maintain the high positive redox potential, which he suggests does exist in photosynthesizing cells; and (*b*) that photosynthesis is not merely reduction (of carbon dioxide) but also oxidation (of water, or of the other reductants used by bacteria), and that whatever one can gain in energy required for reduction only means that correspondingly more energy is required for oxidation.

Rieke (1949) determined the quantum yield of the green alga *Scenedesmus*, which was adapted (by anaerobic incubation) to the use of molecular hydrogen as reductant (*cf.* Vol. I, chapter 6). He found, both in 4% CO_2 and in 0.025 *M* $KHCO_3$, quantum yields of between $\gamma = 0.05$ and 0.12, *i. e.*, close to those determined for ordinary photosynthesis in the same

species. These yields could be measured at light intensities up to 7000 erg/cm.^{2} sec.; at the higher intensities, transition to ordinary photosynthesis occurred too rapidly.

4. Quantum Yield of Oxygen Liberation by Isolated Chloroplasts

French and Rabideau (1945) measured the quantum yield of the "Hill reaction" (photochemical oxygen production from ferric oxalate solution, sensitized by a chloroplast suspension). This reaction was described in chapter 4 (Vol. I) as possibly representing "one half of photosynthesis"—namely, photoxidation of water, with the ferric salt instead of carbon dioxide serving as oxidant. Chloroplast suspensions were obtained from spinach, or from *Tradescantia*, by maceration and centrifugation, and added to 0.5 *M* $K_2C_2O_4$ + 0.01 *M* $FeNH_4(SO_4)_2$ + 0.02 *M* $K_3Fe(CN)_6$. The solution also contained 0.20 *M* sucrose and 0.17 *M* sodium sorbitol borate buffer. A 10% NaOH solution was present in a side arm of the manometric vessel to absorb carbon dioxide (which could be produced by respiration). Figure 29.7 shows the course of pressure changes in a Warburg ap-

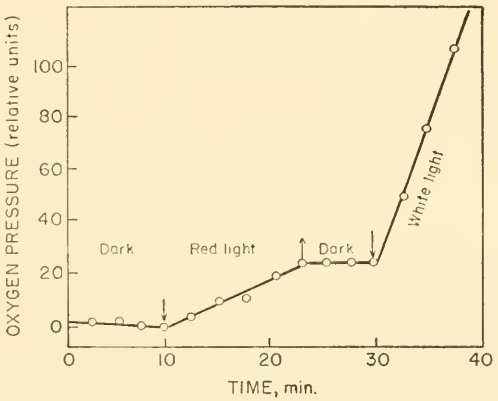

Fig. 29.7. Gas liberation in light from suspension of spinach chloroplasts (after French and Rabideau 1945).

paratus filled with this mixture. The light used was a red band at 660–720 mμ, with a maximum at 685 mμ. The measurements were made at 10° C. Table 29.X gives some typical results obtained with chloroplasts from spinach. Material from *Tradescantia* gave somewhat lower yields.

The quantum yields varied between 0.013 and 0.080, or between 12 and 78 quanta per molecule of liberated oxygen. The average was $\bar{\gamma} = 0.042$ for chloroplasts from spinach, and $\bar{\gamma} = 0.030$ for chloroplasts from *Trades-*

Table 29.X

Quantum Yield of Oxygen Evolution by Illuminated Chloroplast Suspensions from Spinach (After French and Rabideau 1945)

Chloroplast chlorophyll, mg./vessel	Light intensity, micro cal/ cm.²/min.	Fraction of light absorbed	Quantum yield, γ	Quanta required/O_2 molecule, $1/\gamma$	Estimated error, %
0.15	9.0	0.72	0.068	15	10
0.15	3.15	0.71	0.064	16	5
0.34	3.25	0.83	0.030	33	20
0.39	3.3	0.88	0.022	46	20
0.10	3.32	0.57	0.080	12	5
0.10	3.04	0.51	0.037	27	20
0.063	4.13	0.56	0.033	31	20
0.103	4.18	0.545	0.013	78	20
0.09	1.6	0.33	0.058	17	20
0.045	2.8	0.47	0.031	38	5
0.090	2.8	0.79	0.036	28.0	5

cantia (in the same set-up, a value of $\bar{\gamma} = 0.092$ was found for live *Chlorella*). No clear change of $\bar{\gamma}$ with light intensity was noticeable in the range used (1400–6000 erg/cm.² sec., of which 33–72% was absorbed by the suspension).

These quantum yields of the Hill reaction, although markedly lower than the quantum yield of photosynthesis *in vivo*, were nearer the latter than the (much higher) yields of the chlorophyll-sensitized photoxidations *in vivo* (*cf.* Vol. I, page 513, and chapter 35).

The considerable variability of the γ values of the Hill reaction may be caused, at least in part, by rapid deterioration of the material. Several runs, made in succession with one batch, usually showed rapidly declining yields.

New measurements of the quantum yield of the Hill reaction, in whole *Chlorella* cells and in chloroplasts from *Phytolacca americana*, were carried out by Ehrmantraut (1951). They were made relative to the ethyl chlorophyllide-thiorea actinometer; two measurements of the quantum requirement of photosynthesis in carbonate buffer served as an additional, if rough, check on the actinometer, since there is general agreement that this requirement is $1/\gamma_0 = 10 \pm 2$. The results (table 29.XI) are much more consistent than those of French and Rabideau. They were further confirmed by five quantum yield measurements with quinone in whole *Chlorella* cells using a monochromator (λ 669 mμ) and a bolometer which gave, for $1/\gamma$, the values: 10.2; 9.9; 9.3; 10.8; and 12.0 (average: 10.4).

These values, obtained in acid medium (pH 6.5), throw indirect light on the problem of the quantum yield of photosynthesis. Various kinetic evidence points to the Hill reaction having both the primary photochemical process and the rate-limiting dark reaction, in common with photosynthe-

TABLE 29.XI

QUANTUM REQUIREMENT OF THE HILL REACTION (AFTER EHRMANTRAUT AND RABINOWITCH 1951)

(Incident Light Flux 1.5×10^{-7} Einstein per Min.; $t = 10°C.$; 200 μl. Cells)

Material	Oxidant	Quantum requirement
Chlorella	0.5 mg. quinone in 3 cc.	12.4
		12.6
		12.6
		13.1
		13.1
		12.8
	Av.	*12.8*
Chlorella	1.0 mg. quinone in 3 cc.	13.5
		13.4
		14.8
		13.3
	Av.	*13.8*
Chloroplasts (from *Phytolacca americana*)	1.0 mg. quinone in 3 cc.	9.3
		10.2
		11.5
		11.4
		10.6
	Av.	*10.6*
Same	Hill's solution	12.7
Same	Ferricyanide	11.0
Chlorella	Carbon dioxide (No. 9 carbonate buffer)	11.3
		13.0
	Av.	*12.2*

sis. It would be remarkable, under these conditions, if equality of the quantum requirement of the Hill reaction, in whole cells as well as in chloroplast fragments, with the quantum requirement of photosynthesis in alkaline buffers, were to turn out purely coincidental. It seems unlikely that quantitatively the same damage (as measured by a supposedly "substandard" quantum yield) would have been inflicted on the photochemical apparatus by such diverse treatments as immersing cells into alkaline medium, poisoning them with quinone, and smashing them mechanically and separating chloroplasts fragments. A much more plasusible hypothesis is that the photochemical apparatus survives all these treatments without severe damage, and that the quantum requirement of 10 ± 2 represents the true measure of the efficiency of the common primary photochemical process. The quantum requirements of $\ll 8$, reported by Warburg and Burk for photosynthesis in acid media, are the only ones which do not fit into this picture. Whether this discrepancy is caused by a systematic experimental error, as suggested by Emerson and co-workers, or to the substitution, for true photosynthesis, of a partial reversal of respiration, requiring a smaller number of quanta (Kok, Franck) is an independent and controversial question.

5. Maximum Quantum Yield in Relation to Light Curves as a Whole

While much time and ingenuity have been invested in measuring the yield of photosynthesis in very weak light in order to determine directly the maximum quantum yield, no comparable effort has been made to extend these measurements to higher light intensities and to connect them with the determination of the general shape of the light curve, described in chapter 28. This hiatus is worth filling in.

Pitfalls and corrections that loom large in the interpretation of experimental results obtained in very weak light gradually fade into unimportance as light intensity increases. If the light curves, $P = f(I)$, are smooth curves of a comparatively simple and analytically expressible form, it should be possible to determine the initial slope of these curves (*i. e.*, $1/\gamma_0$) by extrapolation from reliable observations in comparatively strong light. At least, it should be possible to use, as an additional criterion of reliability of measurements in very weak light, the requirement that they should be compatible with the results of measurements further up the light curve.

Reversing the above argument, the reliability of results obtained in strong light, can sometime be judged by inquiring into their compatibility with the quantum yield measurements in weak light.

(*a*) *Extrapolation of Maximum Quantum Yield from Measurements at Higher Light Intensities*

Many empirical light curves appear to be practically straight lines up to comparatively high light intensities. If this straight line passes through the zero point of coordinates, it seems safe to assume that its slope actually represents the maximum quantum yield of photosynthesis under the conditions to which the light curve refers. Probably, a more reliable value of γ_0 can be derived from this slope than from single points measured, with all possible accuracy, near the origin of the coordinates, where the per cent error of measurements is high and the respiration correction is larger than the total measured gas exchange.

Whether the slope of light curves which appear as straight lines *not* passing through the zero point, can be used to determine the quantum yield, is less certain. Such a determination implies the assumption that an increment of absorbed light energy produces an increment of true photosynthesis, while the photochemical process (or processes) responsible for the curvature of the light curve near the zero point continue at the same rate at all light intensities above the turning point.

Warburg, Burk and co-workers (1949, 1950), and Moore and Duggar (1949) determined the quantum yield of photosynthesis from the ratio of

the increment of oxygen production and the increment of absorption, in the region above the compensation point, and noticed no systematic difference between the values obtained in this way, and these determined in low light (in other words, the light curve appeared, in these experiments, as a straight line passing through the zero point). Kok (1948, 1949), on the other hand, found for $P = f(I)$, a straight line passing *above* the zero point, and concluded that the quantum yield of true photosynthesis is *lower* than that of a photochemical process ("photorespiration") which predominates in low light, is light-saturated in the neighborhood of the compensation point, and runs at the same saturation speed at all the higher intensities. Finally, French, and Wassink *et al.*, working with purple bacteria, found, in moderate light, approximately straight light curves, whose linear extrapolation passed *below* the zero point. He used the slope in medium light to calculate the "true" quantum yield of bacterial photosynthesis—on the assumption that in weak light a photochemical process occurs which either causes no consumption of hydrogen and carbon dioxide at all, or does it with a much higher quantum requirement than bacterial photosynthesis in stronger light. In this case, too, the "low light process" is supposed to continue at the same rate at all intensities above its light saturation.

The upward curvature of the light curves of bacterial photosynthesis seems to be a generally encountered phenomenon, but the occurrence of an accentuated downward curvature (or even of a sharp turn) of the light curves of ordinary photosynthesis near the compensation point, remains controversial. Equally open to doubt are assertions that the light curves of photosynthesis (with or without a break in the compensation region) remain linear, above this region, almost up to saturation. At least, the most precise experiments in this field—*e.g.*, those by Emerson and Lewis—showed neither of the two phenomena, but indicated that the light curves gradually bend downward with increasing light intensity, the first signs of curvature being noticeable even at light fluxes of the order of only 1 kerg/cm.^{2} sec.

Such curves are most easily interpreted: we recall that all kinetic mechanisms analyzed in chapter 28 lead to hyperbolic light curves. More complicated kinetic mechanisms may lead to light curves of a higher order; but, in any case, these curves will approach the limiting slope asymptotically, and are unlikely to become straight lines at any finite value of light intensity. The most precise method for determining the maximum quantum yield may well be to measure the yields systematically as a function of light intensity in the region where deviations from linearity are small, then to find an equation representing γ adequately as a function of I, and use it for extrapolation to $I = 0$.

A more ambitious and significant undertaking would be to represent by a single equation the whole light curve, including the saturation region, and to calculate γ_0 from the yields observed in high light, using parameters such as the maximum yield ($P^{\max.}$), and the half-saturating light intensity, $_1/_2I$.

We have seen in chapters 27 and 28 that theoretical kinetic curves representing photosynthesis as a function of the supply of "reactants" (carbon dioxide, reductants, light quanta), are *hyperbolae*, even when rather complicated mechanisms are postulated, as long as no "third-order" reactions [such as $2\ CO_2 + A \rightarrow A(CO_2)_2$, or $Chl + 2\ h\nu \rightarrow Chl^{**}$] are considered, and not more than two successive reaction steps are postulated between the external supply of the reactant and the "rate-determining" step. (For example, in the case of the carbon dioxide factor, simultaneous consideration of diffusion *and* carboxylation leads to a hyperbolic carbon dioxide curve; but if one more supply step is interpolated, the resulting equation is of the third order.)

The known light curves are much too unreliable to permit a useful inquiry into the question whether they actually are hyperbolae (*cf.* section 7*f*, chapter 28). If it were possible to demonstrate, by new and more precise measurements, that the light curves *are* hyperbolic, then each curve could be determined completely by three points, *i. e.*, values of P at three known values of I.

Parameters such as γ_0 (*i. e.*, the initial slope), $_1/_2I$ or $P^{\max.}$ could replace one measurement each. The general equation of a hyperbolic light curve in terms of γ_0, $_1/_2I$ and $P^{\max.}$ is:

$$\frac{P}{P^{\max.} - P} + \left(\frac{2\gamma_0\ {}_1/_2I}{(P^{\max.})^2} - \frac{2}{P^{\max.}}\right)\frac{P^2}{P^{\max.} - P} = \frac{\gamma_0}{P^{\max.}}I \tag{29.5}$$

(This equation is obtained from the general equation of a hyperbola by transformation to a set of coordinates with origin in a point on the hyperbola and the abscissa parallel to the asymptote at a distance $-P^{\max.}$ from the latter.)

In treating several particularly simple mechanisms in chapters 27 and 28, we obtained light curves (or carbon dioxide curves) that obeyed an even simpler relation:

$$P/(P^{\max.} - P) = \text{const.} \times I \tag{29.6}$$

or:

$$P/(P_{\max.} - P) = \text{const.} \times [CO_2]$$

Comparison shows that this simplification means the disappearance of the P^2 term in (29.5), *i. e.*, the validity of the relation:

$$\gamma_0 = P^{\max.}/{}_1/_2I \tag{29.6a}$$

The relationship provides a simple way to check whether the kinetic mechanism is of corresponding simplicity.

Whenever this is the case, and equation (29.6) is obeyed, differentiation with respect to I shows that the quantum requirement, $1/\gamma$, is a *linear function* of light intensity, with $1/P^{\max.}$ as the slope:

$$1/\gamma = 1/\gamma_0 + (I/P^{\max.}) \tag{29.7}$$

The question arises whether the experimental value of $1/\gamma$ *is* a linear function of the intensity of irradiation and, if so, whether the slope of the corresponding straight line is equal to the inverse of the saturation value of photosynthesis in strong light, $1/P^{\max.}$

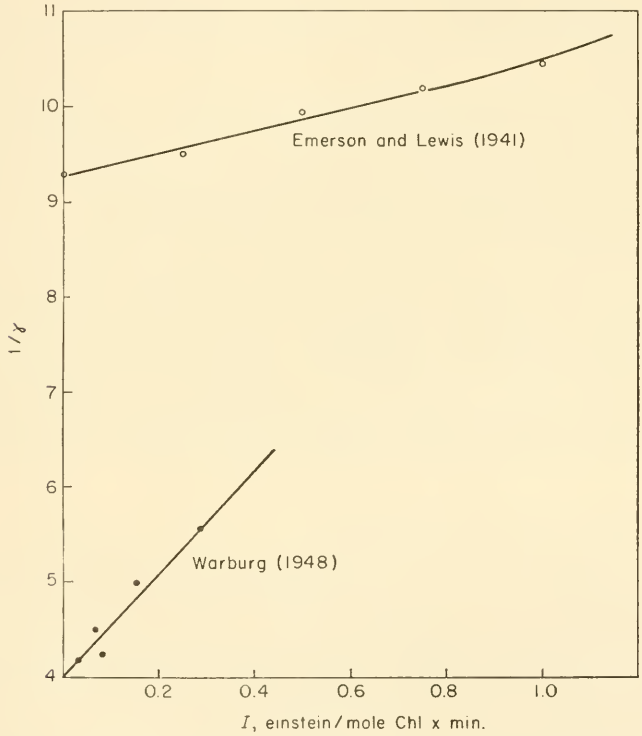

Fig. 29.8. Quantum requirement of *Chlorella* as function of light intensity.

Figure 29.8 represents an attempt to apply equation (29.7) to the quantum yield data of Emerson and Lewis (1943) and of Warburg (1948). Warburg's values scatter too widely to decide whether they lie on a straight line or not; but, if this is assumed to be the case, the slope of the straight line is much higher than in the case of Emerson's measurements (indicating a much more rapid decline of quantum yield with the intensity of illumina-

tion). The abscissae in figure 29.8 are frequencies of absorption of light quanta by each chlorophyll molecule (total number of quanta absorbed by the suspension in unit time, divided by the number of chlorophyll molecules present). The slope of the Warburg curve, equated with $1/P^{max.}$, indicates a maximum production of one oxygen molecule per chlorophyll molecule in about 10 minutes; the slope of the Emerson-Lewis curve, the production of one oxygen molecule per chlorophyll molecule in about 1 minute.

Table 28.V shows that the actual maximum yield in *Chlorella* in steady light is of the order of one molecule oxygen per molecule chlorophyll in 30 seconds. A rapid decline of the apparent quantum yield with I is consistent with the assumption that Warburg's values were affected by the inclusion of the carbon dioxide gush, since the relative importance of this gush must decrease rapidly with increasing light intensity (as was suggested on page 1103).

Franck (1949) suggested that the rapid drop of $1/\gamma_0$ in Warburg's experiments with increasing light intensity, is an indication that they reflect a gradual replacement of a 4-quanta process (half-way reversion of respiration), by an 8-quanta process (true photosynthesis).

On page 1104, we described the more recent experiments of Warburg, Burk and co-workers, who claimed that the highest quantum yields are obtainable by intermittent illumination (which prevails in very rapidly agitated, dense *Chlorella* suspensions). If this were true (it was mentioned on page 1106, that the results of experiments in flashing light do not support the contention of Warburg and Burk), then the relationship between quantum yield and the (average) light intensity must be more complicated than was envisaged in the above derivations.

(*b*) *Quantum Yields in Strong Light*

It was suggested above that the yields of photosynthesis given for high light intensities should not contradict the results obtained in quantum yield measurements in weak light. What we meant can be illustrated by the following examples taken from the work of Willstätter and Stoll (1918).

According to figure 32.2, green leaves of *Sambucus nigra* reduce carbon dioxide, at 6000 lux, at a rate of about 0.23 mg./cm.^{2} hr. or 1.44×10^{-9} mole/cm.^{2} sec. Since 1 lux corresponds roughly to a flux of 5 erg/cm.^{2} sec. in the region 400–700 mμ (*cf.* chapter 25, page 838), and about 80% of this flux is absorbed by a single leaf, we can calculate, for the energy conversion factor:

$$\bar{\epsilon} = \frac{(1.44 \times 10^{-9} \times 112 \times 10^3)\ \text{cal}}{(6 \times 10^3 \times 5 \times 0.24 \times 10^{-7} \times 0.8)\ \text{cal}} = 0.28$$

which corresponds to a quantum yield of about 0.13 (assuming 550 mμ as average wave length). In fig. 32.2, the yellow leaves of *Sambucus* are shown to reduce, at 3000 lux, 0.067 mg. CO_2/cm.^{2} hr., or 4×10^{-10} mole/cm.^{2} sec. These leaves contain less than one tenth the chlorophyll present in green leaves of the same species. Measurements such as those represented in figure 22.10 indicate that the *aurea* leaves absorb, in the region above 500 mμ, not more than 20% of the incident energy. (Blue and violet light do not contribute much to photosynthesis in artificial light; *cf.* page 1163.) Thus, the energy conversion factor of the yellow leaves, can be estimated as:

$$\epsilon \simeq \frac{(4 \times 10^{-10} \times 112 \times 10^3) \text{ cal}}{(3 \times 10^3 \times 5 \times 0.24 \times 10^{-7} \times 0.4) \text{ cal.}} = 0.31$$

corresponding to a quantum yield of about 0.14.

Similar difficulties arise in the interpretation of some of the *maximum* yields of photosynthesis listed in Table 28.V (0.8 or 0.9 mg. CO_2/cm.^{2} hr.). As far as can be judged from the known light curves of land plants, it seems safe to presume that saturation yields can be obtained in light of the order of 40,000 lux. A yield of 0.9 mg. CO_2/cm.^{2} hr. at 40,000 lux means an average quantum yield of the order of 0.1, obtained in a region of almost complete light saturation!

These estimates contain too many approximations to be used as quantitative arguments against the upper limit 0.10 ± 0.02 for the quantum yield of photosynthesis; but they show that it would be well to extend future investigations of the quantum yield to the leaves of higher plants—particularly those of the *aurea* varieties—and to cover the entire length of the light curves.

6. Theoretical and Actual Maximum Quantum Yield

All kinetic theories of photosynthesis agree that the (approximately) linear lower part of the light curves corresponds to the state in which the primary photochemical process is so slow that the nonphotochemical reactions—the "preparatory" as well as the "finishing" ones—can supply the materials and transform the products of this process without delay. It may thus seem as if the maximum quantum yield, calculated from the limiting slope of the light curves, should be equal to the number of quanta actually needed for photosynthesis (except for the practically negligible fraction lost by fluorescence). The fact that the experimentally determined maximum quantum yields often are much lower than 0.1 shows that, in many cases, the photosynthetic apparatus, or parts of it, are in a noneffi-cient state, so as to cause the loss of the predominant fraction of all the

absorbed light quanta. In "aged" cell suspensions, in particular, the maximum quantum yield can be much smaller than in healthy young cells (*cf.* fig. 28.13). The reasons for this inactive state are as yet unknown and may lie in nutritional or enzymatic deficiencies (we recall, for example, van Hille's experiments on the revival of photosynthesis in aged *Chlorella* cultures by a fresh supply of N_2) or in the obstruction of catalytic surfaces by narcotizing metabolites ("chlorellin"; *cf.* page 880). Under the action of external narcotics (*cf.* fig. 28.9C) the initial slope of the light curves is clearly depressed, *i. e.*, no full quantum yield can be obtained even in extremely weak light. This must be attributed to the inactivation of a certain proportion of chlorophyll complexes, probably by adsorption of the narcotic; the light quanta absorbed by these complexes remain unavailable for photosynthesis.

An interesting question—not yet investigated experimentally—is whether a "substandard" quantum yield can be corrected, at least partially, by an increase in temperature. If the low yield is caused by some nonphotochemical process that has become so slow as to depress the rate of photosynthesis even in very weak light, heating should accelerate this process and thus improve the yield; but, if the inefficiency is caused by the fact that a certain proportion of the photosensitive chlorophyll complexes are inactive (partially decomposed, or obstructed by adsorption), heating might have no effect on the yield.

Another interesting question is whether, even in the case when all photosensitive catalytic complexes are fully efficient, the experimental maximum quantum yield must correspond exactly to the number of quanta actually used in the reduction of carbon dioxide.

This question is raised by consideration of schemes 28.1A,B and 28.II, all of which envisage a competition between stabilizing "forward" reactions and primary or secondary back reactions. Examples of primary back reactions are (28.20a′, 28.21a′ and 28.41a′); examples of secondary back reactions are (28.20d and 28.21d).

In scheme 28.IA, the "rate-determining" reaction is (28.20b), and the rate is given by equation (28.23). The quantum yield is:

$$P/I_a = P/k^*I\ \mathrm{Chl}_0 = nk_r[\mathrm{ACO_2}]/(k' + k_r[\mathrm{ACO_2}]) \tag{29.9}$$

The maximum quantum yield (reached when $[\mathrm{ACO_2}] = \mathrm{A}_0$) is:

$$\gamma_0 = k_r\mathrm{A}_0 n/(k' + k_r\mathrm{A}_0) \tag{29.10}$$

In scheme 28.II, the rate is described by equation (28.42), and the maximum quantum yield is given by equation (28.43):

$$\gamma_0 = dP/d(k^*\mathrm{Chl}_0 I) = k_\epsilon \mathrm{E}_\mathrm{B}^0 n/(k' + k_\epsilon \mathrm{E}_\mathrm{B}^0) \tag{29.11}$$

In (29.9), the "forward" reaction of $\mathrm{HX \cdot Chl \cdot Z}$ with $\mathrm{ACO_2}$ competes with the primary back reaction (conversion to $\mathrm{X \cdot Chl \cdot HZ}$); in (29.11) the stabilizing "forward" reaction of $\mathrm{AHCO_2 \cdot Chl \cdot A'HO}$ with the catalyst $\mathrm{E_B}$ competes with the back reaction (conversion to $\mathrm{ACO_2 \cdot Chl \cdot A'H_2O}$). In either case, the "theoretical" quantum yield, n, can be closely approached only if the ratio $k'/k_r \mathrm{A}_0$ (or $k'/k_\epsilon \mathrm{E}_\mathrm{B}^0$) is much smaller than 1.

Recognition that the maximum *observable* quantum yield may be smaller than the *theoretical* quantum yield, n, was first reached in the derivations of Franck and Herzfeld (1941) (*cf.* their Table 1). The reaction mechanism used by them, although more complicated than the ones considered here, also was based on competition between stabilizing forward reaction and back reactions of the immediate reduction products.

The analytical expressions may be more complicated, but the essential result also remains the same if the effective back reaction is a secondary one, such as (28.21d). If this reaction competes with the forward transformation of the intermediate reduction product $\mathrm{AHCO_3}$ by the catalyst $\mathrm{E_B}$ (or of the intermediate oxidation product A'HO by the catalyst $\mathrm{E_C}$), the fraction of the products that undergoes back reaction will remain finite even when the forward reactions have the maximum possible rate constants, *i. e.*, when the full available amounts of the relevant catalysts are free and can be utilized for transformation (as is the case in weak light).

Bibliography to Chapter 29

The Light Factor. II. Maximum Quantum Yield of Photosynthesis

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Chapter 30

THE LIGHT FACTOR. III. PHOTOSYNTHESIS AND LIGHT QUALITY; ROLE OF ACCESSORY PIGMENTS*

1. Action Spectrum

The dependence of photosynthesis on the spectral *quality* of light was the subject of much interest, long before the influence of light *quantity* was first investigated. As early as 1788, Senebier conducted experiments on carbon dioxide assimilation in double-walled vessels, filling the space between the walls with various colored solutions. Since then, the botanical literature has been replete with observations on the behavior of plants in light of different color. (For a review of these investigations, see, for example, Gabrielsen 1940.) This was to be expected, since everything connected with color has always held, and still holds a captivating interest for mankind, even though in scientific photochemistry the qualitative categories of "red," "blue," "yellow" or "green," which were so dear to Goethe (he thought them to be the "primary phenomena" of optics), have been reduced to mere quantitative differences between the energy contents of the light quanta.

About a hundred years ago the effect of color on photosynthesis became a topic of a lively discussion. Its subject was the *position of the maximum of photosynthetic efficiency in the solar spectrum.* In 1844, Draper found that, when the prismatic spectrum of the sun was thrown upon a plant, the largest amount of oxygen was liberated in the yellow-green region; this result was confirmed by such authorities in plant physiology as Sachs (1864) and Pfeffer (1871). Sachs pointed out that the yellow is also the region of maximum "luminosity" of light, *i. e.*, of maximum effect on the human retina. He himself saw in this only a coincidence; but other, less cautious authors thought that such a correspondence must be significant, and attempted to explain it. The belief that photosynthesis proceeds most actively in green light, which is only weakly absorbed by chlorophyll, led to several peculiar hypotheses, such as that light energy is not used in photosynthesis at all (Pfeffer 1871), or that the role of chlorophyll in plants is merely to protect the carbon dioxide–reducing system from injury by light (Pringsheim 1879, 1881, 1882).

Timiriazev (1869, 1875, 1877, 1885) vigorously fought these miscon-

* Bibliography, page 1188.

ceptions. He pointed out that "luminosity" is an anthropomorphic notion, without meaning in objective photometry, that utilization of light energy is the essence of photosynthesis, that this utilization cannot take place unless light is absorbed by a sensitizing pigment, and that this pigment cannot be anything but chlorophyll. Timiriazev was the first to use the concept of sensitization, a phenomenon then recently discovered by Vogel and Becquerel, in the discussion of photosynthesis.

A similar point of view was taken by several physicists, *e. g.*, Jamin, Becquerel and, particularly, Lommel (1871, 1872). The latter pointed out that the basic principle of photochemistry, known as Herschel's law ("no photochemical action without light absorption"), requires that the spectral maximum of photosynthetic efficiency coincide with the absorption maximum of the sensitizing pigment. Timiriazev (1869, 1875), Müller (1872), Engelmann (1882) and Reinke (1884) gave experimental proofs of this coincidence, by showing that the photosynthetic efficiency of green plants decreases steadily from red through yellow to green, parallel with the decline in absorbing capacity of chlorophyll. The error of Draper, Sachs and Pfeffer was attributed by Timiriazev to the use of spectrally impure light. (Timiriazev himself employed light isolated by a monochromator with a narrow slit, and used microanalytical methods to compensate for the weakness of illumination.) Engelmann suggested that the error may have resulted from the use of thick leaves or thalli, which absorb light practically completely even in the minima between the absorption bands of chlorophyll. (He worked with microscopic plant objects, using motile bacteria for the detection and determination of oxygen.)

Engelmann (1882) noticed that, in addition to the main maximum in the red, the photosynthetic "action spectrum" of green plants has a second maximum in the blue or violet, which he associated with the strong absorption band of chlorophyll in this region. This perfectly natural conclusion became the subject of one of the most vitriolic controversies in the history of photosynthesis; it was contested even by such enlightened plant physiologists as Reinke (1884) and Timiriazev (1885). Particularly violent were the criticisms Pringsheim (1886) directed against Engelmann's method and his results; and Engelmann (1887) answered these attacks in language seldom encountered in the pages of scientific journals, even in the quarrelsome nineteenth century. At the same time, Engelmann also sharply rebuked Timiriazev for his attempt (1885) to identify the main maximum of spectroscopic efficiency of photosynthesis with the energy maximum of the solar spectrum. Timiriazev saw in this alleged coincidence a striking example of adaptation of organisms to the prevailing conditions, and thus a triumph of the Darwinian theory. Engelmann answered that a coincidence of the two maxima cannot be postulated without deliberately

twisting experimental evidence: Direct sunlight has an energy maximum in the yellow and not in the red, as the action spectrum of photosynthesis; and the energy maximum of diffuse sky light, which is the second component of the natural "light field" of land plants, lies even further toward the blue-violet end of the spectrum. In this controversy, too, Engelmann was undoubtedly right. Looking backward, one cannot but admire the unfailing correctness of his conclusions, obtained by means of an experimental method most investigators would hesitate to use even for qualitative, not to speak of quantitative, purposes. Engelmann not only established correctly the general parallelism between the action spectrum of photosynthesis and the absorption spectrum of chlorophyll; he also clearly understood the influence on both spectra of the optical density of the specimen. His conclusions concerning the photosynthetic efficiency of the carotenoids and phycobilins, long neglected, appear well on the way to vindication sixty five years later.

Much of the controversy between Engelmann and his opponents can be attributed to a lack of understanding of what is meant by "action spectrum." A primitive definition can be based on the above-mentioned simple experiment of Draper: A spectrum is thrown on a plant or cell suspension, and photosynthesis is measured in different spectral bands of equal width. Such an experiment, performed with a prism in artificial light, may easily lead to the belief that the action spectrum of photosynthesis has only one maximum, because the energy of most artificial light sources declines rapidly toward the violet end of the spectrum, while the dispersion of the prism increases in the same direction. Both factors cooperate in causing a rapid decline of the yield of photosynthesis (related to a given spectral band width) as one proceeds from the red to the violet; this decline may more than offset any increase caused by the renewed rise in the absorption capacity of chlorophyll in the blue-violet region.

Obviously, this definition of the "action spectrum" is arbitrary and irrelevant. An improved definition can be obtained by using light fluxes of equal intensity at all wave lengths, which can be achieved, *e. g.*, by appropriate variation of the width of the monochromator slit, or insertion of neutral gray filters. By using such methods, Kohl (1897, 1906), von Richter (1902) and Kniep and Minder (1909) were able to confirm Engelmann's finding of the existence of a second maximum in the action spectrum of photosynthesis, at the short-wave end of the visible region.

However, even an action spectrum obtained by the use of spectral bands of equal energy is not *universal*, *i. e.*, it cannot claim validity for all plants, not even for all specimens of a given species (*e. g.*, all *Chlorella* suspensions). The first cause of variability of "equienergetic" action spectra is the varying composition of the pigment system (*cf.* chapter 15); but

even for plants with identical contents of all pigments (or suspensions of identical cells) the action spectrum still depends on two individual factors. The importance of one of them—the *optical density* of the sample—was recognized by Engelmann: In a thick leaf or thallus, or a dense cell suspension, the absorption spectrum and the action spectrum both are blurred; in the limiting case of complete absorption (approximately realized in Warburg and Negelein's quantum yield experiments; *cf.* chapter 25, page 844), the action spectrum, too, may lose all structure.

The second factor that affects the action spectrum of an individual plant or cell suspension (without affecting its absorption spectrum) is the *intensity of illumination.* If one would use monochromatic light of such high intensity as to obtain full light saturation at all wave lengths, the rate of photosynthesis (which, in the light-saturated state, is determined only by the velocity of a dark process) will become identical in all parts of the spectrum. The structure of the action spectrum will appear as soon as the intensity of illumination is reduced below the saturating value. Since the saturating intensity depends on wave length (*cf.* sect. 4), the shape of the action spectrum will change with decreasing light intensity, until the latter will fall within the practically linear range for *all* wave lengths. The initial divergence and ultimate convergence of light curves in light of different color is illustrated by figure 30.8B obtained by Gabrielsen (1940) with green leaves of *Sinapis alba.*

In the low intensity range, the shape of the action spectrum becomes constant, and the spectrum thus acquires a definite significance. Here—and only here—can the action spectrum be compared *quantitatively* with the absorption spectrum of the specimen, and the question asked whether the specific photochemical efficiency depends on wave length.

First, however, a "quantum correction" must be applied. According to Einstein's law of photochemical equivalency, one has reason to expect *equal numbers of absorbed quanta* of different wave length to produce the same photochemical effect, but not *equal quantities of absorbed energy.* Thus, action spectra have to be "quantized," *i.e.* expressed in moles per einstein, rather than in moles per erg or calorie. In the linear region, it is legitimate to convert the "equienergetic" action spectrum into the "quantized" spectrum simply by dividing all ordinates by the corresponding wave lengths. In the saturation range, this is impossible, and the quantized action spectrum can be obtained only by direct experiment (*i. e.*, by measuring the rate in bands of equal intensity expressed in einsteins per square centimeter per second), because here the shape of the action spectrum depends on intensity, and spectral bands that have equal intensity if measured in energy units have different intensities if measured in einsteins.

If the maximum quantum yield of photosynthesis is the same for all

wave lengths, the quantized action spectrum can be expected to parallel exactly the absorption spectrum; the "equienergetic" action spectrum, on the other hand, will always be askew, with blue-violet light appearing *ceteris paribus* less efficient than red light.

If the *quantized* action spectrum determined from measurements in low light, differs markedly from the absorption spectrum, this is a definite indication that quanta of different wave length have different photochemical effects in photosynthesis. Recent determinations of the quantum yield of green and colored algae in monochromatic light, carried out by Emerson *et al.*, Dutton and Manning, and Blinks, have established the existence of such differences, and made speculations as to their origin legitimate. Similar conclusions have been drawn previously from experiments under badly controlled conditions, in which broad spectral bands (isolated by means of colored glass filters) and unknown light intensities were used. Conclusions drawn from experiments of this type, *e. g.*, by Montfort, have sometimes proved partially correct, but comparison of Montfort's confused discussions with the concise presentation of Emerson and Lewis, and Dutton and Manning gives a most eloquent demonstration of the progress that can be achieved in plant physiology by the use of better physicochemical tools.

The explanation of the effect of wave length on the maximum quantum yield of photosynthesis can be sought in three phenomena: (*a*) in the *composite nature of the pigment system* and the (qualitatively or quantitatively) different photochemical functions of the individual pigments; (*b*) in the *multiplicity of the excited electronic states* of chlorophyll, two (or three) of which are involved in the light absorption in the visible spectrum (*cf.* fig. 21.20); and (*c*) in the influence that *vibrational energy*, acquired by the sensitizer together with electronic excitation, may have on its sensitizing action. The first factor causes the rate of photosynthesis in different spectral regions to be affected by the apportionment of the absorbed light energy to the several pigments, while the second and third factors can cause changes in efficiency with wave length, even in light absorbed by a single pigment.

Thus, the study of the effect of wave length on photosynthesis should aim, *first*, at the qualitative and quantitative determination of the role of various pigments in sensitization and, *second*, at the analysis of the relation between wave length and photochemical efficiency for each pigment. Needless to say, we are far from having achieved these aims. Even now, the greater part of newly published work on photosynthesis in colored light remains purely descriptive and unsuitable for quantitative interpretation.

In addition to wave length, the quality of light is characterized by its *polarization*. Almost universally, no attention has been paid to this characteristic in the study of the relationship between light and photosynthesis. Dastur and Asana (1932) and Johnson (1937) found no difference in the rate of photosynthesis in ordinary and linearly polarized light of the same intensity; but Dastur and Gunjikar (1934) observed a marked deficiency in the absorption by leaves of elliptically polarized light, and later (1935) also a similar deficiency in the synthesis of carbohydrates. Although we may doubt the correctness of these results, it must be borne in mind that birefringence of the chloroplasts (*cf.* Vol. I, p. 362) and the correlated dichroism may conceivably lead to differences in the capacity to utilize light of a different state of polarization. However, only a very weak dichroism has been observed in the chloroplasts in the natural state (*cf.* Vol. I, p. 366).

2. Quantum Yield and Wave Length in Green Plants. Role of Carotenoids

In chapter 29, we discussed the maximum quantum yield of photosynthesis in green plants, without paying much attention to the quality of light used for its determination, in the tacit assumption that it is either altogether immaterial or has only a secondary influence. Quantum yield measurements with *Chlorella* in monochromatic light were first carried out by Warburg and Negelein (1923). The results are shown in Table 30.I. The quantum yield in blue light is somewhat higher than in red light, if referred to the (estimated) absorption by chlorophyll alone, and somewhat lower, if referred to the total absorption by *all* pigments.

If we assume that the relative yields of Warburg and Negelein in different spectral regions are significant (even though their *absolute* values had been questioned by Emerson *et al.*; *cf.* chapter 29), the figures in Table 30.I point to a participation of the carotenoids as sensitizers in photosynthesis, but with an efficiency inferior to that of the chlorophylls.

Table 30.I

Quantum Yields (after Warburg and Negelein 1923)

Light	λ, mμ	γ	ε
Red	610–690	0.23	0.59
Yellow	578	0.23	0.54
Green	546	0.21	0.44
Blue	436	0.20[a]	0.34[a]
		0.28[b]	0.48[b]

[a] Referred to *all* pigments. [b] Referred to chlorophyll alone.

When Warburg repeated his quantum yields measurements 25 years later (Warburg 1946, 1948), he again found a somewhat lower yield ($\gamma =$ 0.20 and 0.16 in two experiments) in blue light ($\lambda = 436$ mμ) as compared with yellow light.

Briggs (1929) made yield determinations in light of three colors and obtained the results listed in Table 30.IA.

TABLE 30.IA

PHOTOSYNTHESIS YIELD OF LEAVES IN COLORED LIGHT (AFTER BRIGGS 1929)

Species	Yield, ml. O_2/500 cal incident light		
	Yellow light	Green light	Blue light
Phaseolus vulgaris	14–17	9–11	7–8
Ulmus, yellow	8.8	6.5	5.3
green	—	20	12
Sambucus nigra	8.7	9.3	8.7
Sambucus nigra	—	19.0	15.0

These results obtained at light intensities 5–10 times stronger than those used by Warburg and Negelein, show the expected decline in the energy conversion yield with decreasing wave length (the experiment with *Sambucus* is an exception). In other words, the "equienergetic" action spectrum is askew as expected. The decline from yellow to blue is, however, somewhat stronger than could be explained by the quantum correction (the ratio of the yields in yellow and blue is 1.8 to 2.0, instead of 1.4), again indicating a somewhat lower quantum yield in the region of absorption by carotenoids.

Wurmser (1925) found, for *Ulva lactuca*, a much higher quantum yield in the green than in the red, and a comparatively low yield in the blue; but these results cannot be considered reliable (*cf.* page 1118). Gabrielsen's data for *Sinapis alba* (1935), summarized in Table 30.III, (p. 1162) are more significant. The relation between the quantum yields in the red and blue is similar to that found by Warburg and Negelein, and thus allows a similar interpretation. The value in the green—which, in contrast to Wurmser's result, is *lower* than in the red—may perhaps be taken as an indication that the yellow-green filter used by Gabrielsen transmitted much light absorbed by the carotenoids.

The only extensive investigation of the quantum yield of photosynthesis in a green plant as a function of wave length was carried out by Emerson and Lewis (1941, 1943) with *Chlorella pyrenoidosa*. They used bands from 5 to 15 mμ wide, obtained by means of a powerful monochromator. Figure 30.1 shows the results. The scattering of the points is indicative of limitations to which the biological "standardization" of cell cultures is subject. Despite this scattering, it appears certain that the yield is approximately constant between 580 and 685 mμ. (The authors believe that the shallow minimum at 660 mμ is real; for its suggested interpretation, see page 1155.) Below 580 mμ, the yield declines considerably, reaches a minimum at 490 mμ and then recovers. Roughly, the depression of the quantum yield

curve in the green, blue and violet covers the regions where carotene, luteol and other carotenoids contribute markedly to the light absorption by *Chlorella*. An attempt at a *quantitative* interpretation of the γ curve meets with some difficulties. Figure 22.44 shows that, if the absorption by the carotenoids is calculated on the basis of extract spectra (by shifting all bands to make their maxima coincide with the absorption peaks of live cells), significant participation of carotenoids in light absorption cannot be

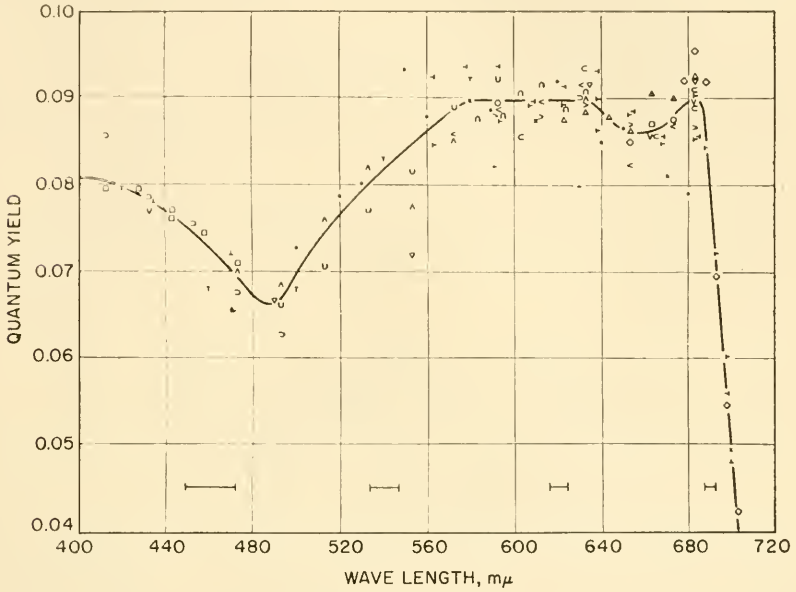

Fig. 30.1. Quantum yield of photosynthesis as a function of wave length for *Chlorella* (after Emerson and Lewis 1943). Points obtained on 19 runs are indicated by distinct symbols. Band half widths used indicated by horizontal lines of corresponding length.

expected above 540 mμ. Thus the decline in γ, which first begins at 580 mμ, cannot be attributed to the carotenoids, unless one assumes that, in the living cell, their absorption bands are not merely shifted, but so strongly broadened as to extend up to 580 mμ. The *minimum* in the quantum yield curve, at 490 mμ, corresponds satisfactorily to the maximum of carotenoid absorption, according to fig. 22.44 (more exactly, to the center of gravity of the two carotenoid peaks); but one notices that in this region the carotenoids can be expected to account for 60 or 70% of the total absorption; while the depression of the γ curve does not exceed 25%. It thus again appears that, even though light quanta absorbed by the carotenoids are *less* efficient than those absorbed by chlorophyll, they are not entirely inefficient.

One may argue that, if the absorption band of the carotenoids *in vivo* is strongly broadened (as suggested above), the maximum of this band may be much lower than *in vitro* (since the *area* of the band—which is proportional to the probability of the corresponding electronic transition—is not likely to be much affected by the state of the pigment). Consequently the proportion of light absorbed by carotenoids in the region of the γ minimum may be smaller than estimated, thus reducing the discrepancy between this proportion (supposedly, 60%) and the deficiency in γ (25%).

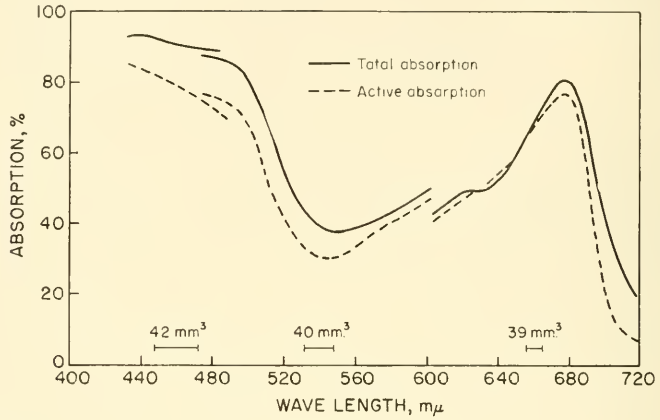

Fig. 30.2. Comparison of total absorption of *Chlorella* with the absorption active in photosynthesis (after Emerson and Lewis, 1943).

It is unlikely, however, that this discrepancy can be completely *eliminated* by this correction, *i.e.*, that the assumption of a strongly broadened and flattened absorption band of the carotenoids will permit the explanation of the quantum yield curve on the basis of *complete* inefficiency of the carotenoids. The *average* quantum yield deficiency between 400 and 580 mμ according to figure 30.1, is about 15%; the average contribution of the carotenoids to light absorption in the same region, according to figure 22.44, is close to 30%. Broadening of the absorption band is more likely to *increase* than to decrease the latter value. Thus, the most likely conclusion to be drawn from the results of Emerson and Lewis is that the sensitizing efficiency of the carotenoids in *Chlorella* is not zero, but about one half that of chlorophyll.

It need hardly be stressed that all conclusions of this type are predicated on the assumption of a uniform distribution of the pigments, and would have to be revised if it were demonstrated that some pigments form "color screens" between the external light source and the other pigments.

The sharp decline in quantum yield, which sets in, according to figure 30.1, above 680 mμ, will be discussed in section 5.

Figure 30.1 was obtained with a dense suspension, which absorbed practically completely at all wave lengths. Emerson and Lewis also made measurements with a thinner suspension, which transmitted about one half of incident light, to be able to compare directly the "action spectrum" of photosynthesis in *Chlorella* with the absorption spectrum of the same specimen. Figure 30.2 shows the results. The two curves were drawn to coincide at 660 mμ. They show a significant divergence above 690 and below 570 mμ. The lower position of the action curve in the green, blue and violet illustrates the relative inefficiency of the carotenoids; but the comparative narrowness of the gap between the two curves confirms the conclusion, reached above, that the carotenoids are not *entirely* inefficient.

The total absorption was measured directly; "active" absorption was calculated from photosynthesis on the assumption that all light used for photosynthesis gives a quantum yield of 0.084. This value was chosen to give agreement between the two curves in the red, where all absorption is assumed to be active. The half widths of the bands used are shown on the figure. The cells used in the run covering the red part of the spectrum were from a separate culture.

The relatively low sensitizing efficiency of the carotenoids of green plants in photosynthesis, indicated by these experiments, may be either a uniform property of all pigments of this group, or it may be an average, *e. g.*, some carotenoids may be as efficient as the chlorophylls, while others are entirely inactive. On the basis of the absorption analysis in figure 22.44 it seems unlikely that the inactive fraction of the yellow pigments does not consist of carotenoids at all, but is formed by pigments such as flavones or anthocyanines.

It can be argued that the shape of the quantum yield curve below 570 mμ could also be explained by assuming that a complete inefficiency of carotenoids is partly compensated by an *enhanced* efficiency of chlorophyll. The possible difference between the photochemical functions of chlorophyll in the three excited states (corresponding to the blue-violet, orange and red band systems, respectively) is an important problem. The available evidence gives little indication of such a difference. In chapters 21 (page 634) and 23 (page 748) we concluded, from the excitation of the same red fluorescence band by light of all wave lengths, that chlorophyll molecules, excited to the electronic states A or B, are rapidly transferred, by a radiationless process, into the lowest electronic excitation state, Y (which is the upper state of the red fluorescence band). However, on page 752, we concluded from Livingston's data, that, in the case of the blue-violet absorption band, the yield of this transformation is far less than 100%—

in other words, that the fate of a large proportion of chlorophyll molecules in state *A* is different from transfer into state *Y* (*e.g.*, they may undergo transition into a metastable state; *cf.* scheme 23.I). Whether this is true not only of chlorophyll in solution but also of chlorophyll in the living cell remains uncertain.

In vivo, direct photochemical dissociation of chlorophyll by blue-violet light appears even less likely than *in vitro* (in consideration of the known photostability of chlorophyll in the living cell). True, the yield of chlorophyll fluorescence *in vivo*, as a function of wave length of the exciting light (represented in fig. 24.5A), shows a slight decline at the violet end of the spectrum; but this decline is most likely due to the presence of carotenoids, and not to a decreased efficiency of light absorbed by chlorophyll itself. As a matter of fact, figure 24.5A was interpreted on page 814 as an indication that the fluorescence of chlorophyll in *Chlorella* can be excited—although with reduced efficiency—also by the light quanta absorbed by the carotenoids; and this interpretation of the fluoresence curve is in agreement with Emerson and Lewis' quantum yield curve of photosynthesis. (However, the fluorescence curve in figure 24.5A shows no minimum at 490 mμ, which is so prominent in the quantum yield curve in figure 30.1.)

To sum up, the results of the quantum yield studies of both photosynthesis and fluorescence are best explained by the assumption that a *considerable fraction—of the order of one half—of the quanta absorbed by the carotenoids in green plants is passed over to chlorophyll, transferring the latter into state Y*. Therefore, these quanta can be used in the same way as those absorbed directly by chlorophyll, either for photosynthesis or for fluorescence. It is furthermore likely that most or all blue-violet quanta absorbed directly by chlorophyll are utilized for conversion into the fluorescent state *Y*.

Quantum yield measurements of Noddack and Eichhoff (1939) and Eichhoff (1939) covered only the range above 515 mμ and therefore reveal nothing concerning the function of the carotenoids. Their only interesting feature is the high yield in the infrared; therefore they will be discussed in the next section.

The action spectrum of the green alga *Ulva taeniata*, measured polarographically by Haxo and Blinks (1950), is represented in figure 30.11A, and is in general agreement with the results of Emerson and Lewis.

3. Photosynthesis of Green Plants in Ultraviolet and Infrared

The extension of the photosynthetically active region into the ultraviolet has not been studied by systematic quantum yield measurements in monochromatic light, although it seems to be generally assumed that the

yield drops rapidly at, or only a little beyond, the violet end of the visible spectrum (400 mμ).

In vitro, the chlorophyll absorption extends throughout the near and medium ultraviolet (*cf.* fig. 21.3). Whether other common components of the plant cells, which absorb in this region, interfere with the light supply to chlorophyll (by acting as "screens"), we do not know (this could perhaps be elucidated by fluorescence measurements). Ursprung (1917, 1918) found evidence of photosynthesis in *Phaseolus vulgaris* down to 330 mμ; Hoover (1937) and Burns (1942) observed it in *Triticum* at 365 mμ. Gabrielsen (1940) calculated a yield of about 1 mg. CO_2/50 cm.^{2} hr. as the possible contribution of the ultraviolet part of the solar spectrum to the photosynthesis of leaves of *Sinapis* and *Corylus.*

Johnson and Levring (1946) found with six marine algae (green and red), a decline in respiration by about 1 mg. O_2 per hr. per g. dry weight (determined by Winkler's methods) in near-ultraviolet light (366 mμ, intensity 5×10^{-4} cal./cm.^{2} min.). This was interpreted as evidence of photosynthetic effectiveness of this light. Further in the ultraviolet (260–320 mμ, intensity about equal to that of the erytheme-producing radiation in sunlight), no such effect could be observed.

Light below 300 mμ is highly injurious to plants. According to Meier (1932, 1934, 1936) an illumination of 1000 erg/cm.^{2} sec. kills *Chlorella* cells in 110 sec. at 260 mμ, and in 10,000 sec. at 302 mμ. Preferential inhibition of photosynthesis (with the cells still alive and respiring) by the mercury resonance line 253.6 mμ was described by Arnold (1933) (*cf.* chapter 13, Vol. I, page 344); it is not associated with a visible destruction of chlorophyll.

The presence of yellow pigments (*e. g.,* flavones, anthocyanines or other hydrophilic compounds, which may be present either in the cell sap or in the cell walls) may cause a decline or complete cessation of photosynthesis at comparatively long wave lengths. This is the probable reason why Burns (1933, 1934) found that the photosynthesis of certain conifers—*e.g.,* spruce and white pine—ceases below 450–465 mμ (*cf.* page 1164).

In the spectrum of extracted chlorophyll, the absorption declines rapidly above 680 mμ; but the absorption spectra of intact cells reveal, in addition to a *shift* of the red absorption band from 660 to approximately 680 mμ, an *extension* of absorption to much longer waves. This spreading of the red band can be recognized, *e. g.,* in figures 22.10–22.24, 22.44, and 22.48; and it has also been observed in aqueous suspensions of chloroplastic matter (Smith, fig. 21.28). In the latter case, the infrared "tail" of the red band disappeared upon clarification of the suspension by digitonin, and was ascribed by Smith to scattering ("false absorption"). However, in other investigations, *e.g.,* those of Noddack and Eichhoff

(fig. 22.21), the absorption in the far red was found also in measurements purported to represent "true" absorption. It may be due either to a genuine broadening of the red chlorophyll band, or to the presence of other, infrared-absorbing components (*e. g.*, ferrous salts). The question of the infrared limit of photosynthesis is closely associated with the interpretation of this infrared absorption "tail." The experimental results of Emerson and Lewis (1943) and Blinks and Haxo (1950), on the one hand, and Eichhoff (1939), on the other hand, are in extreme disagreement. As

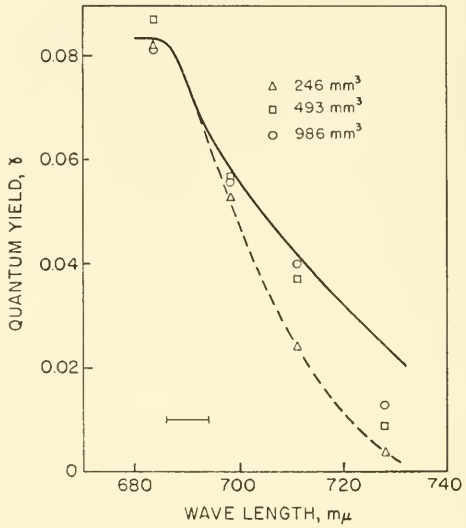

Fig. 30.3. Decline in quantum yield in far red (after Emerson and Lewis 1943). Points show apparent quantum yield with three different suspension densities, calculated for equal incident light. Solid curve extrapolated for true yield, (complete absorption), assuming that differences between the curves obtained with different suspensions is due to incomplete absorption.

shown in figure 30.1, Emerson and Lewis found a sharp drop in quantum yield above 680 mμ. This part of their curve is reproduced in more detail in figure 30.3. To be sure that the decline was not due to incomplete absorption (we recall that the curve in figure 30.1 was obtained by Warburg and Negelein's method which presupposes total absorption), Emerson and Lewis made determinations with several suspensions of increasing density, and extrapolated the results to infinite density; the result is represented by the solid curve in figure 30.3. It shows that, even with a generous allowance for incomplete absorption, there still remains a drop in γ, from about 0.08 at 680 mμ, to as little as 0.02 at 730 mμ.

A similar decline of λ above 630 mμ was noted by Blinks and Haxo (1950) in the green alga *Ulva taniata* (fig. 30.11A), by Emerson and Lewis (1941) with the blue-green alga *Chroococcus* (fig. 30.10A) and by Tanada (1950) with the diatom *Navicula minima* (fig. 30.9A). Ehrmantraut (1950) noted the same phenomenon in the Hill reaction in *Chlorella*. Livingston (*cf.* chapter 23, p. 752) noted a drop in the yield of the chlorophyll fluorescence (in ether and acetone) in the same spectral region.

Emerson and Lewis suggested that the decline in quantum yield on the infrared side of the absorption maximum of chlorophyll *a* (at 680 mμ) is repeated on the red side of the absorption peak of chlorophyll *b*, and that this is the explanation for the shallow minimum in the γ curve that figure 30.1 showed near 660 mμ. (The absorption maximum of chlorophyll *b in vivo* must be situated at about 648 mμ; *cf.* chapter 22, page 702.)

It may be pointed out in passing that the quantum yield curve of Emerson and Lewis provides a direct argument against Seybold's (1941) hypothesis that chlorophyll *b* does not act as a sensitizer in the reduction of carbon dioxide at all, but is a specific sensitizer for the polymerization of sugars to starch.

In sharp contrast to the results of Emerson and Lewis are those of Noddack and Eichhoff (1939) and Eichhoff (1939), who found (*cf.* Table 30.II) that the quantum yield of *Chlorella* remains high even at 832.5 mμ.

Table 30.II

Quantum Yields of Chlorella According to Eichhoff

mμ	γ	mμ	γ
832.5	0.164	598	0.238
780	0.204	576.5	0.263
750	0.244	558	0.238
725	0.228	542	0.204
685	0.179	527.5	0.232
650	0.204	515	0.222
622	0.228		

Figure 30.4 shows a comparison of the action spectrum with the absorption spectrum of *Chlorella*, according to Eichhoff; *the two curves remain closely parallel far above 680 mμ.* Even if we doubt the correctness of the *absolute values* of *Eichhoff's* quantum yields (*cf.* page 1098), the *wave length dependence* of these values could be significant—*e. g.*, it would not be affected by an error in calibration (suggested on page 1098). One possible—but not very probable—explanation of the differences between the two γ curves in the far red is that only Noddack and Eichhoff have measured *true* absorption, while others have related the yield to the sum of absorption and scattering. The elucidation of this point is particularly desirable because of the theoretical implications of a decline in γ with wave length, as

observed by Emerson and Lewis, and Haxo and Blinks. The latter (1950) observed, in the action spectrum of *Ulva* (fig. 30.11), a decline in the far red quite similar to that found by Emerson and Lewis with *Chlorella*. Altogether, the weight of experimental evidence seems to be against the results of Noddack and Eichhoff. This is remarkable, because from the theoretical point of view one would rather expect the quantum yield to remain constant within the red absorption band of chlorophyll. The general experience in photochemistry is that wave length is not important for the photochemical effect, as long as one remains within a single band system, even if this system extends over all colors of the rainbow. The reason is that, within a single band system, the *electronic* excitation energy is constant, and all excess energy absorbed by the molecule serves

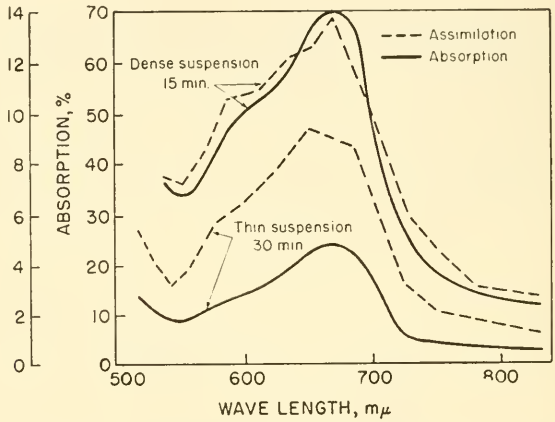

Fig. 30.4. Action spectrum of *Chlorella* (after Eichhoff 1939). Scale at left represents assimilation.

merely to increase its vibrational and rotational energy. If the primary photochemical process is the dissociation of the absorbing molecule, the only effect of variations in wave length is that the dissociation products separate with different relative velocities; usually, this excess energy does not affect the ultimate fate of the dissociation products, because it is lost by collisions before the next reaction step. If the primary photochemical process is electronic excitation, the excess vibrational energy acquired by the absorbing molecules also will usually be lost before the occurrence of the secondary reaction. However, exceptions to this behavior are known. In some cases, the electronic excitation energy is too small to bring about dissociation without the assistance of a certain amount of vibrational energy (an example is the delayed monomolecular photodissociation of large molecules, described by Franck and Herzfeld, 1937, and considered

in chapter 18, page 484, as a possible consequence of the absorption of blue-violet light by chlorophyll). In other cases, the presence of excess kinetic energy may help the dissociation products, formed in a tightly packed medium, to escape immediate recombination (Franck and Rabinowitch 1934). In still others, the vibrational energy of the excited electronic state may contribute directly to chemical activation; this is particularly likely in cases when the reaction partner forms a complex with the absorbing molecule, so that no energy-dissipating collisions intervene between the primary and the secondary photochemical step.

The latter explanation could be suggested for a decline in the γ values of photosynthesis at long waves, if the experimental γ curve would indicate that a certain minimum vibrational energy of the excited electronic state is required for photosynthesis. However, according to Emerson and Lewis, the decline occurs *within the same band* (and not upon transition from one band to another, *e. g.*, from the orange to the red band). This is difficult to understand, since, within a single band, the absorption leads everywhere, not only to the same electronic state, but also to the same vibrational state (at least, in respect to high-frequency band vibrations).

One could suggest that the red band of chlorophyll is not a single band, but contains vibrational bands clustering tightly under its wings. However, vibrational bands situated on the infrared side of the main electronic band most likely originate in the vibrating states of the *ground state*, and lead to the same excited state as the main band; they thus offer no explanation for a diminished quantum yield.

Another possibility is that the red absorption band of chlorophyll conceals a band corresponding to a different *electronic* transition. In figure 21.20, it was suggested that a comparatively weak band $X_0 \rightarrow A_0$ is hidden under the strong $X_0 \rightarrow Y_0$ band; one could suggest that absorption in the far red, exhibited by live cells, is caused by a shift of the $X_0 \rightarrow A_0$ band, rather than by an extension of the $X_0 \rightarrow Y_0$ band. One could also suggest that, in the series of transitions $X_0 \rightarrow A_0$, $X_0 \rightarrow A_1$, $X_0 \rightarrow A_2$, . . ., the latter ones, which lead to the *vibrating* states A_1, A_2, and give rise to chlorophyll bands in the *orange, yellow and green*, can be followed by radiationless transitions into state Y, while the first one, which leads to the nonvibrating state A_0, cannot produce the same result (because of insufficient energy of the excited state), and is therefore ineffective in bringing about photosynthesis.

Before considering any of these hypotheses too seriously, one should ascertain whether the drop in γ above 680 mμ, observed by Emerson and Lewis, is not due to a more trivial cause, such as scattering (which Emerson and Lewis considered unlikely); to the presence of ferrous salts (or other infrared-absorbing inorganic components); or the presence of a photosynthetically inactive pigment with a band greater than 680 mμ (perhaps chlorophyll *d*; see page 1183).

Observations of the low yield of photosynthesis in filtered extreme red or infrared light (Ursprung 1918, Gabrielsen 1940, etc.) without the meas-

urement of absorption have very little significance because of the well-established rapid drop in the absorbing power of leaves in this region. The only real problem is whether the yield of photosynthesis drops *proportionally* with absorption, or *more rapidly* than the latter.

Hoover (1937) was able to observe the photosynthesis of wheat up to 750 mμ and Burns (1933, 1934), that of different conifers up to 740 mμ.

4. Monochromatic Light Curves, and the Action Spectrum of Photosynthesis in Strong Light

When the intensity of monochromatic light is raised, one soon reaches the region in which the shape of the action spectrum becomes variable. The light curves bend earlier or later, and come to saturation more or less suddenly, depending on the value of the absorption coefficient, and on the optical density of the sample, respectively. It was postulated above (page 1145) that, when all curves reach saturation, the rate must become independent of wave length, and the action spectrum must lose all structure. The theoretical and experimental foundations of this postulate will be considered later (pp. 1162, 1165). At present, we will assume it to be valid, and consider only the effect of wave length on the shape of the transition from the linearly ascending part of the light curves (the slope of which at a given wave length is determined by the product of absorption coefficient and maximum quantum yield) to the "saturation plateau," the height of which we assume to be independent of wave length.

The effect of optical density on light curves was discussed in chapter 28, and the results were illustrated by the schematic figure 28.20. A change in wave length is equivalent to a change in optical density; a cell suspension that is "thin" in green light becomes "dense" in red or violet light. However, as far as the rate of photosynthesis is concerned, transition from green to red light is not in all respects equivalent to an increase in cell concentration, since the saturation level remains unchanged in the first case, but increases proportionately with the number of cells in the second case. Thus, the result of a change from strongly absorbed to weakly absorbed light is likely to be more similar to that of the change from green to *aurea* leaves (*cf.* fig. 32.2), where the maximum rate is approximately the same for both varieties.

As mentioned before, the comparison of light curves at different wave lengths should be carried out by plotting the rate against $N_{h\nu}$ (number of incident quanta/cm.^{2} sec.,) rather than against the energy flux, I, in erg (or cal)/cm.^{2} sec. Otherwise, the light curves for the shorter waves will remain below those for the longer waves, even if the photochemical efficiency of both kinds of quanta are identical (fig. 30.6C). An example of

how completely the "quantized" light curves obtained in monochromatic light of different color coincide in their initial sections is given in figure 30.5.

Although the two uppermost points in this figure fall into the region of beginning saturation, they still show no difference between the light curves in green and red light. However, a divergence of these two curves in the

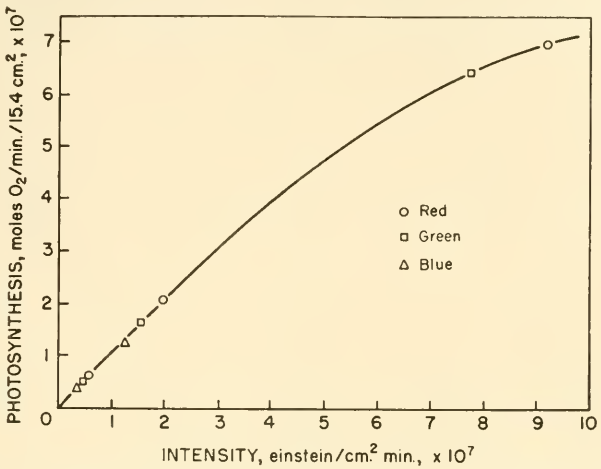

Fig. 30.5. Photosynthesis of *Chlorella* as function of intensity at three wave lengths (after Emerson and Lewis 1943).

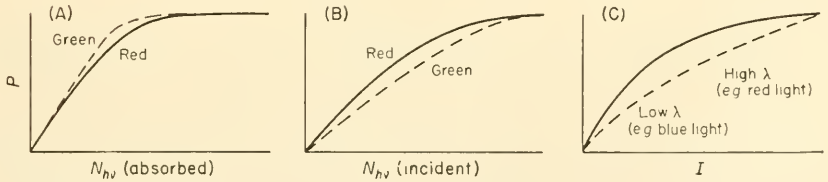

Fig. 30.6. Expected shapes of monochromatic light curves. (A) P *vs.* $N_{h\nu}$ for total absorption, $N_{h\nu}$ (absorbed) = $N_{h\nu}$ (incident). (B) Same for incomplete absorption, $N_{h\nu}$ (absorbed) < $N_{h\nu}$ (incident). (C) For equal absorption, equal quantum yields but different wave lengths; the curves in (C) would coincide if $N_{h\nu}$ were used as abscissa instead of I. In (C), the unbroken curve is for high λ.

saturation region is theoretically inevitable. The theoretical expectations are illustrated by the schematic figure 30.6. In the case of *total absorption* of all wave lengths (*i. e.*, conditions under which fig. 30.5 was obtained), the curve for red light must bend earlier than that for green light (because of the more uniform absorption of the latter throughout the cell layer).

The same picture (fig. 30.6A) should be obtained also for partially absorbing systems, if P is plotted against the absorbed (rather than against the incident) intensity. If $N_{h\nu}$ (incident) is used as the independent variable and the absorption is comparatively weak, the resulting picture must be that shown in figure 30.6B.

If the monochromatic light curves are plotted against light *energy* (I, or A) instead of number of quanta, $N_{h\nu}$, the relationships become unnecessarily obscured (*cf.* fig. 30.6C). (Some investigators, *e. g.*, Montfort, have

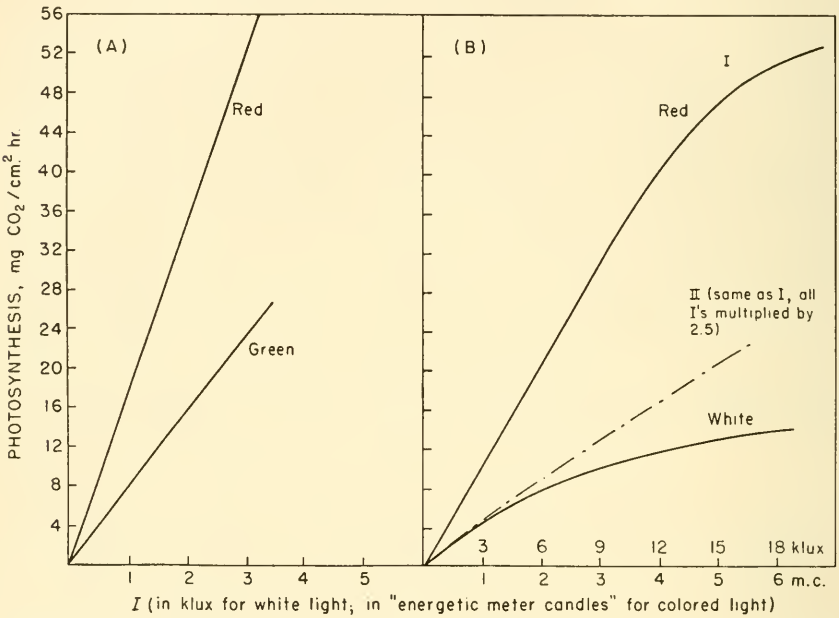

Fig. 30.7. Light curves of *Chlorella* in light of different color. (A) Green *vs.* red light (equal energy flux); "dense" suspension (11×10^6 cells per cc.). (B) Red *vs.* white light (energy flux in klux for white light, in "energetic meter candles" for red light, *cf.* chapter 29, p. 1098). "Thin" suspension, 2×10^6 cells per cc.

used rate measurements in monochromatic light to raise the question whether photosynthesis "is a quantum process at all"; a question no photochemist would ever ask.

The available experimental material to which the above predictions can be applied is very scarce. It is desirable that the precise methods of rate determination, applied as yet almost exclusively to the quantum yield determinations in weak light, should be extended to kinetic studies in strong monochromatic light.

As examples of the few available experimental monochromatic light

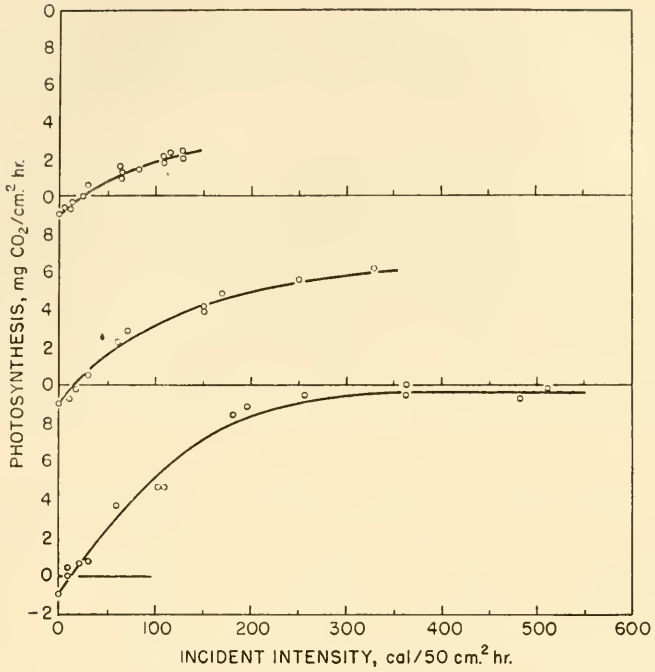

Fig. 30.8A. Light curves of *Sinapis alba* (after Gabrielsen 1935). Top, blue-violet light; center, yellow-green; bottom, orange-red.

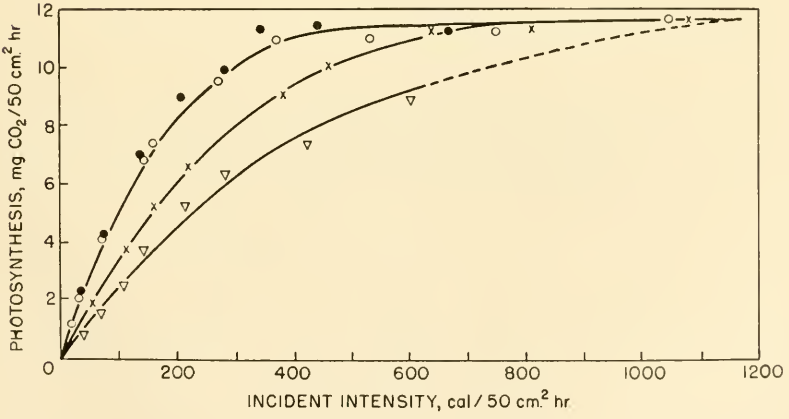

Fig. 30.8B. Light curves of *Sinapis alba* in light of different color (after Gabrielsen 1940). The same saturation yield is approached at all wave lengths. Circles, red-orange light; crosses, yellow-green; triangles, blue-violet.

curves, we consider those in figures 30.7 and 30.8. In figure 30.7A, the relative position of the curves for green and red light is as predicted in figure 30.6B. In figure 30.7B, the curve for *white* light, lies, as predicted, below that for the more strongly absorbed red light, but the difference is much larger than expected.

It was mentioned in chapter 29 (page 1098) that the ratios between photochemically equivalent intensities of red and white light, given by Eichhoff, appeared remarkably small. (Saturation in red light was reached at an intensity equivalent to <2000 lux of white light!) If one arbitrarily multiplies the "monochromatic" intensities by a factor of 2.5 (this would reduce quantum yields from 0.25 to 0.10), fig. 29.7B would be changed as indicated by the dotted line, and acquire a much more plausible appearance.

Figure 30.8A and Table 30.III show the results of Gabrielsen (1935). The difference in the *quantum yield* in the green and in the red is notable

Table 30.III
Photosynthesis in Colored Light (after Gabrielsen 1935)

Light	Av. λ, mμ	Max. quantum yield	Max. P, mg. CO_2/ cm.² hr.	Reached at I = μ cal/cm.² sec.
Red-orange	650	0.100	0.192	1.67
Yellow-green	540	0.083	*ca.* 0.12	1.85
Blue-violet	430	0.071	*ca.* 0.05	0.83

(for a discussion of similar results by Emerson and Lewis, *cf.* page 1148). The *maximum rates* in the blue-violet, and particularly in the green, also are considerably smaller than in the red; however, figure 30.8A shows that the observed maximum rates may be still far from saturation. Figure 30.8B, taken from a later publication by the same author (1940), shows the coincidence of the maximum rates in the three spectral regions very clearly.

It is obvious from the preceding discussion that the action spectra of photosynthesis, obtained by illuminating plants with light of partly saturating intensity, are difficult to interpret—even if precaution has been taken to use the same incident intensity (or, better still, the same number of incident quanta) in all spectral regions. For example, in an optically thin system, the yield per incident quantum should be smaller in the green than in the red, because of weaker absorption (*cf.* fig. 30.6B); while in a dense, completely absorbing system the relation could be reversed, because of the better utilization of the more uniformily absorbed green light (*cf.* fig. 30.6A). Working with systems that are not too dense, and in light that is not too strong, one may obtain a "quantized" action spectrum resembling more or less closely the absorption spectrum of the pigments. An example is given in figure 30.9, which shows the action spectrum of wheat as observed by Hoover (1937) and "quantized" by Burns (1937–38,1942). How-

ever, no *quantitative* agreement between action spectrum and absorption spectrum can be expected under these conditions, and conclusions drawn from differences between them, *e. g.*, as to the role of the accessory pigments, are in the nature of more or less plausible guesses.

Because of the coincidence of the absorption bands of the carotenoids in green plants with the blue-violet bands of the two chlorophylls, correct guessing is in this case much more difficult than in the case of brown or red algae. Engelmann (1887) recognized this and based his suggestion that the carotenoids of green plants also act as sensitizers in photosynthesis not on direct experiments with these plants, but on analogy with the results obtained with colored algae. He quoted, as an additional argument in favor of this suggestion, the observation that leaves of the *aurea* varieties have a comparatively high yield of photosynthesis, despite their deficiency in chlorophyll. However, he did not consider this argument as conclusive, at least not without renewed study; and since then Willstätter and Stoll (1918) have shown that *aurea* leaves possess a high relative efficiency also in light filtered through a yellow filter. Willstätter and Stoll saw

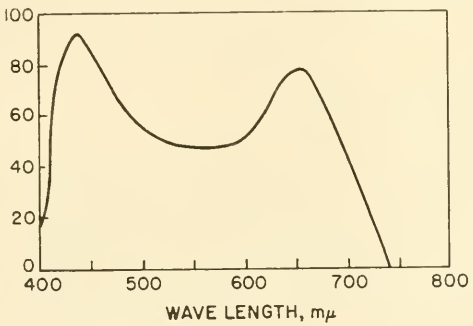

Fig. 30.9. Action spectrum of photosynthesis of wheat "quantized" by Burns (1937, 1938) (after Hoover 1937).

in this proof that leaf carotenoids do not contribute to the sensitization of photosynthesis in *aurea* leaves. However, this conclusion was not convincing because in their experiments not only the relative, but also the absolute, yields in both green and *aurea* leaves were almost unaffected by the interposition of a yellow filter. In other words, the intensity of blue-violet light was negligible; therefore their experiments, while proving that *aurea* leaves are highly efficient in the light absorbed by chlorophyll alone, proved nothing as to the efficiency or inefficiency of the carotenoids.

Wurmser (1921[3]) found the rate of photosynthesis of *Ulva lactuca* in the blue-violet to be lower than in the green, but higher than in the red (calculated for absorption by chlorophyll alone), thus indicating a possible active participation of the carotenoids. Schmücker (1930) found, by bubble-counting experiments with *Cabomba* and *Cryptocoryne*, that the light intensity required to achieve a certain rate of photosynthesis increased from the red to yellow and green inversely proportionately to the wave length—thus indicating a constant quantum yield; in the blue and violet, on the other hand, the increase was about 15% larger than was required by the quantum correction if *all* pigments were assumed to be active, but somewhat less than could be expected if the carotenoids were entirely inefficient.

After the problem of the role of carotenoids in photosynthesis had been brought to the foreground by experiments with *brown algae* (cf. section 5), Montfort (1940) made a new attempt to determine whether light absorption by the carotenoids of green plants also contributes to photosynthesis. He compared the rates of photosynthesis of the green alga *Ulva lactuca* in red and orange light ($\lambda > 550$ mμ) with that in blue-green light (λ 350–625 mμ, maximum at 450–500 mμ) of equal incident intensity. Table 30.IV shows the results. Comparison of the last three figures in the table shows that the

Table 30.IV

Photosynthesis of *Ulva lactuca* in Colored Light (after Montfort 1940)

P = Rate of Photosynthesis; A = Rate of Absorption

P (blue-green)/P (orange-red)	0.89
A (blue-green)/A (orange-red)[a]	1.19
A (blue-green)/A (orange-red)[b]	1.00[c]
(P/A) (blue-green)/(P/A) (orange-red)[a]	0.75
(P/A) (blue-green)/(P/A) (orange-red)[b]	0.89[c]
Quantum correction: λ (orange-red)/λ (blue-green)	0.77[d]

[a] All pigments.
[b] Chlorophyll alone.
[c] Calculated from data on extracts.
[d] For wave lengths 625 and 480 mμ.

quantum yield in the blue-green was only slightly lower than in the red, if referred to the absorption by all pigments, but much higher if referred to chlorophyll alone. This speaks in favor of an almost equal efficiency of chlorophyll and the carotenoids. Thus, the earlier observations of Wurmser, Schmücker and Montfort can all be quoted in support of the conclusions derived by Emerson and Lewis from the much more convincing measurements in weaker and truly monochromatic light, that the carotenoids of green plants do contribute actively, but less efficiently than chlorophyll, to the sensitization of photosynthesis.

In *Chlorella*, the quantum yield deficiency in blue and violet light is not likely to be caused by the presence of a yellow pigment other than the carotenoids. (The comparison of the absorption spectrum of live cells with that of the extracted pigments, *cf.* fig. 22.44, does not indicate the presence of such a pigment.) In some higher plants, on the other hand, pigments of the flavone or anthocyanine class often are present in the cell sap or cell walls and compete with the photosynthetically active pigments for blue-violet quanta, or even serve as "color screens," particularly when they are located in the epidermis, or in the cell walls between the chloroplasts and the external light source. The presence of these pigments should leave the *saturation* yield unaffected, but should depress the quantum yield in the linear range and in the region of partial saturation. Burns (1933, 1942) noted that the quantum yield of photosynthesis of spruce and pine seedlings in the blue-violet (390–470 mμ) was only half as large as in the red (630–720 mμ), or red plus orange (560–720 mμ). This can be attributed to the

presence, in these conifers, of a nonactive yellow pigment. (It was mentioned on page 1153 that photosynthesis in these plants declines to zero below 450 or 465 mμ.)

The same effect should be even more pronounced in leaves of the *purpurea* varieties, or other leaves containing large quantities of red anthocyanine pigments. Engelmann recognized as early as 1887, in an investigation entitled "Leaf Hues and Their Importance for the Decomposition of Carbonic Acid in Light," that the red pigments of land plants do not actively participate in photosynthesis; and Willstätter and Stoll (1918) and Kuilman (1930) confirmed that the presence of these pigments has no influence on the rate of photosynthesis in strong light. Gabrielsen (1940) concluded, from a review of the older work and new experiments with *Corylus* and *Prunus* leaves in red-orange, yellow-green and blue-violet light, that for a given amount of incident energy the red varieties have a minimum yield in yellow-green light, where light absorption by the red pigment has its maximum. This minimum was most pronounced in weak light; in strong light, the monochromatic light curves approached the same saturation level, in conformity with the findings of Willstätter and Stoll. Gabrielsen estimated that, in red *Corylus* leaves, the "screen" absorbed about 37% of incident light in the blue-violet, about 74% in the yellow-green and about 33% in the red-orange region. In *Prunus*, the absorption was somewhat higher, perhaps because in *Corylus* the red pigment was present only in the epidermis cells, while in *Prunus* it was found also in the mesophyll. These results cause us to give little credence to speculations or observations that relate anthocyanines or flavones to photosynthesis.

In 1922 Noack observed a photochemical conversion of flavones into anthocyanines *in vivo* and interpreted this reaction as a chlorophyll-sensitized oxidation-reduction (*cf.* chapter 19, page 541). He suggested that flavones and anthocyanines form a reversible oxidation-reduction system, which may play a catalytic role in photosynthesis. Sen (1942) asserted that anthocyanine-carrying leaves have a *higher* photosynthetic efficiency than ordinary leaves, despite their lower content of chlorophyll.

We now return to the question, raised on page 1158, concerning the third part of the light curves, after the linear range and the transitional region—the saturation plateau. It was postulated there that this plateau should have the same height for all wave lengths. As long as the rate is determined only by the kinetic mechanism of photosynthesis, theoretical arguments in favor of this postulate appear conclusive. Whether saturation is brought about by a limited supply of reactants, or by limited availability of an enzyme, the maximum rate is determined by the velocity of a dark reaction, and should be independent not only of the *quantity*, but also of the *quality* of illumination.

Light quality *could*, however, affect the maximum rate of photosynthe-

sis, if other photochemical processes can interfere with this process. A selective sensitizaton of *oxidative processes* in the photosynthetic apparatus by the light absorbed by the carotenoids, or by chlorophyll in the blue-violet band, offers one possibility of this type. However, in this case, only light curves of the time-dependent "optimum" type (which have been observed, *e. g.*, in some umbrophilic plants; *cf.* page 994) should be affected, since the maximum rate in these curves is determined by the (time-dependent) balance of photosynthesis and photoxidation. Light curves with true saturation plateaus should remain unaffected by an earlier onset of "light inhibition" (except for a shorter extension of the saturation plateau).

The effect of wave length on photoxidation has never been investigated systematically. Franck and French (1941) found that photoxidation occurs, in carbon dioxide-deprived leaves, in red as well as in blue light, but this was merely a qualitative observation. A selective effect of blue light on the *respiration* of *Chlorella*, observed by Emerson and Lewis (1943), was mentioned in chapter 20 (page 568). Another specific function of yellow pigments seems to be well established—the sensitization of phototropic movements. As stated on page 681, the changes in the positions of the chloroplasts in light are caused only by light absorbed by yellow pigments (Voerkel 1933); and the same is true of the phototactic movements of whole cells (*cf.* Castle 1935).

These pigments may be the carotenoids, although Galston attributed this function to riboflavin (because the action spectrum showed only a single peak). In purple bacteria, on the other hand, the action spectrum of phototaxis coincides with that of photosynthesis (*cf.* p. 1188).

Very extensive studies of the effects of light of different colors in photosynthesis and respiration were made by Danilov (1935, 1936), using green, blue-green, and red algae. He reported very complicated results in which the yield was found to depend not only on color (in monochromatic light), but also on the *combination* of colors (in non-monochromatic light). Following the tendency of what Kostychev proclaimed as a new "physiological" approach to photosynthesis (*cf.* chapter 26, p. 872) he discussed these phenomena in vague terms of stimulation and inhibition of different protoplasmic functions by light of different wave length. Thus, yellow and green rays were credited by him with increasing cell sensitivity to red light, and with making it insensitive to infrared light; blue-violet light was said to assist in the utilization of infrared light (supposedly for activation of the "dark" reaction stages in photosynthesis). Blue-green rays were said to counteract the stimulation effects of yellow light, and enhance the stimulating effects of blue rays, and, generally, to create in the cells a "regulator of the

utilization of light energy," and also to determine the reaction of photosynthesis to changes in temperature. In another paper, Danilov (1938) concluded that the effect of various colors of light on photosynthesis depends on the method of cultivation of the algae. In particular, variations in the sensitivity to different colors of light were caused by changes in hydration of the cells (achieved by culturing *Scenedesmus* in 1% sodium chloride solution).

Ursprung (1917, 1918[2]), working with detached leaves by means of Timiriazev's (1890, 1903) "starch spectrum" method (a spectrum is projected on a starved, destarched leaf, and the "latent starch image" formed by light is "developed" by iodine), found, in light of uniform spectral intensity, a continuous decrease in the production of starch from red to violet, without a second maximum, and attributed this result to the closure of the stomata and consequent quenching of photosynthesis in blue-violet light.

Dastur and Samant (1933), Dastur and Mehta (1935) and Dastur and Solomon (1937) described observations that purported to show that pure red light (or pure blue light) is less efficient in photosynthesis than a combination of both. Their experiments were criticized by Montfort (1937), who found that the addition of blue-violet light to red light has no effect on the rate of photosynthesis, provided the red light was in itself of saturating intensity (this agrees with the experiments of Dutton and Manning with diatoms, described on page 1172).

Dastur, Kanitkar and Rao (1938) measured the formation of proteins in leaves in light of different color, and found differences which they related to the above-mentioned observations on photosynthesis in colored light.

The results of Dastur and co-workers probably are trivial, being caused by the use of optically dense tissues. As explained above, such tissues must (and do) utilize moderately intense green or yellow light better than orange-red (or blue-violet) light of the same intensity, because the latter is absorbed in too thin a layer and causes saturation effects there. White light of partly saturating intensity, since it contains green and yellow, will give, in such tissues, a higher yield of photosynthesis than an equally strong red-orange or blue-violet light.

The hypothesis of Baly (1935) that photosynthesis requires a quantum of red plus a quantum of blue light, was mentioned before (Vol. I, p. 554) and characterized as entirely without experimental basis.

In chapter 28 (page 987) we described the different shape of light curves of shade-adapted and light-adapted plants. Since these plants differ in the composition of their pigment systems, they are likely to show differences also in their response to light of different color. Lubimenko (1923) observed that shade-adapted plants often are relatively more efficient in blue-violet light (and relatively easily inhibited by red light). This may be as-

sociated with their higher content of chlorophyll *b* (which permits a better utilization of blue light, 450–500 mμ), or with a higher content of carotenoids (which was indicated by Willstätter and Stoll's figures in Table 15.III, but was not confirmed, as a general rule, by Seybold and Egle's analysis; *cf.* Vol. I, p. 414).

The composition of the pigment system also depends on the color of the light under which the plants were grown. As described in chapter 15 (page 430), chlorophyll is more efficiently synthesized by green plants in red light, and the carotenoids in blue-violet light (although these assertions have been contested, and may represent over-simplifications). This may explain why plants have often been found to be most efficient in the light in which they were grown. *Elodea* plants cultured in red light produced more oxygen in the red, while similar plants grown in blue light gave more oxygen in the blue (Harder, Döring and Simonis 1936, Harder and Simonis 1938, and Simonis 1938). Thus, the physiological chromatic adaptation of photosynthesis may be in this case a consequence of the chemical adaptation of the pigment system.

5. Quantum Yield and Action Spectrum of Photosynthesis in Brown Algae

The study of the relation between wave length and photosynthesis in brown algae and diatoms is of special interest because of the presence in these algae of the carotenoid fucoxanthol, which is not encountered in green plants. The distribution of the light absorption by brown algae and diatoms among the individual pigments was discussed in chapter 22 (page 723) and illustrated by the (very schematic) figures 22.45A, B and Table 22.IX, all taken from Montfort (1940). For diatoms, we also gave the much more adequate figure 22.46 of Dutton and Manning (1941). The two reasons why even this figure is not too reliable are: first, the uncertain (and undoubtedly to a certain extent incorrect) assumptions made regarding the "red shift" of the absorption bands *in vivo*, and, second, the neglect of chlorophyll *c*. According to Tanada's figure 30.9B, chlorophyll *c* adds much to light absorption of pigment extracts from brown algae and diatoms between 450 and 500 mμ. The brown color of these organisms *in vivo* indicates considerable absorption also farther in the green, from 500–550 mμ. Part of this absorption may be due to chlorophyll *c*, but most is probably due to fucoxanthol (to which it has usually been ascribed, *cf.* page 707). Montfort (1940) discussed the experimental action spectra of photosynthesis in different brown algae and concluded that light absorbed by fucoxanthol is fully utilized for photosynthesis; but this conclusion was not very convincing because of the very primitive experimental approach, which included the use of broad spectral regions, and of light of

comparatively high intensity. Dutton and Manning (1941) arrived at a similar conclusion by a procedure which was much more satisfactory—at least, in principle—namely, the determination of quantum yields in weak and truly monochromatic light. Because the method of Dutton and Manning is so much more adequate than that of Montfort (*cf.* the criticism of Emerson 1937) we will discuss their experiments first.

Dutton and Manning used the dropping mercury electrode for the determination of oxygen (*cf.* chapter 25, page 850). The diatom (*Nitzschia closterium*) was found to be more sensitive than *Chlorella* to mercury; however, its resistance was sufficient to permit measurements of 30 minutes duration without marked poisoning.

Chromatographic analysis of the pigments of *Nitzschia closterium* revealed the presence of chlorophyll *a*, carotene, luteol, fucoxanthol and probably flavoxanthol, but showed no trace of chlorophyll *b*. No mention was made of chlorophyll *c*, which was subsequently found by Strain and Manning (Vol. I, p. 406) in diatoms and other brown algae. Analysis of the absorption spectrum of the extract (*cf.* fig. 22.46) indicated that in methanol solution at least one half the absorption between 400 and 550 mμ was due to the carotenoids. This is a much larger proportion than in green plants (*cf.* page 1150); it indicates that the ratio chlorophyll *a*/total carotenoids was, in the investigated diatoms, considerably lower than 3/1 (which is the average for brown algae listed in Table 15.III).

Monochromatic light from a high-pressure a. c. mercury arc was used for illumination (the effect of intermittency being considered unimportant), as well as bands isolated from the light of an incandescent lamp by appropriate filters. The density of the suspensions was such as to give about 50% absorption in the red, and 75% in the blue. Two portions of the same suspension were placed in two vessels, and a simultaneous determination of the quantum yield was made in both of them, the one vessel being illuminated with violet, blue or green light, and the other with red light. Table 30.V shows the results. This table indicates that the individual γ values varied, for each wave length, within wide limits (*e.g.*, in the red, from 0.038 to 0.100); possibly because of various degrees of mercury poisoning, or other factors affecting the vitality of the cells. The conclusions therefore depended entirely on the *ratios* of the yields obtained in light of different color, in simultaneous experiments with two aliquots of the same cells. Table 30.V shows that even these ratios varied widely (*e. g.*, from 0.90 to 1.43 in the comparison of yields in violet and red light). The most consistent was the series of measurements at 496 mμ, where seven determinations all gave, for the ratio (quantum yield in blue-green)/(quantum yield in red) values between 0.07 and 0.08. By averaging the ratios, Dutton and Manning arrived at the values in the last column of Table 30.V.

TABLE 30.V

QUANTUM YIELDS OF DIATOMS (AFTER DUTTON AND MANNING, 1941)

λ, mμ	% of total abs. due to carotenoids,[a]	γ From	γ To	γ Av.	γ/γ (red) From	γ/γ (red) To	γ/γ (red) Av.
404.7 + 407.8 (violet)	38	0.048	0.075	0.075	0.90	1.43	1.08 ± 0.08
665.0 (red band axis)	0	0.048	0.060	0.052			
435.8 (blue)	49	0.048	0.079	0.064	0.77	1.20	1.04 ± 0.05
665.0 (red band axis)	0	0.045	0.092	0.063			
496.0 (blue-green)	93	0.039	0.081	0.059	0.70	0.80	0.75 ± 0.03
665.0 (red band axis)	0	0.054	0.100	0.080			
546.1 (green)	48	0.044	0.080	0.065	0.96	1.27	1.10 ± 0.04
665.0 (red band axis)	0	0.038	0.080	0.060			

[a] Calculated by shifting all curves in figure 22.46 toward the red by 20 mμ (for a criticism of this procedure, see page 726). Chlorophyll *c* absorption neglected!

and concluded that the quantum yields in the violet, blue and green are practically equal to that in the red, despite the fact that in the first two regions 40–50% of the light must be absorbed by the carotenoids, whereas in the last one all absorption is due to chlorophyll. Dutton and Manning therefore suggested that all carotenoids present in diatoms must act as sensitizers of photosynthesis. The value of γ in the blue-green, at 496 mμ, was definitely lower. Dutton and Manning suggested that this may indicate a lesser efficiency of carotene and luteol as compared with fucoxanthol. According to their calculations, the relative contribution of fucoxanthol to the total absorption by carotenoids was less at 496 mμ than at the shorter wave lengths.

This assumption is derived from observations in the extract, in which fucoxanthol shows an absorption spectrum terminating sharply at 500 mμ (fig. 21.35A), and a maximum practically coincident with that of luteol. However, this apparently does not apply to fucoxanthol *in vivo* (p. 706); and some recent observations on extracts also showed absorption extending to 550 mμ (*cf.* fig. 21.36).

The brown color of the algae indicates a considerable spread of the blue-violet absorption region toward the longer waves; and it is plausible—although it cannot be proved—that the pigment responsible for this spread is fucoxanthol. (A possible alternative is to ascribe part of it to chlorophyll *c*, although its band lies, in extracts, on the short-wave side of that of chlorophyll *a*.)

As an alternative, Dutton and Manning suggested that an unknown pigment, with absorption restricted to a narrow region around 500 mμ, may be responsible for the minimum in the yield curve at 496 mμ. This result may also be related to Emerson and Lewis' observations of a selective stimulation of respiration in *Chlorella* by light in the region of 480 mμ.

Dutton and Manning pointed out that the participation of the carotenoids in photosynthesis of *Nitzschia closterium* is indicated, not only by

the *ratios* of the quantum yields in the blue, violet, green and red, but, even more strikingly, by the *absolute value* of the yield at 496 mμ, where, according to their estimates, 93% of absorbed light is taken up by the carotenoids. They argued that, if all photosynthesis observed in this spectral region were attributed to chlorophyll, the quantum yield would be $0.059/(1 - 0.93) = 0.84$, *i. e.*, much larger than the maximum allowed by thermochemical considerations. However, this estimate was based on the distribution data in figure 22.46, and therefore is subject to possible grave errors. True, at 496 mμ, the apportionment of energy is not very sensitive to the postulated specific value of the "red shift"; it would remain almost the same if a shift of 10 or 30 mμ were postulated, instead of 20 mμ (the value used by Dutton and Manning), or if the shift of the carotenoid bands—particularly those of fucoxanthol—were assumed to be twice or three times as large as that of the chlorophyll bands. (A difference of this type is indicated by some data in chapter 22; *cf.* Table 22.VI and page 706.) It may thus seem as if an extreme and unlikely assumption concerning the enhancement of the absorption of blue-green light by chlorophyll *in vivo*, or the assumption of a spatial distribution of pigments strongly favoring absorption by chlorophyll would be required to explain the quantum yield observed at 496 mμ without recourse to sensitization by carotenoids. This argument, however, ceased to be quite conclusive, since Strain and Manning (1942) confirmed the presence in blown algae and diatoms, of a pigment with strong absorption in the blue-green, chlorophyll *c*. According to figure 21.5 this component has an absorption peak at 450 mμ in methanol; in the living cell, its absorption maximum must lie near 470 mμ, if the shift is the same as for chlorophyll *a*. According to figure 30.9C, at 470 mμ, chlorophyll *c* in a methanol extract from diatoms accounts for about ten times more absorption than chlorophyll *a*. The neglect of chlorophyll *c* in the calculations of Dutton and Manning thus may have shifted the ratio of the absorptions by the chlorophyll pigments and the carotenoids, from perhaps about 1 to 1, to the extreme value of 9.3 to 0.7.

To sum up, the average γ values found by Dutton and Manning support the assumption that the carotenoids in diatoms (and fucoxanthol in particular) contribute directly to the sensitization of photosynthesis; but the wide scattering of individual results called for reinvestigation with material and methods giving more consistent results. Furthermore, all results, and particularly the absolute yields at 496 mμ, were in need of reexamination in the light of the possible role of chlorophyll *c*. This reexamination could have conceivably brought the brown algae in line with green algae—organisms in which a distinctly lower quantum yield of photosynthesis was observed in the regions of the carotenoid absorption, but a yield not sufficiently low to permit the assumption of complete in-

activity of the carotenoids. This hypothesis was supported by the action spectrum of the brown alga *Coilodesme*, determined polarographically by Haxo and Blinks (1950) and reproduced, together with the absorption spectrum, in figure 30.11B.

Dutton and Manning also performed experiments in stronger light, in which they first measured photosynthesis in saturating red light, and then added violet light. They found—as one would expect—no appreciable effect of this additional illumination on the yield, and interpreted this as a proof that photosynthesis in blue light, although sensitized by both chlorophyll and the carotenoids, is limited by the same dark reaction as photosynthesis in red light, which is sensitized by chlorophyll alone.

Wassink and Kersten (1946) studied the diatom *Nitzschia dissipata;* the spectroscopic results of this study were presented in Chapter 22 (p. 706). These investigators made measurements of the rate of photosynthesis in

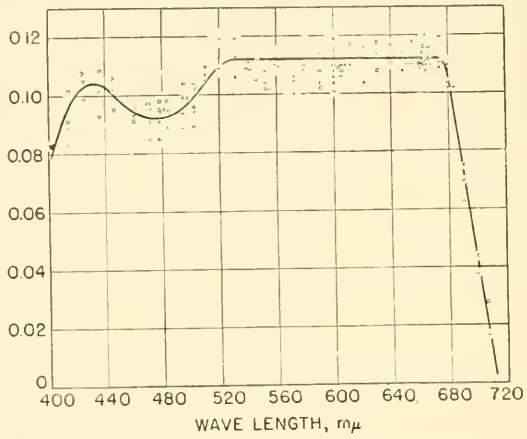

Fig. 30.9A. Quantum yield (ordinate) of photosynthesis as a function of wave length for *N. minima* (after Tanada 1951).

light of different color, isolated by filter combinations. They reduced the data to a common average intensity (7.3 kerg/cm.^{2} sec.) in the assumption that they worked in the linear part of the light curve. (This assumption was based on the type of light curves, showing a very extensive linear part, which had been obtained by the Dutch group for green plants, diatoms and purple bacteria, *cf.* fig. 28.14B and table 28.II; this shape was not confirmed by other investigators.) Wassink and Kersten estimated that the yield per absorbed quantum is the same in *Nitzschia* and in *Chlorella*, and is constant in red, yellow, and yellow-green light; in blue-green light, it is somewhat lower in both organisms. They concluded that in contrast to other carotenoids, fucoxanthol is fully effective as sensitizer of photo-

synthesis. They found, similarly to Dutton, Manning and Duggar (Chapter 24, p. 814), that chlorophyll fluorescence in living diatoms can be excited also by light absorbed by fucoxanthol; from this they concluded that the energy absorbed by fucoxanthol is transferred to chlorophyll before it is used for photosynthesis.

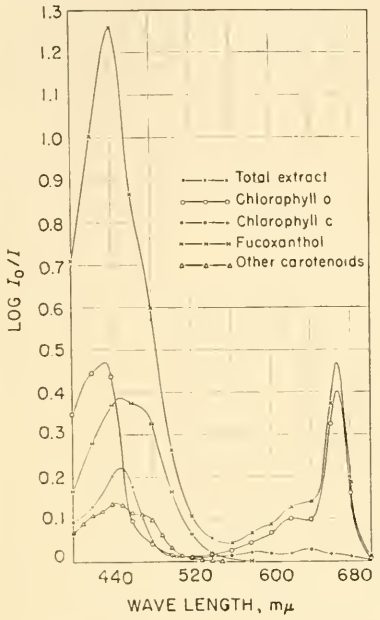

Fig. 30.9B. Absorption spectra of methanol solutions of pigments extracted quantitatively from cells of *Navicula minima* (after Tanada, 1951).

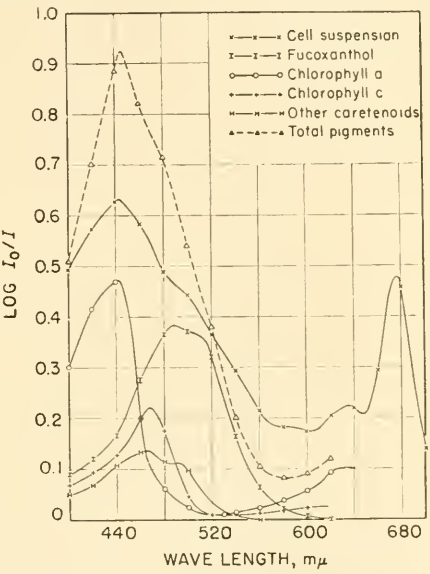

Fig. 30.9C. Comparison of absorption spectra of extracted pigments, and of intact cells of *N. minima* suspended in glycerol. The whole spectrum of each pigment shifted toward the red by an amount equal to that by which the blue maximum shifted by extraction.

A reinvestigation of the quantum yield of brown algae in light of different color was undertaken by Tanada (1951) in Emerson's laboratory. The conclusions of Dutton and Manning were confirmed by much more precise measurements, taking into account the presence of chlorophyll *c*.

Tanada worked with the diatom *Navicula minima* in pure culture. He measured the quantum yield in narrow spectral bands, from 400 to 700 mμ. Fig. 30.9A shows the results: γ is constant between 520 and 680 mμ; as in *Chlorella*, it drops sharply to almost zero above 710 mμ. The

yield dips by about 20% between 520 and 475 mμ (we recall that a minimum of efficiency was found in this region also for *Chlorella* and *Chroococcus*). The yield rises somewhat further in the violet, but declines again toward the ultraviolet, its value at 400 mμ being about 30% below the maximum.

This $\gamma = f(\lambda)$ curve must be compared with the curve showing the absorption by the several pigments in extract from these diatoms (fig. 30.9B), and with the curve (fig. 30.9D), derived from it, showing the contribution of each pigment to the total absorption by the cells. The first figure shows chlorophyll *a*, chlorophyll *c*, and fucoxanthol, as the major components.

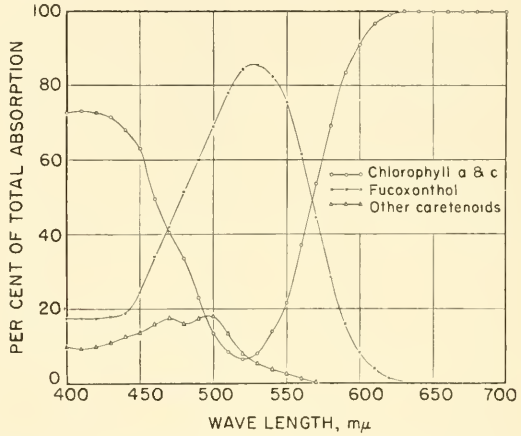

Fig. 30.9D. Curves showing the estimated distribution of light absorption among pigment groups in live cells of *N. minima* as a function of wave length.

Neofucoxanthol (*cf.* chapter 37), β-carotene and an unidentified carotenol (possibly diadinoxanthol) also were identified; their absorption is lumped together under the heading of "other carotenoids."

In deriving, from the spectra of pigments *in vitro*, their contribution to total absorption *in vivo*, corrections for scattering, band shift, and band broadening, must be applied. The unsatisfactory state of this problem was discussed in chapter 22. Tanada made one improvement in the procedure: he found that most of chlorophyll *c* and some fucoxanthol can be extracted with 65% methanol, leaving practically all chlorophyll *a* in the cells; the spectrum of the residue could then be used to locate the position of the blue-violet absorption peak of chlorophyll *a*, and of "other carotenoids" in the cell. The red shift of the blue-violet band of chlorophyll *a*, determined in this way, was 8 mμ; that of "other carotenoids," 20 mμ.

(However, the latter value was derived from a small shoulder on the absorption curve, and is not precise.) The absorption peak of fucoxanthol *in vivo* was calculated from the difference between the absorption curves of the cells before and after extraction with aqueous methanol; it indicated a red shift by as much as 40 mμ for this pigment—from 445 mμ in methanolic solution, to 485 mμ *in vivo*. The blue peak of chlorophyll *c* was calculated similarly from the difference between the spectra of cells before and after extraction with 50% methanol; a red shift of 20 mμ was deduced in this way.

(The shifts deduced by Tanada for the four blue-violet bands—8 mμ for chlorophyll *a*, and 40 mμ for fucoxanthol—can be compared with those derived in chapter 22, on pages 705–706, from earlier investigations. The agreement is good for chlorophyll *a*; for fucoxanthol, the shift found by Tanada—40 mμ—is about twice that estimated previously by Wassink and Kersten.)

When the blue-violet solution bands of all pigments present in the diatoms were shifted as indicated, and superimposed, a composite absorption curve was obtained (see fig. 30.9C). (No attempt was made by Tanada to analyze the region >620 mμ, where all absorption is due to the chlorophylls.) The quantitative agreement is not too good, the composite curve having a higher peak, and a lower valley in the green than the actual absorption curve of the cells. This may be due, at least in part, to scattering—although the cell curve was obtained on Hardy spectrophotometer equipped with an integrating sphere, and with cells immersed in glycerol to reduce scattering. Another likely source of discrepancies is the *broadening* of the absorption bands *in vivo* (particularly of that of fucoxanthol).

The uncertainty implied in the discrepancy between the two curves, had to be accepted in estimating the contribution of the several pigments to total cell absorption at any given wave length. The results of this estimate are shown by fig. 30.9D. It indicates that in the region 500–550 mμ, fucoxanthol takes up most of the quanta absorbed; and yet, fig. 30.9A shows no drop in yield in this region—except below 520 mμ, where absorption by "other carotenoids" sets in. The simplest explanation of the results is that three pigments—chlorophylls *a* and *c* and fucoxanthol—are fully effective in photosynthesis ($\gamma_0 \simeq 0.11$), while the other carotenoids are either altogether ineffective or have a much smaller efficiency.

Tanada found further that γ_0 of *Navicula minima* was the same at 1.5, 10, and 20°C., and that γ was a smooth function of light intensity between 0.9 and 6.9 $\times 10^{-8}$ einstein/cm.^{2} min., the curvature becoming noticeable $>2 \times 10^{-8}$ einstein/cm.^{2} min. Points obtained in red, orange, blue and green light all fell onto the same slightly curved line, similar to that found for *Chlorella* by Emerson and Lewis (fig. 30.5).

If fucoxanthol and other carotenoids are to a certain extent active as sensitizers in green plants and brown algae, the mechanism of their participation is likely to be based on a transfer of energy to chlorophyll, rather than on a direct interaction with the oxidation-reduction system. This hypothesis was suggested by Engelmann over fifty years ago; its first direct confirmation came from the experiments of Dutton, Manning and Duggar on the excitation of chlorophyll fluorescence by light absorbed by the carotenoids, which were described in chapter 24 (page 814). They were carried out with the same organism (*Nitzschia closterium*) that was also used for the measurement of the quantum yield of photosynthesis. The quantitative results of this study also are in need of re-examination for possible effects of chlorophyll *c*.

We will now describe in brief the experiments in light of indefinite (and probably partly saturating) intensity, which can be adduced in support of the hypothesis that the carotenoids of brown algae actively participate in photosynthesis. Conditions in these organisms appeared somewhat more favorable for correct guessing than in green plants, because of the absence of chlorophyll *b*, and consequent enhanced importance of the carotenoids for the absorption in the region between 450 and 500 mμ; here again the contribution of chlorophyll *c* requires consideration.

As early as 1884, Engelmann found that brown algae (*Melosira*, *Navicula*, *Pinnularia*) illuminated by sunlight or gas light, produced the largest amount of oxygen in the *green* part of the spectrum, and concluded that the "orange pigment" of these algae must participate in sensitization.

Fifty years later, Montfort (1934) compared the rate of photosynthesis in white light of a certain standard intensity with its rate in orange-red light. While green algae (*Ulva lactuca*) were equally efficient with both kinds of illuminations, brown algae, *Dictyota dichotoma*, *Alaria* and *Desmarestia*, produced in orange-red light only one half the oxygen liberated in white light. Montfort interpreted this as an indication that *Phaeophyceae* have a higher relative efficiency in blue and violet light, and attributed this to the presence of fucoxanthol. Later, Montfort (1936) and his co-worker Schmidt (1937) found that the removal of blue and violet rays from white light depressed the rate of photosynthesis in brown algae (*Dictyota* and *Laminaria*) much more strongly than in the green *Ulva*. They calculated the ratio P/A (photosynthesis per unit absorbed energy) for different colors, using incident light of equal intensity in all spectral regions.

An arbitrary selection from the confusing abundance of their material is shown in Table 30.VI. Not all the figures in this table may be strictly comparable, but they show the trend of the results.

In green algae, the decrease in P/I from the red to the green and the renewed increase in the blue reflected more or less clearly the changes in absorption and in the size of quanta. (These two factors cooperate to make the yield in green light smaller than in the red; but become antagonistic in the blue.) In brown algae—with the exception of *Fucus*, which Montfort classified as a "xanthophyll alga" (whereas he designated *Laminaria* and *Dictyota* as "fucoxanthol algae")—the yields were invariably 30 or 40% higher in the blue than in the red, and would have become *twice* as high if related to the absorption by chlorophyll alone. In the green, too, the P/I values were relatively higher than they should be according to the absorption of chlorophyll and the size of the

quanta. Montfort and Schmidt concluded from these results that, of all the carotenoids, only fucoxanthol is able to assist in the sensitization of photosynthesis, whereas the carotenoids of green algae are inactive (for a criticism of their methods, see Emerson 1937).

TABLE 30.VI

ENERGY YIELD OF ALGAE IN LIGHT OF DIFFERENT COLORS[a] (AFTER SCHMIDT 1937)

Alga	P/I (green) / P/I (red)	P/I (blue) / P/I (red)
Green		
Cladophora	0.49	0.80
Ulva lactuca	0.46	0.82
Brown		
Laminaria digitata		
Strong light (1)	0	1.29
Medium light (⅓)	0.91	1.32
Weak light (1/9)	1.00	1.23
Phyllitis fascia	—	1.40
Dictyota dichotoma	0.90	1.48
Fucus vesiculosus (brown, but with low fucoxanthol content)	0.38	0.59

[a] P/I for equal incident intensities.

A certain improvement of methods was attempted by Montfort later (1940). The pigments were extracted from the algae and their absorption curves determined (in methanol solution), with results shown in figure 22.45A,B (*cf.* also Table 22.VIII). These absorption data were applied to the results of Gabrielsen and Steemann-Nielsen (1938), who found that, for equal incident light intensity, the rate of oxygen production by diatoms is consistently higher in the blue than in the red. (A similar difference was reported earlier by Mothes, Baatz and Sagromsky 1939.) The difference is particularly strong in low light, as shown by Table 30.VII. The table describes a perfectly under-

TABLE 30.VII

RATIOS OF RATES OF PHOTOSYNTHESIS BY DIATOMS IN BLUE AND RED LIGHT (AFTER GABRIELSEN AND STEEMANN-NIELSEN 1938)

Incident light, cal/cm.² sec.	P (blue) / P (red)	Incident light, cal/cm.² sec.	P (blue) / P (red)
0.25	1.8	1.7	1.4
0.5	1.6	2.4	1.3
1.0	1.5	3.7	1.1

standable transition from the conditions at low light intensities (where the yield is proportional to absorption, and may therefore be higher in the blue than in the red, if the absorption in the blue is so much stronger as to overbalance the larger size of the quanta) to the conditions in strong (saturating) light, where the rate must be (and apparently is) independent of wave length. Montfort (1940) preferred, however, to *average* all the ratios in Table 30.VII and concluded that a ratio of 1.5 between the rates in blue light and red light indicates an active participation of carotenoids in photosynthesis. Using the absorption curves of methanolic extracts he calculated that the energy conversion rate in blue light is 1.03 times larger than in red light, referred to absorption by *all* pig-

ments, and 2.46 times larger referred to chlorophyll alone; while, according to the size of the quanta, it should be *smaller* by a factor of 0.68.

In the case of brown algae (*Laminaria digitata*) a similar calculation (based on Montfort's own measurements) gave energy yield ratios (related to the combined absorption by all pigments) of 0.86 and 1.18 (for two different combinations of filters), as compared with the quantum size ratios of 0.64 and 0.77, respectively, again indicating a *higher* quantum yield in the blue-violet than in the red. At their face value, Montfort's figures appear to indicate that the carotenoids of the diatoms and brown algae are several times more efficient as sensitizers than chlorophyll!

Mothes, Baatz, and Sagromsky (1939), Baatz (1941) and Sagromsky (1943) have described observations of the rate of photosynthesis in filtered red and blue light of equal intensity (in energy units). They found for these two rates, a ratio of 1:1.2 in the diatom *Chaetoceras simplicia centrosperma*, as against 1:0.7 in two unicellular green algae, and attributed the relatively better utilization of blue light by the brown cells to the presence of fucoxanthol. These experiments were more satisfactory than those of Montfont in that unicellular algae were used rather than thick thalli; but it was equally unsatisfactory in the use of broad spectral regions, and even less satisfactory in the absence of any absorption measurements, which would permit approximate allocation of absorption to the several pigments. Mothes and co-workers pointed out the difficulty of the latter problem, caused by the difference of the carotenoid spectrum *in vivo* from that *in vitro*. (This difference is clearly indicated by the change of color from brown to green caused by placing brown algae in hot water—a treatment which, they assumed, disrupts the molecular association of carotenoids with chlorophyll and proteins.)

6. Quantum Yield and Action Spectrum of Red and Blue Algae. Role of Phycobilins

The history of our knowledge of the role of phycobilins in the sensitization of photosynthesis in red and blue algae is similar to that of the role of carotenoids in brown algae. Here too we find the—we now know, correct—guess by Engelmann, made as early as 1883, that the phycobilins are active sensitizers of photosynthesis, as well as a series of vague and indecisive observations and calculations of several authors, mostly tending to confirm this guess, and finally, quantitative analyses of the quantum yield as a function of wave length, carried out by Emerson and Lewis (1941, 1942), and Haxo and Blinks (1950), which brought convincing confirmation of Engelmann's concept. As in the previous section, we will discuss the more recent and reliable experiments first.

Table 29.III showed that Emerson and Lewis (1941), in comparing the quantum yield of different plants in yellow sodium light, found no difference between green plants and the blue-green algae *Chroococcus*, although in the latter, far more than half the absorption in the yellow region was due to phycocyanine. This result of a preliminary observation was in itself a more convincing proof of the activity of phycobilins in photosynthesis than could be derived from all the extensive earlier discussions of this problem. It was confirmed and amplified by the same authors in

1943, by a systematic investigation of the relation between wave length and quantum yield in this *Cyanophycea*.

Chroococcus cells are somewhat smaller than *Chlorella* (2.5 μ in diameter) and are surrounded by a gelatinous sheath. They scatter less light

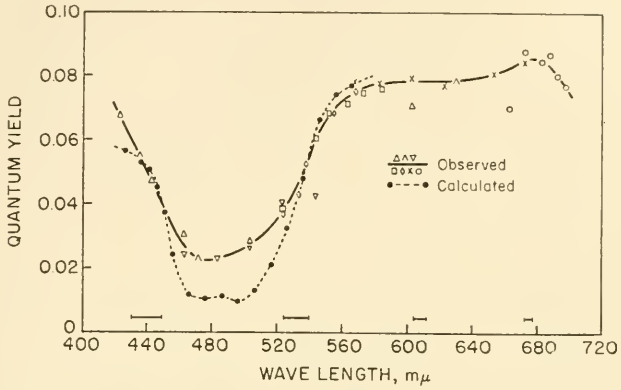

Fig. 30.10A. Quantum yield of *Chroococcus* photosynthesis (after Emerson and Lewis 1942). Solid line is drawn through experimental points, values obtained in different runs being distinguished by different characters. Broken line shows expected dependence of quantum yield on wave length, on assumption that yield for light absorbed by chlorophyll and phycocyanine is 0.08 at all wave lengths, and light absorbed by carotenoids is not available for photosynthesis.

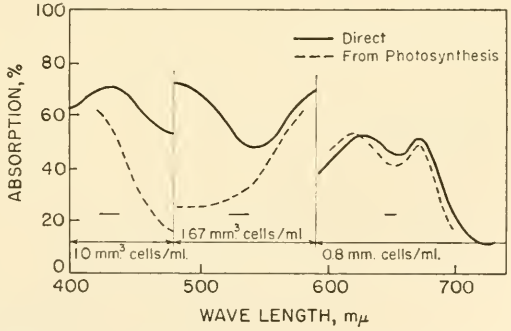

Fig. 30.10B. Comparison of action spectrum (broken curve) and absorption spectrum of *Chroococcus* (after Emerson and Lewis 1942).

than *Chlorella* cells (probably because of the absence of chloroplasts; *cf.* chapter 15, page 355). This makes the determination of the absorption curve easier, and leads to better agreement between the absorption curve of the intact cells and the curve constructed by the combination of the (appropriately shifted) absorption curves of the extracted pigments (illus-

trated by fig. 22.48*B*). The main remaining differences between the "cell spectrum" and the combined extract spectrum is the apparent broadening of the red chlorophyll band in the intact cells, and a somewhat lower absorption of the latter at $\lambda < 510$ mμ.

Chroococcus cells were used in carbonate buffer (85% 0.1 *M* $NaHCO_3$ + 15% 0.1 *M* Na_2CO_3). These algae can live without potassium, but not without sodium. The method of determination of γ was the same as in the work with *Chlorella*. (Bands 6–10 mμ wide were used in the red and 15–20 mμ wide in the blue-violet; photosynthesis and respiration were measured in alternating 10 minute periods of darkness and light, the value of P being derived from the rate of oxygen production in the second half of the illumination period.) Both "dense" (fully absorbing) and "thin" (partially absorbing) suspensions were used.

The quantum yield of photosynthesis in *Chroococcus* as a function of wave length is shown in figure 30.10A. As in *Chlorella*, γ is approximately constant between 570 and 690 mμ (aside from a slight flat maximum at 680 mμ)—despite the fact that in Chlorella *all* absorption in this region is due to chlorophyll, whereas, in *Chroococcus*, more than half the total absorption in the region between 560 and 650 mμ must be attributed to phycocyanin. Judging from figure 22.49, the absorption by phycocyanin at 600 mμ should be at least six times as large as that by chlorophyll; but the quantum yield is the same as at 660–680 mμ, where chlorophyll accounts for practically all absorption. Thus, the photosynthetic efficiency of phycocyanin in *Chroococcus* must *equal that of chlorophyll* (the maximum possible difference being of the order of 10–15%). Another way of representing the same results is shown in figure 30.10B. Here, the absorption spectrum of a thin suspension of *Chroococcus* cells is compared with the quantized action spectrum of photosynthesis. The close parallelism between the two curves in the region above 570 mμ shows the approximately equal availability for photosynthesis of the light absorbed by both chlorophyll and phycocyanin. Particularly convincing are the two separate maxima shown by both curves near 620 and 670 mμ, which must be attributed to phycocyanin and chlorophyll, respectively. The large discrepancy in the region 420–550 mμ indicates the inefficiency (or relatively low efficiency) of the light absorbed by the carotenoids. The dotted line in figure 30.10A shows, however, that assuming *complete* inefficiency of the carotenoids leads to an underestimation of the yield between 450 and 550 mμ; the best agreement between measured and calculated yields can be obtained by assuming a quantum yield $\gamma = 0.08$ for the light absorbed by chlorophyll and phycocyanin, and $\gamma = 0.016$ for the light absorbed by the carotenoids.

The action spectra of several species of *red algae* were studied by Haxo

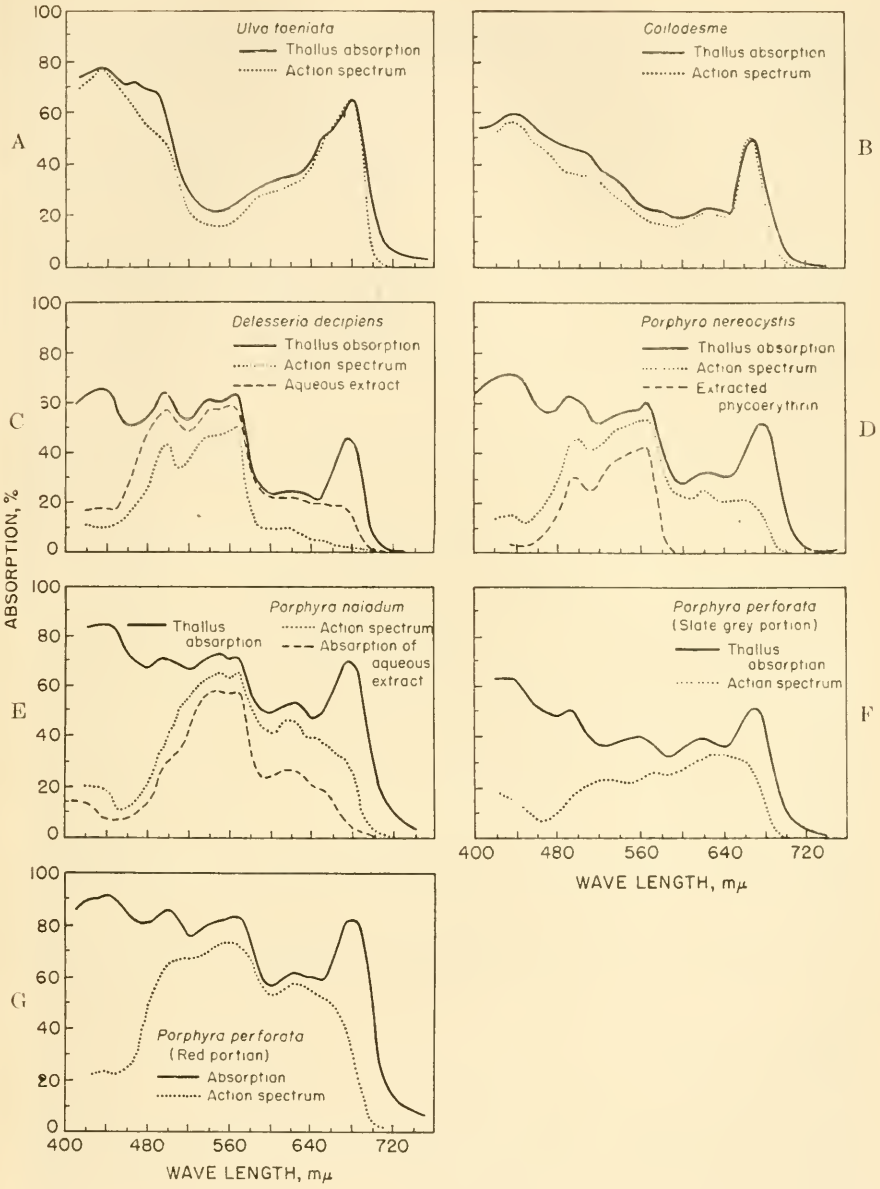

Fig. 30.11. Action spectra after Haxo and Blinks (1950).

and Blinks (1950) with very striking results. The polarographic method (page 850) was used, with occasional checks by oxygen determination and manometric measurements. Light intensities used were low enough (about twice the compensating intensity) for the results to approximate the maximum quantum yields. In the green alga *Ulva taeniata* (fig. 30.11A) the action spectrum was found to follow closely the absorption spectrum, with the exception of a yield deficiency of up to 100% in the far red (>700 mμ), already noticed by Emerson and Lewis (*cf.* fig. 30.3), and of a certain yield deficiency around 480 mμ, also noticed before, and interpreted as evidence of partial inactivity of the carotenoid pigments in green cells. It is, however, to be noted that the yield deficiency disappears at 415 mμ, although a considerable proportion of light (over 50% in extracts) must be absorbed in this region by carotenoids.

In the brown *Coilodesme* (fig. 30.11B), the action spectrum also paralleled rather closely the absorption spectrum with a moderate deficiency (of up to 20%) in the region of strong carotenoid absorption. In the red algae *Delesseria decipiens* (fig. 30.11C), *Porphyra nereocystis* (fig. 30.11D), *Porphyra naiadum* (fig. 30.11E) (purple, indicating relatively high content of phycocyanin) and *Porphyra perforata* (fig. 30.11F, G) (a slate-green vegetative section containing mainly phycocyanin, and a red carposporic section) the action spectra were strikingly different from the absorption spectra. They showed unmistakable maxima corresponding to the absorption peaks of phycoerythrin, at 500 and 565 mμ, and also to those of phycocyanin at 620 mμ (fig. 30.11E, F), but only very little of the chlorophyll maxima in the red as well as in the violet. The quantum yield was estimated to be the order of 0.06 in the phycoerythrin bands, and as low as 0.02 in the chlorophyll bands. Similar results were obtained with several other *Bangiales* and *Florideae*. The absorption by carotenoids appeared to be as little effective as that by chlorophyll. A slight increase in activity shown by some species at $\lambda = 440$ mμ, coinciding as it did with an increase in the absorption of the aqueous phycobilin extract, could not be interpreted as sign of photosynthetic activity of the chlorophylls or the carotenoids.

These unexpected results indicate that in contrast to all other plants direct sensitization by chlorophyll plays only a subordinate role in at least some of the red algae, and that photosynthesis in them is sensitized primarily by phycobilins. If this is the case, it would seem unlikely that the energy quanta absorbed by the phycobilins are transmitted to chlorophyll, since how could indirectly produced excitation of chlorophyll be more effective than excitation due to energy absorbed directly by chlorophyll? Rather, these results would seem to indicate that the phycobilins are sensitizers of photosynthesis in their own right, and that their presence

may perhaps even make that of chlorophyll superfluous, although so far no chlorophyll-free red algae have been found.

We will see below, however, that a different—and no less striking—interpretation of these results is possible.

In one species of *Iridophycus*, which bleaches to almost green color at high tide, a much greater participation of chlorophyll in photosynthesis was noted in rough experiments.

Haxo and Blinks said that, in contrast to Emerson and Lewis's results on *Chroococcus*, they found only a weak chlorophyll activity also in two blue-green algae, *Anaboena* and *Oscillatoria*, whose action spectra were similar to those of *Porphyra perphorata* (fig. 36.11F). They suggested that culture conditions may affect the relative activity of different pigments in algae of the same class or even the same species.

Haxo and Blinks noted that the saturation rate of photosynthesis of *Delesseria* is the same in blue light (565 mμ) as in red light (672 mμ): This seems to indicate that the enzymatic mechanism of photosynthesis—or, at least, the rate-limiting enzymatic reaction—is the same whether the quanta are absorbed by a phycobilin or by a chlorophyll.

In chapter 24 (p. 815) we described the fluorescence studies of French *et al.* (1951) and Duysens (1951) that indicated effective transfer of excitation energy in red algae from carotenoids and phycobilins to chlorophyll *a*, and (in certain of them) from chlorophyll *a* to *d* (despite the low concentration of the latter). If one assumes that transfer to chlorophyll *d* constitutes a "leak" which makes energy unavailable for photosynthesis, the results of Blinks and Haxo become understandable. The question remains why energy transferred to chlorophyll *a* from phycobilins is not also lost to chlorophyll *d* but remains available for photosynthesis. It was noted on p. 815 that this energy stays with chlorophyll *a* long enough to cause its fluorescence (while most of the energy absorbed by chlorophyll *a* itself causes the fluorescence of *d*). Two suggestions were made there as to the possible reasons for this difference in the fate of excitation energy; but further study is needed for a convincing explanation.

Duysens' observations provide the strongest argument at present in favor of assuming that chlorophyll *a* is the one pigment directly participating in photosynthesis, and that not only the carotenoids, but also the phycobilins, sensitize photosynthesis by transferring their excitation energy to chlorophyll *a*. Another argument supporting this view is the observation of French and co-workers (1951) that the yield of fluorescence of the phycobilins in algae shows none of the induction effects and of the peculiar changes with light intensity which were discussed at length in chapters 24 and 28 (part B) and are indicative of an intimate relationship between chlorophyll and the chemical processes of photosynthesis.

Beside the measurements of Emerson and Lewis and of Blinks and Haxo, all earlier observations on the role of phycobilins in photosynthesis have only the weight of corroborative evidence. Most of this evidence pertains to *red* algae, and has been gathered in connection with Engelmann's theory of "complementary chromatic adaptation" of these algae to the blue-green light that prevails under the sea. (Obviously, this color adaptation can only be useful to the algae if the light absorbed by the red pigments can be used for photosynthesis.) Because of the absorption of red and blue-violet light by water, a full utilization of the central part of the visible spectrum—which is only insufficiently absorbed by chlorophyll—is of vital importance for the plants living deep under the sea. This consideration was the basis of the theory of chromatic adaptation, developed by Engelmann in 1884. This subject was almost lost sight of in the first quarter of the new century, while new methods of quantitative study of photosynthesis by green leaves were being developed by Blackman and coworkers and by Willstätter and Stoll. Later, Warburg made the green *Chlorella* cells the favorite subject of photosynthetic studies. In the work on green plants, the presence of accessory yellow pigments was considered to be scarcely more than a nuisance. These pigments were not prominent enough—both in concentration and in the part they took in light absorption—to make them a desirable subject of independent study; but their presence interfered with the quantitative study of chlorophyll-sensitized photosynthesis in the short-wave region of the visible spectrum. The fact that blue and red algae offer a much more promising field for the study of the part played by the "accessory" pigments in photosynthesis was almost forgotten. Engelmann had noticed, however, as early as 1883 (using motile bacteria for the oxygen determination) that the maximum of the photosynthetic efficiency of *red* algae (*Callithamnion* and *Ceramium*) lay in the green part of the spectrum, and that of blue algae (*Oscillatoria* and *Nostoc*), in the yellow. As in the case of green plants, the position of the maximum of photosynthesis coincided roughly with that of the maximum of light absorption. A year later (1884), Engelmann described a "microspectrophotometer," by means of which he was able to show that the parallelism between the absorption spectra and the "photosynthetic action spectra" of the colored algae is quantitative. He concluded that *all* pigments that contribute to light absorption by the algae also contribute to photosynthesis, and expressed this result by the equation $E_{\text{abs.}} = E_{\text{assim.}}$ (E standing for energy), which was a direct challenge to the concept of the exclusive sensitizing role of chlorophyll in photosynthesis.

One of the developments of Engelmann's theory—the concept of "chromatic adaptation" as a factor determining the composition of the pigment system in plants—has been discussed in chapter 15. Here, we are con-

cerned with the other aspect of the same phenomenon—chromatic adaptation as a factor enabling plants to make better use of available light energy. It was mentioned in chapter 15 that Oltmanns (1893) and others (most recently, von Richter 1912, and Sargent 1934) objected to Engelmann's theory (as well as to its extension by Gaidukov to color changes induced artificially in blue-green algae) and insisted that algae responded only to changes in light *intensity*, and not *color*.

Von Richter (1912) raised a further objection and asserted that "chromatic adaptation" could not achieve the purpose that was suggested by Engelmann, because phycobilins do not act as sensitizers in photosynthesis. Although, in comparing the ratio of the photosynthetic productions of the green alga *Ulva lactuca* in red and green light with the corresponding ratios for *Plocamium*, *Callithamnion*, *Delesseria* and other red algae, von Richter could not help confirming the results of Engelmann and finding that red algae are two or three times as efficient in green light as the green algae, he nevertheless denied that this proved the photosynthetic activity of the red pigments. He pointed out that similar differences are obtained also by changing the *intensity* of light, and suggested that the red algae utilize green light better not because of its wave length but because it is only weakly absorbed by chlorophyll— and red algae *are* adapted to weak light.

However, the views of von Richter have not been confirmed by later investigators. Wurmser and Ducleaux (1921) compared the photosynthetic efficiencies of red and green fronds of *Rhodymenia palmata* and *Chondrus crispus* and found that the red varieties give yields two or three times greater than the green ones. Wurmser (1921) compared the photosynthesis of the green alga *Ulva lactuca* with that of the red alga *Rhodymenia palmata* in red, green and violet light. He found that, if the rate in red is taken as unity, that in green is 0.24 in *Ulva* and 0.49 in *Rhodymenia*, and that in violet 0.81 and 0.16, respectively (for equal intensity of incident light). Thus, the red algae are more efficient in the green, but less efficient in the violet than the green ones. Wurmser pointed out that, even if von Richter's intensity effects are real, this does not mean that color effects are only indirect consequences of changes in absorption intensity.

Similar results were obtained by Harder (1923), who concluded that both intensity adaptation and color adaptation are real phenomena. He, as well as Ehrke (1932), interpreted the result of the rate measurements with red algae as indicating active participation of the phycobilins in photosynthesis, and the same conclusions were reached by Montfort (1936) and Schmidt (1937), who found the spectral maximum of the efficiency of phycocyanin algae in yellow light, and that of phycoerythrin algae in green light.

Levring (1947) determined "action spectra" of photosynthesis of a number of marine algae in filtered sunlight. (Only qualitative results can be expected from such measurements, because of the relatively high light intensity and the relatively strong absorption of the thalli). He found evidence of particularly strong photosynthetic efficiency (high ratio yield/absorption) in green light in ten species of red algae, and concluded that the red phycobilin pigment is as active (if not more active) than the green chlorophyll. Combining these results with those of his measurements of spectral distribution of light in different depths, he concluded that because of the presence of the red pigment, the *Rhodophyceae* utilize the blue-green light deep under the sea better than the *Chlorophyceae*, as postulated by the Engelmann-Gaidukov theory. He agreed, however, that adaptation to low light *intensity* is an alternative way of adjustment to life in great depths; an important element of it is low respiration.

Thus, even more uniformly than in the case of brown algae, the crude observations on the relative efficiency of photosynthesis of red algae in light of different color support the assumption that the accessory pigments of these organisms are active sensitizers in photosynthesis, and that Engelmann's theory of chromatic adaptation was fundamentally correct. And one may ask oneself, how could it have been otherwise? Would it not be strange if the appearance of orange or red pigments in deep-water algae would be only a coincidence, and these pigments were helpless in performing the task so obviously set to the plants by the character of the "light field" in which they live—to catch and utilize for their maintenance and propagation radiations in the middle of the visible spectrum, which are the only ones to reach them in some intensity?

It may be argued that not all deep-water algae are red, some green algae being encountered in great depth. In other words, algae *can* survive without phycobilins in the greatest depths where life occurs. However, this in itself is not a convincing argument against Engelmann's theory. Algae could adapt themselves to great depths in two ways: by reducing respiration to a level permitting growth even in extremely weak, and weakly absorbed light; and by adjusting their pigment systems to enhance light absorption. The fact that the first adjustment has been sufficient for some green species does not invalidate the hypothesis that red algae have also used chromatic adaptation for the same purpose.

Another objection to Engelmann's theory is that many red algae live on or near the surface and that phycobilins are found in blue-green algae, which are surface organisms. It is known, however, that red algae often tend to lose their phycobilin and become green when exposed to sunlight (*cf.* Vol. I, Chap. 15); and even if many of them (as well as the blue-green algae) apparently find their phycobilin content useful, or at least not harm-

ful, even on the surface, this does not prove that phycobilins are not pigments primarily intended to permit photosynthesis deep under the sea.

One may speculate—particularly in the light of Blinks' experiments—whether photosynthesis with phycobilins may not be an older process than photosynthesis with chlorophyll; perhaps, the development of the green pigment and its substitution for the phycobilins have been the product of a later development, in which plant life, originating in the depths of the ocean, migrated to the surface and finally spread overland.

7. Action Spectrum of Purple Bacteria

Engelmann found, in his fundamental work on photosynthetically active bacteria (1888) that, if a spectrum was thrown on their cultures, they developed only in the absorption bands of the green pigment (which we now call bacteriochlorophyll). Purple bacteria also contain numerous carotenoids, with absorption bands clearly separated from those of bacteriochlorophyll (*cf.* fig. 22.21). French (1937) found that the action spectrum of *Streptococcus varians* (as determined by the rate of consumption of hydrogen) shown in figure 30.12 closely parallels its absorption spectrum in the yellow and red part of the spectrum but does not show maxima in the green or blue that correspond to the absorption bands of the carotenoids (*cf.* Table 30.VIII). French concluded that the red carotenoid pigments of purple bacteria are photosynthetically inactive. It must be noticed that, from the spectroscopic point of view, the conditions in purple

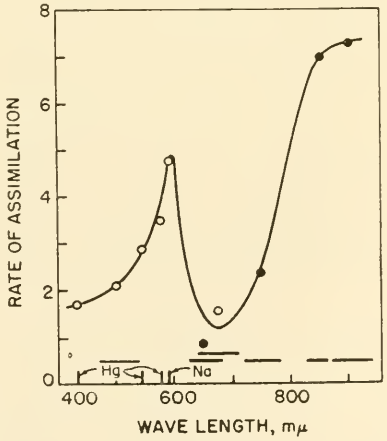

Fig. 30.12. Rate of CO_2 assimilation of a very dilute suspension of *Streptococcus varians* as a function of wave length of incident light (after French 1937). Rate scale represents molecules $CO_2 \times 100$ per incident quantum.

bacteria are particularly favorable for an investigation of the part played by the carotenoids in photosynthesis, since the absorption peaks of the bacterial carotenoids are not concealed behind the absorption bands of chlorophyll, as is the case not only in green plants, but also in "fucoxanthol algae."

TABLE 30.VIII

POSITION OF WAVE LENGTH MAXIMA (mμ) IN SPECTRUM OF *Streptococcus varians* (AFTER FRENCH 1937)

Methanol solution	Cells	Action spectrum
410	420	—
—	490[a]	—
—	510[a]	—
—	550[a]	—
605	590	590
770	880	900

[a] Carotenoid bands.

Vermeulen, Wassink and Reman (1937), too, found that the development of *Chromatium* takes place mainly in light belonging to the absorption bands of bacteriochlorophyll (infrared, red and a narrow band at 590 mμ; *cf.* figs. 22.19 and 22.21), but not in those of the red carotenoid pigments.

More recently, however, Manten and Thomas found the situation to be more complex. First, Manten (1946), in studying the action spectrum of phototaxis of *Rhodospirillum rubrum*, found peaks corresponding to the absorption peaks of bacteriochlorophyll (590 mμ) as well as to those of some of the carotenoids (but not the main carotenoid of these cells, spirilloxanthol). He suggested that the mechanism of phototaxis involves in *Rhodospirillum*, primarily, a stimulation of photosynthesis, and that the action spectrum of phototaxis therefore indicates that some (but not all) carotenoids are photosynthetically active also in purple bacteria. Thomas (1950) investigated the action spectrum of photosynthesis of the same cells manometrically by measuring carbon dioxide consumption in the presence of 0.015 *M* sodium butyrate as hydrogen donor, (*cf.* chapter 5, p. 106). Because of the sigmoid shape of the light curve, the action spectrum was determined by measuring the ratio of ΔCO_2 in light of a given wave length (bands isolated by Christiansen filters) to ΔCO_2 in light of a standard wave length (yellow sodium line). Between 460 and 650 mμ the action spectrum had a peak at 590 mμ belonging to bacteriochlorophyll (see fig. 22.27) and three peaks attributable to carotenoids. However, no maximum of photosynthesis is noticeable at 550 mμ, where a peak is present in the absorption curve of the cells (compare fig. 22.27 and table 30.VIII above); this peak has been attributed to the most abundant bacterial carotenoid,

spirilloxanthol, which is thus shown to be inactive, both in photosynthesis and in phototaxis. The result shows close similarity of the two action spectra, and thus supports Manten's hypothesis that phototaxis is a consequence of enhanced photosynthesis.

The observations by Duysens (1951) of carotenoid-sensitized fluorescence of bacteriochlorophyll in *Chromatium* and *Phodospirillum rubrum*, described in chapter 24 (p. 810) indicates that the capacity of the carotenoids in purple bacteria to sensitize phototaxis and photoreduction of CO_2 may be based on transfer of their excitation energy to bacteriochlorophyll.

Bibliography to Chapter 30

The Light Factor. III. Photosynthesis and Light Quality; Role of Accessory Pigments

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