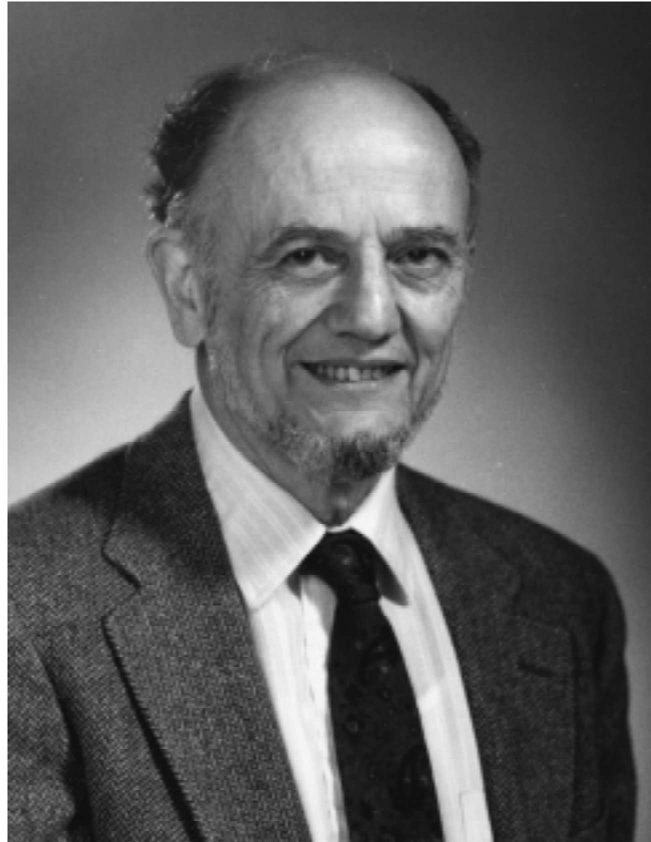

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Personal perspective

Chance, luck and photosynthesis research: An inside story

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Abstract

A major theme in my career has been photophosphorylation; especially contributions to the early work on chemiosmosis, and later involvement in CF_1 activation and function. A second theme has been interest in chloroplast biogenesis, with work ranging from translation in chloroplasts to discovery of the enzyme which may contribute to strand exchange, homologous recombination and DNA repair in chloroplasts. Throughout, I try to point out the major contributions of graduate students and postdocs, and help from friends and colleagues. Without them I would have had no career at all.

The perspective for these recollections comes from the underlying theme in at least one book by Stanislaw Lem (1978). The probability of any one event occurring is amazingly small; in retrospect, each step seems like a minor miracle. Any person's career has to be shaped by interactions with other people; mine was certainly very much shaped by faculty colleagues, postdocs, graduate and undergraduate students. To start with the punch line – whatever happened was very much the result of luck, and chance, and these wonderfully helpful influences.

Starting in early youth – my move into plant sciences was a compromise between two forces. My father, a New York City dentist with broad interests, loved the look of well-kept farms and wanted me to be a farmer. But I was reading science fiction, and was in one of the first classes at the Bronx High School of Science. I compromised, and went into Plant Physiology.

Education, and some deficiencies

In college at Cornell, I was strongly influenced by Loren Petri, teaching General Botany. Later I realized he'd done a fine job of showmanship; and that approach was perfect for fresh-persons. He made Botany come alive for us; a tradition followed in a highly competent way by at least 2 succeeding paleobotanists (Harlan Banks; Karl Niklas) teaching the introductory course at Cornell. Other outstanding teachers helped keep my enthusiasm alive – especially Otis Curtis in Plant Physiology, Lester Sharp in Cytology, and Bob Smock in Pomology.

Going to Yale for graduate work in Plant Science, I was very fortunate to be taken into the group run by David Bonner; essentially because nobody else in that department wanted to take a chance on having me. David Bonner was one of the early founders of Biochemical Genetics. Everybody else in his lab worked with either *Neurospora* or *E. coli* mutants and their biochemistry. However David's PhD thesis, under the guidance of his older brother James Bonner, had been on radish cotyledon growth. He turned me loose on a continuation of that work, which ended up as a thesis describing effects of 2,4-D on seedling growth of cabbage. My training in the lab was aided materially by Aubrey Naylor, in whose tissue culture facilities I did most of my work. While it was tremendously exciting for me at that time, by taking that pathway I did miss out on getting very early into the main stream of genetic engineering and biochemistry (other students in the Bonner group at that time, for instance, included the future distinguished scientists Charlie Yanofsky, Gabriel Lester, Bill Jakoby and Otto Landman). Be-

ing in Bonner's group was a wonderful experience because we had a strong feeling of fellowship, and all of us benefited from David and Miriam Bonner's strong support. And I learned the most important thing a graduate student can learn – doing science is fun.

However, I realized later that my formal education was, at best, spotty. For instance, I was never told I must take a course in Biochemistry, and never did (but I did audit the one given by Joseph Fruton, over in the medical school). It was an era (1948–1951) when exciting events included the discovery of asymmetry in the proportion of bases in DNA (overturning the 'tetranucleotide' theory); the announcement of enzyme 'action at a distance' through a layer of gold leaf (I'll forget the name of the scientist who advocated that); the discovery of the language of the bees; and the beginning of some idea of the diversity and functions of organelles. Certainly, one area that I heard about and felt was really exciting, was the mechanism of oxidative phosphorylation.

David Bonner, not having continued with plant physiology after his thesis, didn't feel entirely comfortable trying to direct my work with cabbages. At one point he sent me to see Kenneth Thimann at Harvard, to consult on some results; then one happy summer I hitchhiked out to Cal Tech to learn Plant Physiology at its true center. I was supposed to work with James Bonner there; but he was busy and I spent my time with Sam G. Wildman, isolating leaf proteins. That 2 month period actually resulted in a paper (Axelrod and Jagendorf 1951); Bernard Axelrod measured some hydrolytic enzymes, and I measured total protein levels, as excised tobacco leaves senesced. The experience was very exciting, and gave me a vision of doing work at a deeper level than just overall plant growth.

The happiest years of my life (postdoctoral)

I received my PhD in 1951 (after 3.5 years) and was lucky enough to receive a Merck Postdoctoral Fellowship. I spent 2 postdoctoral years with Sam Wildman, who had moved from Cal. Tech. to UCLA's greenhouse (we worked in the concrete-floored head house, converted to a genuine laboratory). Again, it was a wonderful, friendly environment in which to work. Together with Al Siegal, Irv Rappaport, Milt Zaitlin and Morris Cohen I discovered the joys of 3-dimensional tic-tac-toe, and checkers, during the lunch breaks. During alternative lunches I attended a 'cynical seminar' – discussion of politics by selected faculty and postdocs, all from the liberal, losing point of view. There I met Jacob Biale, a scholarly, intrepid back-packer and mountain climber, and a very good scientist who introduced me to avocados and respiration. During that first year I also was excited by 'discovering' that leaves had mitochondria (defined by differential centrifugation) as well as chloroplasts.

Finding a job at that time was either easy or difficult, depending on one's connections. One day I received a telephone call – 'Would you like to be an Assistant Professor at the Johns Hopkins University?' I said 'Yes, but could I please come East and give a seminar, first?' (I was engaged, and that would have given me a chance to see my fiancée). The answer was 'No – just come next September.' I was apparently the token botanist they were looking for. I worked with plants, had taken (and enjoyed) Edmund Sinnott's courses on Plant Morphogenesis at Yale and so could be related to the growth and development bias in the Hopkins Biology Department (its chairman was B. Willier, an embryologist). But I had also gotten my degree with Bonner and so in theory should appreciate biochemistry and biochemical genetics. This made me acceptable to the McCollum–Pratt Institute (a biochemical group, devoted to trace element research, funded by Pratt in honor of Dr McCollum) which had just started recently with Bill McElroy in charge. I received a year's reprieve; went East anyway to get married, and then spent a second happy conjugal year at UCLA. This included a full 10 camping trips. During that second year Sam and I actually published a paper declaring among other things that 'chloroplasts have no nucleic acid' (Jagendorf and Wildman 1954). Of course, it was prior to development of really sensitive assays for nucleic acids, and also before we knew the difference between isolated chloroplasts and isolated thylakoids. Dim excuses!

The entry into photosynthesis; The Johns Hopkins University

At The Johns Hopkins, my education in biochemistry really began. From Bill Harrington I learned of density gradient centrifugation; applied it to 'chloroplasts' (really thylakoids), watched them float up on a glycerol gradient, and made them more pure than before (Jagendorf 1955). McCollum–Pratt Institute had a lunch-time journal club in biochemistry, run by Nate Kaplan, Sid Colowick and Al Nason. Sitting in on it,

I began to realize what an exciting field biochemistry could be. Especially, the thrill of rigorous proof of a concept, something often lacking in the plant physiology literature at that time. I also began to understand a little more biochemistry – for instance that enzyme catalyzed reactions are reversible.

Originally, I had thought to use the more pure 'chloroplasts' to generate antibodies, which might be able to pull down proplastids selectively, and help in the study of chloroplast biogenesis. But Bill McElroy gave me a crucial push, by asking whether I might find out what the 'pure chloroplasts' could do. At about that time Wolf Vishniac had presented evidence that TPNH (today's NADP) was an early intermediate in photosynthetic electron flow. I went ahead and showed that the isolated thylakoids could reduce TPN in the light, but not DPN (Jagendorf 1956). And then, having learned that enzymes are reversible, and if TPNH was an important early product of the light reactions – perhaps these chloroplasts might be able to use TPNH as substrate, to reduce something else? That led to the discovery of an enzyme in isolated thylakoids, that did indeed reduce added dyes when fed TPNH.

This discovery was critical, because it permitted me to attract Mordhay Avron to my lab as a postdoc. He had gotten his PhD (in 2.5 years, if you can believe it – Sam Wildman said that if he'd realized, he never would have passed him) with Jacob Biale; so we had a tie in common. At a meeting I offered him the position, to work on this enzyme. The enzymology appealed to him more than working on CO_2 uptake by roots, the heart of a competing postdoctoral offer to work with the much more famous scientist, Kenneth Thimann. Mordhay joined my lab, as my second postdoc. I and Jean were both immediately taken with the wonderful personalities of these exotic people from Israel – Mord and Nira. We remained friends over many years, and Mord did more for me than I can ever repay.

With no real trouble, Mordhay purified the enzyme which turned out to be yellow, have FAD as a cofactor, and was properly described from its function as a 'diaphorase' (Avron (Abramsky) and Jagendorf 1956). An aside – many of the enzymes in photosynthesis were discovered 3 times in a row. This one was discovered just a little later by San Pietro, a colleague of mine in the same department at the Johns Hopkins, as a transhydrogenase: it used NADPH to reduce analogs of NADP. Even later it was discovered for its truly important function *in vivo*, as the Ferredoxin-NADP Reductase, or FNR, by Arnon, Whatley and Allen.

After purifying and characterizing the diaphorase, Mordhay said to me 'André – we have to find its true function. It must be a part of photophosphorylation.' I said 'Mordhay – photophosphorylation does not exist.' This may need a little explanation.

Photophosphorylation had been discovered recently at the time, by Lipmann and Frenkel with bacterial vesicles, and by Arnon, Whatley and Allen with thylakoids. The Arnon group was using AMP as phosphate acceptor (called 'adenylic acid' at that time); perhaps to save money, and probably because it had not yet been proven that ADP was the real acceptor. In trying to repeat their discovery, I went to the shelf having left over chemicals from the previous occupant of the lab, found a bottle labeled 'Yeast Adenylic Acid', and used it. There was no Pi uptake. The point is, I was young and poorly trained at that time, and did not know the difference between yeast adenylic acid (the name at that time for 3′-AMP, made from alkaline hydrolysis of yeast RNA) and muscle adenylic acid (5′-AMP).

Mordhay convinced me to let him try. He'd done his thesis on oxidative phosphorylation with avocado mitochondria. Of course it worked for Mordhay, and we were the first lab in the US to repeat Arnon's discovery. Perhaps in part for a psychological reason. A number of established groups were distressed that in the excitement of their discovery of cell-free ATP synthesis, Arnon et al. had failed to give proper credit to those who had shown by *in vivo* experiments that ATP synthesis *must* be a part of photosynthesis. At least some of these groups spent too much time trying to prove that Arnon was wrong; that he had discovered a mixed reaction between 2 organelles. The chloroplasts would do their thing and produce O_2, and mitochondria in the preparation would use the oxygen to make ATP. Which shows that sometimes it helps not to have any stake in the field to begin with.

Well, our first publication on photophosphorylation was a mistake. It was a note in Nature (Avron and Jagendorf 1957), describing the function of an 'extractable factor' – i.e. protein – in permitting more rapid rates of photophosphorylation. We were still using AMP as substrate. Sid Colowick pointed out to me the protein might just be adenylic kinase (AMP + ATP $\Longleftrightarrow$ 2 ADP). I rushed to the shelf full of hand-me-down chemicals, found a bottle labeled 'ADP', used that as a substrate instead of AMP, and still had a big rate stimulation from the protein. So we said it wasn't adenylic kinase; but we were wrong. Later work showed it was; and no doubt the ADP

in the years old bottle sitting on the shelf at room temperature without desiccation, had largely decomposed to AMP. But at least, the paper got us into the photophosphorylation field.

Another fortuitous event helped move us forward. Symposia were rare in those days; the McCollum–Pratt Institute was pioneering in holding them. At the symposium called 'Light and Life', I heard Howard Gest mention that as the extra electron carrying dye, something called 'phenazine methosulfate' was just terrific, in bacterial photophosphorylation. Please recall that *in vitro* photophosphorylation with isolated thylakoids depends on some added dye to carry electrons around the cycle, for cyclic electron flow. Arnon et al. were using riboflavin and Vitamin K5, both natural products, for this purpose. We threw in PMS and the rates became faster. Then Mordhay noticed that vessels exposed to the middle of the bank of lights (in an illuminated Warburg apparatus, left behind by Conrad Yocum) showed faster rates than those at the ends of the row. This led him to look at light intensities more carefully.(historical note – measured in those days, as foot-candles). Sure enough, by raising the fluence greatly, the rates became much higher (Jagendorf and Avron 1958). This put us way ahead in the 'rate race' with the Arnon crew (they got up to 2 to 3 hundred; we were up to 7 hundred μmoles/mg chlorophyll/hour), and helped show the enormous potentials of the thylakoid system. We now know, of course, that this depends on the formation of a completely artificial energy coupling site, as PMS itself takes electrons away from the reducing end of PS I, is protonated, and moves back into the lumen; generating a proton gradient in the process.

Other work in the early years at the Johns Hopkins included participation in the definition of thylakoid electron transport pathways. Space limitations imposed by a procrustean editor permit me to mention only that the associates doing this work included Giorgio Forti, Maurice Margulies, John Brewer and Tadashi Asahi. Their work included finding an enzyme to reverse inhibition by excess levels of CMU (Asahi), defining the roles for O_2 and ascorbate (Forti), locating sites for inhibition by DCMU and by incubation of bean leaves in the cold and dark (Margulies), and by ferricyanide with thylakoids in the dark (Brewer).

Al Nason, my mentor in plant biochemistry at Hopkins, suggested the way to discover the electron transfer pathway was to spend a week with Britton Chance. With his advanced electronic gadgetry (i.e. double beam spectrophotometers) the problem of the nature and components in chloroplast electron flow would be solved in a trice. I went to Philadelphia, spent hours in the dark lab watching Britt adding components and turn the light on and off. We discovered an enormous peak in the 500 nm region when chloroplasts were illuminated together with ADP and the putative inhibitor of electron flow, *o*-phenanthroline. Which turned out, of course, to be a matter of Fe^{3+} contaminating the ADP. In the light the thylakoids reduced it to Fe^{2+} which forms a deep red complex with *o*-phenanthroline. We also saw large spectrophotometric changes at 518 nm, but didn't understand them. It took Horst Witt to show this was the electrochromic shift, representing a membrane potential. Any life is full of missed opportunities, of course.

The next aspect that we got into was that of 'photosynthetic control' – i.e. the existence of coupled electron flow in the Hill Reaction. Together with David Krogmann, then a graduate student, Mordhay showed that FeCN reduction in the Hill reaction is faster, if photophosphorylation goes on at the same time (Avron et al. 1958). David discovered (just a little before Norman Good did) that ammonium ions were uncouplers for photophosphorylation (Krogmann et al. 1959). Another graduate student, Richard McCarty, found that free fatty acids (generated in bean 1° leaves during grinding, by active lipases) were also uncouplers of photophosphorylation (McCarty and Jagendorf 1965). Later McCarty and his students, among others, provided massive evidence for the existence of the ΔpH in illuminated thylakoids, for the stoichiometry of protons/ATP formed, for the nature of coupling, and for structure and function of CF_1, in many publications over the years.

The existence of coupling in thylakoids sounds like old hat today; and indeed it was that for oxidative phosphorylation at that time. But oxidative phosphorylation had been discovered 18 years before (in 1937) and the Arnon group had just discovered photophosphorylation by thylakoids only a year or two before. I remember going to a Brookhaven Symposium, telling about the chloroplast reactions, and getting a truly startled reaction from Britton Chance. He could hardly believe that photosynthetic control had never been noticed before.

Even more surprising, perhaps, was the reaction of Otto Warburg. For younger readers – he was the world's pre-eminent biochemist, without a doubt, for several decades. He discovered respiratory enzymes, studied many different biochemical reactions, and had won a Nobel prize. While a marvelous laboratory

worker, his theories, especially later in his life, tended to be way out of line. He had convinced himself, for instance, that CO_2 attached to chlorophyll, and was reduced directly by energy from chlorophyll in the light. So to have electron transport generating ATP seemed a bit beside the point to him.

Warburg was dedicated to manometric methods to measure O_2 exchanges and electron transport. These take time, and Warburg found no difference between the 30 minute output of O_2 with and that without added ADP, Pi & Mg^{2+}. Probably the thylakoids were in bad shape due to their prolonged incubation with FeCN and at room temperature in the light. Warburg published that there was no such stimulation, and also wrote me a letter to that effect. In a footnote to his table, he noted briefly and cryptically, that our observation was probably due to a failure to control the pH.

Birgit Vennesland, a highly competent biochemist in Chicago, and Mary Stiller worked hard to find out just what 'failure to control the pH' might do. They figured it out. Without proper buffering, if the pH was too low, the Hill reaction was rather slow. But ATP synthesis leads to the consumption of protons; so the pH rises. (This rise in pH is often used as an indirect way to measure phosphorylation rates.) Vennesland thought that we had used a too low pH, and the rise due to phosphorylation would then make the Hill reaction run faster.

Their concept was published in Nature (Vennesland and Stiller 1961). Luckily, Nature gave both Norman Good (1961) and me (Jagendorf 1961) the chance to answer in accompanying letters. This I did in a chivalrous, and therefore gentle and mealy-mouthed way, and Norman Good in a highly straightforward way. The point is, of course, we *had* controlled the pH; and the Warburg/Vennesland criticism was totally unjustified.

Mordhay Avron finally had to leave, and went on to a most productive and distinguished career at the Weizmann Institute of Science in Israel. While there, among other things, he discovered the coupling factor of chloroplast thylakoids, CF_1. I had discovered how to uncouple thylakoids using EDTA at low ionic strength (Jagendorf and Smith 1962) but never found how to recouple them. Racker and Vambutas had found an extractable ATPase in thylakoids, and presumed it would be the coupling factor; but Avron was the first one to really show that it worked in ATP synthesis (Avron 1963).

Looking into the mechanism of ATP synthesis: post-illumination, pH rise, and chemiosmosis

At that point in the history of bioenergetics, the shining goal was to discover the link between electron flow, and the chemical reaction of ATP synthesis. There were many theories; but all of them involved some sort of high energy intermediate of one of the electron carriers bonded to some other molecule (see, for instance, Chance and Williams 1956).

Every year, somebody else seemed to have an experimental demonstration of the true high energy intermediate. A friend described it to me by saying that going to the Federation meetings was reminiscent of going to the Alchemists' convention. Each year, somebody else would stand up and say that **he** had discovered a fool-proof way of turning lead into gold. And of course, nobody did.

Well, I thought that using chloroplasts might make the job of capturing a high energy intermediate a little easier, because you could turn off the light so quickly. So I thought I'd try to jump into the act.

First, I spent a summer at Brookhaven, trying and failing to set up a stop-flow apparatus where the thylakoids would go from light to dark and meet the substrates (ADP, Pi, Mg^{2+}) at the same time. However, I did learn a great deal about photosynthesis that summer; because Marty Gibbs was there also. Lunch after lunch we would eat outside on the grass, and Marty would ramble on and on about people, events, theories and ideas in photosynthesis. It was the first time I had actually had somebody talking about photosynthesis, and it was very educational.

Then later, it occurred to me to use a simpler system - just a syringe in the light, with the thylakoids pushed down into an aluminum-foil covered dark test tube containing ADP, ^{32}Pi and Mg^{2+}. That worked, and I discovered the existence of 'X_E' – an unknown, high energy intermediate. Shortly after, I was fortunate to have Geoffrey Hind join my laboratory and help with this discovery. Now the only thing was, the *amount* of ATP made in the post-illumination dark (up to 50 nmoles/mg chlorophyll) was too high to fit with the hypotheses current at that time. It represented up to 50 times the amount of any one electron transport enzyme. So turnover must have occurred, in the dark (Hind and Jagendorf 1963).

Geoffrey did scrupulous experiments defining the kinetics of rise and fall of X_E. Of course, we never used anything but spinach from the local A&P. Perhaps I shouldn't have been surprised when the dark

decay constant for this hypothetical high energy intermediate in ATP formation decreased 3-fold, in experiments before and after April 1. I tried, and failed, to get an explanation (let alone an apology) from the A&P. They said it was a trade secret.

Keeping the record straight – X_E was not a unique discovery. A little sooner, Y.-K. Shen in China had also discovered post-illumination ATP synthesis by thylakoids (Shen and Shen 1962). We did not know of their work until we had sent our paper off for publication. I corresponded with Shen a little bit; about 2 letters in each direction. I remember, in the last letter, writing that I hoped we could meet, once political passions died down. I didn't hear from him again for many years – the Cultural Revolution had hit, and he was unable to do research for at least 10 years. After, Shen rose high, headed the Shanghai Institute of Plant Physiology, and led a distinguished group, exploring many details of photophosphorylation. We did meet – Shen's group of photosynthesis workers were the first to make a tour of the U.S. after the Cultural Revolution was over (see Shen, 1994 for a more complete description of his fascinating career).

It was both fortunate and critical that Geoffrey Hind was in my lab at that time. I had heard Peter Mitchell talk about chemiosmosis at a bioenergetics meeting in Sweden. His words went into one of my ears and out the other, leaving me feeling annoyed they had allowed such a ridiculous and incomprehensible speaker in. But – Geoffrey read Nature. Geoffrey was from England, both better trained and more intelligent than I was. He read Peter Mitchell's paper, came to me, and said 'André. could this possibly explain X_E?'

During the discussion, it occurred to us that we might be able to see the pH in the medium rise during light-driven electron flow. I stayed in the lab late that same evening, put thylakoids in a beaker together with PMS, inserted a glass electrode, and watched the needle of the meter rise in the light and fall in the dark. It was the first time I remember an immediately successful test of a working hypothesis – a most exciting event! We published this first in the photosynthesis symposium at Airlie House (Jagendorf and Hind 1963).

I should point out that we were lucky on 3 accounts. First is that Mitchell, writing about mitochondria, postulated electron flow causing H^+ uptake. That's true for chloroplasts, but not for right side out mitochondria. If we had seen the pH change in the wrong direction, it would have been too puzzling to follow up. Secondly, we were lucky in that thylakoids, unlike mitochondria, have active ion transporters in their membranes. So that many protons can go in, their + charges neutralized by exiting cations but especially by entering Cl^-. Thylakoids accumulate HCl, rather like the stomach. Mitochondria, not having the appropriate translocator(s), rapidly form a membrane potential, and only a very few protons can move before the whole system stops.

The third point is, we were lucky in having pyocyanine (the light-driven product of PMS non-enzymatic oxidation) to support a rapid cyclic electron flow, with no obscuring scalar production of H^+ in the medium as with ferricyanide or $NADP^+$ reduction. The perceived change in pH of the (weakly or unbuffered) medium came to 0.5 pH unit, or more. This was true if the medium pH was about 6.0 to begin with; much less change is seen at pH 8. The extent of proton movement did seem large enough to suggest a real basis for the high amount of ATP that could be formed in the post-illumination darkness.

This discovery of H^+ uptake in the light and release in the dark was explored further by Joseph Neumann, who was able to tie H^+ uptake definitively to the coupling phenomenon. It only occurred in the light, was prevented by uncouplers, and uncouplers added once the protons were in allowed them to leak out very rapidly (Neumann and Jagendorf 1964).

At this point, I became rather convinced – although without completely rigorous evidence – that the chemiosmotic explanation was the correct one for the connection between electron flow and ATP synthesis. This conviction helped me to avoid a fruitless search for a chemical intermediate. I remember more than one biochemical colleague (for instance, Israel Zelitch) saying 'You have your fingers on the real intermediate, André. What you should do is go in there and fish it out!' To which my response was – 'Yes, but maybe it's just a pH gradient, and you can't fish that out.' Of course he laughed.

Geoffrey Hind looked for spectral changes that might lead us to the nature of X_E. The only thing he found were changes in light scattering. Apparently in the light, thylakoids shrink together and scatter light more strongly than when they're swollen. This effect was greater as the pH decreased, reminiscent of the yield of ATP in post-illumination experiments. Being a careful scientist, Geoffrey ran a dark control. He was amazed to see that a little bit of ATP was indeed formed entirely in the dark, just because the thylakoids had been moved from pH 4.6 to pH 8 (see Table 1, from Hind and Jagendorf 1965). The amount was only

Table 1. Effect of pH on post-illumination phosphorylation by spinach thylakoids. Chloroplasts with 40 μg chlorophyll per ml were illuminated for either 5 sec or 1 min. Adjustment of pH was made by dilution of a concentrated particle suspension in the appropriate buffer, just before use. Samples for determining the dark X_E level were kept in total darkness at pH 8.0 and 5° for 1 h before use, and were maipulated in total darkness. Results are shown in nmoles ATP per Mg chlorophyll. Modified from Hind and Jagendorf (1965)

Row	Measurement	pH					
		4.5	5.3	6.0	6.7	7.2	7.7
A	Yield from 5-sec flash	15	17	18	7.1	4.3	1.9
B	Maximal yield	52	54	37	7.8	4.3	1.9
C	Initial dark X_E level	5.5	1.4	1.6	1.6	0.6	0
D	Maximal X_E level	58	55	39	8.0	4.9	1.9

5.5 nmoles of ATP/mg chlorophyll (see Row C, in Table 1). This was so little compared to the amounts of post-illumination ATP ('X_E') that we hardly knew whether to consider it significant. Now in retrospect, it was a very logical thing to look for. But if it had not been for the unlikely result in one control point, we probably would not have discovered acid-base ATP synthesis.

Geoffrey moved on to Brookhaven, anxious to get into spectrophotometric analyses of thylakoids doing photosynthesis. I thought it would be important to look a little closer at this low level of dark X_E. Geoffrey had used hydrochloric acid to adjust the pH of the thylakoid suspensions. Being a mystical minded biologist instead of a trained biochemist, I worried whether the strong acid might be intrinsically harmful to the thylakoids. So I started using organic acids – first of all, the highly synthetic one, phthallic acid. The difference was startling. Instead of 5 nmoles of ATP, I worked up to 50, 60 and eventually 100 or more nmoles of ATP per Mg chlorophyll. Explanation from hindsight – the divalent acid at a pH where it was 50% protonated, moving into the thylakoid, brought along a store of protons far beyond the number of protonatable groups present endogenously in the thylakoid lumen.

At this point I began to communicate with Peter Mitchell himself. He had recently moved into his new laboratory and home in Cornwall (the Glynn Research Foundation) and invited me to spend a week there so he could educate me about the chemiosmotic hypothesis in more detail. I was happy to go, and enjoyed very much meeting his family and the family donkey, and seeing his fascinating Regency house (half home, half lab) and tree-high rhododendrons in bloom in Cornwall in the spring. I doubt that I learned enough about chemiosmosis, however.

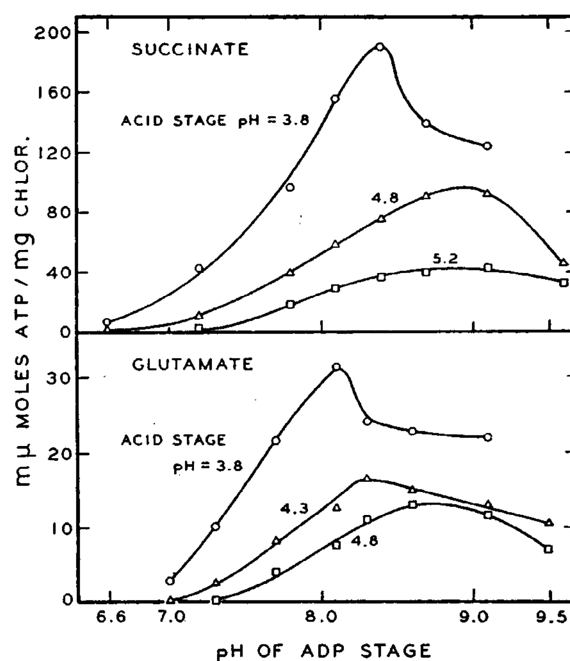

Figure 1. Yield of ATP in acid-base phosphorylation as a function of varying pH in the acid stage, at different base stage pHs ('pH of ADP stage'). Reproduced with permission from Jagendorf and Uribe (1966).

Later that summer I did the experiment that convinced me (and I hoped, others) that we were really seeing a chemiosmotic mechanism at work. The amount of ATP that was made depended on the height of the pH difference between acid and base stages, more than on their absolute values (Figure 1, from Jagendorf and Uribe 1966).

These data helped very much in the scientific community's acceptance of the chemiosmotic principles. I reported them first at a Gordon Conference in 1965. At the Federation meetings in the spring of the following year Paul Boyer was able to report that they were indeed repeatable. With acceptance from important quarters, the rest of the world had to pay attention.

Another bit of sociological fallout – it was good for the morale of plant scientists at that time. It showed that work with plant materials could contribute to problems of importance for the general biological community. We didn't have to be restricted to either plant-specific problems, or trying to see if plants did the same things as animals or yeast or bacteria, in an area of general interest.

Cornell again: Chemiosmosis, CF_1 action, and chloroplast biogenesis

Just after this (1966) I was enticed to move back to Cornell, especially by the persuasive Bob Morison, who was building the revitalized Biology Division. Of course, part of the attraction was the memory from my undergraduate days of what a beautiful spot Ithaca is. Indeed, the continuity extended to the point of my being given, for my office, the room in which I was advised as an undergraduate.

A pH gradient is not simple to demonstrate directly. Showing it was the true basis for ATP synthesis required ruling out alternatives as rigorously as possible. Ernest Uribe, moving with me from Baltimore to Ithaca, showed that entry of the organic acid was the only parameter that really mattered, for ATP formation (Uribe and Jagendorf 1967a, b). His results emphasized another lucky break in this project – Geoffrey Hind had gotten into the habit of washing the thylakoids in 10 mm NaCl prior to use, and this meant we were using swollen vesicles, with plenty of internal space to store organic acids.

Another point – Donald Miles found that inhibitors of electron flow (DCMU, HOQNO, *o*-phenanthroline, BDHB, simazine) either had no effect, or actually *increased* the ATP yield (Miles and Jagendorf 1970). We can ascribe that to blocking of reversed electron flow, which is driven by and would help use up, a protonmotive force, as discovered by Mordahay Avron's lab (Rienits et al. 1973). Also relevant – Alice Grebanier demonstrated that 2 different light-dependent inhibitions could be driven by either PS I only or PS II only. Then, the subsequent photophosphorylation supported by the opposite photosystem was equally inhibited (Grebanier and Jagendorf 1977). This clearly implied the absence of a specific association between the electron flow and one particular ATP synthase population. Many other laboratories contributed to the evidence for chemiosmosis. Maybe most prominent was the demonstration that liposomes containing both the H^+-pumping purple membrane patch of *Halobacterium halobium,* a non-photosynthetic bacterium, together with the F_o/F_1 of mitochondria, accomplished photophosphorylation (Racker and Stoeckenius 1974) There was hardly any question left that this ruled out a direct, necessary interaction between mitochondrial electron carrying enzymes (which were not there) and the ATP synthase.

All in all, the bioenergetics community has done a good job of showing the reality of Peter Mitchell's brilliant concept of the nature of coupling between electron flow and ATP synthesis. There are some controversial areas – it's not totally clear that thylakoids *in vivo* are swollen enough to drive ATP synthesis via a bulk pH gradient. This, and some other data, leads some to wonder whether there are "localized" protons along the membrane, communicating directly from the electron carriers to the ATP synthase (see, for instance, Chiang and Dilley 1987). But this may be a matter of definition, as to what a 'local proton' really is. Basically, however, Mitchell's concept of protonmotive force as the driving force for ATP synthesis stands, and I've been very pleased to have played some part in showing its validity.

On the photophosphorylation trail, I eventually developed a stronger interest in CF_1, and in its conformational changes in the light when bound to thylakoids. A major contribution for that time was the tedious but effective determination of the amino acid content of all of the 5 CF_1 subunits, by Andres Binder (Binder et al. 1978). I was inspired to think about conformational movements of these subunits by the earlier finding of R. McCarty that one of the free -SH groups of CF_1 is attacked by N-ethylmaleimide only when the thylakoids are illuminated (McCarty et al. 1972)). A primary finding was that made by Ivan Ryrie, who showed that exchange between H atoms on CF_1 and medium tritiated water was more extensive in the light than in the dark, and returning thylakoids to the dark put up to 100 atoms per mole into hidden pockets where exchange out was very much slower (Ryrie and Jagendorf 1971, 1972). This was primary evidence for a major conformational change in thylakoid bound CF_1. Studies of light-enhanced or -dependent

inhibition by chemical reagents were also made by Dipak Datta (permanganate inhibition), David Oliver (TNBS), and Ivan Ryrie and Alice Grebanier (sulfate, which uncouples in the light if ADP and mg are present). Other contributions with chemical modifiers were made by Emanuel DeBenedetti and Joan Garbarino, Roland Schmid and Tetsuko Takabe (Takabe et al.1982). Lawrence Posorske found significant effects of adenylates in protecting CF_1 against inactivation in the cold. Some of the ionic interactions of thylakoids as related to energy status were explored by Bill Cohen, and by Yuichiro Nishizaki, who built a successful small stop-flow apparatus to look at the kinetics of proton efflux from thylakoids.

Studies of CF_1 activation is another direction we followed. Joel Kaplan had found that the acid-base transition activated thylakoid ATPase (Kaplan et al. 1967). Gordon Anthon found a remarkable stimulation of the basal activity by methanol, with an incredibly sharp optimum at 35% (v/v) during the assay (Anthon and Jagendorf 1983). With solubilized CF_1, a major inhibition of the methanol-driven ATPase by free Mg^{++} could be reversed by sulfite ions (Anthon and Jagendorf 1986). Eric Larson looked at the sulfite stimulation in much greater detail, and found that it had remarkable effects on the reduced form of either thylakoid-bound or solubilized CF_1 (Larson and Jagendorf 1989). Part of the effect is probably due to release of the bound, inhibitory ADP (Larson et al. 1989); but in any case it permitted observing ATPase rates 5 times faster than any seen before. Others working in this area included Jia-mian Wei, Bruce Howlett, Ann Umbach and Rong-Long Pan.

Other work with CF_1 has included experiments by Geoffrey Chen, showing assembly of the full, active protein from over-expressed, denatured subunits only with the help of chloroplast chaperonins (Chen and Jagendorf 1994). Unisite hydrolysis of ATP by thylakoids was found by Shiying Zhang to differ in surprising ways from that shown by mitochondrial particles (Zhang and Jagendorf 1995).

Another strong interest for me has been understanding something of chloroplast biogenesis. Alva App, in Baltimore, showed that the development of chloroplasts in *Euglena* could be repressed by metabolizable substrates, such as ethanol. We had a quick fling at chloroplast RNA polymerase, with Gideon Polya (Poly and Jagendorf 1971), and Holly Doremus demonstrated synthesis of pyrimidine nucleotides in isolated chloroplasts (Doremus and Jagendorf, 1985). Quite a few students and postdocs worked on protein synthesis by isolated chloroplasts, with special emphasis on activity of thylakoid-bound ribosomes. These included A. Gnanam, Mahtab Bamji, Linda Gooding, Harry Roy, Kar-Ling Tao, Richard Patterson, Len Fish, Josh Hurewitz, Helen Nivison, Ruth Alscher, Taibo Yamamoto and Devaki Bhaya. While pursuing protein translation, Xiang-Qiu Liu discovered the ATP-dependent proteolysis of some of the newly formed polypeptides in chloroplasts (Liu and Jagendorf 1984). Unfortunately, the activity was not stable once the chloroplast envelopes were opened, and Liu studied other peptidases in chloroplast stroma, using synthetic substrates. An endopeptidase which may be able to attack Rubisco was purified further by the brothers Bushnell (Bushnell et al. 1993), a pair of very talented undergraduates.

Probably no scientific career is without its near-misses. I had at least two, both related to my interest in chloroplast biogenesis. On sabbatic leave at the Weizmann Institute of Science in Rehovot, my ambition was to attach antibodies covalently to a solid matrix, in order to capture the specific antigen. Michael Sela's department was developing solid matrices which permitted covalent attachment of proteins. I eventually tried brom-acetyl-bromide, which could attach to cellulose, and the second bromide could bind a protein. Six weeks before I had to leave Rehovot, I had a happy accident. I left a magnetic stirrer run overnight in the cold room, and it ran amok. That way I discovered that if immunoglobulins are partially denatured, they can bind to such a column (Jagendorf et al. 1963). In retrospect – what was needed was a spacer arm (see Cuatrecasas 1970). We showed that the column with bound antibodies could in turn bind the antigen, egg albumen (Jagendorf et al., 1963). While the work was successful, it was a near-miss because I never did anything further about it. Fortunately for science, more astute people such as Cuatrecasas developed affinity chromatography to the useful state we all know about now. The moral is, follow-through, and publishing more than one paper on a subject, is very often needed to allow something to become a real contribution to science.

The other near-miss was coming close to, but failing to, discover that proteins targeted for the chloroplast are synthesized as a larger precursor. Linda Gooding and Harry Roy, using antibodies, had shown that isolated cytosol ribosomes apparently made a protein of the expected size (12 kDa) for the small subunit of Rubisco (Gooding et al. 1973). But Roy also found a product that was quit a bit larger (about 20 kDa).

Both proteins were isolated from the SDS gel, and lyophilized prior to analyzing peptides to see if they corresponded to the expected primary sequence of amino acids. During the lyophilizing, the flask containing the larger protein imploded, and the analysis was never run. Roy had to leave my lab to go to an academic job, and I never had anyone follow up on that discovery.

Eventually, the era of DNA technology caught up with me. I thought it would be interesting to try chloroplast transformation; this interest was shared by a new graduate student, Heriberto Cerutti. He first attempted to see if DNA could actually enter intact chloroplasts. This turned out to be the case if they are subject to heat shock, probably forming hydrophilic pores in the envelope via lipid Hex_{II} phase formation (Cerutti and Jagendorf 1995). Trying to see if the imported DNA might function in chloroplast metabolism, Heriberto discovered DNA strand exchange activity in stroma extracts, with characteristics very much like those of bacterial RecA protein (Cerutti and Jagendorf 1993). This led to a successful search for the gene for a RecA homolog in an *Arabidopsis* cDNA library. This was sequenced, and its close relationship to bacterial genes noted (Cerutti et al. 1992). The genomic form of the gene was sequenced by Marie-Noëlle Binet (Binet et al. 1993), and further definition of its function with the help of a magnificent series of DNA constructs was made by Jun Cao (Cao and Jagendorf 1997). This was the pathway that brought my lab activities into the current era, and incidentally gave a little more insight into the capabilities of chloroplasts.

Concluding remarks

All of us in science know the deep satisfaction of doing a practical experiment to test a concept or model. Certainly those with more than a year or two of experience know the feeling of wonder and pleasure at seeing more and more of the workings of the plant world exposed and understood; and the continual process of change and refinement of our understanding. Adding to these pleasures I have had warm, supportive and genial companions in the arena of photosynthetic science. I have certainly heard and seen the dog-eat-dog atmosphere in some other fields, but rarely if ever in photosynthesis. Maybe some colorful characters here and there; but it's been great to know, and interact with, kind and thoughtful people like Jack Myers, Bessel Kok, William Arnold, Takashi Akazawa, Lou Duysens, Marty Gibbs, Kazuo Shibata, George Hoch, Rod Clayton, Norman Good and so very many others, too numerous to mention.

Science is an activity done by human beings. In any human activity, there are many turning points, chance events, lucky and unlucky accidents. There are some brilliant, hard-driving individuals who make enormous contributions based on clear hypotheses and logical reasoning. But others of us noodle along and by chance can add an important bit here and there. In my own career, chance and luck have had starring roles. I never would have accomplished much at all, except for the help of postdocs, graduate students and friends. Most prominent among these were Mordhay Avron and Geoffrey Hind. My thanks to them, and to the United States public for its support of basic science over the years spanning my career. May these happy conditions, or at least a reasonable facsimile, continue for all the patient readers of this wandering memoir.

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Postscript

Photophosphorylation and the greenhouse at Johns Hopkins University, 1959–1964

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The headhouse (storage and working space) for the Johns Hopkins greenhouse should have a plaque commemorating a scientific breakthrough. Converted to lab space, it was the original home of the McCollum–Pratt Institute (starting faculty: W. McElroy, S. Colowick, N. Kaplan, A. Nason and R. Ballentine). André Jagendorf, after a 2-year start in one room of the main building, was moved to the greenhouse headhouse. Then, one by one, the McCollum–Pratt faculty moved to fancier, renovated labs in the main building, Mergenthaler Hall. By the late 1950s, André had inherited all three floors of this small, separate building. It was here that André with his postdoctoral associates, Geoffrey Hind, Joseph Neumann and Ernest Uribe, carried out the remarkable series of experiments that provided strong evidence in support of Peter Mitchell's chemiosmotic hypothesis.

I started as an undergraduate in the Jagendorf laboratory as a result of a happy accident. I was the only person to sign up for a laboratory course in plant physiology that André was offering during the spring semester that year. I stayed on to do my thesis work and left the lab in the summer of 1964. I was thus in André's lab when Geoff Hind was investigating the light and dark stages of photophosphorylation, work that led to the finding that ATP formation by thylakoids in the dark could be driven by acid to base transitions. Joe Neumann was in the process of showing that electron transport caused a rise in pH that was clearly a consequence of proton transport into what we now call the thylakoid lumen.

I would like to say that I was in the thick of this exciting work, but I was not. André's space in the greenhouse was distributed over all three floors of the greenhouse. His main lab was adjacent to his office on the first floor. There was a wonderfully air conditioned lab in the basement that housed equipment, including the Radiometer pH meter and pH-stat and a spectro-

graph. The graduate students were housed upstairs in what is essentially a garret. I was working on a project of my own making, one much less significant than those being worked on downstairs. André was a wonderful mentor for graduate students. He gave us free rein and expected us to have our PhD theses completed in four years. It was the postdoctoral associates and André who did the most significant work in André's lab at the time.

Despite the fact that I was not directly involved in the work on protons and photophosphorylation, I have clear memories of several aspects of this work. I recall that the two-stage phosphorylation and acid bath experiments were carried out in near darkness in a coldroom. Many of these experiments were carried out by the exceptionally able and good natured technician, Marie Smith. Marie would spend hours groping around in the coldroom clad in a parka that looked as though it was meant for use in the Arctic and that seemed entirely out of place in Baltimore, especially during the torrid summer months.

I also recall Joe Neumann working in the semi-darkness of the instrument lab in the basement, shining light from a slide projector on thylakoid membranes and measuring pH changes. Only after he left the lab did I realize the significance of his experiments. I could always tell when Geoff Hind's work was going well. He would sing what I think were motets in a clear, tenor voice. He sang very frequently.

André has a long standing interest in cell, molecular and developmental biology. He and Alva App, a postdoctoral associate, were investigating protein synthesis by what we then called intact chloroplasts but were really thylakoids. I had more interactions with Al App than with either Joe or Geoff perhaps because I had aspired to do my thesis work on the development of the two photosystems and of photophosphorylation in greening etiolated beans. I never got that far.

André was actively involved in all aspects of the lab. In addition to contributing his keen insights to the work of others in the lab, André spent as much time as his often hectic schedule would allow working at the bench. He still does.

By the time I left André's lab I had assimilated a great deal of advanced knowledge about photophosphorylation and how to study the process. Thanks to my wonderful experiences there, I was able make rapid progress in defining the role of a coupling factor for photophosphorylation discovered by Vida Vambutas, a graduate student in Ef Racker's lab. Thus, the excitement of the results from the labs in the greenhouse rubbed off on a graduate student more interested then in developmental biology than in biochemistry.

Every chance I get I point out the greenhouse to students and visitors to the Hopkins campus and inform them that the greenhouse is the place where the famous acid bath and related experiments were carried out. Many seem incredulous. The building is small and not that impressive. Today, the greenhouse is occupied by Facilities Management, although one of the two glasshouses still is sometimes used. André's main lab on the first floor has been converted to a conference room. I like to attend meetings in this room as it is a wonderful place to evoke the extraordinary experiences we all had in André's lab.