REFLECTING ON THE PIONEERS OF MITOCHONRIAL RESEARCH: THE FAB FIVE.

Collected short, anecdotal biographies of the pioneers of bioenergetics first presented in the MitoAlmanac I wrote for the Mitochondrial Interest Group of NIH.

1). DAVID EZRA GREEN.

A comprehensive review of Green's career has been published and this provides an indication of the vast number of important studies that came from Green's lab, and equally important, the number of distinguished scientists who trained under him. This biography gives not only the facts but conveys very well the personality of the man: http://www.Nap.edu/readingroom.php?book=biomems&page=dgreen.htm Everyone who worked with David has anecdotes of their time with him. I arrived to work with Green knowing something about membrane proteins but nothing about mitochondria. It seemed logical on my first morning at work to go into the library next to Green's office and read up on the new topic. I'd been in there about an hour when Green came in and said, "Rod, we don't read the literature, we make it" and marched me into a lab to learn how to measure ATP-Pi exchange with what at the time seemed like several millicuries of $P-^{32}$.

My wife and I arrived in Madison recently married and with very little money. We were given a few furnishings for our apartment and for reasons not immediately apparent Green was very keen that we get a telephone (so we could keep in touch with our families in England?) On about the third night after we moved in, and at around 10pm, the phone rang for the first time. It was David; he wanted to discuss an idea with me. Next night at around 11pm we got another phone call, it was David with a new idea. The following night at midnight the phone rang again; it was David, but this time my wife answered. To this day I do not know what she said but he never called me at home again.

Green took a daily constitutional from the Enzyme Institute along University Avenue to the Student Union for lunch a distance of about a mile and a half. He usually asked his favorite post–doc of the moment to

accompany him. My time came in January, when the snow lay thick and the temperature was below freezing. I soon learned that the intense cold could be endured by stopping for a minute in the entry of each of the several stores along the route that had hot air blowers over the front door, and then running to catch David up. Green was most of all a gentleman, and never made mention of my odd behavior.

2). PETER MITCHELL

Let me provide you a fairy tale. Once upon a time, not long ago (by my standards), there was a man who liked biology and worked at one of the premier universities in England but became disillusioned with the academic life as practiced at such institutions. So, he bought an old run down house in the middle of nowhere (Bodmin, Cornwall) which he made his home and in which he built a laboratory. From there he set out to prove a revolutionary idea he had about energy transduction, using his own money and donations to fund the research. For many, many years other researchers in the field were rude and nasty to our hero and even had meetings to plot common strategies to discredit his ideas. But slowly, the evidence amassed, and our hero won out and was celebrated all over the Queen-dom and the world more widely.

Detailed evidence that the above is not a fairy tale is provided in a beautifully written book “Peter Mitchell and the Vital Force” by Milton Saier who sums up Mitchell as follows; “he was the brilliant but apparently nutty intellectual dynamo who nearly single-handedly provided conceptual advances which exceeded those of almost any other biologist, living or dead. He revolutionized our ideas about how cells couple oxidative energy-releasing reactions to the synthesis of chemical energy in the form of ATP. He represents to vectorial molecular biology what Einstein represents to nuclear physics and Fischer represents to organic chemistry”.

There are several brief reviews of Mitchell and his work that are well worth reading. The one I recommend is by Peter Rich who took over from Mitchell as Director of the Glynn Institute (A PERSPECTIVE ON PETER MITCHELL AND THE CHEMIOSMOTIC THEORY. RICH P.R J. BIOENERG. BIOMEMBR. 40. 407-10. 2008). Peter Rich kindly provided a couple of stories about Mitchell, the man, below;

Peter had a wide range of interests outside science. For example, he had a keen interest in economics, often traveling to London to deal with his investments in what he called a 'glorified day at the races'. This interest extended to direct practices at Glynn where, at one time, he minted his own silver 'Glynns' and even offered them to sometimes bemused staff in lieu of their cash salaries on the basis that they would keep up with, or beat, inflation (which sadly, and dramatically, did not live up to expectation). He also introduced a system that adjusted salaries each month to take into account inflation; a great system for staff but a nightmare for administration. His advice and personal practice on pensions were rather insightful given the present economic climate – instead of formal pension schemes he instead recommended buying works of art and antiques that could be stored and resold in retirement.

Peter had a great, rather mischievous, sense of humor. For example, he was fond of recounting his tale of a meeting of Nobel Laureates with the Queen, where he took one of his silver Glynns to show her. When his turn came in line to meet Her Majesty, he proudly showed her the Glynn, which to his horror, she took, put into her handbag and moved on.

This humor appeared in many other ways. He had a lifelong hearing problem and could often be seen readjusting his hearing aid at conferences. I commented one time about how inconvenient this must have been for him and he answered 'but I can also tune out instead of in'. On another occasion, we were returning together from a magnificent dinner, hosted by a drug company, which extended over very many courses and hours. Peter's summarising comment whilst we were returning to the hotel very late at night and exhausted was that he had 'never before been subjected to so much hospitality'.

3) BRITTON CHANCE.

In a career that spanned 60 years Britton Chance developed some of the most important technology, and used this to obtain some of the most incisive observations that have been made on mitochondrial structure and functioning. Details of his scientific career are well covered in a web site describing the man and his science that has been built by his colleagues in Philadephia

http://www.med.upenn.edu/biocbiop/chance/

As were Green and Mitchell (described in previous editions of the MitoAlmanac), Brit was a larger-than-life character. Besides his scientific achievements he was a master sailor and Olympic gold medal winner in the sport. His life is well described in the above web site.

Brit got his start in enzymology in Cambridge and early on invented the now-standard stop flow device. David Green told me the story of his meeting Chance in Cambridge in those early years. Brit had the prototype stop flow machine working and needed an interesting sample to examine. He persuaded Green to let him "use" the batch of old yellow enzyme (now called NADPH dehydrogenase) that David had spent a year in purifying. As Brit pointed out, you stick the enzyme in at one end and catch the effluent at the other end of the machine and have your enzyme back...no problem...unless you forget to use a receptacle to catch the effluent in, which Brit did. Green was not a happy man!

In the reflections part of the website cited above, Maurizio Brunori remembers a time that he visited Brit's lab, where he collaborated on experiments using low temperature triple trapping methods to monitor intermediates in the reaction of cytochrome c oxidase. Briefly, the enzyme was saturated with CO in the active site and then the CO was removed by a light flash to allow oxygen binding, the rate of which was slow enough at the very low temperatures, that intermediates in the oxygen binding reaction could be detected and characterized. Brunori remembers the enthusiasm Brit always had for doing experiments himself. I took the same pilgrimage, and did the same experiments in the early 1980s. The thing I remember most is going into Brit's office and seeing his notebooks of research, all beautifully bound, covering all of the walls. I also remember going into the spectrophotometer room through black curtains to the sound of animals afraid of the light scurrying to their lairs. I only felt comfortable in there by wearing two pairs of socks with my trousers tucked in the outer set.

Chance later focused his efforts on developing approaches for monitoring non-invasively the metabolism of living tissues. This included his making major contributions to the use of phosphorus NMR .

4) ALBERT LEHNINGER.

It was my first Gordon Conference in New Hampshire. Things were not

going well. It was hot and very humid, there were mosquitoes everywhere and my room smelled strongly of dirty feet, as did most in the boarding school that was to be my home for 5 days. My bed was 6 inches shorter than me, and its regular occupant had stuck fluorescent stars on the ceiling above the bed to mock me through the long sleepless nights. My talk, the first of the morning, had gone badly: half of my slides had become stuck in the carousel (remember slides?) making for a choppy presentation, and now the speaker after me was trying to convince the audience that the proton to electron ratio in OXPHOS was more than 2. I went out for fresh air. Within 5 minutes a helicopter came over and then landed quite close to where I was sitting, a military-looking man got out, walked toward me and the auditorium, stuck out his hand and said "Hi I'm Al Lehninger, has Dr B..... started to speak yet?" I responded that it was some fellow droning on about proton to electron ratios. His face reddened and he rushed in to listen. How was I supposed to know Dr B. was Al's colleague?

Albert Lehninger is remembered by most for his books, including Principals of Biochemistry, which even now is regularly used and is still a very authoritative source after recent updates. However, he also made many seminal contributions to our understanding of mitochondria. Specifically, it was he along with Eugene Kennedy in 1948, who first showed that mitochondria were the site of oxidative phosphorylation and of fatty acid oxidation, thereby ushering in the modern study of energy transduction. The website at http://www.tc.umn.edu/%7Eallch001/papers/lehninger.pdf provides an excellent review of the man and his work.

Pete Pedersen remembers his time as a student with Lehninger as follows:

I first met Albert Lehninger in the spring of 1964 at which time he was Professor and Chair of the Department of Physiological Chemistry (now Biological Chemistry) at Johns Hopkins University, School of Medicine, Baltimore, MD. This was shortly before I received my Ph.D at the University of Arkansas working with the late Professor Jacob Sacks. Sacks encouraged me to start seeking Postdoctoral positions. After searching for some time, I decided that Dr. David Green at the Enzyme Institute at the University of Wisconsin would be my best choice. I was very impressed with Dr. Green's work on mitochondria perhaps because

of his Scientific American article (1964. Vol 210, pages 67-74) near that time. My mentor Dr. Sacks at the University of Arkansas was not happy about my decision and encouraged me to consider Albert Lehninger at Johns Hopkins in Baltimore. Dr. Sacks set the trip up for me and informed Lehninger that I was coming.

The trip from Fayetteville, Arkansas to Baltimore Maryland in 1964 was my first plane ride and although an enjoyable experience, my first impression of Baltimore was not. It was at the time, in contrast to today, a depressing city and the Johns Hopkins Medical School where I would meet Lehninger was in the most depressing area. Upon entering Lehninger's Department of Physiological Chemistry (now Biological Chemistry) at Johns Hopkins and being greeted by his secretary, I was told I would not be able to see him until late in the day as his schedule was so busy. So, I was shunted to other faculty throughout the day until late in the afternoon my moment came.

I found Lehninger to be a very low key and nice person, a true "gentleman and scholar". Although the interview seemed to be going well, I was not sure that Lehninger was too impressed with me, and the thought occurred that I had better do something soon to hit a "home run" or I would be out the door and on my way back to Arkansas. Then, Lehninger ask me if I had read his new book on Bioenergetics. I was happy to say that I had, at which time he said that if I found any typos or other mistakes to please let him know. When I pulled out a piece of paper listing several typos and mistakes, Lehninger was a little embarrassed but knew that I had read his book. My foot was now in the door but it could slam at any minute.

The subject with Lehninger then turned to whether I was considering other biochemists with whom to do a Postdoc. I said only one, David Green at the University of Wisconsin. I could see Lehninger's expression changing, his face becoming a little red at which time he said something to the effect "so why are you coming here to interview and not Wisconsin". I said "because Dr. Sacks, my Ph.D. mentor at Arkansas said that Dr. Green is (words not said here)." Lehninger almost fell over laughing. A few days after returning to the University of Arkansas, I received a nice letter from Lehninger accepting me as a Postdoctoral Fellow. I came to Johns Hopkins in 1964 to work with Lehninger, after which in 1967 he invited me to join the faculty of his Department. To

this day, I do not know whether I landed the Postdoc with Lehninger because I had read his book, because of my comments about his rival David Green, or both.

E.C (BILL) SLATER.

As referenced above Bill Slater was one of the fab five who published the 1977 paper on chemiosmosis with Mitchell, referred to above, i.e. the cease-fire declaration. Bill deserved his place at the table as a contributing antagonist, and all good theories need good antagonists to evolve. If you isolate mitochondria, if you do spectroscopy of the respiratory chain, if you assay electron transfer activity, and if you use inhibitors to dissect respiration or modulate the ATP synthase, you are using methods developed from Slater's lab in Amsterdam. What makes the man an icon at least in my mind is that he built a Department and large teaching program at the University of Amsterdam from the ground up, and more broadly was involved with expanding biochemistry and bioenergetics throughout Europe, AND YET HE DID HIS OWN EXPERIMENTS WITH THE HELP ONLY OF A TECHNICIAN. An excellent summary of Slater, the man, and his many contributions, was provided by Piet Borst in 2007 in celebration of Bill's ninetieth birthday. This can be found at: www.iubmb.org/index.php?id=174

If history provides lessons, what can we learn from the observation that Slater and Chance, both of whom loved the bench and the excitement and urgency of a discovery,....each lived into their 90s?

PAUL BOYER.

Paul Boyer won the Nobel Prize for Chemistry in 1997 for his contributions to the understanding of the mechanism of ATP synthesis by the F1F0ATP synthase (ATP synthase). The work that led him to the alternating site mechanism of this enzyme is well

documented in a biography provided by the Nobel Foundation.
www.nobelprize.org/nobel_prizes/chemistry/.../**boyer**-autobio.html
But there is much more to this man that is not fully documented.
I first met Paul in Madison Wisconsin when I was a post doc as he regularly visited to spend time with his good friend Henry Lardy. Later we met up at Conferences where Paul would inevitably be carrying a tennis racket and looking for a game. It is important to realize that the alternating site hypothesis preceded any evidence of the rotary mechanism of the ATP synthase. Paul was quick to marry the two ideas and this was followed relatively quickly by the X Ray structure of the F1 part of the complex, which confirmed the differences in the 3 catalytic sites that had been long championed by Boyer.
Our field of bioenergetics has been blessed in that it has attracted many gentlemen and few sharks. Paul is the very model of a modern gentleman. He has time for everyone, actually listens and provides feedback in discussions of science, and gives credit where credit is due and sometimes when not.
In reviewing materials for this note I read a number of articles and looked up several websites that I did not know existed.
For example I was surprised to learn that Paul said "I remember such things as picnics in Provo Canyon, and the anticipation that I might get to lick the dasher after cranking the ice-cream freezer." I know of this quote because I looked it up at www.bellaonline quotations.com. It is listed under ice cream quotations.
Another quote from Boyer I did not know about is "Most of our accomplishments [in science] are the coal we mine while looking for diamonds." I found this in an article written in 2002 and published in the J. of the History of Biology by Douglas Allchin and entitled "To err and win a Nobel Prize: Paul Boyer, ATP Synthase and the emergence of bioenergetics".
I encourage young scientists, particularly the more timid of you, to read this article.

Paul Boyer is now in his 90s, further establishing how healthy it is to study bioenergetics (Bill Slater and Britt Chance are other examples; see earlier MitoAlmanacs), or is it the passion for tennis that equates with long life?