Advances in Membrane Biochemistry and Bioenergetics

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DEDICATION

Whenever and wherever research on mitochondrial respiration is discussed, the name of Tsoo E. King looms large. He boldly resolved at the onset of his notable career to base his researches on the proposition that critical membrane-bound components of the respiratory electron transport chain in mitochondria could be solubilized, purified and characterized, while still remaining essentially completely functional and demonstrably capable of incorporation to accomplish the classical biochemical goal of reconstruction. This objective at the time was regarded by most biochemists as impossible of attainment, but King and his associates showed brilliantly over the years that it could be achieved. From his laboratory there came a flood of studies establishing the molecular structure of crucially important membrane-bound components of the mitochondrial electron transport system, as for example, solubilized functional succinate dehydrogenase, and the primary structure determination of cytochrome c_1. He predicted the existence of non-heme iron moieties as comprising most of the mitochondrial iron content, amply confirmed by experiments in his laboratory and elsewhere. He was quick to appreciate the potential of the new spectroscopies and physical biochemical procedures to probe details of structure in cytochrome *c* oxidase, especially techniques based on the use of optical rotatory dispersion and circular dichroism. Another of his important findings centers on the demonstration that quinones function only when linked to protein in complexes, of which several exist acting at sites determined specifically by the protein component.

It would be fatuous to attempt a detailed evaluation of King's contributions, even if space were available. His impact in the field of mitochondrial respiration, and bioenergetics generally, is evident from the contents of this Festschrift which is a compilation of papers contributed by most of the leading investigators in the relevant fields of research.

There is no question that Tsoo E. King is one of the principal architects of the structure of knowledge pertaining to mitochondrial respiration. It is a privilege to write these few lines in tribute to him and his achievements.

Martin D. Kamen

December 10, 1986

PREFACE

This book is formulated from the papers presented at the International Symposium on "Membrane Biochemistry and Bioenergetics," held at the Rensselaerville Institute, Rensselaerville, New York, August 1986, in honor of Tsoo E. King on the occasion of the 30th anniversary of reconstitution of a respiratory chain system by Professor David Keilin and Tsoo E. King.

Professor Tsoo E. King, to whom this volume is dedicated, has made enormous contributions to the field of isolation and reconstitution of membrane proteins and has continued to explore the frontiers of bioenergetics. In particular, his persistent proposals on the existence of ubiquinone binding proteins from conceptualization to experimentation eventually convinced many scientists to study these proteins further. Professor King's preparation of reconstitutively active succinate dehydrogenase opened a new avenue in the field of membrane bioenergetics, and his work has been greatly appreciated.

The purpose of the symposium was to bring together scientists from diverse disciplines related to membrane bioenergetics to discuss the recent developments in the field. This symposium, initiated by the Capital District Bioenergetics Group, was attended by 100 scientists, 80 of whom presented their recent discoveries. The symposium was arranged in a sequence of platform lectures, poster presentations and discussion sessions so that all the participants had opportunities to discuss the subjects presented. Most of the participants contributed a chapter to this volume. We would like to express our regret to many other scientists including Professor King's friends, colleagues and students who could not attend due to various reasons.

This symposium could not have been possible without generous financial support from Rensselaer Polytechnic Institute and the State University of New York at Albany. We also appreciate support from General Electric Co. Most of all, we are grateful to all participants who enabled us to have a great scientific gathering in honor of Professor King and his contribution to "Reconstitution". Special thanks are extended to Michael Seaman and Stephen Chace of the Laboratory of Bioenergetics for their enormous assistance in proof-reading of retyped manuscripts. We also thank Laura Waelder and her staff at Words and Images for their excellent word processing services.

Finally, we are pleased that we were able to have this memorable symposium in honor of Professor King's pioneering contributions to membrane biochemistry and bioenergetics.

Chong H. Kim
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Tsoo E. King

MY TEACHERS AND I

By Tsoo E. King

First, I must apologize as I have not greeted all and each of you due to my physician's advice that I need more rest because of my recent bout with pneumonia. Indeed I still use oxygen once in awhile. I was superstitious when my fever was high because of the Lehninger event as you know. He would be here otherwise. About this symposium, I first vigorously objected to having it. But the organizers were very persuasive; through their persistent and strong urge and push, I have reluctantly gone along with them. Honestly, if I've made a little contribution in bioenergetics it is due to the benefits I received from my teachers, associates and indeed students.

I must mention here a few of them. First, I should emphasize that I owe much to Professor Keilin. From him, I learned the background of his discovery of the cytochrome, the respiratory chain concept and, more importantly perhaps, his method of attacking scientific problems. A few days ago I received a lovely letter from his daughter, Joan. I will tell you more about the Professor in a moment. I came to this country with practically a brand new bachelor's degree in Chemistry and Biology. I entered the department of chemistry of a then rather small college which did not even have a refrigerated centrifuge or a biochemistry department (now it is much larger than SUNY here, both the university and the departments of chemistry and biochemistry). The reason I entered the school in Oregon, rather than supposedly the University of Minnesota that was much more well known in China, was actually quite simple. The college is near the port of my debarkation, so I stopped there for a few days just to look around. But Dr. Cheldelin who was only a few years older than I was most amiable. Furthermore, I was warned that Minnesota was extremely cold in winter and full of mosquitoes in summer. Because of these factors I stayed in Oregon and continued for a long time. It must be fate that I still moved from Oregon to here where it is as cold as Minneapolis, although there are not so many mosquitoes. A fate?

Anyway, after passing French in my last year of graduate study at Oregon, and a few months before my Ph.D. oral, I overheard Professor Karl Folkers, who was visiting Oregon, telling my adviser, Dr. Vernon H. Cheldelin, that one must see those institutions outside of this country -- to see world science and get a Guggenheim, which was quite prestigious. Mind you, this was what I overheard in a hallway as I was only a graduate student. In Oregon just a year or so after the war, there were not many scientists, not like Berkeley with E. O. Lawrence, even a physicist; his group had some biochemists although Martin Kamen had already left the Radiation Lab. Albert Barker, Zev Hassid, and quite a few other biochemists were in Berkeley. However, in Oregon, I most benefited from my adviser from whom I learned English -- his English was

almost beautiful -- and the American way of life. He always told me that he advised his sons if they were hit by their peers they must hit back instead of crying. This advice was so alien to my Chinese tradition. I also learned from him how to apply for grants because he had a good friend, an old timer in NIH; some of you may remember the late Donald Larsen. Larsen came to Oregon often, at least twice a year either for the site visits for Cheldelin's grant or to make a side trip during some visits to nearby schools, such as the State of Washington or California. Cheldelin sometimes let me chat with Dr. Larsen and even occasionally invited me to lunch with the two of them. Don was a very likeable and helpful person. He and his wife had a hobby collecting gladioli. Eventually he and I became good friends. He even took a special trip from London to Cambridge to visit me.

My first trip to Europe for a scientific purpose was in 1952 when my adviser and I went together to attend the second International Congress of Biochemistry. On our way back we stopped in London, Cambridge and Oxford. Surprising enough many "scientists-tourists" were in those cities too. Professor Keilin then had just passed his 65th birthday but was still working at his famous microspectroscope in his office-lab. The first person with whom I met for a significant conversation was Emanuel Margoliash who was working in the Molteno with Professor Keilin. I saw buckets and buckets (I think at least 15 liters each in capacity) of pink fluid and occasionally yellow fluid. The former, he intoned, was cytochrome and the latter was flavins, his byproduct from horse heart extract. His preparation of cytochrome in buckets, sequencing *c*'s of so many phylla to his recent studies of cloning and site specific mutagenesis of the cytochrome *c*, epitomizes one fact that impinged on me deeply, that is, biochemists really work themselves. It was quite a contrast for me to see more than half of the biochemists in Wisconsin working though my adviser did not even touch a test tube. In Madison, however, one professor and concurrently the department chairman, directed more than 200 graduate students at one time, I was told! I know my adviser was too busy searching for funds by telephoning 3,000 miles away to Washington, D.C. One must admire him; from literally one biochemist at the time I arrived, he established all by himself a separate department of biochemistry, with at present a total of 40 or so faculty and he obtained one of the first NIH construction funds to pay for part of the cost of construction of a huge 5-story building of some 100,000 square feet. In addition, the American Chemical Society (Rochester, N.Y. Section) bestowed the Harrison Howe Award on Dr. Cheldelin and he also gave an E. R. Squibb lecture. At any rate, this European trip furthered my intention to study abroad even though I worked as a postdoctoral with Frank Strong, an antimycin man in the biochemistry department at Wisconsin. Wisconsin was and is a world class university. Nevertheless, comparing Cambridge with Madison, for example, the latter still lacked some undescribable ingredients, call it tradition, call it intellectual atmosphere, whatever you want.

Back in 1953, I paid my own way to attend the 19th International Physiological Congress in Montreal. On the great lawn of McGill University, I was intercepted by an already famous biochemist. Do you know who he was? Of course, it was Britton Chance who interrupted his afternoon tea with his friends or associates on the great lawn. But surprising enough, the questions he directed to me were not related to my reports at the meeting but rather were technical questions about cytochromes of a particulate fraction of *Acetobacter suboxydans*. In subsequent years, I found out that Brit is a truly innovative

experimentalist. Sure enough, 21 years later, I went to Station 7 of SSRL and the first person I saw was Chance working at two (!) stations at the same time. He had been in a quonset for already 16 hours without sleep. There were two tape recorders, one for the experimental results and the other for correspondence, while the always-carried notebook was used not only for taking notes for conversations, but also for writing his theory, schemes, and perhaps some speculative hypothesis. He is not here right now because he is working at Brookhaven with the new but more powerful EXAFS equipment. On the other hand, I must confess that nowadays in 1986 working in the lab is a privilege. Writing proposals, etc. takes a great deal of time. Another hazardous machine is the telephone which I used to forbid having in the lab. But because of colleagues' pressure, I had to yield. A person like Brit is truly lucky; he has capable administrative assistants, such as Sally Congdon. Thus, he does not do anything himself except experiment and think.

From Professor Keilin to Emanuel Margoliash to Britton Chance, I know that biochemists were not just writing proposals, calling NIH, the Senate, or even the White House. Another person I must mention is Peter Mitchell. From him, I learned more about his way of thinking. Peter, sitting now on my left, emphasizes thorough thinking. He is an early riser, getting up when it is still dark. When I was at Bodmin, we would talk until midnight or later. But he still got up as early as usual. He searches out all the possibilities of many problems, ponders them, excludes many, and winds up with only one or two. I recall that his Q-cycle was a result of being awake a full night or several nights. The cycle at the beginning was only a theory. Now many experiments from other laboratories have confirmed and extended the original Q cycle, which he first published in FEBS Letter, volume 56, page 1 in 1975. Another characteristic of Peter is that his thinking can truly be labeled unconventional; literally he can visualize what other people never even dream of. The Q cycle, vectorial transfer in the chemiosmotic hypothesis seems to be so logical, but they are nearly the same as the egg story of Columbus. Parenthetically I must emphasize he is not only just a theorist but does experiments himself. A strong experimental support of the chemiosmotic hypothesis was done by himself with Jennifer Moyle. In avocation he is also a dexterous worker. He designed, made the template, and fabricated the medallions of Glynn Research Laboratory at Bodmin House. Now, these medallions are truly collectors' items.

Finally, I must tell you what I received from Professor Keilin. The first time I met him was 1952 when we did not talk much as he was busily working with his well-known microspectroscope while a bottle of liquid air or nitrogen was evaporating on the bench along the window. The second time was in 1955 on my way back from the Brussels Congress where I learned two completely different methods that were reported in the meeting about the solubilization of succinate dehydrogenase and had been worked out concurrently in Detroit and Shanghai. I did not know the Detroit group then, but I knew Y. L. Wang who was at the meeting and C. L. Tsou; both of them had worked with the Professor. At this visit I presented my idea of reconstitution which I first had several years earlier, as Peter Nicholls narrated eloquently the night before last. I told the Professor, "whenever I get a chance I would like to work with you on this subject". He welcomed this suggestion but as usual he was very objective. However, I emphasized that the method should not be empirical or trial and error. Since the Brussels Congress in August, practically all the time I had been pondering these two methods and of course the main theme, reconstitution. The preparation by the Detroit

group lead by Tom Singer caused me a bit of confusion because of the number of irons in the enzyme -- four or two -- per flavin; and as I remember they later claimed pure with a molecular weight of 200,000 using a sedimentation rate in the ultracentrifugation and a diffusion constant as well as calculated from the flavin content. The method by Singer *et al.* was of course an extension of Hogeboom's; Hogeboom used acetone powder from guinea pig liver mitochondria and extracted with alkaline buffer. The Chinese method was derived from the late Morton's. It was much simpler. At any rate, in 1957, I not only got a Guggenheim Fellowship but also a well paid National Science Foundation Senior Postdoctoral Fellowship. I suppose in those days it was very easy to get any kind of fellowship in science, although my publication of more than 15 papers in a short stay at Madison might have helped. Therefore, I went to the Professor's lab. At first I tried the American method for making succinate dehydrogenase. Oh, it certainly gave me a hard time. Molteno had only one Spinco ultracentrifuge and a large capacity French-made centrifuge but it was not refrigerated and was rather slow. In order to obtain sufficient amounts of soluble enzyme, I had to beg to use centrifuges in the other buildings. In addition, it was rather dangerous to handle many gallons of inflammable solvent such as acetone which must be further removed by a more volatile solvent, ether. A fire could happen at any time. Percy went to a slaughterhouse by bicycle for horse heart. Eventually, some soluble succinate dehydrogenase was obtained but its activity was lower than reported. I decided to try the Chinese method. It was so easy to obtain the Keilin-Hartree preparation and the method was and still is much simpler. I did not have to use a Spinco centrifuge. The enzyme obtained showed about the same activity as Wang *et al.* reported. It was dark sherry color. I could finish the preparation and the activity assays from the Keilin-Hartree preparation in less than one afternoon.

However, at that time the Professor had already told me the Hopkins' effect and my good friend, Heinz Frankel-Conrat of Berkeley, a character—so people labeled him, told me more than once about the cyanide reaction of disulfide producing two groups, one -SH and one -SCN. I interpreted that the dehydrogenase by the Wang *et al.* method which contained a rather high concentration of cyanide arising from prior treatment of the Keilin-Hartree preparation might have been modified. I also knew that one of the coauthors, C. L. Tsou, was fond of cyanide as his contribution of the cyanide reaction of cytochrome *c*. So after a lengthy deliberation and deep meditation, I wanted to try a procedure to see what would happen if I omitted cyanide. To my great delight, the soluble succinate dehydrogenase thus obtained exhibited even higher specific activity with ferricyanide as electron acceptor. I had some three kinds of succinate dehydrogenase for realization of my reconstitution idea, but I still needed a particle which contained all respiratory components except succinate dehydrogenase. In this aspect, I spent considerable time, more than working on preparations of the dehydrogenase. I worked literally day and night and eventually succeeded after many failures. (Whenever I could not borrow a key while working at night, I had to climb a medieval-type fence.) The professor called these preparations "cytochrome system" or particle; at that time, the other respiratory proteins were not yet known. The first reconstitution was done on Wednesday, 6 October, 1957, of course using my "unmodified enzyme". In my notebook the comment said, "the reconstitution (#53, #66) was very successful and the activity inhibited was 75% by 0.03 mM hemin and 100% by 0.06 mM".