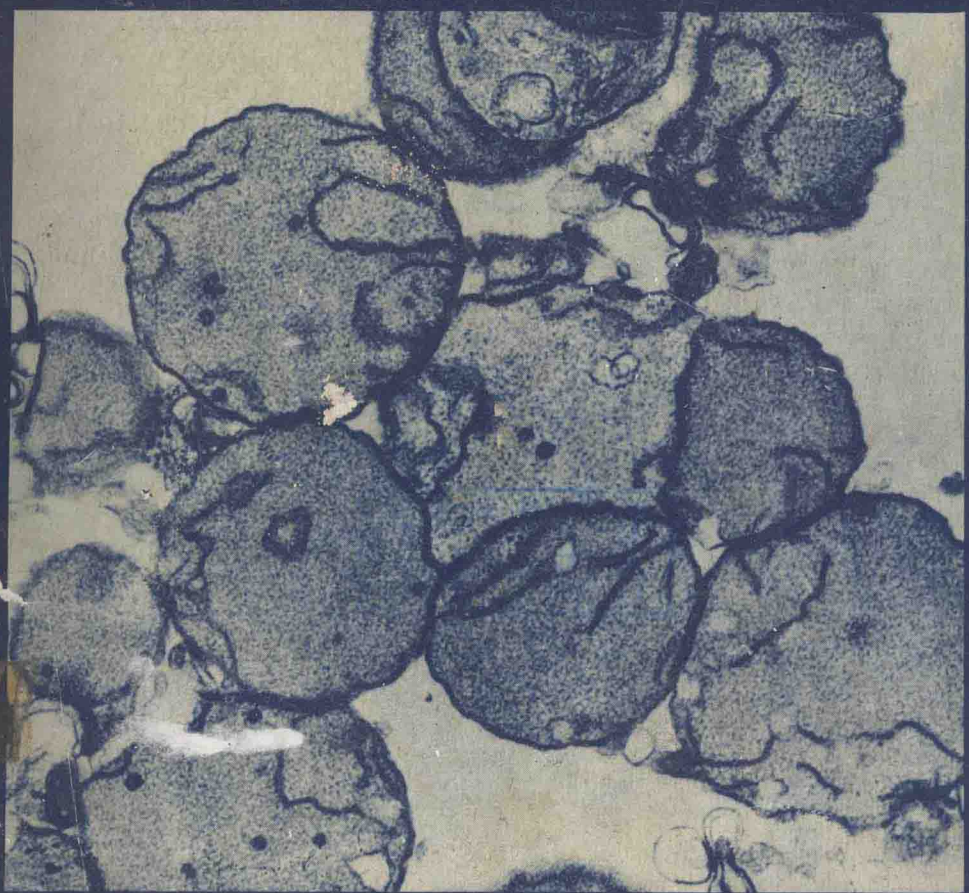
The Molecular Biology of Membranes

Edited by Sidney Fleischer, Youssef Hatefi, David H. MacLennan, and Alexander Tzagoloff



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Preface

On February 14, 1977, a symposium entitled "The Molecular Biology of Membranes" was held in New Orleans in honor of Professor David E. Green, whose many contributions in mitochondrial structure and metabolism have influenced and guided research in this important area of biochemistry for many years. The symposium was attended by many former and present-day colleagues, friends, and interested scientists. The contents of this volume represent papers that were delivered at the symposium and other contributions from individuals who have been associates of Professor Green.

We wish to thank Plenum Press for their help in making the symposium and publication of this book possible.

Sidney Fleischer
Youssef Hatefi
David MacLennan
Alexander Tzagoloff

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Impressions of David E. Green by His Colleagues

1930—1932

Events have shown that the brash, 20-year-old Dave Green, whom I met at the Marine Biological Laboratory during the summer of 1930, had a great deal to be brash about. The friendship which he gave me during the following year at Washington Square College, New York University, and during the summer of 1931 at the MBL, where we both worked with Professor Robert Chambers, played a great role in helping me maintain my morale during this most frustrating year of my professional life.

The following summer (1932), I learned from Dave that he had saved enough money for a year of graduate work at Cambridge University, England. With supreme self-confidence, he informed me that during that year he would make his mark to the point where Cambridge University would itself provide support to time of completion of work for his doctoral degree. His self-confidence was obviously not misplaced.

The Dave Green whom I knew in the early 1930s had a remarkable ability to read and critically evaluate pertinent literature with great rapidity and insight. In addition, he had a photographic memory—while I was groping for a reference, he would give me more than one, often complete with names of authors, journal, date, page numbers, and summary. Finally, it is my recollection that he had already arrived at the idea that to understand any particular biochemical activity of a living cell, it was necessary to elucidate the pertinent molecular structures.

Lyle V. Beck

1932–1940

David Green joined the Department of Biochemistry of Cambridge University in 1932 as a Research Student for the Ph.D. degree. Sir F. G. Hopkins, the Head of the Department, suggested that he should work under my supervision on the enzymes and other catalysts concerned with biological oxidation processes and respiration. Research on that subject was then in a very interesting stage, when it was becoming possible to bring together the results of a number of then recent discoveries, and at Cambridge research was actively proceeding on a number of different aspects, e.g., study of separated enzymes, especially dehydrogenases, cytochromes and cell respiration, thiol compounds, "coenzymes I and II" (now NAD and NADP), flavins and flavoproteins, redox potential, respiratory inhibitors, and reactions in muscle, bacteria, plants, etc.

David threw himself into this research with great enthusiasm, energy, and enterprise. He was full of ideas which he expressed freely, and although not everybody agreed with all of them, they were always interesting and characterized by freshness and vitality. His research proved fruitful from the beginning, and he soon began the production of a continuous flow of important publications, which has continued now for about 45 years. He very soon obtained the Cambridge Ph.D. degree, and was enabled to continue his research in Cambridge for a further 6 years by the award of a Beit Memorial Fellowship, and later a Senior Beit Fellowship. During that time, he contributed very considerably to the growth of our knowledge of biological oxidation systems. He obtained a series of preparations of particular dehydrogenases, and although at that early date they could not be expected to be homogeneous, they were a great improvement on the extracts which had been available previously. He quickly realized the importance of starting with comparatively large amounts of material to obtain a reasonable yield, and this usually depended on teamwork. David had a gift of attracting other research workers, both senior and junior, to collaborate with him to form small research groups. This made it possible to carry out a series of important studies on the role of coenzymes, flavoproteins, cytochromes, etc., in the linking of dehydrogenases with O_2 (in respiration) and with other dehydrogenases (in fermentations). He also gave us much help in devising interesting experiments for our advanced practical classes.

His work, however, did not stop at systems of soluble enzymes. Our relations with Professor Keilin were close, and David soon became familiar with Keilin's particulate preparation of cytochrome oxidase from heart muscle mitochondria. Keilin drew attention to the possible importance of the physical interrelationships of the catalysts within the particle. David saw the importance of this, and it became the basis of

much of his work for many years after his return to the United States, particularly with reference to the "cyclophorase system."

I must not fail to mention the book of essays *Perspectives in Biochemistry*, written in 1937 by over 30 distinguished writers, who were or had been connected with the Laboratory, in honor of the 75th birthday of Sir F. G. Hopkins. It was edited jointly by Joseph Needham and David Green, and attracted considerable attention at the time. It seems likely that it may have formed a model for the two later volumes *Currents in Biochemical Research*, edited by David in 1946 and 1956.

Malcolm Dixon

1940–1946

I met Green first in the spring of 1940 when he came from Cambridge to Harvard Medical School, an expatriate forced home by the war. I was a medical student being introduced to biochemistry. I later realized he must have experienced a kind of culture shock in coming from the fountainhead of biochemistry and enzymology that Cambridge then was, and being there one of the most enterprising of the younger men, to discover that the United States, and even Harvard, was a biochemical desert. Other refugees also experienced this, and their presence in the United States produced a flowering of the science by the end of the war that needs to be detailed. Green was a dominant influence among this now illustrious group. Because they called on him as they arrived, I saw that these future leaders of biochemistry placed a much higher value on Green and his work than did his colleagues at Harvard. This slanted my own evaluation of the subject. Green had been responsible for the famous Part II biochemistry honors course at Cambridge. He went through my Harvard course notes and assignments, with astonishment. "My God," he said, "at Harvard enzymes are the hair and fingernails of biochemistry!"

The balance began to change, but of course too slowly. His book, the first to present enzymes operationally, grouped by coenzymes, came in through the U-boats. It was to read this together that a group of eminent physicians formed the first Enzyme Club, and invited Green to New York City to preside at their learning, and to work at P & S. His trace substance hypothesis was published in Volume IV of *Advances in Enzymology*, the most stained and used volume in the series, which also contains Lipmann's high-energy phosphate paper.

Finally, there was some recognition. The Paul Lewis Award was created, and the first of these went to him without question. Wisconsin,

never as enzymically desolate as most of the country, pioneered in starting an Enzyme Institute, and chose Green to organize it. I played a significant role at that time because I came from the Middle West. I reassured him that it was plausible to live west of the Hudson, a fate that Green, ever the New Yorker, looked at with some foreboding. When his appointment was announced in Madison, he brought me, rather quizzically, the newspaper clippings: "Enzymes," said the subheadline, "are in beer, cheese and wine."

W. Eugene Knox

1940–1946

During my last year at Harvard, I was required to prepare a research thesis to fulfill the honor requirements in biochemistry. Since I had become interested in enzymes, and since, in 1940, no enzymologist was on the staff in Cambridge, I made an appointment to see Professor A. Baird Hastings, at that time Chairman of the Department of Biological Chemistry at the Medical School at Brookline. At the appointed hour, I was ushered into the august and wood-paneled chambers of Hastings, and, after a brief series of questions, Hastings said that he no longer was active in this field but that a young chap just back from Cambridge, England, was downstairs and it would be a worthwhile experience to at least meet him. Hastings took me down to the high-ceilinged, darkish, cupboard-lined laboratory and introduced me to David E. Green, who promptly suggested, in a broad English accent, that I roll up my sleeves and go to work.

Thus, in September of 1940, I began a 6-year involvement with David E. Green. At the end of the 1940–1941 academic year, Green obtained a position as Instructor of Biochemistry at Columbia University College of Physicians and Surgeons. He suggested I apply for my graduate work at Columbia; so September 1941 found me in New York City with David as my major professor. I had the unique distinction of being Green's only graduate student in America. The period from 1941 to 1946 was an exciting one. With the war at full swing throughout this time, with research funds still severely limited, with supplies and equipment at a premium, today I am still struck by the highly successful operation that David ran in his two small laboratories on Floor "G." One of the two laboratories was occupied by Sarah Ratner, Marian Blanchard, and myself, and the other lab by David, his technician, Violet Nocito, Joe Dolan—a most unusual dishwasher—and visiting scientists who, during the war years, included Luis Leloir and later W. Farnsworth Loomis. The lab was the center of information on enzyme chemistry for

all of New York City. David Nachmansohn, Konrad Bloch, David Rittenberg, and David Shemin were constantly wandering up for advice; S. Ochoa and E. Racker from N. Y. U. were frequent visitors, as were Fritz Lipmann, Otto Myerhof, and many others. At this time, Green organized a group of people who had interests in common and called it the "Enzyme Club" with monthly meetings at the Columbia University Faculty Club. After the war, this idea caught on all over the country, and for many years these "clubs" could be found at any large urban academic center.

Green had his desk in his small lab where he carried out all his own experiments, administered his laboratory, met people, ordered equipment, and wrote his papers. He was therefore a "lab" man. There is no question in my mind that Green was the best experimentalist I ever met. As we used to say, he had a "Green thumb," an intuitive sense to set up relevant experiments; he had a knack in isolating difficult enzymes. He was always enthusiastic, impetuous, perhaps stubborn, always available for advice and encouragement. We developed one of the first ultrasonic devices for the purpose of disintegrating bacteria; Green purchased one of the first Beckman DU spectrophotometers; he was the first, to my knowledge, to make use of Waring blenders for extraction of enzymes from tissues. One time, during the war, he needed a supply of xanthine oxidase. He managed to obtain about 10 liters of raw heavy cream—how he obtained this rare dairy product, I have no idea—and we extracted the milk xanthine oxidase and, more important, a very large amount of butter as a most desirable by-product.

P. K. Stumpf

1950–1953

I was fortunate in joining David Green's team in October 1950, at the start of a very exciting period of research. A few months earlier, the group had transferred to the new laboratory space on University Avenue, into what was then the new Enzyme Research Institute. David Green was now able to fully realize the dreams which he had developed during his time in Cambridge, England, that is, to build up a facility for large-scale enzyme preparative work, on a level that had not previously been attempted. He went about this with tremendous verve and enthusiasm, and I have fond memories of him, in a white coat, energetically supervising the installation of the 13-liter low-speed centrifuge and the Sharples centrifuge in the somewhat overheated boiler-room premises.

David had relatively few administrative duties at the time, and he was able to spend a good deal of this time at the bench, and, more

importantly, with his younger colleagues. Discussion ranged widely; and although we mulled over the events of the day, the main topics concerned the science which was our chief interest. During the fall of 1951, discussion turned on the structure of the mitochondrion and of "cyclophorase." David insisted that his critics, who claimed the mitochondrion to be a "little bag of soluble enzymes," were entirely wrong, since the properties of the cyclophorase enzymes indicated the presence of a defined structure. I was so bold as to interpret David's theorizing in a diagram of a "cycloforasarus," which was photographed into multiple copies by Shirley Schweet, and which provided the Enzyme Institute group with an informal Christmas card for 1951.

Priscilla Hele

1958–1959

Those were the days when coenzyme Q had just been discovered by Fred Crane and his colleagues, which made Fred walk with his head a few feet higher. Absolute contrast was the case with another group in the same Institute, who were working on fatty acid synthesis. The carboxylation reaction was playing hide and seek with them, leading to unlimited gloom and frustration. Then, when one fine morning Wakil isolated malonate as an intermediate in fatty acid synthesis, the gloom suddenly disappeared and Wakil found himself firmly lodged in the hall of fame.

J. Ganguly

He was always a generous man to his young scientific colleagues in terms of publications—if anything, overgenerous; for I believe that he would have certainly been awarded the Nobel Prize for his work in the lipid field if the contribution that he made to fatty acid synthesis had been justly acknowledged. However, because of his generosity, this was not to be.

Anthony W. Linnane

1959–1962

The greatest aim of Dr. Green and myself, at that time, was the discovery and isolation of the hypothetical unit of the mitochondrial energy-transducing system. In the spring of 1961, Dr. Green and I

visited Dr. Fernandez-Moran at Boston, carrying an ice-box with mitochondria in it, to ask him for the electron microscopic observation of mitochondria by negative staining with phosphotungstic acid. I cannot forget my dramatic emotion when we saw the repeating particles in the inner mitochondrial membranes (the headpieces of the elementary particles).

Takuzo Oda

1962–1967

I am very pleased to supply some personal recollections of the exciting scientific atmosphere at the Enzyme Institute when I was there. This was a period shortly following Hatefi's work on the isolation of the respiratory complexes and the demonstration by means of reconstitution and other criteria that these are genuine enzymes of the electron transfer chain. In my view, this was one of the major contributions that came out of Green's laboratory. The reality of the complexes as *bona fide* mitochondrial enzymes has withstood the test of time and is now generally accepted in the mitochondrial field.

My own exposure to these enzymes occurred when I first arrived at the Enzyme Institute and began working with David Wharton on the copper component of cytochrome oxidase. Later, David McConnell, our electron microscopist in residence, came, and the rather important discovery was made that the purified respiratory complexes were capable of forming membranes when appropriately supplemented with phospholipids. David Green, with his keen eye for ultrastructural details, was quick to recognize the significance of this phenomenon which became a strong experimental basis for his interpretation of the inner membrane of mitochondria.

One meeting I had with him at that time may be worth recounting, since it illustrates his analytical skill at connecting experimental observations with phenomena that at first glance might seem unrelated. On this particular occasion, we were discussing membrane formation by the complexes and Green quite suddenly realized that the conditions which had been found earlier by Hatefi to be necessary for the reconstitution of the electron transfer chain would obligatorily lead to membrane formation. Without spelling out all the details, he then suggested the idea that in order for two or more complexes to reconstitute an integrated activity, the sole structural requirement would be that they be present in the same membrane. In other words, Green was able to make the connection between Hatefi's conditions for reconstitution and a

more abstract concept having to do with the assembly of the complexes into membranes. In fact, I was able subsequently to show that Green's idea was correct.

Alexander Tzagoloff

There are four essential things I learned from David Green. One is that before you can talk about the function of a biological component, you have to isolate it and characterize it as it functions *in vitro*, away from other components with whose functions its own could be confused. The second is that before you can appreciate the function of the component in its natural environment, you have to put it back together with other components to see how they work in concert. The third thing is that if you just run experiments without thinking carefully and at length about why you're doing them, they may all turn out to be trivial. The fourth is that when you make a mistake, you should make a quick and graceful acknowledgment of it, but be prepared to hear your critics bring it up the rest of your life.

David Green made numerous mistakes, almost always *en route* to a new and better synthesis of ideas. His energetic mind continually leaped from crag to crag in the cloudy heights of biological phenomenology. His leaps were intrepid and magnificient. The timid were sometimes left behind in the well-cultivated valleys of more pedestrian ideas. The uncritical were sometimes plunged into the abyss, from which Green himself seemed to escape, although not without scars. There was a sense of heady, almost extravagant excitement about research with this extraordinary scientist.

Among the many significant contributions he had made to biology, one that I recall most personally was his early realization and demonstration that the electron microscope was not the exotic plaything of a scientific elite, but a powerful interpretive tool to be put at the disposal of every working biochemist. In the early 1960s, to bring this about, he traveled repeatedly—thermos jug in hand to transport the ever-present mitochondria—to the laboratory of a world-renowed electron microscopist for instruction and collaborative research. Soon thereafter, with the merest of credentials in this new field, he was calumnied by morphologists and biochemists alike for venturing into the structure–function arena. Yet he persisted and triumphed in his goal. Characteristically, it was of less interest to David Green that his inductive generalizations fit completely the available facts than that he enter forbidden temples with searchlight and rapier in hand, his mop-up troops to follow.

David G. McConnell