

Lehninger, Albert Lester (*b.* Bridgeport, Connecticut, 17 February 1917; *d.* Baltimore, Maryland, 4 March 1986), *biochemistry; energy metabolism* [OR *bioenergetics*].

Albert Lehninger is perhaps most widely known for his synoptic and lucid textbook, *Biochemistry*, which inspired many students in the field. Through his research, as well as through substantive contributions in leadership roles and education (including two other books, *The Mitochondrion* and *Bioenergetics*), he helped pioneer a new interdisciplinary field, bioenergetics, in the mid-twentieth century. His most significant research achievements were to identify the organelle, the mitochondrion, as the site of the most important energy reactions in the cell and to characterize and quantify many features of that system (including the burning of fats, calcium transport and proton stoichiometries). His focus on the mitochondrion also laid a foundation for studies on the movement, storing and regulation of calcium in the cell.

From Writing to Science

Albert grew up in relative economic security in Bridgeport and Hartford, Connecticut. He attended nearby Wesleyan University, a small liberal arts college for men, from 1935 to 1939. Originally he intended to write stories and poetry. He was a member of the Scrawlers' Club. However, one of his teachers, Ross Fortner, Jr., introduced him to the emerging field of biochemistry and to the recent discoveries of Otto Warburg and Hans Krebs on cellular metabolism. Inspired, Albert's interests and major soon shifted to chemistry and he targeted a new career in medicine and biochemistry.

Lehninger earned his Ph.D. from the University of Wisconsin in 1942. His dissertation research focused on the metabolism of fats. When World War II started, he joined the Plasma Fractionation Program. His task was to develop methods to extend blood plasma by modifying its globulin proteins (a project later abandoned as ill conceived). After the war, Lehninger settled into a position at the University of Chicago, where he enjoyed the mentorship of Charles Huggins (who later won a Nobel Prize for work on cancer treatment).

From Fatty Acids to the Mitochondrion

At Chicago, Lehninger continued his investigations on fatty acids, the long chain molecules that make up fats. Fats supply roughly one-third of the body's energy, yet in the mid-1940s no one yet knew quite how. Lehninger methodically characterized all the conditions (reactants, temperature, pH, cofactors, etc.) that affect the reactions. In the process he found in 1945 that the breakdown of fats linked to another set of well known energy reactions: Krebs' tricarboxylic acid cycle (the discovery of which had earlier inspired Lehninger). Lehninger had discovered how fat and carbohydrate metabolisms importantly converge. Each breaks food molecules into two-carbon fragments which then share a common energy pathway.

Lehninger had been working with whole cell extracts. A major aim next was to isolate the specific set of enzymes that catalyzed fat metabolism. In 1948 George Palade's lab at the Rockefeller Institute reported a new method for suspending broken cells in a dense sugar solution. They had successfully separated one organelle, the mitochondrion, undamaged, using differential centrifugation. Lehninger saw an opportunity. He and one of his first graduate students, Eugene Kennedy, set up their own chilled centrifuge — in a refrigerator normally used

for storing urine samples. Within weeks they found that isolated mitochondria could break down the fatty acid chains, while the remaining parts of the cell did not. The mitochondria also contained the enzymes for the tricarboxylic acid cycle, as well as for the subsequent production of adenosine triphosphate, or ATP, the unit molecule of energy in the cell. The mitochondria could not, however, begin the breakdown of glucose. In a remarkably short period, Lehninger had identified the location of nearly all the major energy reactions in the cell. It was a major discovery, foundational for further studies.

Lehninger's characterization of mitochondrial reactions was also significant conceptually for cell biology. For the first time, a specialized function of a cellular organelle had been experimentally demonstrated. The discovery supported the widespread view that particular functions of the cell were sorted in different parts of the cell (lysosomes, ribosomes, etc.), and that one could investigate them separately. At the same time, as Lehninger would note in subsequent publications, compartmentalization was important to understand on its own. How did the structure of reactions organized in a membraned unit relate to their function and integration in the cell?

The Mitochondrion: Energy

Lehninger would ultimately devote over three decades to the study of the mitochondrion, and his expertise on it earned him wide acclaim. First, he continued to further localize and identify the vital energy reactions. The primary focus became the final stage of the energy pathways, oxidative phosphorylation (or ox phos). Here, a highly energized phosphate is added to adenosine diphosphate (ADP) to generate the final ATP while, at the same time, cells consume oxygen. Severo Ochoa had already determined in 1943 that for each oxygen used, three ATP were produced. Yet the nature of the reactions was still unclear. In 1948 Lehninger showed definitively, with his student Morris Friedkin, the suspected role of electron transport through the cytochrome system. They thereby clarified just how the Krebs cycle yielded ATP: via the high-energy electrons of NADH. In 1949 Lehninger further showed how one could generate NADH without the Krebs cycle by using β -hydroxybutyrate; the method would be applied widely in subsequent investigations. In both cases, to measure oxygen consumption, Lehninger had relied on a classic apparatus designed by Warburg, who had earlier inspired his turn to biochemistry. In his typically methodical style, Lehninger had also noted inhibition by calcium and the effect of ion concentration on reaction rates, apparently modest findings that would later become central to his studies.

Lehninger's renown was growing and in 1952, after a year as Guggenheim fellow and Fulbright scholar in England, he moved to The Johns Hopkins University Medical School. There, at only age 35, while continuing his research, he assumed leadership of a department and the development of educational programs.

Lehninger added another important method to the biochemists' toolkit in 1956. He and post-doctoral fellow Cecil Cooper and student Thomas Devlin were searching for ways to further disassemble the mitochondrion into functional parts. They tried extracts obtained by "vibration, exposure to butanol-water mixtures, drying with acetone, exposure to hypotonic media, grinding, and treatment with cholate or deoxycholate," but all were found to be totally inactive. However, digitonin effectively disrupted the membrane. The resulting fragments, or submitochondrial particles (SMPs) exhibited oxidative phosphorylation, but not the Krebs cycle. They concluded that the electron transport chain and ATP-synthesizing enzyme were located in the mitochondrial membrane, which they had isolated, and that the Krebs cycle was in the fluid interior, or

matrix—another landmark localization. At the same time, they began to identify functional segments of the electron transport chain. Later that year Britton Chance and his lab, using spectrographic data, would complete this task, identifying three sites that each yielded one ATP.

By the late 1950s, ox phos had become the premier research topic among biochemists. Lehninger's lab was a leading contributor. In 1958, Lehninger and Charles Wadkins developed evidence for the terminal reaction in ox phos. Their claim of a phosphorylated high-energy intermediate (based on the ADP-ATP and ATP-P exchange reactions) was widely accepted, a welcome benchmark in an increasingly perplexing field. (Much later the data would be reinterpreted in an alternative theoretical context.) Beginning in 1959, Lehninger also drew attention to the swelling of the mitochondrion and its relation to energy changes, indicating that ion movements and osmotic changes were also significant in interpreting mitochondrial function. In 1963 such concerns led to precise measurements of calcium transport across the mitochondrial membrane fueled by electron transport system (see section below). Based on these findings and earlier conclusions about compartmentalization, Lehninger prominently advocated a role for mitochondrial structure in understanding the energy reactions.

As a respected leader in the field and an effective communicator, Lehninger became an important interpreter of research on how cells transform energy. He led important co-authored review articles for *Science* in 1958 and for the *Annual Review of Biochemistry* in 1962. He also wrote for a more general audience in *Scientific American* in 1960 and 1961. In 1964 he published *The Mitochondrion*, the first monograph on the organelle, widely noted for its completeness and clarity. Lehninger expanded his focus the following year in another book, *Bioenergetics*. The trim 258-page volume would serve as a reference and advanced text both. Lehninger valuably consolidated and organized information, effectively profiling an emerging scientific field, which ultimately adopted the name he had given it.

As the study of oxidative phosphorylation unfolded, intense controversy emerged. While Lehninger typically addressed theoretical issues thoughtfully, he distanced himself from the theoretical fray and focused instead on what experiments could concretely demonstrate. Nevertheless, Lehninger's theoretical impact was significant, especially in contributing to the development of the chemiosmotic hypothesis. Lehninger's influence began even before the concept was published by Peter Mitchell in 1961. Mitchell's ideas were still incomplete when he attended a conference in Stockholm in 1960. There he met Lehninger and quizzed him about several aspects of oxidative phosphorylation, then outside his own expertise. Not long after, Mitchell wrote a colleague about the value of the exchange. Lehninger, steeped in research on mitochondrial swelling and ion movements, was well primed to appreciate Mitchell's unconventional idea that electron transport might be coupled to proton movements across the mitochondrial membrane. In 1962, when most investigators were still unaware of Mitchell's ideas, Lehninger endorsed their significance in his review. He discussed them again in his 1964 book, lucidly explaining even Mitchell's generalized concept of vectorial metabolism.

Lehninger was not without criticism, however. Here, his important role was to stimulate further research. Lehninger — ever one to get the numbers straight — noted that Mitchell's original proposal did not properly account for the energy levels as actually measured. That prompted Mitchell and his colleague Jennifer Moyle to redo their measurements with more care (and ultimately to articulate the theory further). Lehninger also observed that according to Mitchell's model, based on membrane gradients, ox phos should occur only if the membranes remained intact and unbroken. Yet Lehninger's own submitochondrial particles, presumably membrane fragments, seemed obvious counterexamples. In response, Mitchell and Moyle

would soon argue, ultimately correctly, that the SMPs were indeed sealed vesicles, albeit everted from the mitochondrion's natural orientation. Closed compartments were indeed critical, as Lehninger had originally claimed.

Lehninger's lab also developed evidence for key chemiosmotic claims. In 1966 — when Mitchell's ideas were still largely considered peripheral — Lehninger, with Carlo Rossi and Jozef Bielawski, showed that electron transport (induced by calcium) led not only to an increase in external protons (already documented), but also to a decrease in internal protons: the net shift supported Mitchell's notion of a proton "pump," or the separation of H^+ and OH^- across the membrane. That same year Lehninger, Bielawski and Thomas Thompson addressed the functional role of membrane integrity. They showed that dinitrophenol, an insecticide regularly used to disrupt ox phos, increased the electrical conduction of simple membranes. That would, as Mitchell had claimed, allow protons to "leak" across the membrane, dissipating the postulated energy gradient and thereby interfering with ox phos. Lehninger's work thus provided important support for elements of chemiosmotic theory in its early development. Yet Lehninger himself guarded against broad theoretical conclusions.

Lehninger's caution was perhaps warranted, as illustrated in his later and perhaps most challenging work on characterizing the energy reactions of ox phos. In earlier work Lehninger had studied how electron transport could fuel both calcium movements and the production of ATP. In the early 1970s discrepancies on the magnitude of the intermediate proton gradient led him to reevaluate their relationship. In particular, Lehninger wondered whether undocumented ion movements invalidated earlier measurements of proton extrusion. Assisted by Baltazar Reynafarje and Martin Brand, he remeasured the proton movement with additional controls. Whereas earlier measurements had indicated two protons for each electron transport site, Lehninger now found an $H^+/2e^-$ ratio of three, possibly four. That would require radical revision in Mitchell's theoretical models. The team cross-checked the measurements with two other methods. They thoroughly analyzed the flaws in earlier methods. When they presented their findings at a conference in Bari, Italy, in 1975, Mitchell disputed them, remarking on the need to distinguish "fact" from "opinion." Lehninger felt his experimental competence had been challenged and his once cordial relationship with Mitchell soon became formal and strictly professional. The two tried to resolve their differences over the next decade. The mitochondrial reactions were quite complex and it was not easy finding methods and controls for calcium flux that satisfied everyone. Details were pursued on many fronts in many labs. By 1986 Mitchell had finally conceded. By then, over ten years later, the ox phos community had generated a much deeper and more robust characterization of proton movements. For example, the $H^+/2e^-$ ratio was found to differ for each site of electron transport, reflecting different mechanisms for translocating protons. The ultimate results certainly reflected Lehninger's lifelong standards for precision and experimental rigor.

The Mitochondrion: Calcium

As reflected in the controversy over proton measurements, biochemists recognized the important of calcium in the energy economy of the mitochondrion. The capacity of the mitochondrion to accumulate calcium was discovered in the early 1950s and its link to electron transport, in the early 1960s. Lehninger had encountered a role for calcium in ox phos as early as 1949, and then noted in 1956 that it did not affect his digitonin particles. Calcium uptake would affect osmotic balance, possibly accounting for the mitochondrial swelling that Lehninger observed. So, beginning in 1963, with students Carlo Rossi and John Greenawalt, Lehninger set about

precisely quantifying the energy used for calcium uptake. They dramatically confirmed the connection to electron transport. They also discovered that the energy from electron transport was more readily used for calcium transport than for ATP production. That was puzzling on the prevailing view of the mitochondrion as "the power plant of the cell." Calcium was not a mere peripheral reaction, Lehninger concluded, but likely integral to the mitochondrion's function. Interpreting the role of mitochondrial calcium became another major theme in his research.

In 1963 Lehninger's lab showed further that phosphate is taken up along with the calcium. Over the next several years they found that inside the mitochondria the two substances formed amorphous granules of tricalcium phosphate, a precursor to hydroxyapatite (the stuff of bones and teeth). That led to speculation about the role of the mitochondrion in regulation of cell calcium and perhaps in biological calcification.

For further clues, Lehninger turned to other species. One colleague suggested land crabs, as they salvage calcium from their exoskeletons before molting. Lehninger ultimately found the common blue crab more practical, since it was readily obtainable from a fish market on the Baltimore waterfront. In 1974 he, Chung-ho Chen and Gerald Becker found that the crab's liver mitochondria indeed concentrated a great deal of calcium. Moreover, as in rats, the cells stored calcium phosphate in an amorphous form and contained a substantial amount of tightly bound ATP. (ATP, biochemists later learned, helped bind calcium phosphate.) Lehninger and another student also showed that the mitochondrion could concentrate calcium carbonate, indicating its prospective contribution to calcification in mollusks and corals. The role of the mitochondrion in cellular calcium was phylogenetically widespread.

Another thread of investigation considered the fate of the mitochondrion's calcium phosphate granules: why did they not spontaneously crystallize, forming bone? A clue came unexpectedly from a colleague at the Johns Hopkins Medical School. His research indicated that patients with urinary stones seemed deficient in a calcification inhibitor normally present in urine. Lehninger saw the analogy with mitochondria: was a similar inhibitor at work? Several colleagues were indeed able to extract such a substance from both urine and the mitochondrion — and the blue crab as well — and show their similarities. In 1981, after the urine inhibitor was identified as phosphocitrate, Lehninger helped show that it could inhibit calcification in mitochondria *in vivo*.

Lehninger consolidated the available information into a prospective account of biological mineralization. He stressed the unique ability of mitochondria to concentrate calcium, along with phosphate, yet to not crystallize them irreversibly. However, Lehninger never saw evidence for his scheme fully realized. His work left important ideas (and measurements) for others to pursue.

Teacher, Author, Leader

Lehninger's research was paralleled by equally significant contributions in education and professional service. He led the Department of Physiological Chemistry at The Johns Hopkins University Medical School for twenty-five years. During his early years there, he helped modernize their teaching of medicine and strengthened the school's graduate program across all the sciences. In 1958 he earned the distinction of receiving the very first graduate teaching grant from National Institute of Health. Lehninger's skill for well organized and vivid lectures was renowned. He also understood unique teachable moments. On the first day of class one year, he set aside his prepared lecture to discuss how the newly announced discovery of reverse transcriptase would revolutionize biochemistry. In 1977 Lehninger was named University

Professor of Medical Science, a position created specially to honor his outstanding service.

Lehninger's influence extended well beyond his own students and home institution. In 1970 he wrote a textbook on *Biochemistry* that became the standard in the field for many years. It was translated into several languages. Over a half million copies of the first two editions were sold. Lehninger had become a writer after all, and reviewers acknowledged his extraordinary skills. They found the book "eminently readable" and "vibrant with scientific enthusiasm." "With a felicity of diction," one wrote, "he has woven a meaningful pattern into the fabric of the science which is characterized by beauty and accuracy." Even the opening lines evoked a sense of appreciation for the topic:

Living things are composed of lifeless molecules. . . . Yet living organisms possess extraordinary attributes not shown by collections of inanimate matter.

By the time a second edition appeared, the first edition had achieved "an almost unprecedented world-wide popularity and acclaim." His editor, too, was deeply impressed on another level with his "skill and attention to details of content, level of discussion, writing style, illustrations, and all the other elements that shape a book and determine its success." Lehninger was writing the third edition when he died. His book remained in print (with supplemental revisions, but still exhibiting Lehninger's character) at least two decades after his death.

Lehninger's skills in organization, his discipline, and his sense of thoroughness led to numerous leadership roles. At different times, he served on the editorial boards of eight journals, most notably the *Journal of Biological Chemistry* (1954-66) and the *Journal of Membrane Biology* (1968-1986). He served on various advisory groups, including panels at the National Academy of Sciences, a Presidential panel on biomedical research, and the board of trustees of his alma mater, Wesleyan University, as well as assuming leadership positions in several professional organizations. He was elected to the National Academy of Sciences (1956), the American Academy of Arts and Sciences (1959) and the American Philosophical Society (1970), and received seven honorary degrees, among numerous other awards.

Although congenial with students, Lehninger was a private person. When he died of complications from asthma, few colleagues even knew that he had managed the disease for many years. While he worked in downtown Baltimore, he resided with his wife and two children in a rural area north of the city. He enjoyed sailing on the Chesapeake Bay.

DOUGLAS ALLCHIN

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Lehninger's personal papers from 1952 to 1984 are archived at the Alan Mason Chesney Medical Archives, The Johns Hopkins Medical Institutions, Baltimore, MD. See URL:
www.medicalarchives.jhmi.edu/sgml/lehninger.html