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SAILING TO BYZANTIUM

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EARLY EDUCATION

I was born on September 4, 1919, in Chicago, the fourth of five children of Catherine Frawley Kennedy and Michael Kennedy. My parents were Irish immigrants who had been born within a few miles of each other in County Clare, near Miltown Malbay, but as was not infrequently the case with such couples, had not met before coming to America. My mother told me later that I had been named Eugene, not an uncommon name, after the great socialist

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Eugene V. Debs. This was not a political statement on her part; she simply liked the sound of the name.

My father was a motorman on the streetcars of the Chicago Surface Lines. With five children, and a motorman's salary of about $35 per week in the 1930s, our family was distinctly on the lower end of the lower middle-class. Although plagued by a chronic shortage of money, we were never really poor, as urban poverty is now known. My father's education had stopped at about the fifth grade; my mother had somewhat more schooling, with even a smattering of French. Both urged their children to strive for better education and better opportunities than they had had.

I began my education in Catholic parochial schools. I did not at first easily learn to read, but rather as many children do, tried to memorize the simple text of the primer rather than puzzling out the words. We had boxes of letters that we were told to arrange into words, and I distinctly remember the shock of recognition when it dawned on me that the letter "y" at the end of a word was pronounced "eee." Somehow that seemed the key to all of phonetics, and I was soon reading easily.

Not long thereafter, I was introduced to the local branch of the public library, a store-front operation on North Avenue, by my brother Joseph, six years older, and in this as in other ways a strong influence on my early life. He also lent me his "intermediate" library card so that I was not confined to the children's section in borrowing books. I became a voracious, indiscriminate reader. While still in grammar school, I happily read my way through a ton of junk, such stuff as the Fu Manchu novels of Sax Rohmer, the detective stories of S. S. Van Dine, all the available works of Zane Grey, James Oliver Curwood, and on a somewhat higher level, Jack London.

In 1933, having completed grammar school, I entered St. Philip High School, taught by priests of the Servite order, a few of whom made a lasting impression on me. The principal of the school was Father Wolfe, a fierce disciplinarian, who was held in awe and some terror by the students, not only from fear of corporal punishment, which he was not slow to administer, but also from a kind of intensity that seemed to simmer just below the surface of his personality.

My teacher of English was Father Peter Doherty. Sandy-haired, rather pale, he was soft-spoken, but given to outbursts of rage at infractions of order or defective preparation of an assigned task, so that even the most indifferent or refractory students took care not to come to class unprepared as they might in other courses. His instruction stressed fundamental English grammar, which was reviewed meticulously from start to finish, and expository writing. He was a regular reader of the *New Yorker*, then considered rather sophisticated fare, and was a strong advocate of the simple, clear prose for which the style of that magazine's E. B. White became celebrated. When I became editor of

the school newspaper, which he supervised, I saw a little more of him. As a year-end treat for the staff he took us to dinner in a downtown restaurant, followed by a play, *Three Men on a Horse*, the first I had ever seen. It was a bit strange to encounter him outside the context of the school in a more relaxed mood. Father Doherty was rumored to be a poet of considerable powers, but I never saw any of his verse. I had the feeling that he lived as much removed from his fellow Servites as from the students.

In 1937, I entered De Paul University, to which I had been awarded a scholarship. Located in Chicago, not far from Lincoln Park, it was known then as now principally for its basketball team. Nearly all of the students were residents of Chicago and continued to live at home, as I did, of economic necessity.

I enrolled as a chemistry major, although my strongest bent was toward literature. At that time, the Department of Chemistry was very small, with a staff of only three or four, headed by Malcolm Dull, who was, however a first-rate teacher of organic chemistry. Later I was to regret that, diverted by other pursuits including the editorship of the university newspaper, I gave only a divided attention to the courses in chemistry.

Events unfolding on the international scene during my college years, 1937–1941, were surely the most dismal of this century, perhaps of any century. A child of the Great Depression, I was an enthusiastic Roosevelt Democrat, and followed international politics closely, particularly of course after the beginning of World War II. It is hard now to convey the nightmare quality of May, 1940, as one watched, helpless, what appeared to be the inexorable triumph of Hitlerism in Europe.

After graduation in June 1941, I was uneasily aware that my training in chemistry had suffered from the diversity of my other interests. I decided nevertheless to enroll as candidate for the PhD degree in organic chemistry at the University of Chicago, which in that simpler time I did simply by writing a letter of application, together with a transcript of college credits. I was accepted also by mail, without the formality of a interview, which was perhaps just as well from the point of view of my candidacy. Of course, the Department of Chemistry was making no commitment whatever to me since I would be paying full tuition. If as a graduate student I sank, there was no loss to them; if I swam, so much the better.

THE PLASMA FRACTIONATION PROJECT

It was now necessary to find a full-time job. The expenses of college had been modest, because I had had a full scholarship and lived at home, but had nevertheless been a strain on the very limited resources of my family. Through an employment agency, I applied for a job in the chemical research

department of Armour and Company, one of the giant meat-packers in the world-famous stockyards that dominated the South Side of Chicago at that time. I was interviewed by Lemuel C. Curlin, a biochemist who had only recently received his PhD degree from the University of Chicago.

"What do you know about proteins?" he asked. I fleetingly considered assembling the few pitiful scraps of what I knew about proteins into some kind of a response, but fortunately opted for candor, and said firmly that I knew nothing about proteins. Curlin took this in good part, perhaps concluding that my answer indicated some sophistication rather than dismal ignorance as was the fact. He said that not much was known about proteins in any case, which was certainly true in 1941, and without further interrogation went on to outline the project for which he was assembling a team.

The laboratory of Edward J. Cohn in the Harvard Medical School, of which John Edsall has given a valuable account (1), was then one of the few great centers for the study of proteins. By 1941, many foresaw that the United States would soon be forced into war. The Cohn laboratory was diverted to an all-out effort to meet the anticipated need for blood plasma and plasma substitutes by developing new methods for the fractionation of plasma. Because serum albumin is the most abundant protein in plasma, it makes a major contribution to the maintenance of normal blood volume, a problem of special importance to patients in shock. The strategy therefore was to fractionate large volumes of plasma to isolate the serum albumin in purified, stable form to administer as needed under battlefield conditions. The fractionation procedure was further designed to make available the other valuable proteins of plasma, notably the gamma globulins and fibrinogen.

It was also hypothesized that bovine serum albumin might be an effective plasma substitute for administration to human subjects, based on the theory that, if absolutely pure and completely free from highly antigenic globulins, it so closely resembled human serum albumin that it would not provoke a dangerous immune response. This was the reason for the entry of the Armour laboratories into the picture. Through its pharmaceutical division, Armour had much experience in the fractionation of extracts of animal tissues on a large scale, and therefore undertook the large-scale fractionation of bovine blood with the aim of preparing bovine serum albumin of sufficient purity for adminstration to human subjects.

Curlin hired me as a member of his "plasma gang" at a salary of $40 per week, which I thought quite generous, because entry-level chemists with a B.Sc. degree at that time usually received $25–30. The job provided a jolting introduction to reality. The laboratories were located in the stockyards, a roaring bustling place. To reach the laboratories from Ashland Avenue one passed the loading-dock of one of the nation's largest knackers, specialists in converting the carcasses of animals condemned for use as food into soap and

fertilizer. On a summer day, the smell was staggering but was rivalled by the sight of scattered parts of animals, often the heads of horses and cattle, which had spilled onto the dock as they were unloaded from freight cars. When I see the paintings of Georgia O'Keeffe, depicting the spare, clean skulls of animals dried in the pure desert sun of New Mexico, I sometimes think that there can hardly be a more striking example of the transforming power of art.

The work that we underlings carried out on the plasma project was not intellectually demanding but required patience and care. The fractionation procedures, rigidly stipulated by the Cohn laboratory in Boston, were based on control of temperature, pH, ionic strength, and ethanol concentration. Much of the work was done at -5 °C in a large cold-room equipped with large Sharples continuous centrifuges and glass-lined tanks.

In September of 1941, I began graduate studies in chemistry at the University of Chicago. Work on the plasma project was now being pushed forward in shifts around the clock, and my new schedule found me working from midnight to 8:00 AM in the cold-rooms at Armour, followed by morning classes at the University of Chicago, not far distant. The folly of attempting such a schedule while I was acutely aware of the need for extensive review of my background in chemistry is apparent, but the very magnitude of the challenge lent it some of the glamor to which the young are so susceptible. I managed to get through the courses in organic chemistry which were prerequisite for the qualifying examination before beginning research, but I really enjoyed only one course, that in organic qualitative analysis. The instructor was a young chemist named Frank Westheimer. The star pupil was Dan Koshland, and the teaching assistant was Aaron Novick, who was given to cryptic hints as to the identity of the compounds we were required to identify. All three of course became leaders in biochemistry and molecular biology in their later careers, and our paths fortunately were to cross again and again.

Our entry into the war, followed by the almost unrelieved series of military disasters in 1942 that brought the United States and its allies to the very brink of defeat, greatly increased the urgency of the plasma project. Despite sporadic encouraging results, by the end of 1942, hope had almost faded that bovine serum albumin would provide an effective treatment for human patients in shock. The Red Cross was now engaged in collecting blood from human volunteers on a vast scale, and it was decided that Armour would open a new facility in Fort Worth, Texas, where it already had extensive meat-packing operations, for the fractionation of human blood from donors in a large catchment area of the Southwest.

I was one of the team sent to Fort Worth in October, 1943 to begin this new effort. Fortunately, I was soon joined by Adelaide Majewski, who had been a classmate in college, and we were married in Fort Worth on October 27, 1943. Our family was later to be completed by the births of Lisa (1950), now

a lawyer and married to the writer Mark Helprin; Sheila (1957), now an architect and married to Frano Violich, also an architect; and Katherine (1960), now a lawyer married to Matthew Diller, also a lawyer.

The brand-new laboratory at Fort Worth was equipped with huge cold-rooms, containing glass-lined tanks with capacities up to a thousand liters, and was expressly designed for fractionation of plasma by the Cohn procedures. As in Chicago, we worked in three shifts around the clock, and my task was to supervise the work of one such shift, staffed mostly by women recruited from the local labor force, and presumed to have some minimum training in chemistry. Some proved to have only a very limited grasp of the fundamentals of chemistry and required very close supervision, but were highly motivated and conscientious.

As in Chicago, the work offered little opportunity for real research. I was, however, given some scope in the design of large-scale procedures for the production of fibrin foam, sponge-like materials designed for use by neurosurgeons in the treatment of massive head-wounds to control bleeding and to promote healing. Never an admirer of high technology, I managed to do this with a minimum of expense. I was particularly pleased with an apparatus that I designed for the large-scale lyophilization of fibrin foams, which had been assembled in the local Armour shop for a few hundred dollars, equipped with a thermocouple device that I assembled myself to follow the internal temperature of the foams, and thus their moisture content. When the chief engineer on a inspection visit from headquarters in Chicago saw this make-shift apparatus, his face plainly revealed what he thought of it, although he could hardly object since it was working quite well. I later surmised that part of his displeasure arose from the fact that I had spent only a very small fraction of the funds that he had earlier estimated would be needed.

In the spring of 1945, World War II was clearly approaching its end, and large amounts of human plasma proteins had been stockpiled. I returned to Chicago, where I began work on a quasi-independent project on the crystallization of enzymes (particularly pepsin) in which the company was interested.

GRADUATE STUDIES: MITOCHONDRIAL FUNCTION

With the generous support of my supervisors, James Lesh and Jules Porsche, I was now able to arrange a much more flexible schedule to pursue my studies at the University of Chicago. I immediately transferred from the Department of Chemistry to the Department of Biochemistry. Experience on the plasma project, lowly as it was, had offered some glimpse of the fascination of biochemistry.

The Department of Biochemistry at the University of Chicago at that time

was headed by Earl Alison Evans Jr., who in 1942 at the age of 32 had succeeded F. C. Koch as chairman. Evans had assembled a brilliant group of colleagues, including Konrad Bloch, Herbert Anker, Albert Lehninger, Birgit Vennesland, Frank Putnam, Hans Gaffron, James Moulder, and until his untimely death in a mountaineering accident, John Speck. Like Bloch and Anker, Evans was a product of Hans Clarke's great Department of Biochemistry at Columbia University, one of the earliest centers for the application of the isotope tracer technique, which was soon to transform biochemistry. Evans later worked with Hans Krebs on the newly formulated Krebs tricarboxylic acid cycle. He had become well known for his studies on the fixation of carbon dioxide in animal tissues. While continuing work along these lines, his laboratory was making a transition to the study of the replication of bacteriophage, thus revealing in the 1940s a remarkable insight into the importance of an approach that was to prove to be one of the precursors of molecular biology, a discipline then still slouching toward Bethlehem to be born. Evans, a widely cultured and urbane man, rather formal in speech and manner, was held in some awe by students in the Department.

It is not easy to convey the heady sense of excitement and adventure that caught up those entering biochemistry at that time. The feeling was that of an intellectual gold rush. Everyone was interested in and more or less knowledgeable about the work of everyone else. At Chicago, Konrad Bloch had begun the series of studies on the biosynthesis of steroids that was to win him a Nobel prize. Herbert Anker, whose critical insight and remarkable technical abilities have perhaps never been sufficiently recognized, was also working on the biosynthesis of lipids but was further attempting to develop approaches to probe the biosynthesis of proteins, an enterprise in which he was soon to be joined by Howard Green. Bloch was also interested in protein synthesis, and began the studies that led to his elucidation of the biosynthesis of the tripeptide glutathione, perhaps as a kind of warm-up for the main event. Lehninger, deeply immersed in his studies of oxidative phosphorylation and fatty acid oxidation, had very wide interests and regarded the problem of photosynthesis, then under vigorous attack at Chicago by Hans Gaffron and James Franck, and later by Birgit Vennesland, as one of the central problems of biology.

Graduate students in the Department worked for the most part in a single large laboratory on the third floor of the Abbott building rather than in the laboratories of their mentors. They were a lively group, including at this time Irving Zabin, Eugene Goldwasser, Robert Johnston, Lloyd Kozloff, and Ray Koppleman. Morris Friedkin, another graduate student, was a teaching assistant in the introductory biochemistry course that I took. His abilities and personality, marked by a kind of sunny integrity, greatly impressed me. He

and his wife Bert, and Adelaide and I became fast friends, and have remained so for almost five decades. Friedkin had also been involved in war work, on the development of penicillin production, but was a year or so ahead of me in graduate studies and had already begun a research project in Lehninger's laboratory.

In 1947, when I was ready to begin research for a dissertation, upon Friedkin's recommendation, I went to Lehninger to discuss the possibility of working under his direction. With staggering naiveté, I suggested to him that the proper approach would be to purify the various enzymes undoubtedly involved in fatty acid oxidation and crystallize them. He agreed that this would be desirable, but went on to point out rather gently that fatty acid oxidation had not yet been demonstrated in a soluble extract from which individual enzymes might be isolated. To reach that stage, it would first be necessary to discover the nature of the energy-requiring activation or "sparking" of fatty acid oxidation and the special dependence of the process on particulate structures. He agreed, however, to take me on as a graduate student to work on this project, perhaps in part because he felt that experience in the fractionation of proteins might in fact be useful at a later stage in the research.

Lehninger was only a few years older than Friedkin and I, his only students. Although he held an appointment in the Department of Biochemistry, his laboratory was in the Department of Surgery, to which he had been recruited from the University of Wisconsin by Charles Huggins at the recommendation of Earl Evans. This was the beginning of the research enterprise fostered by Huggins that was to grow rapidly and culminate in the establishment of the Ben May Laboratory for Cancer Research. Huggins, already a famed cancer researcher, and a brilliant, idiosyncratic and sardonic man, was a formidable figure to a neophyte entering his domain. He possessed and cherished a semi-micro balance, the only one available, which was kept not in the laboratory but in his own office. It was a bit of a test to knock, enter, and weigh out a reagent under his watchful eye.

Lehninger had set Friedkin to work on the problem of oxidative phosphorylation, then one of the most baffling in all of biochemistry. Friedkin was able to show clearly that the reoxidation of reduced carriers such as NADH was linked to the phosphorylation of ADP to ATP, an important conceptual advance (2). To do so, he used $^{32}P_i$ as a tracer, and found that it was converted not only to ATP, but also further metabolized in this cell-free preparation, with the formation of labeled phospholipid, nucleic acid, and phosphoprotein (3). Friedkin had uncovered a marvelous set of leads for explorations in biosynthesis, but some time was to pass before they were explored.

On June 30, 1947 I began work on the problem of fatty acid oxidation. The

central approach to intermediary metabolism in that day was through the measurement of oxygen uptake or carbon dioxide production in a highly sensitive manometric procedure brought to perfection by Otto Warburg. The Warburg apparatus was then the centerpiece of nearly every laboratory. It is now as defunct as the dodo, its passing yet another reminder of the rapid obsolescence of experimental techniques in biochemistry. Our enzyme source was rat liver, and because of the great lability of the systems under study, it was necessary to prepare fresh enzyme every day. Killing the rats was a necessary but odious task, to which my experience collecting blood in a slaughterhouse had by no means inured me. Although Lehninger had become allergic to rats, and had already begun to suffer from the asthma that was so serious an affliction in his last years, on that first day, he prepared the enzyme and taught me the art of Warburg manometry.

Lehninger had a very clear idea of the central importance of structure in the biological processes we were studying, and long before methods became available for the isolation of functionally as well morphologically intact mitochondria, he was convinced that mitochondria were in fact the organelles in which the oxidative and energy-conserving systems were localized. He had read and also set me to reading much of the old literature on mitochondria. One of the clues guiding Lehninger's thinking arose from his observation that both fatty acid oxidation and oxidative phosphorylation were inhibited in strikingly parallel fashion by exposure of our particulate enzyme preparations to hypotonic buffers. The activity could be preserved by adding to such buffers either salts such as KCl or iso-osmotic amounts of sucrose. My first project in Lehninger's laboratory (4) was a detailed study of these effects, which led us to surmise that fatty acid oxidation, oxidative phosphorylation, and the accompanying reactions of the Krebs tricarboxylic acid cycle must all be taking place in a single organelle, bounded by a membrane impermeable to certain solutes. Although our particulate preparations were still quite crude, we were convinced that this organelle was the mitochondrion.

Palade and his collaborators in the meantime were developing the methods for the separation and identification of cell organelles that were to prove so important in linking biochemistry to cell biology, work which was later to be recognized by a Nobel prize. They described a procedure for the isolation of purified mitochondria by differential centrifugation in 0.88 M sucrose (5). I immediately tested mitochondria isolated by this method and obtained convincing evidence that oxidative phosphorylation, fatty acid oxidation, and the reactions of the Krebs tricarboxylic acid cycle are indeed localized in mitochondria (6, 7).

I also worked hard on the problem of fatty acid oxidation. Previous work by Leloir as well as by Lehninger showed that the initiation of fatty acid oxidation requires metabolic energy, presumably in the form of ATP. It was

further known on the basis of evidence dating from the classical studies of Knoop that degradation of fatty acids via beta-oxidation led to the formation of a "two-carbon unit" or "active acetate," which could either be oxidized via the Krebs tricarboxylic acid cycle, or yield acetoacetate, long known to be a characteristic end-product of fat metabolism under certain conditions. A shrewd suspicion was also spreading that Lipmann's coenzyme A must be involved, but the pure cofactor was not available and there was still much uncertainty about its structure.

My studies consisted principally of a detailed examination of the conditions regulating the relative yield of acetoacetate and of carbon dioxide during the oxidation of fatty acids of varying chain-length (8).

WORK WITH H. A. BARKER AT BERKELEY

During my last year of graduate study, I obtained a fellowship from the Nutrition Foundation upon the recommendation of Lehninger. This enabled me to apply for a leave of absence from Armour, which was generously granted, to work full time on research for the dissertation. Up to this time, I had not very seriously considered the possibility of an academic career, but Lehninger now suggested that I undertake a year of postdoctoral study, which entailed resignation from Armour and considerable uncertainty for the future. He recommended that I apply to the laboratory of H. A. Barker, at the University of California in Berkeley. He greatly admired Barker, whom (I believe) he had not yet met, and said: "Whatever he touches turns to gold," a judgment later amply confirmed. Barker and his brilliant student Earl Stadtman had just set the world of enzymology on its ear with their discovery of an extraordinary enzyme preparation derived from cells of the obligate anaerobe *Clostridium kluyveri*, an organism discovered by Barker.

Stadtman and Barker discovered that soluble extracts of dried autolyzed cells of *C. kluyveri* when shaken in air would oxidize fatty acids of chain-length up to eight carbon atoms to a mixture of acetyl phosphate and acetate (9). Acetyl phosphate had been found by Lipmann (10) to be a form of "active acetate" in bacteria, but (disappointingly) not in animal tissues. The same enzyme preparation, when shaken under an atmosphere of hydrogen, would resynthesize short-chain fatty acids from acetyl phosphate and acetate! To those toiling away at comparable problems with feeble and unstable preparations from animal tissues, this seemed almost too good to be true, like the legendary Shmoo in the comic strip, which when fried, tastes like chicken and when broiled, tastes like steak . . .

I was accepted by Barker for a year's study, and my wife and I drove west from Chicago in a new automobile, picked up from a dealer in Chicago to be delivered to another dealer in San Francisco. This was a common mode of

shipping new automobiles to the West Coast in those days, and since even the cost of gasoline was repaid by the dealer, it represented the cheapest mode of travel, an important consideration for us. The drive itself was a memorable experience, through parts of the country we had never visited, and we found Lake Tahoe, then almost pristine and now so sadly overdeveloped, and the mountains around it especially attractive.

Arrived in Barker's laboratory, not much larger than Lehninger's, I found him to be a mild-mannered, soft-spoken man, who with all his great abilities was simple, direct, and friendly. Barker and his wife Margaret were warm and generous in their hospitality to Adelaide and to me, as they were to others who worked in his laboratory. I later found that Barker rarely appeared at national meetings, avoided service on committees, and was completely devoid of the arts by which some workers in science think it necessary to attract notice. It is pleasant to observe that these traits have not prevented the wide recognition of his work.

Barker asked me to study the oxidation of butyrate in extracts of *C. kluyveri* in greater detail. In Stadtman's experiments, the oxidation of fatty acids had been completely dependent upon the addition of inorganic phosphate. With the extracts I studied, however, the oxidation of butyrate proceeded in the absence of added phosphate at about one-third the maximum rate. This might perhaps be thought to reflect the presence of low levels of phosphate in the enzyme preparation itself, but I also found that the product of oxidation of butyrate in the absence of phosphate was acetoacetate, not acetyl phosphate (11). This was interesting because Stadtman had shown that neither free acetoacetate nor β-hydroxybutyrate were intermediates in the oxidation of butyrate. This led us to formulate a scheme, which also fit much that was known about β-oxidation in animal tissues, in which a derivative of acetoacetate (designated CH_3COCH_2CO - X) was the true intermediate. In the presence of phosphate, it could be converted very rapidly to acetyl phosphate. In the absence of phosphate, it could be hydrolyzed more slowly and irreversibly to free acetocetate. We suggested that X represented CoA, but definitive evidence on this point was tantalizingly elusive.

In 1949, Berkeley was already a great center for the biological sciences. Through Barker, I became acquainted with his colleagues Michael Doudoroff and Zev Hassid, who had worked with him on the study of sucrose phosphorylase, which had won them a prize awarded for a successful synthesis of sucrose, which was indeed catalyzed by their enzyme, although not alas on a scale that could compete with the processing of sugar cane. Roger Stanier, like Barker a representative of the van Niel school of microbiology, was also an occasional visitor to the laboratory. Paul Stumpf had laboratory space directly adjacent to Barker. He had just bought a new house perched on a hill at the edge of Berkeley. When the winter rains began, the hillside began to

slide, and for a time, each morning began with an appraisal of the ominous weather reports and the progress of the erosion. The house finally had to be abandoned, but fortunately Paul did not have to bear the entire financial loss.

During this time, my wife Adelaide and I lived in San Francisco where she had a job on the *San Francisco Chronicle*, working in the so-called morgue, where prewritten obituary notices of notable people were filed, ready for publication as needed. I commuted to Berkeley each day on the interurban trains that then linked Berkeley and San Francisco via the Bay Bridge. San Francisco was experiencing a mild economic downturn after the boom period of war. We were able to rent an apartment atop Russian hill for $60 per month. Restaurants in the largely Italian area around us offered delicious meals for one or two dollars. We enjoyed the city thoroughly.

At this point, quite out of the blue, a letter arrived from William Mansfield Clark, head of the Department of Physiological Chemistry at Johns Hopkins Medical School, offering me a job as instructor in his department. In those innocent days, such an offer could be made without an interview, and without elaborate screening by committees. The work on mitochondrial function had attracted some attention, and presumably I had also been recommended to him by someone at Chicago. If I were indeed to undertake an academic career, this seemed to be its start. I sought Barker's advice, however, and was surprised to hear him suggest that I decline the offer and go instead to Lipmann's laboratory for another year of postdoctoral work, suggesting that it would not be difficult to find another job at the end of that time. There was a strong link between Lipmann and Barker, who at one time had worked in Lipmann's laboratory, and had recommended Stadtman to him. Lipmann was already recognized as one of the great figures in biochemistry, and I gratefully accepted Barker's good offices in arranging for me to work with Lipmann.

WITH FRITZ LIPMANN AT THE MASSACHUSETTS GENERAL HOSPITAL

In 1950, when I joined Lipmann's laboratory, Earl and Thressa Stadtman had just departed to take up positions at the National Institutes of Health (NIH). The group then included David Novelli, John Gregory, Morris Soodak, Harold Klein, Charles Du Toit, and Lipmann's research assistant Ruth Flynn. We were crammed into a tiny laboratory in the Massachusetts General Hospital next to the famous Ether Dome, the scene of the first (or perhaps only almost the first) use of diethyl ether as an anesthetic. In the course of the year, we were to move into much more spacious, even elegant, quarters in a new research building.

With abundant hair just becoming a little gray, and usually wearing a soft bow tie, Lipmann presented a figure closer to the stereotype of the artist than of the scientist. He spoke softly, and his sentences often trailed off into the

distance. He thought not only in terms of the essential technical details of an experimental approach but also deeply about the fundamental biology underlying a problem. He was originally trained as a physician, and I believe the breadth of this training helped give him the feeling that nothing in biology was beyond his range. Again and again, he proved ready to tackle new problems, no matter how far removed from previous work in his laboratory.

Lipmann had a great respect for the chemical approach to biology, and perhaps regretted his own rather limited background in chemistry of which he made nevertheless quite effective use. He discovered the role of acetyl phosphate as a product of the oxidation of pyruvate and an "energy-rich" precursor of ATP in bacterial extracts, not by attempting the isolation of small amounts of the labile compound from the bacterial enzyme preparations, but by synthesizing acetyl phosphate, which he then added to the enzyme preparation and showed that it was a precursor of ATP (10). I imitated this strategy in later research of my own.

Lipmann's manner toward those who worked in his laboratory was rather formal. He was friendly, but a little aloof. He inspired nevertheless not only loyalty and admiration but also lasting affection in those who worked under his direction.

Loomis & Lipmann (12) had discovered that 2,4-dinitrophenol prevented the phosphorylation of ADP to form ATP and at the same time stimulated the rate of oxygen uptake of cell-free preparations containing mitochondria. It was soon learned that a large number of other compounds, some not obviously related in structure to 2,4-dinitrophenol, also had this uncoupling effect, which Lipmann rightly regarded as fundamental to an understanding of oxidative phosphorylation. The uncoupling phenomenon was later to be brilliantly explained by Mitchell (13) as an important part of his formulation of the chemiosmotic theory of oxidative phosphorylation.

Lipmann had noted with interest that di-iodophenols were uncoupling agents similar to dinitrophenols, and had the idea that the hormonal action of thyroxine, with its di-iodophenol residues, might be due to its action as an uncoupling agent. Indeed, poisoning of human subjects with 2,4-dinitrophenol induces heightened metabolic rate and wasting of tissue somewhat similar to that seen in hyperthyroidism. Further, there was some evidence that treatment of mitochondria with very high levels of thyroxine in vitro sometimes brought about uncoupling. Lipmann therefore suggested that Du Toit and I look for an uncoupling action of thyroxine in intact animals and in isolated mitochondria, respectively. We could find no evidence to support Lipmann's idea. Du Toit's experiments I thought had special force. Mitochondria from the tissues of rats made severely thyrotoxic by the adminstration of huge doses of thyroxine were not significantly uncoupled, nor did tissue slices from such animals incorporate amino acid into protein at a lower rate. Lipmann readily accepted these negative findings, but was charac-

teristically unwilling to discard completely an idea that he felt had appealing elements. He would concede the validity of our data, and then at the end of a long discussion would murmur: "But still . . . but still . . ."

Lipmann also had the notion that there might be two distinct pathways of electron transport in mitochondria, a pathway coupled to phosphorylation, and another for uncoupled oxidation. Britton Chance was the master of the spectroscopic methods that might perhaps detect differences in the putative two pathways. Arrangements were made for me to go to Chance's laboratory in Philadelphia to test this idea. I believe that I was the first person in Chance's laboratory to prepare mitochondria, later to be so important a subject of his research. Certainly I had to borrow most of the needed equipment from other laboratories. The spectral studies were done under the tutelage of Chance's colleague Lucille Smith. The spectrophotometer was in an improvised dark-room not much larger than a closet, closed off by heavy, dark curtains. It was midsummer in Philadelphia, without air-conditioning, and sweat dripped from my face as we made the readings. We could find no evidence for spectral differences between intact and uncoupled mitochondria. Years later, when Lucille Smith introduced me in a seminar at Dartmouth, I startled the audience by informing them that she and I had once spent a week together in a small room in Philadelphia.

Although Lipmann was very interested in the work on oxidative phosphorylation, the principal focus of the laboratory was on the isolation and characterization of coenzyme A, on which progress was discouragingly slow. We were convinced that "active acetate," of central importance to intermediary metabolism, was an adduct of some kind of acetate and CoA, but every effort to isolate it was unsuccessful. Because acetyl phosphate, known to be active in bacterial systems, is quite labile, we surmised that acetyl CoA, whatever its structure might be, was even more labile, and this supposed lability seemed to stand in the way of most schemes we tried to design to isolate it.

Pursuing a habit of wide, although rather haphazard, reading I came one day upon an article in *Angewande Chemie* (14) from the laboratory of Feodor Lynen, then unknown to me. He and his student Ernestine Reichert reported definitive evidence for an essential sulfhydryl residue on CoA, and had isolated acetyl CoA and proved it to be a thioester! I brought the article at once to Lipmann, who apparently had no previous notice of this work. Although the Munich laboratory had obviously stolen some of his thunder, Lipmann was generous in his praise of the work. He was particularly impressed by the fact that in isolating acetyl CoA from yeast, they had begun by boiling the yeast. So much for the hyperlability that we had so much feared! We should have realized, Lipmann pointed out, that an intermediate that plays such varied roles must have a certain stability.

RETURN TO CHICAGO: THE PHOSPHORYLATION OF PROTEINS

In 1951, after a year in Lipmann's laboratory, I returned to the University of Chicago with a joint appointment in the Department of Biochemistry and in the newly organized Ben May Laboratory for Cancer Research, founded by Charles Huggins with the financial support of Mr. Ben May of Mobile Alabama. Lehninger, who was a leading member of the new Ben May research group, was about to depart for a sabbatical year in England, and made his laboratory as well as his research assistant Sylvia Smith available to me during his absence, while space that I would later occupy was to be readied on another floor. While on sabbatical leave, however, he accepted an invitation to succeed William Mansfield Clark as De La Mar Professor and head of the Department of Physiological Chemistry at the Johns Hopkins Medical School, and I inherited his facilities on a more permanent basis.

Charles Huggins had gathered into the Ben May a number of talented young people, including Paul Russell, Paul Talalay, Guy Williams-Ashman, Jean Sice, and Elwood Jensen. The atmosphere among these juniors was easy and friendly, and enthusiasm for research was high. Huggins, in his 90th year and still active at the time of this writing, was then barely 50 but was already "the old man," around whom legends were beginning to gather. He was master of a deadpan delivery, which added effect to occasional outrageous pronouncements on subjects sacred or profane. Himself completely committed to research, he was caustic in his appraisal of others he thought to be less so, and particularly scathing in his dissection of a colleague in another department who was said to spend some time each day on his stamp collection. Huggins was helpful and generous to the young scientists in the Ben May, and strongly fostered their careers. In a more Puckish mood, however, he had a trick of treating someone with mockingly exaggerated courtesy and flattery so overblown that it made its object squirm. It was more pleasant for the junior staff to be invited to drink tea with him, one on one. Tea would be brewed by him in his laboratory, directly connected to his office, in beakers, accompanied by sugar in paper packets thriftily saved from airline dinners. He would inquire about one's research, which he clearly followed with great interest, and tell about his own, which had two notable themes: the hormonal regulation of tumor growth (for which he was to receive a Nobel prize in 1966) and the induction of tumors in experimental animals by highly specific carcinogens. Although he had many irons in the fire, as head of the Ben May and professor of surgery with continuing involvement in the care of patients, often critically ill with cancer of the breast, he continued always to do research with his own hands. Like many other surgeons, he was an early riser, and had a great part of a day's work accomplished by the time we later arrivals

appeared. He always worked on Sunday morning, and perhaps took pleasure in the absence of weekday bustle. Because we all lived within an easy walk of the laboratory in the Hyde Park district of Chicago, many of the rest of us also turned up on Sunday, although less regularly, and it was felt that this was a good time to approach Huggins with some special request.

Following some of the leads that had emerged from Friedkin's work, I began a study of the incorporation of $^{32}P_i$ into phosphoproteins catalyzed by cell-free, particulate preparations from liver and from tumors. Some credence was still given to the doctrine of Otto Warburg that the energy metabolism of tumors was largely anaerobic. To test this idea, Williams-Ashman and I (15) carried out a study of oxidative phosphorylation in particulate preparations from highly malignant tumors, and found them to be almost as active as those from normal tissues. Further, tumor preparations also actively incorporated $^{32}P_i$ into lipid, nucleic acid, and phosphoprotein. The specific activity of the phosphoprotein fraction was an order of magnitude higher than that of the other fractions, which I took to be clear proof of the biological importance of the phosphorylation of proteins. I undertook a further study, and found that only phosphoserine could be identified amongst the labeled products of the partial acid hydrolysis of phosphoproteins derived from Ehrlic ascites cells (16).

George Burnett and I then searched for an enzyme that would catalyze the phosphorylation of proteins, and found that a soluble enzyme could be extracted from acetone powder extracts of our particulate enzyme preparations, and partially purified by ammonium sulfate fractionation (17). The enzyme was quite specific for casein, and we further found that casein stripped of its phosphate by treatment with alkali under relatively mild conditions was inactive, although its primary structure must have remained largely intact, suggesting that specific conformations as well as sequences were recognized by the enzyme. This was the first demonstration of a protein kinase. In the discussion we stated: "The physiological significance of this new type of enzymatic action still remains to be discovered. The great biological reactivity of the phosphoproteins of a wide variety of tissues, particularly tumors, as evidenced by the very high rate of renewal of phosphoprotein phosphorus appears to point to some role of importance." I dropped the study of protein kinases, and like the base Indian, cast a pearl away, else richer than all his tribe.

THE BIOSYNTHESIS OF PHOSPHOLIPIDS IN ANIMAL TISSUES

A brief account of the origins of the work on phospholipid biosynthesis has been given elsewhere (18). My interest from the start was centered on the

mechanism of formation of the phosphodiester bond of phosphatidylcholine because of the hope that it might shed light on the formation of the phosphodiester bond in nucleic acids. Arthur Kornberg had the same idea, and for a time his studies on phospholipid biosynthesis ran parallel to my own.

The incorporation of ^{32}P into phospholipid in our preparations was greatly stimulated by the addition of glycerol, an observation that promptly led to the finding that *sn*-3-glycerophosphate was an intermediate in the process (19), which in turn led to the discovery of the enzyme glycerokinase (20). The principal labeled phospholipid formed in this system was phosphatidic acid. The important studies of Kornberg & Pricer (21) established that acyl CoA was the activated form of fatty acid for the acylation of glycerophosphate.

An essential first step in the biosyntheses carried out by our type of enzyme preparation was the conversion of labeled phosphate to ATP via oxidative phosphorylation, and this of course required mitochondria, leading to the mistaken impression that the subsequent biosyntheses were also mitochondrial. Only later did it become clear that microsomes, derived from endoplasmic reticulum, and also present in these enzyme preparations, were a major, although not the sole, site of phospholipid biosynthesis.

In 1952, I began to study the origins of the phosphodiester bond of phosphatidylcholine in investigations with labeled choline, and immediately fell into error, from which I was later fortunately able to retrieve myself. I found that free choline, but not phosphocholine, was converted to lipid in an enzymic reaction dependent upon the generation of ATP via oxidative phosphorylation (22). The lipid product cochromatographed with phosphatidylcholine on columns of alumina, and was alkali-labile, and therefore not sphingomyelin, the only other lipid form of choline known at that time. I therefore concluded, wrongly as it turned out, that the labeled product was phosphatidylcholine. Only later did I establish (23) that the labeled product was in fact a long-chain fatty acyl ester of choline, the exact biological function of which remains uncertain.

In the meantime, Kornberg & Pricer (24) had reported experiments in which phosphocholine, doubly labeled with ^{32}P and ^{14}C, was converted to phosphatidylcholine with little alteration of the isotope ratio, in a reaction that required added ATP. Their formulation of the synthesis of phosphatidylcholine was:

$$\text{Phosphatidic acid} + [^{32}P]\text{phosphocholine} \rightarrow [^{32}P]\text{phosphatidylcholine} + P_i \qquad 1.$$

This was the state of affairs in July 1954, when Samuel Weiss, my first postdoctoral fellow, arrived on the scene. Sam proved to be a gifted re-

searcher, as was to be amply confirmed by his later brilliant studies leading to the discovery of DNA-directed RNA polymerase in animal tissues and in *Micrococcus luteus*. We decided to make a detailed examination of the difference between my previous studies of choline incorporation and those of Kornberg & Pricer. ATP in our system was generated from added AMP via oxidative phosphorylation. Sam first carefully checked to make sure that oxidative phosphorylation was in fact generating ATP and that under these conditions phosphocholine could not be converted to phospholipid. When ATP was added from the bottle, however, phosphocholine was indeed converted to phosphatidylcholine, confirming the findings of Kornberg & Pricer.

ATP was needed at very high levels for the incorporation of phosphocholine at maximum rates, giving rise to our suspicion that it might contain some more active impurity. Crystalline ATP had just come on the market, and we were able to obtain some through the generosity of Drs. S. A. Morrel, S. Lipton, and A. Frieden of the Pabst Laboratories. It was quite inactive, making it certain that some impurity in the amorphous ATP was the true cofactor. At this point, we considered fractionating the amorphous ATP to isolate the active substance, but decided first to test other nucleoside triphosphates, then rare substances. CTP proved to be the active triphosphate.

We formulated many schemes that might account for the participation of CTP. By analogy with the uridine coenzymes then only recently discovered by Leloir, the intermediary formation of cytidine diphosphate choline seemed an attractive candidate. Just at this time, again through rather random reading, I came upon a paper from Khorana's laboratory (25) describing an elegant new method for the synthesis of ATP by the carbodiimide method, which offered a new approach to the synthesis of substituted pyrophosphates. It occurred to me that it might be applied to the synthesis of asymmetical, di-substituted pyrophosphates such as cytidine diphosphate choline. At this stage, we had no evidence whatever that CDP-choline was in fact involved, and the synthesis of the reagent, dicyclohexylcarbodiimide, required several steps that might be time-consuming for a chemist of my limited skills. On the other hand, I was encouraged by our success thus far in arriving at the truth by conjecture, and both ^{32}P-phosphocholine and CMP needed for the synthesis were already available.

I was deeply involved at a critical point in my first attempt to prepare dicyclohexylcarbodiimide when I received an urgent telephone call. It was my wife Adelaide's mother, babysitting with our daughter Lisa, and she informed me that the house was on fire! I lived in an apartment in a house on Drexel avenue, owned by the University, only a few hundred yards from the laboratory. I raced down the street, lab coat flying, only to find that Adelaide had just returned. She and her mother and Lisa were on the sidewalk outside the house. Fire trucks were arriving, and the small blaze in the basement caused

by an overheated furnace was soon extinguished. After hasty greetings to my family, I raced back to the laboratory and finished the preparation without further incident.

CDP-choline, synthesized by the carbodiimide method (26), proved to be the key intermediate in the biosynthesis of phosphatidylcholine that we had been seeking (27). We immediately synthesized CDP-ethanolamine, although in very poor yield, and showed that it was similarly the precursor of phosphatidylethanolamine. A little later, Henry Paulus synthesized CDP-diacylglycerol by a similar approach (28), and showed that it is the activated form of phosphatidic acid and the essential intermediate needed for the synthesis of phosphatidylinositol, as Agronoff (29) had earlier suggested. In each case, the coenzyme was synthesized before it was isolated from nature. As a happy consequence of this small venture into nucleotide chemistry, I became acquainted with Gobind Khorana, whose procedures I had adapted, and whose friendship I have valued now for many years.

The pace now quickened, and a rather complete picture of the biosynthesis of phosphatidylcholine and of phosphatidylethanolamine began to emerge (Figure 1). An important piece of the puzzle was the function of a specific phosphatase (30) that catalyzed the dephosphorylation of phosphatidic acid, the precursor of all other glycerophosphatides, to generate *sn*-1,2-diacylglycerol, which Sam Weiss showed was the precursor of triacylglycerol (31) as well as of glycerophosphatides. Shortly thereafter, Weiss left the laboratory to work for a time with Fritz Lipmann, to whom I had enthusiastically recommended him. He was to return to the University of Chicago in 1959 to begin his pioneering studies on RNA synthesis.

In 1959, while I was on sabbatical leave at Oxford, I was invited to succeed Baird Hastings as Hamilton Kuhn professor and head of the Department of Biological Chemistry at the Harvard Medical School, an offer which, attractive as it was, I accepted only after considerable soul-searching. I felt strong affection for the University of Chicago, which when I became a student in 1941 could claim with some plausibility to be the nation's leading university, and I realized how much I owed to the support not only of Lehninger and of Huggins but also of Evans, to whose Department of Biochemistry I had moved in 1956.

The move to Harvard was considerably eased by the kind agreement of Louise Fencil Borkenhagen, my colleague at Chicago, to come with me for a few months to help set up the laboratory and to transplant to it the research we had been pursuing on the biosynthesis of phosphatidylserine, which stubbornly resisted our efforts to fit it into the same pattern as that established for phosphatidylcholine and phosphatidylethanolamine. Our search for a serine kinase, expected by analogy with choline and ethanolamine kinases, had instead led to the discovery of a specific phosphatase that converts

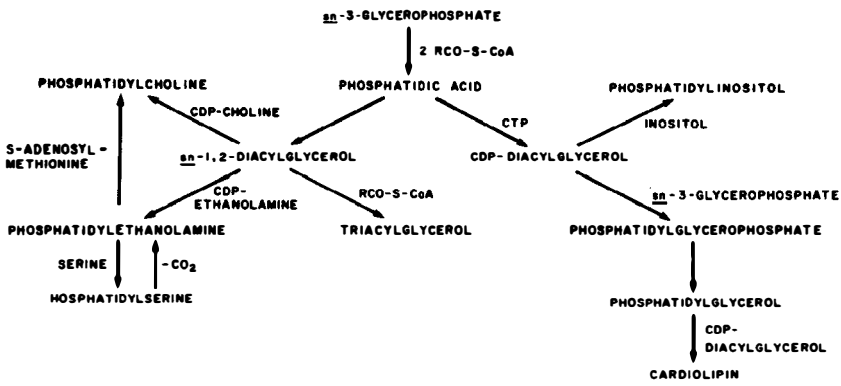

Figure 1 Biosynthesis of phosphatidylcholine and phosphatidylethanolamine

phosphoserine to serine, the final step in the biosynthesis of serine. The phosphatase is inhibited by serine, which it readily equilibrates with phosphoserine, simulating the action of a kinase (32). We could find no evidence for a requirement of cytidine nucleotides for the conversion of serine to phosphatidylserine, which we found to occur instead by an exchange of free serine with the ethanolamine moiety of pre-formed phosphatidylethanolamine. By now thoroughly convinced that even a modest investment in organic synthesis might pay big dividends in enzymology of this kind, we synthesized phosphatidylserine labeled with ^{14}C in the serine carboxyl group. With this substrate, the missing link, phosphatidylserine decarboxylase, was discovered (33) that enabled us to formulate a phospholipid cycle, driven by the decarboxylation reaction, in which free serine may be converted to free ethanolamine.

It was possible by 1961 to formulate (34) a rather detailed picture (Figure 1) of the pathways of biosynthesis of the principal glycerophosphatides and of triacylglycerol in animal tissues. It has stood the test of time.

There has been some confusion in textbooks and elsewhere as to which are de novo and which are scavenger reactions in schemes such as Figure 1. The term de novo denotes those pathways that lead to a net, new formation of glycerophosphodiester bonds in phospholipids. In Figure 1, the stepwise methylation of phosphatidylethanolamine to phosphatidylcholine, discovered by Bremer & Greenberg (35), is highly important because it provides the only known pathway for the synthesis of choline. In mammalian tissues, however, it occurs at a significant rate only in liver. In all other tissues, phosphatidylcholine is synthesized almost entirely from CDP-choline. In animal tissues, so far as is known, the sole reaction leading to the synthesis of phosphatidylserine is the exchange reaction depicted in Figure 1. Therefore both phospha-

tidylserine and phosphatidylethanolamine are entirely derived from CDP-ethanolamine in all animal tissues. In yeast, on the other hand, phosphatidylserine can be synthesized from CDP-diacylglycerol and serine. Its decarboxylation to phosphatidylethanolamine, which is then methylated to phosphatidylcholine, provides a de novo pathway that does not require CDP-choline or CDP-ethanolamine. In yeast, but not in mammalian tissues, the CDP-choline and CDP-ethanolamine reactions may be regarded as a scavenger pathway for the re-utilization of choline and ethanolamine, respectively.

It seems likely that this difference in metabolic pathways reflects the fact that in the course of evolution, mammals have become almost entirely dependent upon choline in the diet as a precursor of phospholipid and of acetylcholine, and most tissues have lost the capacity to synthesize it at a significant rate.

MEMBRANE BIOGENESIS AND FUNCTION IN BACTERIA

In 1963, while continuing to work on some aspects of lipid biosynthesis in mammal tissues, I turned to the study of the membrane biogenesis and function in *Escherichia coli.* At this point, I was joined by Marilyn Rumley, whose contributions were to prove so important to the life and work of the laboratory. We have now been friends and collaborators for almost 30 years.

The rapidly growing body of information on the genetics of *E. coli* made it extremely attractive for the study of membrane function as well as the biosynthesis of membrane lipids. The very rapid rate of growth of bacterial cells also makes them a rich source of biosynthetic enzymes. In contrast, the extraction and purification of lipid biosynthetic enzymes from animal tissues was frustratingly difficult, a situation that was to persist for decades.

The pathways for the synthesis of the principal membrane phospholipids of *E. coli* have been reviewed elsewhere (36), and are shown in abbreviated form in Figure 2. CDP-diacylglycerol derived, as in animal tissues, from a reaction of CTP with phosphatidic acid, plays a central role. The reactions of Figure 2 are also found in many other bacteria and in yeast. As a result of the work of many laboratories as well as our own, nearly every enzyme involved in phospholipid bioysnthesis in *E. coli* has been purified to homogeneity. The structural genes for these enzymes have been identified and many have been cloned. More is now known about the enzymology and genetic regulation of the biosynthesis of phospholipids in *E. coli* than in any other organism.

Those who contributed to this work in our laboratory during the 1960s included Julian Kanfer, Ying-ying Chang, Ronald Pieringer, and James Carter, to be followed in the 1970s by William Wickner, William Dowhan,

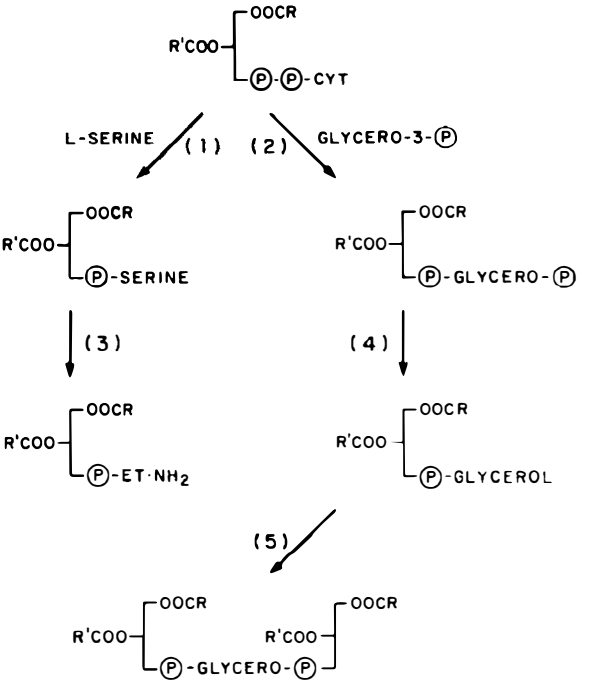

Figure 2 Pathways for the synthesis of the principal membrane phospholipids of *E. coli*

Edward Hawrot, Christopher Raetz, Keith Langley, Martin Snider, Gayle Schneider, Richard Tyhach, Michel Satre, and Carlos Hirschberg.

PHOSPHOLIPID TRANSLOCATION AND THE BIOGENESIS OF ORGANELLES

In contrast to the much-studied translocation and targetting of proteins to destinations in the cell, little is known about translocation of membrane phospholipids, which obviously must be coordinated with that of proteins in the synthesis of organelle membranes. Studies with Wilgram (37) and Dennis (38) on the localization of specific steps in the biosynthesis of phospholipids in liver and in *Tetrahymena* showed the need for specific mechanisms for the translocation of phospholipids to mitochondria from sites of synthesis in the endoplasmic reticulum. It is widely assumed that soluble "phospholipid transfer proteins," which have been shown to bind phospholipids and interchange them between different membranes in the test tube, carry out this function in

vivo. The experiments of Yaffe (39), which revealed that the specificity of the phospholipid transfer proteins of BHK cells does not correspond to the specificity of translocation within the cell, cast doubt on this dogma. Recent work in Dowhan's laboratory has led to the isolation of the structural gene for one such "phospholipid transfer protein" in yeast (40), an essential step towards a definitive test of its function.

James Rothman devised an experimental approach to measure the "flip-flop" or transverse movement of newly synthesized phosphatidylethanolamine from inner to outer face of the membrane in cells of *Bacillus megaterium*, and found that its rate is orders of magnitude greater than in model lipid bilayers (41). Further investigation by Keith Langley showed that this rapid movement is independent of sources of metabolic energy (42). Despite much work in a number of laboratories, the fundamental mechanisms involved in phospholipid "flip-flop" remain obscure.

LACTOSE PERMEASE AND THE DOUBLE LABEL EXPERIMENT

In 1965, Hiroshi Nikaido, then working in Herman Kalckar's laboratory at the Massachusetts General Hospital, reported that the function of the lactose permease in *E. coli* caused a "phospholipid effect" revealed by an increased rate of incorporation of $^{32}P_i$ into phospholipid (43). This phenomenon appeared to be fundamentally similar to the effects discovered in animal tissues in the now classic studies of the Hokins (44).

Alvin Tarlov, a young physician from the Department of Medicine of the University of Chicago, where he was later to become chairman, had just arrived for a period of postdoctoral work. I found him to be an able experimentalist, so that I half-seriously advised him that he was too smart to end as a professor of medicine and he should switch completely to biochemistry, a suggestion that he wisely ignored. Tarlov, like others who came to the laboratory, was surprised at the simplicity of the equipment, arising partly from my own adherence to the wax-and-string school of experiment, and partly from the vigilance of Phyllis Elfman, my secretary for more than 30 years, who screened requested expenditures with the parsimony of a New England Yankee. Tarlov was particularly annoyed that he was forced to scrounge in other laboratories for crushed ice. He finally won a hard-fought campaign to get an ice-maker, which for some years after his departure was known as the Alvin Tarlov Memorial Ice Machine.

Tarlov confirmed Nikaido's findings that the operation of the permease increased the labeling of phospholipids from $^{32}P_i$. In a more detailed analysis,

however, Tarlov showed that the effect was not specific for phosphatidic acid; all of the phospholipids showed increased ^{32}P-labeling. Further, a similar effect could be noted with labeled glycerol, or with labeled serine (45). The cells in these experiments were suspended in a medium containing no source of utilizable nitrogen. Under these conditions, the rate of phospholipid biosynthesis is very severely reduced, presumably in part at least because of "stringent" regulation. The initiation of lactose transport causes a transient release from this regulation by a mechanism that is not known but does not involve a specific "phospholipid effect."

If the mechanism of lactose transport did not involve a phospholipid effect, what was the mechanism? It is now hard to credit, but at that time the role of proteins in membrane transport was by no means certain. To test the idea that the specific recognition of a β-galactoside such as lactose by the transport system must involve a protein, Fred Fox and I first tried affinity labeling. I prepared the diazonium derivative of p-aminophenyl-β-galactoside. Fox tested it and found that it was indeed an irreversible inhibitor of the permease, but also found that other diazonium compounds unrelated to β-galactoside structure were equally effective. They were not affinity but sulfhydryl reagents. Kepes (46) had already found that the lactose permease was inhibited by treatment with sulfhydryl reagents.

Some years earlier, an elegant paper by Cohen et al (47) had caught my eye. These workers showed that the irreversible inhibition of acetylcholine esterase by diisopropylfluorophosphate may be prevented by saturating the enzyme with substrate, and exploited this finding to develop a method for the specific labeling of the enzyme with ^{32}P-diisopropylfluorophosphate. Driving back home to Brookline from Woods Hole one Sunday evening, I recalled this paper, and it suggested another approach to the specific labeling of the sugar-binding component of the lactose permease.

As Fred and I finally worked it out, the experiment involved two forms of the sulfhydryl reagent *N*-ethylmaleimide (NEM), one labeled with ^{3}H and the other with ^{14}C. Two cultures of cells of *E. coli* were prepared, one induced and therefore containing the lactose permease, and the other uninduced. In stage 1, these cultures were incubated with unlabeled NEM in the presence of saturating amounts of thiodigalactoside, a specific substrate for the lactose permease that protects it from reaction with NEM. The great bulk (but not all) of the proteins accessible to NEM were therefore blocked in this step, except the lactose permease, which was protected by the substrate thiodigalactoside, which was then removed by washing the cells.

In the second stage, induced cells, containing the permease protein, were incubated with ^{14}C-NEM in the absence of thiodigalactoside, resulting in the labeling of the permease protein with ^{14}C-NEM. Other "background" proteins that had not been completely blocked in the first stage also become labeled.

The uninduced cells were treated in identical fashion with ^{3}H-NEM. Because the permease was not present in these cells, only the "background" proteins became labeled with tritium. When portions of the two types of cells were mixed, all "background" proteins contained both tritium and ^{14}C, but the permease protein contained only ^{14}C. Its presence in partially purified fractions extracted from the mixed cells was therefore revealed by an increase in the ratio of ^{14}C/^{3}H.

This double-label approach led to the identification of a membrane protein component of the lactose permease (48), which later was identified by electrophoresis in gels containing sodium dodecyl sulfate as a protein apparently of molecular weight 30,000 (49). When the sequence of the protein finally became known from the sequence of its gene (50), its size was found to be 46,500; its behavior on gels, like that of some other membrane proteins, is quite anomalous.

Our procedure specifically labeled the lactose permease by a step that also inactivated it. We now began a series of attempts to extract and purify the permease in functional form. Despite vigorous efforts by students and postdoctoral fellows whose later careers proved their ingenuity, we were completely unsuccessful. It was small consolation for this great disappointment that the efforts of other laboratories for almost 20 years were also unsuccessful. It remained for Michael Newman in Thomas Wilson's laboratory (51) to solve this problem, which opened a new era in the study of the lactose permease. Aided by recombinant DNA technology, the analysis of structure and function of the permease is now proceeding at a level of resolution and sophistication undreamt of in our early studies.

PERIPLASMIC GLUCANS AND CELL SIGNALLING IN BACTERIA

Early pulse-chase experiments with $^{32}P_i$ revealed a continuous turnover of the headgroups of phospholipids in *E. coli* (52). Later work by van Golde (53) showed that this turnover is the result of the transfer of phosphoglycerol and phosphoethanolamine residues from membrane phospholipids to a novel type of cell-constituent, termed membrane-derived oligosaccharides (MDO). These are periplasmic glucans, closely related to the cyclic glucans of the Rhizobiaceae.

Both in *E. coli* and in the Rhizobiaceae the synthesis of periplasmic glucans is a striking instance of osmotic adaptation, being 10–20 times higher in cells growing in medium of low osmolarity than in similar medium of high osmolarity (54,55). Periplasmic glucans also have important although poorly

understood functions in cell signalling. Fiedler & Rotering (56) reported that mutants of *E. coli* defective in the production of MDO have greatly impaired motility, an increased production of capsular polysaccharide, and a decreased production of outer membrane protein OmpF when grown in medium of low osmolarity. These are all properties expected during growth in high osmolarity, consistent with a model in which the presence of MDO in the periplasm is needed to signal to certain osmoregulatory systems that the osmolarity of the medium is low, but much further work is needed to understand the real basis of these effects.

Howard Schulman helped to outline the framework of the biosynthesis of MDO, followed by later studies of Daniel Goldberg, Barbara Jackson, and Jean-Pierre Bohin. Audrey Weissborn (57) discovered a membrane-bound glucosyltransferase and found that it also requires a soluble factor, which Helene Therisod (58) identified as acyl carrier protein. This completely unexpected role of acyl carrier protein is presently under intensive scrutiny, as is the requirement for polyprenol phosphate in this surprisingly complex system.

The work of Karen Miller (59) strongly underscored the fundamental similarity between the MDO of *E. coli* and the cyclic glucans of *Rhizobium* and *Agrobacterium*. In Rhizobia, cyclic glucans appear to play a part in the complex interchange of signals between plant and bacterium that leads to the mutual recognition needed for symbiosis. Mutations that block the synthesis of glucans also prevent the formation of normal root nodules.

Stimulated by the discovery of the unusual role of acyl carrier protein in MDO synthesis, we recently turned to an examination of the acyl carrier proteins of *Rhizobium*. Mark Platt isolated the constitutive acyl carrier protein responsible for the synthesis of cell lipids needed for growth (60); Otto Geiger and Herman Spaink isolated the inducible acyl carrier protein, product of the *nodF* gene, which functions in cell signalling (61). The two proved to be distinctly different. In an exciting recent development, Spaink and his collaborators (62) have isolated rhizobial signalling compounds containing a novel fatty acid for the synthesis of which the NodF acyl carrier protein is needed.

Because membranes of every type of living cell are freely permeable to water, osmotic regulation is a fundamental problem in biology as is cell signalling in symbiotic nitrogen fixation. How does the cell detect the osmolarity of the medium in which it is growing? Is there a hierarchy of regulation of osmotic adaptation in which one system derives information from another? Are periplasmic glucans important in these processes? What is their role in symbiotic nitrogen fixation? What is the significance of the involvement of membrane phospholipids in their biosynthesis?

Fortunately, no problem is ever really solved; it simply opens to reveal another.

SAILING TO BYZANTIUM

An aged man is but a paltry thing,
A tattered coat upon a stick, unless
Soul clap its hands and sing, and louder sing
For every tatter in its mortal dress,
Nor is there singing school but studying
Monuments of its own magnificence;
And therefore I have sailed the seas and come
To the holy city of Byzantium.

Yeats

What are these monuments of the soul's magnificence that we must study to transcend age and physical decay? Yeats meant the monuments of art, but surely there is another Byzantium to which we may sail and it contains the monuments of science. For those to whom fame is the spur, that last infirmity of noble minds, to labor on the monuments of art seems to hold a distinct advantage. Mozart, Beethoven, Yeats himself produced works that seem forever stamped with their personalities, like thumbprints on pottery. In contrast, scientists are interchangeable, with the the rarest exceptions, perhaps none. The towering figure of Otto Warburg dominated biochemistry during the first half of this century. Today it is not easy to find a graduate student who can give a meaningful synopsis of his work.

The anonymity that is the fate of nearly every scientist as the work of one generation blends almost without a trace into that of the next is a small price to pay for its unending progress, the great long-march of human reason. In his Nobel address, Fritz Lipmann said of scientists: "Their purpose often may be none but just to push back a little the limits of our comprehension. Their findings mostly have to be expressed in a scientific language that is understood only by a few. We feel, nevertheless, that the drive and urge to explore nature in all its facets is one of the most important functions of humanity."

To feel that one has contributed to this splendid enterprise, on however small a scale, is reward enough for labor at the end of a day.

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