Reflections
A PAPER IN A SERIES COMMISSIONED TO CELEBRATE THE CENTENARY OF THE JBC IN 2005

Hitler’s Gift and the Era of Biosynthesis

Published, JBC Papers in Press, September 14, 2001, DOI 10.1074/jbc.R100051200

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Before the Second World War biochemistry in the United States had a strong flavor of clinical chemistry. It was much occupied with problems of analysis of blood and tissues and the determination of the structures of body constituents. This was important and indeed essential work, but American students had to go abroad to Germany or to England for training in what came to be called dynamic aspects of biochemistry. After the war, the flow of students was largely reversed. This transformation was in considerable part the result of new insights and new approaches brought to America by immigrant scientists.

It is a remarkable fact that as late as 1945 when I began graduate studies in biochemistry at the University of Chicago almost nothing was known about the linked reactions leading to the biosynthesis of any of the major types of cell constituents, carbohydrates, lipids, proteins, or nucleic acids. However, this picture was about to change with dramatic rapidity. The latter half of the 20th century became the era of biosynthesis. Now, in 2001, we know in great detail the patterns of reactions leading to the formation of each of these classes of cellular materials, although to be sure much remains to be learned about the regulation and integration of biosynthetic processes in living organisms.

The achievements of three biochemists, Fritz Lipmann, Rudolf Schoenheimer, and Konrad Bloch, greatly stimulated this flowering of biosynthetic studies in the United States at the mid-20th century. Each had been driven out of Germany by the brutal anti-Semitism of the Nazi regime. Each was an important part of what has been called Hitler’s gift (1) to American and British science.

In helping to bring about the transition to the era of biosynthesis, Fritz Lipmann made clear the crucial role of “energy-rich” phosphates in driving biosynthetic reactions and showed how this principle operated in the formation of the much sought and highly elusive “active acetate” involved in so many pathways. Rudolf Schoenheimer helped put into the hands of biochemists their most subtle and versatile approach, that of the isotope tracer technique, and with its aid revealed the dynamic state of body constituents. Konrad Bloch’s work on the formation of cholesterol illustrated how the insights of Lipmann and Schoenheimer could be combined in a masterpiece of biochemistry to solve a problem of great medical as well as biological significance.

Fritz Lipmann: The Energetics of Biosynthesis

Fritz Lipmann (Fig. 1), who helped to shape the development of modern biochemistry, was born in Koenigsberg, East Prussia in 1899 into a Jewish family of the professional class (2). In 1917, he began the study of medicine. In 1918, while still a medical student, he was drafted into the German army and spent the rest of the war in the medical corps in France. Released from the army, Lipmann resumed his medical studies and received the M.D. degree in 1921. He soon abandoned plans for the practice of medicine in favor of biochemical research, but he always valued the broad view of biology his medical education had given him, concluding: “The

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biological education to which the observant student is exposed in medicine is a superior preparation for any career.” Indeed, the study of medicine offered the most comprehensive view of biology then available. Many of the greatest figures in biochemistry early in the 20th century, including Warburg, Meyerhof, and Krebs, were trained as physicians. The breadth of his background helped give Lipmann the confidence 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.

Turning to a career in research rather than the practice of medicine, Lipmann realized that the most fruitful approach to biological problems was through chemistry. He began a program leading to a Ph.D. in chemistry. His work for the dissertation, begun in 1927, was carried out in the laboratory of Otto Meyerhof. Meyerhof, whose work on glycolysis in muscle earned him a Nobel Prize, had a laboratory on the first floor of the Kaiser-Wilhelm Institute for Biology in Berlin, a city that was then the leading center of science in the world. Lipmann felt that his experience in Meyerhof’s laboratory was in many ways the origin of all his later work. His most intense admiration, however, was reserved for Warburg. As Lipmann later recalled (3): “At the top of everything, on the uppermost floor, was Otto Warburg. Warburg already had a mystery about him. We admired him boundlessly but saw little of him . . .”

In Meyerhof’s laboratory, Lipmann worked on the role of creatine phosphate in muscle contraction. It was of course known that muscle contraction, with its attendant production of lactic acid, is intimately linked to glycolysis. The energetics of this linkage, however, remained obscure. Lipmann (3) commented on “… the vagueness of the understanding, then prevalent, of both the intermediary path of glycolysis and the mechanism of action of energy-rich phosphate.” This work did much to turn Lipmann’s thinking to the role of phosphorylated intermediates in energy transduction.

In 1930, Lipmann was already aware that a career for a Jewish scientist in Germany was fraught with difficulty and peril. There began a period of Wanderjahre before he finally found a position that offered both independence and scope. However, he wished to remain in Berlin at least for a time to be near his fiancee Freda Hall (3). He became an assistant to Albert Fischer, working on problems of tissue culture. In 1931 during a hiatus in the work of Fischer’s laboratory caused by its move from Berlin to Copenhagen, Lipmann, newly married to Freda Hall, traveled to the United States to work at the Rockefeller Institute in New York in the laboratory of Phoebus A. Levene on the biochemistry of phosphoproteins. Here he succeeded in isolating phosphoserine from partial acid hydrolysates of egg phosphoprotein.
In 1932, Lipmann rejoined Fischer’s group in its new quarters in the Carlsberg Laboratories in Copenhagen, where he was to remain until 1939. He was free to work independently in pursuit of his own ideas. At about this time, Otto Warburg was making his great discoveries elucidating the central mechanisms of glycolysis. Why the splitting of glucose involved phosphorylated intermediates had long been a great puzzle, which Warburg now solved.

In 1905 Arthur Harden, working in London, had discovered that glycolysis requires a heat-stable, organic cofactor, which he termed “cozymase.” This cofactor proved to be remarkably elusive. In 1929, more than two decades later, Hans von Euler received a Nobel Prize for his work on its isolation and characterization, but it is clear from his Nobel lecture that he had at that time no real idea of its true structure and function. It was Warburg and his collaborators (4) who isolated “cozymase” and showed that it contains a pyridine ring that undergoes alternate reduction and re-oxidation. It is of course the famous coenzyme NAD now known to function in many hundreds of enzyme-catalyzed redox reactions. Warburg also discovered that the oxidation of 3-phosphoglyceraldehyde by NAD is linked to the uptake of orthophosphate and the formation of 1,3-diphosphoglyceric acid. This acyl phosphate may then react with ADP to form ATP. For the first time, the bioenergetic function of glycolysis became clear. A portion of the free energy released during the breakdown of glucose is made available to the cell as ATP.

Lipmann followed these developments closely and they deeply influenced his thinking. In 1939, he turned to an investigation of the role of phosphate in the oxidation of pyruvate in extracts of the organism then called *Bacterium acidificans longissimum* (Delbrueckii). He discovered (5) that the oxidation of pyruvate was coupled to the uptake of orthophosphate and the phosphorylation of AMP (presumably with the formation of ATP).

By analogy with the role of 1,3-diphosphoglyceric acid in glycolysis, he formulated the following reactions.

\[
\text{Pyruvate} + \text{phosphate} \rightarrow \text{acetyl phosphate} + 2[\text{H}]
\]

\[
\text{Acetyl phosphate} + \text{AMP} \rightarrow \text{"adenosine polyphosphate"}
\]

**Reactions 1 and 2**

The isolation from these enzyme preparations of highly labile acetyl phosphate present only in very small amounts was really not feasible with the methods then available. Lipmann neatly got around this difficulty by synthesizing acetyl phosphate from acetyl chloride and trisilver phosphate. He then showed that this synthetic compound, like the presumed intermediate, was effectively utilized for the formation of “adenosine polyphosphate” in these extracts. (Much later, when I worked in Lipmann’s laboratory and read his early papers, I was greatly taken by this strategy. I learned from it that it is sometimes easier to synthesize a suspected intermediate in an enzyme system than to isolate it, a lesson that led me to synthesize CDP-choline first and then demonstrate its role as coenzyme.)

The work on acetyl phosphate marked the beginning of Lipmann’s long and productive engagement with both the role of phosphate esters in energy transduction and the problem of “active acetate.” In July of 1939, with the Nazi menace ever more threatening, Fritz and Freda Lipmann left Copenhagen for the United States. There followed a difficult period in which he sought without success a position that would offer him security and scope commensurate with his talents. In 1940, he was invited to present a talk in a symposium at the University of Wisconsin in Madison attended by many of the leading figures in American biochemistry. Lipmann, never a facile or polished speaker, vastly underestimated the time needed for the material he wished to present and finally, midway through his discourse, had to be interrupted by the chairman of the session (3). Later he felt that this painful episode was one of the factors that made it difficult for him to secure a suitable position.

In 1940, Lipmann was invited by F. F. Nord to contribute a chapter to the first volume of the series, *Advances in Enzymology*. As Lipmann later (3) wrote: “... I was happy when he accepted my suggestion that I write about the role of phosphate bonds as carriers in energy transformations and in biosynthesis. This had begun to impress me as an extension of my experience with acetyl phosphate. Some of the propositions made in that article must have been more novel than I realized.”

Now, in 2001, it is very difficult to realize the impact of this article (6), particularly on American biochemists who had not closely followed the work in European laboratories.
Lipmann clearly distinguished between two classes of phosphate compounds in living cells. The first class, phosphate esters of alcohols such as glycerophosphate with a free energy of hydrolysis of 2–4 kcal, was termed by Lipmann as “energy-poor” phosphates, designated in the shorthand which he introduced as (−ph). These were to be sharply distinguished from another class comprising pyrophosphates, acyl phosphates, enol phosphates, and nitrogen-linked phosphates such as phosphocreatine. The free energy of hydrolysis of phosphates of this class is of the order of 8–12 kcal. In Lipmann’s terminology these are energy-rich phosphate bonds, designated with a symbol that was to become famous as the “wiggle bond” (−wph).

A great generalization was stressed in his essay. Photosynthesis and the breakdown of organic foodstuffs provide energy to living cells, some part of which is captured in useful form as “energy-rich” phosphates, leading to the formation of ATP. Lipmann pointed out: “Indications are found that the phosphate current can be utilized to carry out mechanical work... (and) to synthesize protoplasmic material as lecithin, nucleic acid, and so forth.” Lipmann made it clear that the energy needed to drive biosynthetic processes must come from ATP either directly or, as was soon to be found, indirectly. Before this time biosynthetic processes could be studied only in intact animals or in preparations such as tissue slices (developed by Warburg) in which cellular structure remained intact. The principal conceptual barrier to the study of cell-free systems was now removed. Biochemists began to add ATP (of varying degrees of purity!) to their enzyme systems when searching for biosynthetic reactions.

The 1941 essay is revealing in many ways of Lipmann’s style, which had a personal flavor even when dealing with chemical thermodynamics. These were problems about which he had thought deeply, and he conveyed his ideas in striking and forceful metaphors. Thus he spoke of a “phosphate potential” in analogy to an electrical potential and a “phosphate current” that conveys energy as “energy-rich” phosphates are hydrolyzed. Critics pointed out that his use of the term “bond energy” to denote the energy released in breaking a bond was the opposite of the conventional use to denote the energy of bond formation, but Lipmann (3) tended to wave aside such criticism. “The physical chemist remains aloof. He may be forced to accept the usage, but he usually refrains from referring to the dilettante who originated it.”

Lipmann’s search for a suitable position now found a happy outcome in a rather unusual way. In 1941 Dr. Oliver Cope offered him an appointment in the Department of Surgery at the Massachusetts General Hospital. Although the space made available was at first quite limited, he was given complete freedom to follow his own ideas. Lipmann’s years at the Massachusetts General Hospital were highly productive and led him to a Nobel Prize in 1953.

In 1941 the identity of “active acetate,” also described as the “two-carbon unit,” was one of the most pressing problems in intermediary metabolism. A growing body of evidence suggested that “active acetate” was the fundamental building block for the synthesis of sterols and fatty acids. Derived from the oxidation of pyruvate or of fatty acids, it could also react with oxalacetate to form citrate and thus enter the Krebs cycle for the final common pathway of oxidative metabolism. Strongly encouraged by his success in identifying acetyl phosphate as an intermediate in the bacterial oxidation of pyruvate, Lipmann set out to examine its possible role as the elusive “active acetate” in animal tissues.

He chose to study the acetylation of sulfanilamide, known to occur in liver, because of the ease with which this aromatic amine could be diazotized and coupled with a chromogen to form an intensely colored dye. The conversion of sulfanilamide to the unreactive N-acetyl derivative could thus be easily measured. He succeeded in obtaining preparations from pigeon liver that actively acetylated sulfanilamide but to his considerable disappointment found that acetyl phosphate did not stimulate acetylation but instead was rapidly hydrolyzed (7). Significantly, however, he found that ATP as well as acetate was required for acetylation and further reported that enzyme preparations, inactivated by storage overnight at 7 °C, could be restored to activity by the addition of boiled liver extract.

Nachmansohn and Machado had previously described a cofactor needed for the acetylation of choline. With the arrival of Kaplan in his laboratory, Lipmann’s cofactor was purified about 100-fold and shown to be active also in the acetylation of choline (8). It appeared to be a general coenzyme for acetylation and hence the designation coenzyme A or CoA. The next step was the discovery (9) in 1947 that CoA, by then purified about 700-fold, contains the vitamin pantothenic acid. This was a very great advance.

A little later, in 1950, recommended by H. A. Barker, I entered Lipmann’s laboratory as a postdoctoral fellow following the footsteps of Earl Stadtman, who had just departed to take up...
a position at the National Institutes of Health. Stadtman had also come to Lipmann from Barker’s laboratory. Lipmann’s group at this time included David Novelli, John Gregory, Morris Soodak, Harold Klein, Charles Du Toit, and Lipmann’s research assistant, Ruth Flynn. We were crammed into a single, tiny laboratory in the Massachusetts General Hospital next to the famous Ether Dome, scene of the first (or so it was claimed) use of diethyl ether as an anesthetic. In the course of the year we were to move into spacious, even rather elegant, quarters in a newly constructed research building.

With abundant hair just turning gray and usually wearing a soft bow tie and a dark blue shirt, Lipmann presented a figure closer to that expected of an artist rather than a scientist. He spoke softly, and his sentences often trailed off into the distance. 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.

At this time, Lipmann’s chief goal was the final purification of coenzyme A, which was proving very difficult, and the determination of the structure of “active acetate,” the intermediate with so many crucial roles in metabolism. Because acetyl phosphate, shown to be an activated form of acetate in bacteria, was so labile, we surmised that acetyl-CoA, whatever its structure might be, would be even more labile, and this supposed lability was assumed to explain the failure of our efforts to isolate it.

One day in 1951, I came upon an article in Angewandte Chemie (10) from the laboratory of Feodor Lynen. He and his student Ernestine Reichert reported evidence for an essential sulphydryl residue in CoA. They had isolated acetyl-CoA and proved it to be a thioester! I brought the article at once to Lipmann who had not learned previously of this development. He was generous in praise of the work although Lynen had stolen some of his thunder. He was particularly impressed by the fact that in isolating acetyl-CoA from yeast, they had begun by boiling the yeast. We should have realized, Lipmann pointed out, that an intermediate that plays such varied roles is unlikely to be so extremely labile as we had feared. Lipmann also noted that thioesters must be added to the list of biologically active “energy-rich” compounds.

“Yes,” he mused in a discussion at this time, “there is a world of sulfur, like the world of phosphorus, only smaller!”

In 1953 Lipmann shared the Nobel Prize with H. A. Krebs. Although the citation for the prize emphasized his work on CoA, Lipmann placed greater stress on his contributions to bioenergetics. “In my own judgment,” he wrote (3), “there was greater scope in the recognition that ~P, as I had dubbed it, was acting as a biological energy quantum, carrying energy packages to metabolic function and biosynthesis.”

In 1957, he moved to the Rockefeller Institute. He continued to be remarkably productive in a wide variety of biosynthetic problems, further developing his grand themes of group activation and the energetics of biosynthesis until his death in 1986 at the age of eighty-seven.

Rudolf Schoenheimer and the Dynamic State of Body Constituents

The single most important technical advance that transformed biochemistry in the 20th century was the isotope tracer technique. Without it, the rapid growth of our knowledge of biosynthesis would be simply inconceivable. Georg Hevesy was the first to explore the biological usefulness of radioactive tracers in studies of the uptake of radiolead and its movement into tissues of plants (11). It is to Rudolf Schoenheimer (Fig. 2), however, that we owe the brilliant exploitation of the concept of isotopic tagging, that is the introduction of isotopes into specific positions of organic molecules, whose metabolic transformations could then be traced.

Valuable accounts of Schoenheimer’s career have been published by Kohler (12) and by Young and Ajami (13). He was born in Berlin in 1898 (12). Like Lipmann, he studied medicine and received the M.D. degree from the University of Berlin in 1922. Again like Lipmann, he recognized the need for deeper knowledge of chemistry and spent 3 years in the laboratory of Karl Thomas in Leipzig, working largely on problems such as the chemical synthesis of peptides.

In 1926, Schoenheimer went to the Institute of Pathological Anatomy in Freiburg as assistant to Ludwig Aschoff, a leading expert on atherosclerosis (12). Schoenheimer began an investigation on the deposition of cholesterol into the arteries of rabbits fed a high level of cholesterol in the diet. He was to pursue his interests in cholesterol metabolism for the rest of his life.
It was here in Freiburg in 1930 that Schoenheimer encountered Hevesy, who wished to study the partition of labeled lead between normal and tumor tissue (12). Realizing his inadequate background in biology, Hevesy asked Aschoff to suggest a collaborator for this work. Aschoff suggested Schoenheimer. Later, Hevesy (14) wrote: “It was in the course of these investigations that Schoenheimer became familiar with the method of isotopic indicators, which he applied several years later with such great success... Never were more beautiful investigations carried out with isotopic indicators than those of the late Professor Schoenheimer...”

Although the collaboration with Hevesy was undoubtedly significant for Schoenheimer’s thinking, his development of the use of isotopes was to go far beyond the scope of Hevesy’s approach. In 1933 Schoenheimer, like so many others, was forced to leave Germany. The Josiah Macy Foundation in the United States had begun in 1931 to support Schoenheimer’s research, and the director of the foundation, Ludwig Kast, now arranged an appointment for Schoenheimer in the Department of Biological Chemistry at Columbia, with salary and research funds supplied by the Foundation (12).

Hans T. Clarke, an organic chemist by training, had assumed the direction of the Department of Biological Chemistry in 1928, and he proceeded to make it the finest department in the United States. In an account of his career (15), Clarke stated: “Among the many benefits which accrued to Columbia University from the racial policy adopted by the Germans under the Third Reich was the arrival in our laboratory of various European-trained biochemists, notably Erwin Chargaff, Zacharias Dische, Karl Meyer, Rudolf Schoenheimer and Heinrich Waelsch. Erwin Brand, who joined our group during the same period, reached this country somewhat earlier. The scientific achievements subsequently made by these men are so well known that their enumeration is unnecessary.” Clarke modestly omitted to mention that his own vision and humane instincts in welcoming these gifted refugees were by no means to be found in every American academic institution.
In 1932, also at Columbia University in the Department of Chemistry, Harold Urey discovered deuterium, the heavy isotope of hydrogen, by demonstrating the presence of new bands in the positions predicted for a form of hydrogen of mass 2, in the spectrum of a sample of hydrogen enriched in the heavier isotope by fractional distillation of liquid hydrogen. In 1934, Urey received a Nobel Prize for this work. Because separation of the isotopes of an element is a function of the ratio of their masses, isotopes of the heavier elements are very difficult to separate. Deuterium, however, has twice the mass of ordinary hydrogen, and its preparation in pure form or as D₂O (immediately dubbed “heavy water”) is comparatively straightforward and was very soon undertaken in the laboratories of Urey and G. N. Lewis among many others.

The discovery of a completely new form of a substance of such universal importance as water immediately attracted great public interest all over the world. When Urey received his Nobel Prize in 1934, Palmer, in his laudatory introduction of Urey, mentioned that large amounts of heavy water were already being produced by an electrolytic process at the Norsk Hydro Concern in Norway at the rate of about a half-liter per day (16). In 1940 after a more sinister use of heavy water as the moderator for atomic piles had emerged, this Norwegian heavy water production facility was taken over by the German army of occupation. It then became the target for heroic and tragic efforts of Norwegian patriot saboteurs and the allied air forces to destroy it. The Germans finally dismantled it in 1945. The first biological experiments with D₂O were relatively crude. For example, Lewis (17) reported that tobacco seeds suspended in pure D₂O failed to germinate, and flatworms died when placed in water containing more than 90% D₂O. In these and other early experiments, the emphasis was on replacement of hydrogen by deuterium in molecules of biological importance.

Urey, a physical chemist, stated that he was a biologist at heart. Indeed, at a later stage of his career at the University of Chicago he turned to fundamental biological research. With his gifted collaborator Stanley Miller, he designed experiments that demonstrated the ready synthesis (under conditions that simulated the atmosphere of the early earth) of molecules that might plausibly be considered to be building blocks for the formation of cell substances. These studies greatly influenced many later investigations of the origin of life.

To promote the applications of the deuterium isotope to biological research, Urey persuaded Warren Weaver, head of the Rockefeller Foundation, to provide funds to permit David Rittenberg, a recent Ph.D. in physical chemistry in Urey’s department, to come to the Department of Biological Chemistry (12). As Hans Clarke commented (15): “In 1934, Schoenheimer made a new contact which proved to exert a fundamental influence on the nature of his work . . . David Rittenberg came from Urey’s group to the laboratory in which Schoenheimer had been working for a year. From their association there developed the idea of employing a stable isotope as a label in organic compounds, destined for experiments in intermediary metabolism, which should be biochemically indistinguishable from their natural analogs . . .”

This new conception of Schoenheimer and his collaborators was a far cry from the simple measurement of the movement of a radioactive ion from one part of a plant or animal to another, as had been done by Hevesy. In the new approach, the fate of the molecule into which the isotope had been incorporated was studied, not simply the isotope itself. Perhaps the nearest intellectual predecessor of this idea was the approach of Knoop, who in 1904 “labeled” fatty acids by the attachment of a phenyl residue to the ω-carbon atom. Knoop found that if the fatty acid had an even number of carbon atoms, phenylacetic acid (linked to glycine in a so-called detoxification reaction) was recovered from the urine of dogs to which it had been fed. If on the other hand, the fatty acid had an odd number of carbon atoms, benzoic acid was similarly recovered. Knoop concluded that the phenyl residue could not be cleaved from the ω carbon to which it was linked and more significantly correctly concluded that fatty acid oxidation in animal tissues must involve oxidation at the β-position. This result strongly influenced later studies of fatty acid oxidation, but the work was subject to the objections that phenyl-substituted fatty acids are very different from natural fatty acids, and a more serious limitation was that this type of labeling was not generally suitable for substances other than fatty acids.

Schoenheimer was well aware of Knoop’s work. In a brief review in 1935 (18), Schoenheimer and Rittenberg pointed out: “Many attempts have been made to label physiological substances by the introduction of easily detectable groups such as halogens and benzene nuclei. However, the physical and chemical properties of the resulting compounds differ so markedly from those
of their natural analogs that they are treated differently by the organism. The interpretation of metabolic experiments involving such substances is therefore strictly limited. We have found the hydrogen isotope deuterium to be a valuable indicator for this purpose . . . We have prepared several physiological compounds (fatty acids and sterol derivatives) containing one or more deuterium atoms linked to carbon, as in methyl or methylene groups . . . The number of possible applications of this method appear to be almost unlimited."

At this period, mass spectrometers were still rare and finicky instruments. It was an advantage of these early experiments that the content of deuterium in organic compounds could be determined comparatively simply by combustion of the compound and very precise measurement of the density of the water so produced.

In 1935, it was a widely held doctrine that the bodily constituents of an adult animal were quite stable, while foodstuffs in the diet were immediately metabolized to provide energy and the end products excreted. In their earliest experiments, Schoenheimer and Rittenberg found evidence to overturn this doctrine. When fatty acids labeled with deuterium were fed to mice, most of the deuterated fat was first deposited in the fat depots. The fat burned in the body was not taken directly from the diet but from adipose tissue. Schoenheimer (19) concluded: "These first experiments with isotopes showed that the fats of the depots are not inert storage materials but are constantly involved in metabolic reactions."

To study the synthesis of fatty acids, Bernhard and Schoenheimer (20) administered D2O to mice and later measured the isotope content of their fatty acids. The saturated fatty acids were found to contain relatively high levels of deuterium, but the polyunsaturated linoleic and linolenic acids, known to be essential components of the diet, contained only traces. They concluded that the mice carried out a very active de novo synthesis of saturated but not of essential fatty acids. Because the total fat content of the mice did not change, the results indicated a rapid breakdown of body fats, equal to the rate of synthesis.

As might be expected, an important objective of Schoenheimer’s new program was an investigation of the metabolism of cholesterol. When cholesterol was isolated from mice given D2O, Rittenberg and Schoenheimer (21) found from the rate of incorporation of deuterium into it that cholesterol must be continually renewed with a half-time of the order of 3 weeks. To account for the extensive incorporation of stably bound deuterium into the cholesterol molecule, it was concluded that its synthesis, like that of fatty acids, must involve the condensation of many small molecules.

A major extension of the range Schoenheimer’s investigations came with the concentration of the isotope 15N by Urey and his collaborators in 1937. It was immediately applied to studies of the metabolism of amino acids and proteins. In 1938, Schoenheimer et al. (22) reported the first experiments in which an amino acid in the diet, tyrosine, was labeled with 15N. "The original aim of this exploratory experiment was merely to find out whether in nitrogen equilibrium, the nitrogen in the urine is derived from the food proteins directly, or whether dietary nitrogen is deposited, with liberation of an equivalent amount of tissue nitrogen for excretion . . . The results indicate that in our rat the nitrogen of at least one amino acid, tyrosine, was only partly excreted in the urine, while almost half of it was retained in the body proteins."

Here was another blow at the doctrine that ingested foods were immediately metabolized and the products promptly excreted. Schoenheimer now found this view very naïve. If one puts a penny into a gumball machine, he asked, and a gumball comes out, does the machine turn copper into gum?

Schoenheimer had now become the central figure in Clarke’s Department of Biological Chemistry. New and larger laboratory facilities were made available for him. His enthusiasm and vision attracted collaborators and students. As Kohler (12) has pointed out, he had become the leader of perhaps the first multidisciplinary biochemical laboratory. A physicist was needed for the preparation and measurement of isotopes. An organic chemist was employed for the synthesis of isotopically labeled compounds, because of course none were available commercially. Biochemists were required for the separation and analysis of cell constituents. Technicians for animal care were also needed. Schoenheimer’s background in chemistry as well as in biology and medicine made him especially effective in the leadership of this disparate group.

Schoenheimer’s investigations of protein metabolism, carried out with amino acids containing 15N in the amino group and deuterium on the carbon chains provided results that had the
greatest impact on biochemical thought. Briefly summarized (19), body proteins were found to be in a state of continuous turnover. “The peptide bonds have to be considered as essential parts of the proteins and one may conclude that they are rapidly and continually opened and closed in the proteins of normal animals. The experiments give no direct indication as to whether the rupture is complete or partial.” The work thus raised questions that were to challenge the next generation of biochemists.

Together with the earlier work on fat metabolism, a new and remarkable picture of the overall metabolism of animals emerged. Schoenheimer summarized his conclusions (19): “The large and complex molecules and their component units, fatty acids, amino acids, and nucleic acids, are constantly involved in rapid chemical reactions. Ester, peptide, and other linkages open; the fragments thereby liberated merge with those derived from other large molecules and with those absorbed from the intestinal tract to form a metabolic pool of components indistinguishable as to origin ... This idea can scarcely be reconciled with the classical comparison of a living being to a combustion engine nor with the theory of independent exogenous and endogenous types of metabolism ... The classical picture must thus be replaced by one which takes account of the dynamic state of body structure.”

In 1941, Schoenheimer was invited to give the prestigious Dunham Lectures at the Harvard Medical School. The materials and notes that he prepared for the lectures, from which some of the quotations above are taken, were later published (19) under the title “The Dynamic State of Body Constituents.” This lucid summary of his innovative work made a deep impression on the biochemists of the generation to follow.

Schoenheimer had apparently been subject to attacks of depression and was undergoing a period of considerable personal stress when tragically in September of 1941 he ended his own life (12). Forty-three years of age at the time of his death, he was at the height of his powers. Fortunately many of the projects that he had begun were carried forward by very able collaborators, one whom took up the cholesterol problem.

Konrad Bloch and the Biosynthesis of Cholesterol

Konrad Bloch (Fig. 3) was born in 1902 in Neisse, a town in the eastern German province of Silesia, the second child of a prosperous Jewish family (23). In his boyhood, Bloch evinced little interest in science other than nature studies, but his attendance in a course of organic chemistry at the Munich Technische Hochschule taught by Hans Fischer marked a turning point for him. Fischer, later to receive a Nobel Prize, was one of the remarkable group of gifted German chemists who then dominated the study of natural products. Although Fischer’s lectures were delivered in a monotone, Bloch found the material fascinating and he realized that he had found his field (23).

In 1934, the brutal Nazification of Germany prevented Bloch from continuing his studies there. Hans Fischer came to his rescue by recommending his appointment at the Schweizerisches Hoehensforschungs Institut in Davos, Switzerland, the scene where Thomas Mann placed the tuberculosis sanitarium in his novel The Magic Mountain.

In Davos, Bloch worked for a time on the lipids of the tubercle bacillus. In 1936, however, he was refused permission to continue to reside in Switzerland. Desperate, he applied to R. J. Anderson at Yale, with whom he had some correspondence concerning his research. He promptly received two letters, the first from the Dean of the Medical School of Yale University informing him that he had been appointed assistant in Biological Chemistry and the second from Anderson informing him that there was no salary attached to this position. He showed the first letter, but not the second, to the United States consul in Frankfurt and received a life-saving visa to immigrate to the United States.

Upon arrival in New York, Bloch applied to Hans Clarke’s department for admission as a graduate student. The sole formality in those happy days was an interview with Clarke himself. The most important question, Bloch later jested, was: “Do you play a musical instrument?” Fortunately, Bloch could say that he played the cello, an answer agreeable to Clarke, who loved chamber music.

Shortly after completion of his work for the Ph.D. degree under Clarke’s supervision, Bloch joined Schoenheimer’s group. In 1940, Schoenheimer suggested that he investigate the origin of the hydroxyl oxygen in cholesterol. Was it water or O₂? The thought that it might be molecular oxygen showed the remarkable prescience of Schoenheimer because direct oxygenation was without precedent at that time. Unfortunately, Bloch found the technical problems...
of the mass spectrometry of oxygen compounds intractable in the state of technology of 1940 and was forced to give up the project. In 1956, however, he returned to the problem and with his student Tchen (24) showed that molecular oxygen is indeed the source of the hydroxyl oxygen.

As Bloch (23) recalled: “Schoenheimer’s untimely death in 1941 left his associates without the leader and the inspired leadership they so admired. We feared that we might have to look for jobs elsewhere, but Hans Clarke encouraged us to continue as heirs to the wealth of projects Schoenheimer had begun and developed . . . How the division of ‘spoils’ came about I do not recall—it may have been by drawing lots. At any rate, David Shemin ‘drew’ amino acid metabolism, which led to his classic work on heme biosynthesis. David Rittenberg was to continue his interest in protein synthesis and turnover, and lipids were to be my territory.”

Bloch now began his independent studies of the biosynthesis of cholesterol. It was a formidable enterprise. In the era before NMR, infrared, and mass spectroscopy, the determination even of the chemical structure of cholesterol, with its 27 carbon atoms arranged in four rings and with a branched hydrocarbon side chain, had been a challenge to the world’s greatest chemists of natural products. The Nobel Prizes in chemistry for 1927 and 1928 had been awarded to Heinrich Wieland and Adolf Windaus, respectively, for their work on the structure of cholesterol and the closely related bile acids, but it was not until 1932 that the fully correct structure was established.

In his 1928 Nobel lecture (25), Windaus stated: “This formula [of cholesterol] is very complicated and has no similarity to the formulae of sugars, fatty acids, or the amino acids which occur in protein. The synthesis of such a substance appears to the chemist particularly difficult, and up to now I have not dared to attempt it, as success is extremely improbable. Furthermore, the majority of physiologists have not been inclined to believe the animal organism capable of such a synthesis, for it is known that other seemingly simpler syntheses—e.g. that of tyrosine and tryptophane—have not succeeded in the animal organism.”
Bloch of course knew that Windaus’ pessimistic view of the capabilities of the animal organism was unfounded. Schoenheimer and Rittenberg had demonstrated the extensive incorporation of deuterium from D₂O into cholesterol in the mouse and concluded that cholesterol must be synthesized by the joining of a number of small molecules. The pathway for the biosynthesis of cholesterol from acetate, involving more than 30 separate enzyme-catalyzed reactions, can now be found in every textbook of biochemistry. A detailed review is beyond the scope of this essay. Here we will consider only the principal landmarks in its three major stages: 1) acetate to “activated isoprene”; 2) “activated isoprene” to squalene; and 3) squalene to cholesterol.

Bloch’s studies began with investigations of the overall process of formation of cholesterol in the intact organism. Stimulated by a report from the German workers Sonderhoff and Thomas (26), indicating that acetate is efficiently converted into the sterols of yeast, Bloch began a series of studies demonstrating the incorporation of specifically labeled acetate into cholesterol in the intact animal. These studies were continued and expanded after his move in 1946 to the Department of Biochemistry at the University of Chicago, where his good friend Earl Evans, also a product of Hans Clarke’s department, had become chairman.

I was a graduate student in the Department at this time, and so I came to know Konrad Bloch, first as a teacher and later as a colleague and friend. He was a man of personal qualities commensurate with his great abilities. His manner with students was friendly and easy. He was painstakingly generous in acknowledging the research contributions of his colleagues and of other laboratories. He was widely cultured, devoted to music, literature, and art.

In the mid-1940s, Bloch (23) was completely convinced of the truth of Lipmann’s dictum that energy-requiring biosynthetic reactions are driven by ATP, directly or indirectly. Before this period the synthesis of peptide bonds had been observed only by reversal of the reactions catalyzed by proteases. In a project quite unrelated to the cholesterol problem, he and his students began to investigate the synthesis of the tripeptide glutathione as a possible model of protein synthesis. They were indeed able to show that the assembly of glutathione requires the successive activation of glutamate and glutamylcysteine by ATP, but unfortunately the mechanism proved to shed little light on the ribosomal synthesis of proteins.

Bloch was also very much aware of the potential power of microbial genetics for the analysis of metabolic pathways, and he enrolled as a student in the famous course in microbiology taught by C. B. Van Niel at the Hopkins Marine Station in Pacific Grove, CA. When a mutant of the mold Neurospora crassa was isolated in Tatum’s laboratory that grew only when acetate was added to the medium, Bloch was eager to follow this lead. He and his collaborators found that isotopically labeled acetate was converted to ergosterol in this mutant essentially without dilution of the isotope. Clearly the sterol could be built up entirely from acetate.

In the conversion of acetate to cholesterol, which of the carbon atoms of cholesterol were derived from the carboxyl group and which from the methyl group? Studies carried out over a number of years in the laboratories of Cornforth and of Popjak, as well as of Bloch, achieved the ambitious goal of defining the origin of each of the 27 carbon atoms of cholesterol as either the methyl or the carboxyl carbon of acetate. This work placed important constraints on possible structures of intermediates in the scheme.

It had been known for some time that squalene (a branched, acyclic hydrocarbon found in abundance in the livers of sharks) when fed to animals increases the levels of cholesterol in their tissues. To test the idea that squalene might be a precursor of cholesterol, Bloch went to the Biological Research Station in Bermuda to attempt the preparation of isotopically labeled squalene in shark liver, but the shark proved to be an intractable subject for study (23). “All I was able to learn was that sharks of manageable length are very difficult to catch and their oily livers impossible to slice.” Back at the University of Chicago, however, his student Robert Langdon was able to prepare labeled squalene by feeding rats labeled acetate along with unlabeled squalene as an isotopic trap. Labeled squalene so obtained was then fed to rats and found to be converted to cholesterol (27). This was an important result. In the dissection of every biosynthetic pathway, it is particularly helpful to identify an intermediate in the middle of the chain of reactions; the researcher can then trace the pathway both backwards and forwards. At this stage in his work, in 1954 Bloch moved to the Department of Chemistry at Harvard, where he was to remain for the rest of his career.

Squalene, containing 30 carbon atoms, could plausibly be considered to be built up from 6 units of isoprene, a branched, unsaturated compound containing five carbon atoms. Isoprene
was already known to be a building block of other naturally occurring hydrocarbons such as rubber, although the nature of the biologically active “isoprene donor” remained unknown.

Robinson (28) had suggested that squalene might be folded to form the basic structure of cholesterol directly. Bloch, however, after illuminating discussions with his Harvard colleague Robert Woodward considered that lanosterol, with a structure closely similar to cholesterol but with three “extra” methyl groups, was likely to be an intermediate in this transformation.

Up to this point, Bloch’s experimental approach to the cholesterol problem had been largely confined to isotopic tracer studies with intact animals or with tissue slices in which cellular structure was preserved intact, but now he turned increasingly to the study of cell-free enzyme systems. Rat liver homogenates, prepared by the methods developed by Nancy Bucher, were found to catalyze the transformation of labeled squalene to lanosterol and of lanosterol to cholesterol. Although much work remained to be done, Bloch had established the landmarks for the final stages of the biosynthesis of cholesterol (29).

The focus now was turned to the first stages of the pathway, the conversion of acetate to the “active isoprene donor.” A mutant strain of *Lactobacillus acidophilus* had been found to grow only when acetate was added to the medium. A substance that very efficiently replaced the acetate requirement was identified by workers at Merck, Sharpe and Dohme (30) as mevalonic acid (isolated as the lactone). Mevalonic acid was then shown to be a very efficient precursor of squalene and of cholesterol in homogenates of liver (31). These findings opened the way for the elucidation of the reactions leading to the formation of the “active isoprene unit” of which mevalonate was clearly the precursor. Progress in this area now became fast and furious with important contributions from the laboratories of Rudney, Lynen, Cornforth, and Popjak among others.

Bloch and his collaborators showed that the overall conversion of labeled mevalonic acid to squalene in extracts of bakers’ yeast required ATP as well as reduced pyridine nucleotide and manganese ions. His colleague Chen then discovered the phosphorylation of mevalonate to a monophosphate. The further conversion of this monophosphate to the important intermediates isopentenylpyrophosphate and dimethylallylpyrophosphate was elucidated largely by work in Lynen’s laboratory.

The synthesis of squalene via geranyl pyrophosphate and farnesyl pyrophosphate was next documented. As shown by the early studies of Bloch, squalene is converted in a series of steps to lanosterol, which after several further transformations gives rise to cholesterol.

It is impossible, of course, in this highly condensed account to do justice to the vast amount of work, still ongoing in laboratories over the world, that has led to our present knowledge of the biosynthesis of cholesterol. It was Bloch, however, who was a prime mover in all three phases of the problem. For this work he was awarded a Nobel Prize, with Feodor Lynen, in 1964.

Working out the pathway for the assembly of the complex structure of cholesterol was an exemplary achievement of the era of biosynthesis, important not only because of the intrinsic interest of its enzymology but also because of its significance for medicine. High levels of blood cholesterol, characteristic of populations in developed countries, strongly increase the danger of heart disease and stroke. An understanding of the detailed route of biosynthesis made it possible to determine that the synthesis of mevalonate from HMG-CoA is a rate-making step in the production of cholesterol. This advance made possible the development of drugs, the family of statins, that reduce levels of blood cholesterol with a minimum of toxic side effects. These drugs are among the most useful in modern medicine.

Konrad Bloch made outstanding contributions to fields other than the biosynthesis of cholesterol, including the enzymic synthesis of fatty acids and the mechanism of enzyme action (23). He died on October 15, 2000 at the age of eighty-eight.

The development of any field of science is inevitably the work of many hands. Obviously, Lipmann, Schoenheimer, and Bloch cannot be regarded as single handedly transforming American biochemistry. Their work was nonetheless a great gift to their adopted country and a shining manifestation of the international character of science.

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