# H.A. BARKER 1907-2000

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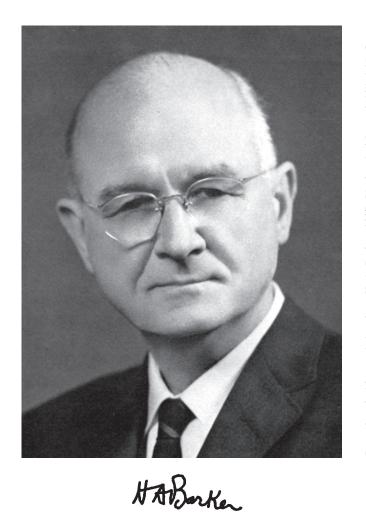
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# H. A. BARKER

November 29, 1907–December 24, 2000

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46 T XPLORATIONS OF MICROBIAL METABOLISM" was the title Lichosen by H. A. Barker for his summary of his lifelong scientific accomplishments in 1978. His title was descriptive and accurate, but characteristically for this modest man, it significantly understated the extraordinary breadth and depth of the impact of his discoveries on central concepts in the intermediary metabolism of all organisms, the mechanism of enzymatic catalysis and of coenzyme function, as well as on microbial physiology, taxonomy, and ecology. Barker's contributions are today so much a part of the fundamental fabric of modern biochemistry and microbiology that it is easy for younger scientists to overlook the modest and gentle man who made them. Also important is the exceptional influence Barker had on the many scientists who worked with him, whether as students, postdoctoral researchers, or in one of many collaborations, research visits, and sabbaticals. He taught the conduct of insightful, thoughtful, carefully executed scientific experimentation by quiet example, and he was admired and respected by all who worked with him.

Horace Albert Barker, known as "Nook" by his intimate friends but as H. A. Barker or simply "Dr. Barker" by

everyone else, grew up in California and by his own description came slowly to a career in science. Although he was originally more interested in music and literature, his love of the outdoors and the natural world and his undergraduate studies of biology at Stanford University, completed in 1929, led him to choose graduate study first in biology; he then switched to chemistry as his interest in biochemistry developed at Stanford, which awarded him the Ph.D. degree in 1933. During his graduate studies Barker began an association with the Hopkins Marine Station at Pacific Grove that was to profoundly affect the direction of his future scientific research. Two summer research periods were followed by a postdoctoral fellowship from 1933 to 1935 at the Hopkins Marine Station, where Barker began his studies of microbiology under the direction of the distinguished Dutch microbiologist C. B. van Niel. Van Niel's mastery of this discipline, his enthusiasm, and his gifts as a teacher and researcher soon persuaded Barker to choose microbiology for his own career. From van Niel he absorbed the concepts so strongly associated with the "Delft school" of microbiology, namely, that microorganisms can be best understood and classified by the chemical activities they carry out, that understanding these biochemical processes provides the key to the ecological niches microorganisms occupy, and the unity of biochemistry (i.e., that the fundamental nature of the metabolic reactions in bacteria was the same as was being unraveled by other biochemists in the study of yeast and animal tissues).

Barker's commitment to a lifelong study of the chemical activities of microorganisms was consolidated during a year's study (1935-36) with van Niel's mentor A. J. Kluyver in the Delft Microbiology Laboratory, which was supported by a fellowship from the Rockefeller Foundation. In the course of this single year he initiated studies on three top-

ics that he was to pursue throughout his career and that would yield many of his most significant discoveries. These were production of fatty acids by microbial fermentation, the biochemistry of methanogenesis, and the anaerobic degradation of glutamate. Barker adopted from Kluyver and van Niel the practice of characterizing fermentative pathways by quantitative analysis of the amounts of substrates consumed and products formed. From a determination of equations that account for the carbon, nitrogen, and redox balance of a fermentative process much about the nature of the fermentation itself could be deduced. In his later research Barker was to add the use of radiocarbon tracers and identification of individual enzymatic steps in cell-free extracts to this fundamental method. The combination led to the many remarkable discoveries we discuss below.

In 1936 Barker returned to California to take a position as a soil microbiologist in the Agricultural Experiment Station at the University of California, Berkeley. He was to remain on the Berkeley faculty for his entire career, holding appointments first in plant nutrition, then plant and microbial biochemistry, and finally in the reorganized Department of Biochemistry after 1959. His students came from many disciplines, however, and many earned their degrees in an interdepartmental program called comparative biochemistry. Through the years of the Great Depression and World War II to the great flowering of federal support of basic research in the postwar years, Barker pursued his fascination with microbial fermentations. "In lean years we did microbiology," he told one of us (R.L.S.), "and in good years we did biochemistry." Because of the proximity of the Berkeley Radiation Laboratory, radioactive carbon isotopes became available to biological researchers. As early as 1939 Barker, Zev Hassid, Sam Ruben, and Martin Kamen began experiments on the fate of <sup>11</sup>CO<sub>2</sub> during methanogenesis.

When [<sup>14</sup>C] with its much more convenient long half-life became available in 1944, the use of radiotracer technology for the investigation of metabolic pathways began almost immediately to lead to important and often unexpected findings.

It was evident to Barker that to elucidate the individual biochemical steps involved in the fermentations he was studying, it was necessary to isolate and characterize the enzymes that catalyze each step from cell-free systems. This became all the more crucial as his studies led again and again to the involvement of cofactors and the formation of cofactor-bound intermediates—cofactors of universal importance in intermediary metabolism. His interest and skill in working with cell-free systems were stimulated during time spent with Fritz Lipmann in 1941-42 while supported by a Guggenheim Fellowship, by collaborative research with Michael Doudoroff and Zev Hassid on the catalytic mechanism of sucrose phosphorylase, and later during research in 1951 and 1952 with Arthur Kornberg at the National Institutes of Health.

Some of H. A. Barker's most significant scientific contributions will be discussed in individual sections below. Although this modest man did not actively seek public recognition, recognition came to him as the impact of his discoveries became widely appreciated. He was elected a member of the National Academy of Sciences and the American Academy of Arts and Sciences. He received the 1965 Borden Award in Nutrition and the Hopkins Medal from the Biochemical Society (U.K.). He was named California Scientist of the Year in 1966, and President Lyndon Johnson presented the National Medal of Science to him in 1968. The Biochemistry Building on the Berkeley campus was renamed H. A. Barker Hall in 1988, a rare recognition for a living scholar. Professor Barker retired to emeritus status in

1975, but he remained active in the Biochemistry Department's research and intellectual life for many years thereafter. He died peacefully at his home in Berkeley at the age of 93.

During his years at Stanford, Barker married Margaret McDowell, and they were together for 62 years until her death in 1995. They had three children: Barbara Friede, Elizabeth ("Betsy") Mark, and Robert Barker. We have noted that Barker's love of science was interwoven with his love of nature and the outdoors. He and his family carefully reserved a portion of their summers for holidays at their cabin in the Sierras, and Barker's fondness for fishing in remote locations was well known to his many friends.

## BIOCHEMISTRY OF METHANE FORMATION

Methane fermentations are now known to serve as important anaerobic processes in which the decomposition of a variety of alcohols, amines, and fatty acids is coupled to the reduction of carbon dioxide to methane. C. B. van Niel, who was trained in the famous Microbiology Department of the Technical University in Delft, Holland, and later became professor of microbiology at Stanford University's Hopkins Marine Station in Pacific Grove, California, became interested in the anaerobic fermentation of specific compounds in nature and the origin of the methane generated in these processes. Barker was exposed to van Niel's theories about the chemistry of the overall methane fermentation when he spent summers at the Hopkins Marine Station while a graduate student in chemistry at Stanford and later as a postdoctoral fellow with van Niel.

During this period van Niel proposed his carbon dioxide reduction theory to explain the origin of methane as a common product in the diverse reactions known at the time. Stimulated by these ideas Barker became interested in meth-

ane bacteria. During a later postdoctoral appointment in the laboratory of A. J. Kluyver in Delft he obtained enrichment cultures from canal mud that converted ethanol-bicarbonate mixtures to acetate and methane. The microorganism in these cultures responsible for this reaction was named Methanobacillus omelianski. Although it was shown many years later to actually be a consortium of two mutually dependent species, the culture proved to be very useful biological material for detailed studies of carbon dioxide reduction to methane. In particular, this culture was used for Barker's groundbreaking experiments that demonstrated conversion of <sup>11</sup>CO<sub>2</sub> to radioactive methane, even though the half-life of this new radioactive element was only 20 minutes. When the long-lived [14C] isotope became available in 1944, it enabled Barker and his associates to conduct many elegant studies on the chemical details of methane biosynthesis. An important exception to the general theory that methane originates exclusively from carbon dioxide was reported in the literature while one of us (T.C.S.) was a graduate student in Barker's laboratory in Berkeley. The authors reported that when [14C]-labeled carbon dioxide was added to cultures that were fermenting acetate and producing methane, little or no radioactivity was found in the methane evolved. Under Barker's direction these results were confirmed, and furthermore it was established that during acetate fermentation methane was derived exclusively from the methyl group of acetate and the carboxyl group was converted to carbon dioxide. Likewise, during fermentation of [14C]-labeled methanol three equivalents of radioactive methanol were reduced to radioactive methane at the expense of the oxidation of one equivalent of methanol to radioactive carbon dioxide and water. Later Pine and Barker showed that when CD<sub>3</sub>OH was used as the substrate, all three deuterium atoms were retained in the

methane product. With deuterium-labeled solvent and unlabeled methanol one deuterium atom from solvent was incorporated into the methane product.

The demonstration from Barker's laboratory of methane generation from the methyl groups of acetate and methanol led to a considerably expanded and modified view of the biochemistry of methane fermentation in general. In 1956 Barker proposed a unifying conceptual scheme for methanogenesis from carbon dioxide, acetate, and methanol. A central feature of this scheme was the postulate that carbon dioxide becomes attached to an unspecified carrier prior to stepwise reduction to methane. Furthermore, the methyl groups of acetate and methanol could be transferred to this or additional carriers and be reduced to methane or oxidized to carbon dioxide. This concept was verified by the discoveries by B. Blaylock and T. Stadtman and of D. Grahame of the intermediary role of methyl corrinoids as methyl group carriers and the discovery in the laboratories of R. S. Wolfe, R. K. Thauer, and G. Vogels of several novel cofactors that function as C-1 carriers in methanogenesis. These findings have permitted the detailed biochemistry of methanogenesis to be described.

# STUDIES OF FATTY ACID METABOLISM WITH CLOSTRIDIUM KLUYVERI

During the year he spent in Kluyver's laboratory Barker embarked upon a study designed to test van Niel's theory that the reduction of  $\mathrm{CO}_2$  to methane might be involved in the fermentation of organic compounds by methane bacteria. To this end Barker prepared anaerobic enrichment cultures containing  $\mathrm{CaCO}_3$  and ethanol and a generous inoculum of mud from the Delft canal outside Kluyver's laboratory. Microscopic examination of a culture producing a mixture of acetic, butyric, and caproic acids and methane disclosed the presence of two different types of bacteria, which Barker

separated and purified. One of these catalyzed the conversion of ethanol and  $\mathrm{CO}_2$  to methane and acetic acid and was given the name *Methanobacterium omelianski*, as described above. A pure culture of the other microorganism catalyzed the conversion of ethanol and acetic acid to shortchain fatty acids and was given the name *Clostridium kluyveri*. In subsequent studies one of Barker's students B. T. Bornstein established that *C. kluyveri* catalyzes the conversion of one equivalent each of ethanol and acetate to butyrate, and the further reaction of the butyrate with a second equivalent of ethanol to form caproate.

In 1944 a more detailed investigation of the mechanism involved in fatty acid synthesis by C. kluyveri became possible with the availability of the long-lived isotope of carbon [14C]. In a collaborative study with Martin D. Kamen, co-discoverer of [14C], Barker and Bornstein demonstrated that the fermentation of [carboxyl-14C] acetate and unlabeled alcohol led to the production of [14C]-butyrate that was almost equally labeled in the carboxyl and beta carbon atoms and to [14C]-caproate that was labeled in the carboxyl, beta, and delta carbon atoms. Furthermore, during fermentation the specific radioactivity of the added acetate was decreased by an amount equivalent to the amount of ethanol used. This established that the formation of butyrate and caproate from ethanol and acetate is a coupled oxidation-reduction process in which the ethanol is oxidized to acetate (or a compound in equilibrium with acetate) and that the consumption of two or three equivalents of acetate leads to formation of the 4- and 6-carbon derivative (viz. βketo acids) that are reduced to butyrate and caproate. In view of Lipmann's calculations showing that the condensation of two moles of acetate to form acetoacetate is strongly endergonic ( $\Delta G^o = +16,000 \text{ cal}$ ) and Lipmann's demonstration that acetyl-P is formed in the decomposition of pyruvate by *Lactobacillus delbruckii*, Barker proposed that acetyl-P might be the active acetate formed in the oxidation of ethanol by *C. kluyveri*.

It became possible to test this hypothesis with the discovery that dried cell preparations of C. kluyveri undergo autolysis in phosphate buffer, yielding cell-free extracts that contained all of the enzymes involved in the conversion of ethanol and acetate to lower fatty acids. These extracts also could utilize molecular oxygen as an electron acceptor for the oxidation of butyrate and caproate to acetate. Studies with these extracts confirmed that ethanol is oxidized to acetyl-P by a mechanism in which acetaldehyde is an intermediate. It was shown further that the cell-free extracts contained an enzyme system that catalyzed the transfer of the phosphoryl group of an unlabeled acetyl-P molecule to [14 C]-acetate to form [14C]-acetyl-P, thus accounting for the equilibration of ethanol-derived active acetate with ordinary acetate. Of particular significance was the finding that C. kluyveri contained an enzyme (phosphotransacetylase) that catalyzed the reversible transfer of the acetyl moiety of unlabeled acetyl-P to [32P]-labeled inorganic phosphate to form acetyl-32P. Moreover, in the presence of arsenate the enzyme catalyzed rapid hydrolysis of acetyl-P to acetate and inorganic phosphate. These results were reminiscent of the demonstration in 1947 by Doudoroff, Barker, and Hassid that sucrose phosphorylase catalyzes the arsenolysis of glucose-1-phosphate. This led them to postulate that the reaction proceeds by a mechanism in which the glucose moiety of glucose-1-phosphate is transferred to a site on the enzyme with release of phosphate and that substitution of arsenate for phosphate in the reverse reaction leads to the unstable glucose-1-arsenate derivative that undergoes spontaneous hydrolysis to form glucose. By analogy Barker suggested that the arsenolysis of acetyl-P might involve the formation of an acetyl-enzyme intermediate. However, he recognized the possibility that the results could also be explained if phosphotransacetylase catalyzed the reversible transfer of the acetyl group to an undefined cofactor in the cell extracts, possibly coenzyme A (discussed below).

It was subsequently found that molecular hydrogen could serve as the electron donor for the reduction of acetyl-P and acetate to butyrate and that molecular oxygen could serve as an electron acceptor for the oxidation of butyrate to acetate and acetyl-P. Thus, it became evident that the involvement of postulated intermediates in fatty acid synthesis could be determined by manometric measurements of hydrogen or oxygen consumption when the postulated intermediate was incubated with crude cell-free extracts. Of 15 possible intermediates examined, only 2—acetoacetate and vinylacetate—were metabolized by the extracts, however the roles of these two substances as free intermediates in either butyrate synthesis or oxidation were excluded by a number of criteria. Most important was the fact that there was no incorporation of radioactivity in either substance when added to incubation mixtures catalyzing the overall oxygen-dependent oxidation of [14C]-butyrate to [14C]-acetyl-P and [14C]-acetate or during the hydrogen-dependent reduction of the latter labeled compounds to [14C]-butyrate. It was therefore concluded that 4-carbon carboxylic acids at various states of oxidation do not normally occur as free intermediates but are present only as activated derivatives or as enzyme complexes that do not readily equilibrate with the free acids.

While Barker's studies on fatty acid metabolism were in progress, studies in Lipmann's laboratory (1945-49) on the mechanism of sulfonamide acetylation by pigeon liver extracts led to discovery of a new form of active acetate that was produced by a reaction of ATP with acetate and a new

coenzyme, which he named coenzyme A (CoA). Further studies in Lipmann's laboratory by G. D. Novelli, M. Soodak, and N. O. Kaplan showed that CoA is composed of adenosine-5'-phosphate pantothenic acid and a sulfhydryl moiety. The biochemical importance of acetyl-CoA became evident from studies in the laboratories of F. Lipmann, S. Ochoa, and D. Nachmansohn showing that acetyl-CoA is implicated in the acetylation of choline, in the synthesis of citrate and acetoacetate, and in pyruvate metabolism. In view of the fact that extracts of C. kluyveri were found to contain high concentrations of pantothenic acid, Barker proposed that CoA might be implicated in the fatty acid metabolism of this organism. Subsequently, studies in Lipmann's laboratory by E. Stadtman and G. D. Novelli showed that the arsenolysis of acetyl-P by phosphotransacetylase from C. kluyveri is dependent upon the presence of CoA, thus confirming Barker's suggestion that acetyl-CoA might be involved in this reaction. About the same time F. Lynen and E. Reichert (in 1951) succeeded in isolating acetyl-CoA and demonstrated that its synthesis involves acetylation of the free sulfhydryl group of CoA to form a thiolester derivative.

At the first Symposium on Phosphorous Metabolism held in Baltimore, Maryland, in the spring of 1951 Barker reviewed the results of studies with enzyme preparations of *C. kluyveri* as well as complementary studies in the field of CoA metabolism. Based on a most impressive critical analysis of the available information he proposed that the oxidation of butyrate by extracts of *C. kluyveri* occurs by a mechanism in which butyrate is first converted to butyryl-CoA and in which all of the 4-carbon intermediates involved in its oxidation exist as their CoA derivatives. Then the last of these to be formed, acetoacetyl-CoA, is cleaved by reaction with a molecule of free CoA to form two molecules of acetyl-CoA, which in the presence of phosphate is converted by

phosphotransetylase to acetyl-P and CoA. Significantly, when Barker first proposed this scheme for fatty acid oxidation, there was only inferential evidence for a role of CoA in the oxidation of butyrate by extracts of *C. kluyveri* and there was no evidence of any kind to implicate CoA in the oxidation of fatty acids by animal enzyme systems. It is therefore a tribute to Barker's imagination and conceptual analysis that within a few years after his report his hypothesis was shown to be correct in every significant detail, not only in *C. kluyveri* but in animals as well.

# GLUTAMATE FERMENTATION AND DISCOVERY $\mbox{OF THE $B_{12}$ COENZYMES}$

During his postdoctoral year in Delft in 1936 Barker isolated the glutamate-fermenting bacterium Clostridium tetanomorphum and determined that the ratio of products of the fermentation—acetate, butyrate, ammonia, and CO<sub>9</sub> was incompatible with the degradation of glutamate via the reactions associated with the tricarboxylic acid cycle. In the 1950s he and his student J. T. Wachsman returned to the problem of glutamate fermentation and showed by analysis of the products of fermentation of [14C]glutamate that the degradation must involve a previously unknown pathway. Studies with cell-free extracts led to the isolation and identification of mesaconic acid and later β-methyl-L-aspartate as intermediates in the fermentation. Chemical degradation of [14C]mesaconate formed from [4-14C]glutamate led to the surprising conclusion that the methyl group of mesaconate (and β-methylaspartate) originated from C-3 of glutamate and that a novel isomerization of the carbon skeleton of glutamate, namely, migration of the C-1 + C-2 "glycyl" moiety from C-3 to C-4, must occur during its conversion to β-methylaspartate. In 1956 A. Munch-Petersen found that the isomerization was inhibited by charcoal treatment of cell-free extracts and began attempts to isolate and identify the charcoal-absorbable cofactor. After considerable difficulty the identification of the cofactor as a novel form of pseudovitamin B<sub>19</sub> in 1958 by H. Weissbach and R. D. Smyth was made possible by two advances: development of a rapid spectrophotometric assay for glutamate mutase—the enzyme catalyzing the reversible isomerization of glutamate and βmethylaspartate—and most important of all the discovery that the coenzyme forms of vitamin B<sub>19</sub> are rapidly destroyed by light. Barker and his coworkers soon isolated several forms of the new coenzyme in pure form and showed that it contained the elements of adenine and a pentose linked to the corrinoid. Lenhert and Hodgkin demonstrated in 1961 by X-ray diffraction analysis that the coenzyme was formed by the direct ligation of C-5 of 5'-deoxyadenosine to the Co atom at the center of the corrinoid ring of vitamin B<sub>19</sub>. This was the first demonstration of the existence of a biologically stable and functional carbon-metal bond. Barker has described the detailed path of this beautiful series of discoveries; they could serve as a textbook example of the careful, thoughtful, and insightful conduct of research.

The discovery of the coenzyme forms of vitamin  $B_{12}$  was quickly followed by the demonstration of their involvement in the methylmalonyl coenzyme A mutase, diol dehydratase, and ethanolamine dehydratase reactions. R. L. Blakley and Barker discovered the involvement of coenzyme  $B_{12}$  (now called deoxyadenosylcobalamin) in the ribonucleotide reductase reaction of *Lactobacillus leichmanii* in 1965. The discovery of the involvement of the  $B_{12}$  coenzymes in these and other enzymatic reactions led to a period of intensive study of the mechanism of the coenzyme's involvement in catalysis. By a combination of experiments with isotopic tracers and EPR spectroscopy and use of substrate analogues,

R. H. Abeles, J. Stubbe, and others established our current view of these reactions. Transient homolytic cleavage of the 5'-deoxyadenosyl carbon-corrinoid cobalt bond in the enzyme-bound coenzyme leads to formation of a carbon free radical, which in turn abstracts a hydrogen atom from the substrate, leaving a carbon radical form of the substrate, which undergoes rearrangement of the carbon skeleton or dehydration. The substrate product radical then accepts a hydrogen atom from the 5'-carbon of the 5'-deoxyadenosyl coenzyme intermediate, and the coenzyme returns to its original state.

The discovery of the carbon-cobalt bond in deoxyadenosylcobalamin also led to the recognition that C-alkylcorrinoids could play roles in reactions of methionine biosynthesis and methanogenesis involving Co-methyl- $B_{12}$  intermediates and in biogenesis of acetate from  $CO_2$  via enzyme-bound Co-carboxymethyl- $B_{12}$ . The structures of a number of coenzyme  $B_{12}$ -dependent enzymes, including glutamate mutase from C. tetanomorphum, have been determined at high resolution. While questions of detailed mechanism of catalysis remain, the biochemical roles of corrinoids are well understood today, thanks to Barker's discoveries.

#### LYSINE FERMENTATION AND THE "RADICAL SAM" ENZYME FAMILY

Toward the end of his research career Barker turned his attention to the pathway of lysine fermentation by anaerobic bacteria. When Olga Rochovansky, a student of Sarah Ratner's, came to his laboratory in the mid-1960s, Rochovansky selected the lysine fermentation for study. Her interest in this fermentation had been stimulated by discussions of this topic with Sarah Ratner and Thressa Stadtman, a visitor in the New York laboratory. In Barker's laboratory a new *Clostridium* species that converted lysine to fatty acids and ammonia was isolated. Cell-free extracts were shown

to form acetate from carbons 1 and 2 of lysine, which followed one of the two types of lysine cleavage reactions previously established in 1954. Costilow, Rochovansky, and Barker basic intermediate—3,6discovered that a more diaminohexanoate, or \beta-lysine—was initially formed from L-lysine and could be separated by electrophoresis from it. The isolation of  $\beta$ -lysine provided the first lead in the elucidation of a novel pathway of lysine metabolism. The enzyme that catalyzes conversion of L-lysine to β-lysine—lysine 2,3-aminomutase—was oxygen labile and was stimulated by a combination of pyridoxal phosphate, ferrous iron, and Sadenosylmethionine. The full biochemical significance of these initial findings was revealed only in 1990s by an elegant series of investigations by Perry Frey and his collaborators. Remarkably, lysine 2,3-aminomutase resembles the coenzyme B<sub>19</sub>-dependent enzymes in that it involves enzymebound cobalt and the transient formation of an enzymebound 5'-deoxyadenosyl free radical. The latter initiates formation of free-radical forms of lysine (bound in Schiff's base form to pyridoxal phosphate). Radical migration accompanies isomerization between lysine and β-lysine. The role of S-adenosylmethionine in radical formation has led to its description as a "poor man's adenosylcobalamin." Now it is recognized that the mechanism of lysine 2,3-aminomutase is also found in a number of other diverse enzymes, which constitute the so-called "radical SAM" enzyme family. Members include the pyruvate formate-lyase activating enzyme, anaerobic ribonucleotide reductase, biotin synthase, and probably lipoate synthase and benzylsuccinate synthase or its activating enzyme. As had occurred so often previously the careful analysis of the biochemistry of a seemingly obscure metabolic pathway in a little studied anaerobic bacterium by Barker and his coworkers led to the discovery of novel and highly unexpected biochemical mechanisms of great general significance.

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