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The Emergence of Biochemistry

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George Sarton was a tireless advocate of professionalism in the history of science, and took a dim view of the scientist "who has become sufficiently interested in the genesis of his knowledge to wish to investigate it, but has no idea whatsoever how such investigations should be conducted and is not even aware of his shortcomings. . . . He generally lacks the humility of the beginner, and publishes his results with blind and fatuous assurance" (*1*). This stern stricture was not unjust, and its recollection is well calculated to dismay a biochemist who proposes to discuss the history of his subject. So in thanking the History of Science Society for having chosen me to deliver this George Sarton Memorial Lecture, I must confess that the sense of honor is tinged with a measure of disquiet.

In part, this uneasiness arises from the knowledge that all too often a scientist who tries to tell the history of his specialty is tempted to celebrate its successes, and that his uncertain and self-serving recollections (should I say anecdotage?) have frequently muddied the historical record. Nevertheless, I venture to suggest that, although Sarton's hopes for a professional history of science have been realized to a considerable degree, scientists can still make a useful contribution to its advancement, especially through critical study of the historical record of theories, observations, experiments, and methods within their field. Such so-called internal history, no matter how accurate or complete, does not of course constitute the history of science and must be seen in relation to the lives of scientists and to the societies in which they worked. Also, the relative importance of the internal factors in a scientific development and those arising from social circumstances is not the same in different branches of science. The fact remains, however, that, when we study the history of modern science, our interest in the lives and social environments of particular people is usually related to the significance we attach to the work they did as scientists. The intensive examination of the scientific development in which that work played a role is clearly an important part of a professional history of science. I submit therefore that, although internal history does not suffice to illuminate the scientific enterprise as a human and social activity, without it the illumination is likely to be superficial and uncertain.

My subject is the historical development of efforts to explain biological phenomena in terms of the specific properties of chemical substances present in living organisms. Today, few informed people doubt that such explanation is possible, and indeed useful. Recent achievements in the study of the chemical structure and biological function of such things as nucleic acids or hormones have been in the public eye, and chemical concepts and methods have permeated the fabric of biology, medicine, and agriculture (*2–6*). As a consequence, much favor has been shown to the area of science now known as biochemistry, which I take to include much of what some prefer to call molecular biology.

A century ago, there was less confidence in the power of chemistry to explain the phenomena of life and, in contrast to the situation today, there were few outward marks of an independent discipline devoted to this pursuit, in the form of academic departments, professional societies, or research journals (*7*). It is not my aim, however, to describe the institutional rise of biochemistry or to trace the adoption of its present name (*8*). Rather, I wish to examine some of the various attitudes and approaches since about 1800 to the study of what we now call biochemical problems. I hope to indicate that there has been continuity in the effort to solve these problems, not as a succession of conjectures and refutations within a defined scientific discipline, but as a complex and often tortuous interplay of chemistry and biology in which many kinds of scientists took part (*9*). In particular, I suggest that a prominent feature of this interplay has been a competition between two styles of molecular explanation of biological phenomena, one in which molecules were considered to be units of physical motion while in the other they were viewed as units of chemical reaction.

Immediate Principles of Biological Organisms

If we begin around 1800, it is with the recognition that historians of science have found the roots of biochemistry in ancient Greece and China (*6*), and that they have identified as the fathers of modern biochemistry Paracelsus (*6*, p. 80) in the 16th century or van Helmont (*10*) or Sylvius (*11*) in the 17th century. Whatever merits such attributions of paternity may have, all accounts agree that, by the first decade of the 19th century, many scientists, largely trained as physicians or pharmacists, were engaged in the study of the chemistry of living things. One of their objectives was to characterize chemical substances isolated from biological fluids or from extracts of solid plant and animal tissues. Methods were sought to obtain such substances in a state that had not been changed by the process of isolation, so that they could be considered to represent natural chemical constituents—"immediate principles"—of the plants and animals from which they had been derived (*12*). This

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kind of separation chemistry continued a tradition established by 18th-century pharmacists (*13*) whom we would perhaps call chemical craftsmen (*14*). Aside from the obvious commercial advantage to be gained in preparing drugs in as pure a state as possible, with enhanced and reproducible potency, there was also the conviction that the identification of the immediate principles of plants and animals would throw light on biological organization and physiological function.

It is a truism that all empirical research is laden with theory, and the seemingly dull plant and animal chemistry of the early 19th century is no exception. The lack of time prevents me from developing this theme, but I must note the concern of the chemical craftsmen with the problem of purity, a central philosophical concept of chemistry (*15*). Also, I think it is important to emphasize that throughout the development of what we now call biochemistry, to this day, the craftsmanship of separation chemistry has played a decisive role in the discovery and isolation of new immediate principles whose existence was not predicted (*16*), but whose biological function could then be studied. Obviously the separation chemistry of today is based on a vastly greater fund of theoretical and empirical knowledge and is aided by valuable instruments; but it is still a part of science in which the skill, industry, and ingenuity that we associate with individual craftsmanship are human qualities essential for success.

By the first decade of the 19th century, a sizable number of immediate principles had been identified in plants and animals. Some of them, like cane sugar and urea, were called organic substances because they were believed to be made under the influence of the vital force of organized living things. Within a few years it became clear, however, that, as with inorganic compounds, the molecules of organic materials are composed of elements present in fixed and multiple proportions (*17*). Improved methods of analysis provided an increasing volume of knowledge about the elementary composition of the many new organic substances that were being discovered and of the products derived from them by chemical modification. The manpower for the elaboration of this new organic chemistry came largely from pharmacy. It was a time of professional opportunity, when a pharmacist's apprentice could aspire to a university professorship in chemistry after working in a laboratory such as that of Liebig (*18*).

By about 1860, the unification of inorganic and organic chemistry had been completed (*19*), largely as a consequence of the chemical insight of a group of young organic chemists led by Laurent. I do injustice to a complex and important intellectual synthesis in merely saying that these men set the stage for the development of concepts of chemical structure based on valence and stereochemistry. Nor is it possible in this article to indicate the magnitude of the subsequent theoretical and experimental achievements that led not only to the determination of the structural arrangement of atoms in the molecules of many plant and animal substances, but also to the rational synthesis of known organic compounds and of substances not previously found in nature (*20*). Among these new substances were artificial dyes and drugs, and during the latter half of the 19th century this service to industry brought to organic chemistry public prestige and generous financial support.

This emergence of organic chemistry as an independent branch of science weakened the tie that had linked it to physiology at the beginning of the century. To indicate the intimacy of this early connection, we need only recall that in 1806 Berzelius defined organic chemistry as "the part of physiology that describes the composition of living bodies, together with the chemical processes that occur in them" (*21*). Indeed, by the 1820's there had been several notable achievements in the study of the chemical dynamics of physiological function, among them the experiments of Prévost and Dumas on the formation of urea in the animal body (*22*). The new chemical knowledge about immediate principles was also applied to studies on digestion (*23*) and to the problem of the substances oxidized during animal respiration (*24*). In the experimental physiology advocated by Magendie in France and by Müller in Germany, chemical concepts and methods played a central role, and by 1850 this emphasis had promoted the emergence of a physiological chemistry more closely linked to medical physiology than to pure chemistry (*25*).

The Albuminoid Substances

The immediate principles identified by the middle of the 19th century included substances such as uric acid or lactic acid, whose chemical structure was soon elucidated by the new organic chemistry. There were more complex materials known to be important in animal nutrition, notably the fats and starch, and their constitution also yielded relatively easily to chemical analysis (*26*). Later in the century, new kinds of complex immediate principles such as lecithin and nuclein were discovered, and, although their physiological role was unclear, by 1900 important advances had been made in the study of their chemical nature. There was one class of immediate principles, however, that occupied the center of biological attention throughout the 19th century, but which posed awkward problems to the new organic chemistry. They were the so-called albuminoid substances—albumin, casein, fibrin—later to be known as proteins. These materials were considered to be uncrystallizable, their elementary composition suggested chemical complexity, and they appeared to be labile structures susceptible to alteration by heat or mild chemical treatment. Indeed, by mid-century, some chemists had excluded them from organic chemistry, and had denoted them "organized" substances rather than organic compounds (*27*). Nevertheless, largely because of the importance biologists attached to proteins, efforts were made throughout the rest of the century to determine their chemical constitution. The methods that had been effective in the study of more respectable organic compounds gave results that were usually inconclusive and often bewildering. The prospects changed somewhat at the turn of the century when Emil Fischer, fresh from his success in the elucidation of the structure of sugars, partially dispelled some of the uncertainty about the constitution of the proteins. I emphasize this uncertainty, because it made itself evident in research on many physiological problems.

Among these problems, few were more important to 19th-century medical physiologists than those arising from the conversion of food materials to the constituents of animal tissues. They had inherited from the previous century the view that this conversion is a sequential process in which food materials are dissolved in the digestive organs to form a chyme that is converted into soluble blood substances which are then assimilated into the solid matter of the tissues. The total chemical change was termed nutrition, which included what later came to be called metabolism (*28*). For example, during the 1830's, it was shown that the gastric dissolution of insoluble proteins is effected by an agent with the properties of an albuminoid substance. Schwann named it pepsin and considered it to be a catalyst. The identification of pepsin thus solved a part of the physi-

ological problem of nutrition, but it raised many questions that were pursued indecisively for a century thereafter (*29*). What was the chemical nature of this pepsin, how did it work, and what relation did it have to other catalytic agents derived from biological sources, such as those known to cause alcoholic fermentation or the conversion of starch to sugar? Such problems could not be fitted into the fabric of the new organic chemistry, and were largely pursued in medical laboratories rather than in chemical institutes, thus accentuating the separation of organic chemistry from physiology (*30*).

The uncertainty about the chemical constitution of the albuminoid substances is also evident in the development of the protoplasmic theory of life. During the 1830's, Schwann had proposed that all living organisms are composed of and derived from independent cellular units in which an organized layer of matter surrounds a nucleus and is bounded by a membrane. He considered cells to grow by virtue of their "plastic" power to attract nutrient material that is deposited around the nucleus, and of their "metabolic" power to effect chemical change both in the nutrient material and in their own constituents (*31*). He likened the plastic phenomena to the crystallization of chemical substances (*32*), and, in his idea of metabolic power, the cell was thought to be a dynamic unit whose components were endowed with chemical force. Although Schwann's theory of cell formation was replaced during the 1850's by the view that cells arise solely by the division of preexisting ones, the concept that the cell represents an organized unit of metabolic activity was widely adopted. In the subsequent development of the cell theory, the dynamic portion of the cell became protoplasm (*33*), whose most characteristic chemical property was its resemblance to albumin (*34*). This albuminous character of protoplasm was elevated, in Huxley's famous phrase, to the physical basis of life (*35*); and according to Haeckel, the discovery that it "is the original substratum of all vital phenomena is one of the greatest achievements of modern biology, and one of the richest in results" (*36*).

Protoplasmic Molecules

The impact of the protoplasmic theory of life on physiological thought was far-reaching, and by the 1870's, processes such as animal respiration, muscular contraction, electrical conduction in nerve, or the fertilization of egg by sperm were considered expressions of the properties of protoplasmic molecules. But what did physiologists mean by the term "protoplasmic molecule"?

I simplify the complex development of 19th-century scientific thought in suggesting that, after about 1860, the molecular explanations that biologists offered for physiological phenomena tended to distinguish between molecules as physical or chemical entities. One style of speculation was based on the definition of a molecule as the smallest unit of a substance that moves as a whole, and specific biological function was seen as the expression of differences in a continuously variable motion within a specific arrangement of molecular units (*37*, *38*). The other defined a molecule as the smallest portion of a substance that retains its properties in chemical reactions, and biological phenomena were considered to be a consequence of the specific properties of different kinds of discrete interacting molecules. This approach required, however, some knowledge of the chemical structure of these molecules. The two definitions of molecules as physical or chemical entities were not mutually exclusive, and occasionally attempts were made to combine them. Nonetheless, the dichotomy between the two styles of speculation is a striking feature of biological thought during the latter half of the 19th century and carried forward into the 20th century. This dichotomy has a more ancient lineage, as in the relation of iatrochemistry to iatrophysics in the 17th and 18th centuries (*39*).

After about 1860, the undoubted success of men like Helmholtz and duBois-Reymond in demonstrating the explanatory power of the new biophysics (*40*), and the apparent inability of organic chemistry to elucidate the constitution of albuminoid protoplasm, clearly made the physicalist mode of biological thought the more attractive one. Even for physiological processes that did not appear to lend themselves experimentally to the biophysical approach, the molecular explanations of protoplasmic activity that were offered during the latter half of the 19th century reflected a predilection for this style of speculation. For example, the embryologist His considered that in the fertilization of the egg by sperm there was a transmitted motion rather than a transfer of specific material, and the cytologist Strasburger used similar language in describing the influence of the cell nucleus on the cytoplasm (*41*).

At the turn of the century, this preference for the physicalist mode of molecular explanation found expression in the welcome extended by biologists to the new physical chemistry based on thermodynamics and kinetics (*42*). Aside from its great importance in the development of chemical theory, this new branch of chemistry gave a quantitative character to the colloid chemistry founded during the 1860's by Graham. Among the noncrystalline materials of large molecular size that he called colloids were the albuminoid substances (*43*), and he defined the colloidal state as "a dynamical state of matter, the crystalloidal being the static condition. The colloid possesses ENERGIA. It may be looked upon as the probable primary source of the force appearing in the phenomena of vitality" (*44*). Not only were colloids retained by membranes that allowed the passage of water and salts, but like protoplasm they also imbibed water and adsorbed substances on their surface. Such adsorption phenomena had been studied qualitatively at mid-century, but the new physical chemistry provided a consistent theory to explain them. It is not surprising therefore, that, at a time when albuminoid protoplasm was considered to be the dynamic component of living cells (*45*) but when there was only confusion and uncertainty about the chemical structure of cellular proteins, the colloid chemistry of protoplasm offered to biologists a more satisfying guide to the molecular explanation of physiological phenomena than did the organic chemistry of Emil Fischer.

Having traced, all too sketchily, what I have called the physicalist style of speculation about protoplasmic molecules, I turn to the 19th-century attempts to explain their intracellular activity in more chemical terms. The hypothesis that appears to have received the most respectful attention was offered by the physiologist Pflüger in 1875 (*46*). He suggested that intracellular oxidation is effected by a labile energy-rich protoplasmic protein that contains cyano groups, which combine explosively with molecular oxygen to liberate CO_2. In Pflüger's hypothesis there was an admixture of physicalist speculation, since he proposed that these explosions create intramolecular vibrations. The popularity of his idea encouraged others to produce variants, as in the case of Loew's proposal that the chemical group responsible for the dynamic properties of protoplasmic protein is not the cyano group but the aldehyde group (*47*, *48*). Thus, to explain the energy-rich character of protoplasm, Pflüger and Loew had drawn analogies from the

available knowledge about the high reactivity of particular chemical groups in small organic molecules of known structure. In the same chemical tradition is the hypothesis advanced by Ehrlich that living protoplasm attracts to itself "side chains" that represent the agents of intracellular processes (*49*). The ideas of Pflüger and Ehrlich were then put together by the physiologist Verworn, who coined the term "biogen" to denote the labile energy-rich protein material of living protoplasm (*50*).

The Enzyme Theory of Life

During the last quarter of the 19th century, chemically inclined biologists held such speculations in higher regard than a competing view which assigned a central role in the dynamics of biological processes to intracellular catalysts not very different from pepsin. After the term "enzyme" had been introduced in 1876 to denote such catalysts (*51*), this hypothesis was often termed the enzyme theory of life. The chemical nature of the catalysts was unclear, but it was widely believed that they are breakdown products of protoplasmic proteins, and thus outside the orbit of respectable organic chemistry. Whatever the nature of enzymes might be, most biologists agreed with Pflüger that the assumption of intracellular enzymes "is not only unnecessary, but indeed highly implausible" (*52*). Even the leading cluster of physiological chemists, associated with Hoppe-Seyler, took a cautious view of the enzyme theory of life, although they roundly condemned the speculations about living proteins endowed with cyano or aldehyde groups (*53*).

Much of the 19th-century debate about the enzyme theory of life revolved about the process whereby yeast ferments glucose to alcohol and carbon dioxide. During the 1830's microscopic observations had identified the agent of alcoholic fermentation as a living microorganism that nourishes itself at the expense of the sugar it ferments. This view had been advanced earlier in the century, but had been discounted in favor of Liebig's idea that fermentation arises from the decomposition of albuminoid matter by oxygen, whereby molecular vibrations are communicated to sugar molecules and thus cause their cleavage to alcohol and carbon dioxide. Liebig's theory of fermentation had no place for soluble catalytic agents analogous to Schwann's pepsin. The role of such agents in fermentation was advocated around 1860 by some chemists, notably Traube (*54*) and Berthelot, but throughout the rest of the century this idea met the objection that despite repeated efforts no one had prepared from yeast a cell-free extract that could ferment sugar.

The decisive blow to Liebig's idea that fermentation is caused by the oxidation of albuminoid substances was delivered by Pasteur when he showed that yeast grows in the absence of such substances and that it ferments best in the absence of oxygen. Pasteur did much more, of course. By studying carefully the effects of changes in the composition of the medium on the growth and fermentative activity of various microorganisms, and by applying criteria of purity to the cultivation of microbial forms of life, he brought chemistry into microbiology. As a consequence of his work, and that of Koch, there was an explosive development of medical microbiology, with considerable impact on 19th-century physiological chemistry. By 1890 it was generally accepted that infectious microorganisms produce toxins which, like the enzymes, appeared to be degradation products of protoplasmic proteins. Clusters of physiological chemists began work on the chemical activities of bacteria and on the toxins and enzymes they produce (*55, 56*). After the discovery of antitoxins and the rise of serum therapy during the last decade of the century, a new discipline, immunology, emerged as part of a bacteriology allied to physiological chemistry.

These close ties are an important part of the story of 19th-century work on the nature of alcoholic fermentation. They form the background of Eduard Buchner's success in preparing a cell-free extract of yeast that converted sugar to alcohol and carbon dioxide (*57*), thus bringing the controversy about the enzyme theory of life to a sharp focus. In his justly famous paper of 1897, Buchner wrote: ". . . the initiation of the fermentation process does not require so complicated an apparatus as is represented by the yeast cell. The agent responsible . . . is rather to be regarded as a dissolved substance, doubtless as protein; this will be denoted zymase" (*58*).

Buchner's discovery has historical interest for many reasons. It was made by an organic chemist of modest talent who stumbled into it while helping his brother Hans, a bacteriologist, to prepare microbial extracts that Hans thought might contain antitoxins. Although it was hailed by Eduard's fellow organic chemists as proof that alcoholic fermentation is a "chemical" and not a "vital" process, the validity of his claim was questioned by many biologists (*59*). Among those who accepted it were groups of physiological chemists who proceeded to study the chemical changes effected by zymase. Thirty years later, their efforts had led to the recognition that zymase is not a unitary chemical principle but a mixture of 12 separate catalytic proteins, and that alcoholic fermentation requires inorganic phosphate and several organic compounds that turned out to be related to vitamins. Also, it had been known since the middle of the 19th century that the chemical changes during muscular contraction resembled those observed in the microbial fermentation in which glucose is converted into lactic acid (*60*). After 1910 several groups, notably those associated with Embden and Meyerhof, began to apply the results from yeast to muscle, and to explain the chemical changes in both biological systems in terms of a unified sequence of enzyme-catalyzed reactions.

In the light of these later successes, it is tempting to see in Buchner's zymase the breakthrough that tilted the scale in favor of the enzyme theory of life. Such hindsight oversimplifies, I believe, a more complex and more interesting interplay of biology and chemistry during the first three decades of this century. There can be no doubt that for many scientists Buchner's achievement had erased the 19th-century division between the living agents of fermentation and soluble enzymes such as pepsin, and thus had brought fermentation processes into the stream of enzyme research. During the three decades that followed, however, there was uncertainty about the chemical nature of enzymes, and their study was dominated by the molecular-physical approach of colloid chemistry. The view that enzymes are proteins was questioned, and enzymes, along with vitamins and hormones, were widely thought to be small bioactive molecules adsorbed on nonspecific colloidal carriers. For example, during the 1890's, Fischer explained his results on the specificity of enzymes by speaking of the combination of a protein enzyme with a substrate as a lock accepting a key. Twenty years later, this analogy was denied by the colloid chemist Bayliss (*61*), who reflected the prevailing idea that enzymic catalysis is a phenomenon arising from physical adsorption on surfaces, rather than from specific chemical combination. The dominance of such views during the 1920's suggests an explanation for the dismissal of Sumner's claim to have isolated the enzyme urease in the form of a crystalline protein. Indeed, many leading biochemists questioned the biological significance of discrete isolated enzymes and, in keeping

with current opinions about the colloidal nature of protoplasm, considered them to be artifacts arising from "the transformation and decomposition of a single homogeneous substance present in life" (*62*). The situation changed after 1930 with Northrop's crystallization of pepsin, and the acceptance of his massive evidence for its identity as a protein (*63*). There soon followed the isolation of many enzymes in the form of crystalline proteins, and after 1945 enzyme chemistry became a branch of protein chemistry. Today, the limits to the understanding of enzymic catalysis are largely imposed by the limitations of the available methods for the study of the intimate molecular structure of proteins.

Aside from the uncertainty about the chemical nature of enzymes, and of their relation to the protoplasm of living cells, the enzyme theory of life encountered other objections that were not removed by Buchner's discovery. Foremost among them was the argument that nearly all the processes catalyzed by discrete enzymes, including zymase, involved chemical breakdown and that no enzyme had been shown to effect the synthesis of a cell constituent (*64*). According to this view, only the part of metabolism that involves the degradation of such substances as proteins is catalyzed by enzymes like pepsin, and probably occurs outside living cells, but the constructive part of metabolism, whereby proteins and other complex cell constituents are formed, is a biological property of living protoplasm and is not an enzyme-catalyzed process. The example most often cited in support of this position came from the work of Claude Bernard. In 1855, he discovered that the liver can convert blood constituents derived from food proteins into a starchlike substance he named glycogen, because it was the source of blood sugar. Bernard considered the formation of glycogen in the liver, like the formation of starch in plants, to be linked to the activity of living cells. On the other hand, starch had long been known to be cleaved by an enzyme extractable from plants, and Bernard believed that he had obtained from liver a similar soluble agent that converts glycogen into sugar. He therefore drew the generalization that, whereas the metabolic breakdown of complex immediate principles such as starch, glycogen, or albumin are purely chemical reactions independent of life, the synthesis of such principles requires physiological processes linked to life.

By 1900, several other biosyntheses, such as the formation of urea in the liver, were also thought to be linked to life because they could only be effected by living or surviving liver tissue in the presence of oxygen, but not by disintegrated tissue or by liver extracts. These synthetic processes were considered to occur within tissue cells, and intracellular oxidations were understood to provide the requisite chemical energy (*65*). Biochemical syntheses thus appeared to have distinctive requirements not evident for Buchner's zymase, and this was taken by some biochemists to indicate that the enzyme theory of life was irrelevant to the study of protoplasmic activity (*66*).

Intermediary Metabolism

For others, however, Buchner's discovery provided an impetus to the study of the metabolism of relatively simple organic compounds whose transformations in the animal organism might be explained on the basis of the new organic chemistry based on valence and stereochemistry (*67*). This approach continued a tradition established after 1860 by medical physiologists and pharmacologists (*68*), who had attempted to study chemical processes either in intact organisms or in isolated perfused animal organs. Also, during the course of such work, new compounds provided by organic chemistry were administered to animals, and often were found to have been transformed to hitherto unknown excretory products. The aim of this approach was to understand what came to be called "intermediary metabolism," and indeed by 1900 it had provided clues to the nature of intermediates in the metabolic transformation of sugars, fatty acids, and amino acids. These metabolic studies differed fundamentally from the kind of black-box physiological chemistry in which the heat produced by an animal organism was related to its oxygen uptake and CO_2 output, and to the amount and chemical composition of its dietary constituents and excretory products (*69*). For Bernard, and for those influenced by his work, such experiments told nothing about what happens in the animal body. The proper strategy, according to their view, was the use of experimental surgery and chemical methods to unravel the sequence of events in the metabolic breakdown or synthesis of important cell constituents.

From studies of this kind there began to emerge, during the latter half of the 19th century, hypotheses about metabolic pathways. In addition, indications of possible intermediate steps in metabolism came from two other sources. An especially important one was the observation of the appearance of abnormal chemical products during particular human diseases. The lack of time forces me to limit my examples to the case of diabetes, where the finding of the so-called ketone bodies gave important clues to the intermediary metabolism of fatty acids and sugars (*70*). A rather different source of hypotheses about metabolic pathways was chemical speculation by analogy to the structure and behavior of known organic compounds. Many of these chemical hypotheses led physiologists down blind alleys, but others turned out to be fruitful, and indeed influenced the chemical dissection of the process of alcoholic fermentation (*71*).

At a time, therefore, when the physical-chemical approach of colloid chemistry was prominent in biological thought, the study of metabolic processes such as the one catalyzed by Buchner's zymase strengthened an alternative strategy more closely allied to organic chemistry. As Hopkins put it in 1913, it was a matter of studying the metabolism of "simple substances undergoing comprehensible reactions" (*72, 73*), rather than one of speculation about large protoplasmic molecules (*74*). Hopkins reflected, of course, the uncertainty of his time about the nature of enzymes (*75*), but his emphasis on the utility of organic chemistry in the study of intermediary metabolism represents an attitude that was to find increasing favor in biochemistry. This became especially evident during the 1930's, when Krebs (*76*) elucidated the pathway of urea biosynthesis, and Schoenheimer (*77*) and Hevesy (*78*) introduced the isotope technique for the study of intermediary metabolism. By that time, enzymes were recognized to be catalytic proteins, and metabolism, including biosynthesis, was seen as a coordinated set of sequences of enzyme-catalyzed reactions. Moreover, during the 1930's the concepts and techniques of organic chemistry were being applied with notable success to the elucidation of the structure of many important cell constituents, such as nucleotides and steroids. Although the impact of colloid chemistry was on the wane, the physical chemistry on which it had been based continued to influence the development of research on the structure of proteins and other macromolecules, on biological oxidations, and on the mechanism of enzyme action (*79*). Also, the dichotomy between the chemical molecule and the physical molecule that characterized 19th-century thought had been partly erased by the application of quantum theory and of the electronic theory of valence to chemical reactions.

Conclusion

As one looks back on the interplay of chemistry and biology from 1800 to the 1930's, perhaps its most striking feature is the extent to which the limits of molecular explanation of biological phenomena were set by the state of the contemporary chemical knowledge. I believe that this is no less true today, and for the future of biochemistry it is important, therefore, that chemistry still is an open science, subject to further fundamental change. In part, this character of chemistry is a consequence of its power to create, through synthesis, new forms of matter. Here, in my opinion, lies the enormous importance of chemistry for biology. Biologists study complex assemblies of chemical materials that already exist in living organisms. Chemists seek not only to elucidate the molecular structure of these materials, but also to create new forms of matter that mimic the behavior of natural biochemical substances, and thereby to enlarge and clarify the scope of biological inquiry. I believe that this contribution of chemistry to biology is likely to continue in the future, through refinements in the art of synthesis and through the development of the theory of chemical reactions.

Having offered this optimistic hope, I must also call attention to the oft-repeated objection that efforts to explain the phenomena of life in terms of the specific chemical properties of such things as enzymes or nucleic acids are doomed to fail because biochemical analysis cannot discern the complex integration necessary for specific biological organization and for the purposeful behavior of living things. This objection has received renewed prominence, though perhaps less acceptance, with the successive appearance of striking new developments in the chemical study of life. We have seen it most recently in response to the important discoveries that followed the appearance of the Watson-Crick model of DNA (*80*). It is tempting, in the flush of biochemical success, to dismiss all such objections as vitalist obscurantism, but the historical record suggests they often served as a fruitful goad in the development of modern biochemistry. For example, around 1900 leading physiological chemists were skeptical about the achievements in cytology during the previous quarter-century. They questioned the validity of conclusions about cell dynamics drawn from microscopic observation (*81, 82*), and were unmoved by the debate among biologists whether the constitution of protoplasm is homogeneous, reticular, fibrillar, granular, or foamlike. This attitude is evident in a lecture delivered in 1901 on the chemical constitution of the cell by Hofmeister, who responded to the prominence of cytology by advocating the study of cellular reactions promoted by colloidal catalysts (*83*). Indeed, the aloofness of biochemists toward cytological studies continued through most of the first half of this century. After World War II, however, the use of electron microscopy and differential centrifugation erased this separation, and the mode of assembly of the macromolecular components of living cells is now a subject of intense biochemical study. Similarly, before 1940 most biochemists were indifferent to the achievements in genetics that came from studies on *Drosophila* or maize. Today, the interface of biochemistry and genetics is one of the most flourishing areas of science, largely as a consequence of work on plant and bacterial viruses, on the transformation of microbial types on *Neurospora* mutants, and on the structure of nucleic acids (*84*).

Of course, some of the most important phenomena of life, such as embryonic development or human thought, have been more resistant to chemical investigation and still provide an arena of debate between adherents of biological reductionism and holism. If there is more confidence today than at the beginning of this century in the power of chemistry to illuminate such biological problems, it is a consequence of the fruitful interaction of many kinds of chemists and many kinds of biologists. I have tried to discuss some of the history of this interaction in terms of changes in the relative prominence of various styles of speculation about the molecular basis of biological phenomena, and of various modes of experimental attack on biochemical problems. I have touched on several aspects of the interplay of chemistry and biology since 1800—the craftsmanship of separation chemistry, the physicalist conception of protoplasmic molecules, the enzyme theory of life, the concern with comprehensible chemical reactions in biological processes. All have been historically significant in the emergence of what we now call biochemistry, as have been the various kinds of scientists I have mentioned, whether identified as pharmacists or physicians, as organic or physical chemists, or as reductionist or holistic physiologists. There is little evidence of a linear historical progression within a single scientific discipline toward the so-called mature biochemistry of today, and the continuity of the biochemical enterprise may be seen rather in the competition among attitudes and approaches derived from different parts of chemistry and biology. Inevitably, such competition is attended by tensions among the participants (*85*). I venture to suggest that this competition and these tensions are the principal source of the vitality of biochemistry and are likely to lead to unexpected and exciting novelties in the future, as they have in the past.

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5. N. G. Coley, *From Animal Chemistry to Biochemistry* (Hulton Educational, Amersham, England, 1973).
6. H. M. Leicester, *Development of Biochemical Concepts from Ancient to Modern Times* (Harvard Univ. Press, Cambridge, Mass., 1974).
7. In large part, the emergence during the past century of separate departments, societies, and journals devoted to biochemistry is another sign, among many, of the specialization of a growing population of professional scientists for whom research facilities were provided in response to the economic and political needs of the wealthier nations. Some of the specific factors that promoted the institutional development of biochemistry have been discussed (*4*, pp. 2–15).
8. During the 19th century, the term biochemistry was used frequently. An early example is in the title of the book by V. Kletzinsky [*Compendium der Biochemie* (Braumüller, Vienna, 1858)]; he distinguished biochemistry from biophysics and biomorphology. In France, the more common term was "chimie biologique," whereas in Germany it was "physiologische Chemie," but others, such as "chemical physiology" or "cell physiology," also appeared. The definitions of the scope of the field varied considerably, and it is incorrect to state [R. E. Kohler, *Isis* **64**, 183 (1973)] that physiological chemistry referred to the chemical statics and biochemistry to the chemical dynamics of living organisms. A plausible reason for the increasing use of "biochemistry" or "biological chemistry" during the early years of this century was the wish to emphasize its separation from medical physiology. Some prominent biochemists (for example, F. G. Hopkins) found the term biochemistry unattractive, and in the United States some leading university departments (as at Yale until 1952, and at Johns Hopkins to the present) retained the name "physiological chemistry."
9. Throughout the development of this interplay, the kind of biology that interacted most directly with chemistry was practiced in animal and plant physiology, and the pathology, pharmacology, and embryology associated with them. With the rise of the cell theory and the germ theory of disease, other specialties such as cytology or bacteriology were added. As has been emphasized repeatedly [see E. Mayr, *Science* **134**, 1501 (1961)], this kind of functional biology has differed in aim and method from evolutionary biology, which until recently has been relatively unaffected by parallel developments in chemistry.
10. W. Pagel, *Bull. Hist. Med. Suppl.*, No. 2 (1944); p. v.
11. E. A. Underwood, *Endeavour* **31**, 73 (1974).
12. D. C. Goodman, *Med. Hist.* **16**, 113 (1972).
13. E. N. Hiebert, in *Mélanges Alexandre Koyré: L'Aventure de la Science* (Hermann, Paris, 1964), vol. 1, pp. 303–325; A. Berman, *Bull. Hist. Med.* **40**, 101 (1966); F. L. Holmes, *Isis* **62**, 129 (1971).
14. During the first two decades of the 19th century,

the most influential cluster of these chemical craftsmen was in Paris, where A. F. Fourcroy and his successors L. N. Vauquelin and M. E. Chevreul nurtured a tradition that carried on into the 20th century. A glimpse of the activity of several generations of Parisian pharmaceutical chemists is provided by A. Goris [*Centenaire de l'Internat en Pharmacie des Hopitaux et Hospices Civils de Paris* (Imprimerie de la Cour d'Appel, Paris, 1920)]. These men have fared rather poorly at the hands of modern historians of science, possibly for reasons similar to those of contemporary "philosophical" chemists like Humphry Davy, who disparaged the efforts of Vauquelin on the ground that his methods were those of an industrious tradesman (*5*, p. 32).

15. E. F. Caldin, *The Structure of Chemistry* (Sheed & Ward, New York, 1961); N. W. Pirie, *Br. J. Philos. Sci.* **2**, 269 (1951–1952); G. Bachelard, *Le Matiéralisme Rationel* (Presses Universitaires de France, Paris, 1953).
16. The discovery of such new immediate principles often followed the introduction of new analytical methods, as in the identification of adenosine triphosphate (ATP) by C. H. Fiske and Y. Subbarow and by K. Lohmann during the late 1920's, shortly after the former had developed a new method for the quantitative determination of phosphate. For a valuable discussion of unpredicted scientific discoveries, see T. S. Kuhn, *Science* **136**, 760 (1962).
17. During the 18th century, the continuing demands of mining and metallurgy, as well as such developments as the growth of the textile industry or the importance attached to the medicinal value of mineral waters, had furthered the analytical chemistry of inorganic compounds. Eighteenth-century chemists tacitly assumed much of what later emerged from the ideas of J. Dalton and L. J. Proust about chemical composition, but more than anyone else it was J. J. Berzelius who combined analytical skill with theoretical insight to convince most of his contemporaries that these ideas were correct for both inorganic and organic compounds.
18. The most comprehensive available account of the early history of elementary organic analysis is by M. Dennstedt [*Samml. Chem. Chem.-Tech. Vort.* **4**, 1 (1899)]. It is less well treated in the otherwise excellent book by F. Szabadváry [*Geschichte der Analytischen Chemie*, G. Kerstein, Transl. (Akadémiai Kiadó, Budapest, 1966)]; for a perceptive review of this book and a valuable brief summary of the importance of analytical chemistry in the development of chemical thought, see W. H. Brock [*Hist. Sci.* **6**, 156 (1967)]. A valuable account of the role of pharmacists in the early development of German chemistry, and of Liebig's place in the story, has been provided by B. H. Gustin [thesis, University of Chicago (1975)].
19. J. H. Brooke, *Br. J. Hist. Sci.* **5**, 363 (1971).
20. The impact of the artificial synthesis of organic compounds on 19th-century thought has been the subject of many recent articles, especially those by J. Jacques [*Rev. Hist. Sci. Leurs Appl.* **3**, 32 (1950)] and by J. H. Brooke [*Ambix* **15**, 84 (1968)]. F. Wöhler's synthesis of urea, or those later effected by A. W. H. Kolbe and P. E. M. Berthelot, did not kill vitalism, nor did the prevalence of vitalist ideas stop the chemical study of life.
21. J. J. Berzelius, *Föreläsningar i Djurkemien* (Delén, Stockholm, 1806), p. 6.
22. J. L. Prévost and J. B. Dumas, *Ann. Chim. Phys.* **23**, 90 (1823).
23. The observations of R. A. F. Réaumur and L. Spallanzani during the 18th century provided the background for chemical studies on digestion during the first three decades of the 19th century, notably those of F. Tiedemann and L. Gmelin [see N. Mani, *Gesnerus* **13**, 190 (1956)].
24. A. L. Lavoisier's famous experiments (with P. S. Laplace and A. Séguin) on respiration opened the question of the nature of the carbon compounds burned in the animal body. Although initially more attention was given to the relation of respiration to the generation of animal heat [see E. Mendelsohn, *Heat and Life* (Harvard Univ. Press, Cambridge, Mass., 1964)], the problem of the chemical nature of the substrates of respiratory combustion came to the forefront with the physiological speculations of J. Liebig and J. B. A. Dumas around 1840.
25. In addition to the accounts of the rise of physiological chemistry given above (*2*–*6*), see H. Simmer [*Sudhoffs Arch.* **39**, 216 (1955); *Ciba Z.* **8**, 3013 (1958)]. One of the early textbooks was by F. L. Hünefeld [*Physiologische Chemie des Menschlichen Organismus* (Voss, Leipzig, 1826)].
26. One of the early achievements, which served as an exemplar to 19th-century chemists, was the elucidation of the constitution of the fats by M. E. Chevreul [*Recherches Chimiques sur les Corps Gras d'Origine Animale* (Levrault, Paris, 1823)]. His book *Considérations sur l'Analyse Organique et sur ses Applications* (Levrault, Paris, 1824) provides one of the clearest contemporary accounts of the strategy then used for the chemical study of the immediate principles of plants and animals.
27. An extreme statement of this view is that "albuminoid substances . . . properly speaking, do not constitute a chemical species; they are organs, or the remains of organs, whose history belongs to biology rather than to chemistry" [A. Naquet, *Principles of Chemistry Founded on Modern Theories*, translated from the second edition by M. Cortis and revised by T. Stevenson (Renshaw, London, 1868), p. 721]. During the course of the emergence of biochemistry, other terms—ferment, virus, gene, antitoxin—were used, often ambiguously, to denote entities with properties more akin to biological units than to known chemical substances. In a sense, the history of biochemistry may be looked upon as a succession of efforts to translate the terms used to define biological units into the language used to define chemical molecules.
28. F. C. Bing, *J. Hist. Med. Allied Sci.* **26**, 158 (1971).
29. E. Hickel, *Naturwiss. Rundsch.* **28**, 14 (1975).
30. After 1850, as organic chemists became increasingly preoccupied with the promotion of their subject for its own sake and for the benefit of the chemical industry, decisions about the academic status of what we now call biochemistry were largely made by professors in the medical faculty. Although differences are evident in the institutional development of biochemistry in Germany, France, England, and the United States, this dependence is seen in all these countries, even after physiological chemists had begun to free themselves from direct domination by medical physiologists. See the lecture given in 1886 by G. Hüfner [*Über den Ursprung und die Berechtigung besonderer Lehrstühle für physiologische Chemie* (Pietzcker, Tübingen, 1899)].
31. T. Schwann, *Mikroskopische Untersuchungen* (Sander, Berlin, 1839), pp. 229–239.
32. R. C. Maulitz, *J. Hist. Med. Allied Sci.* **26**, 422 (1971). The analogy between crystal growth and biological organization had been drawn before T. Schwann, and was to be reiterated many times later in appropriate new language; for example, see H. Przibram [*Arch. Entwicklungsmech. Org.* **22**, 207 (1906); *Die anorganische Grenzgebiete der Biologie* (Borntraeger, Berlin, 1926)] or E. Schrödinger [*What is Life?* (Cambridge Univ. Press, Cambridge, 1941), p. 61].
33. H. Güttler, *Rete* **1**, 365 (1972).
34. Earlier in the century, some biologists believed that animal tissues are composed of globules of coagulated albumin and drew an analogy between blood coagulation and the conversion of soluble blood albumin to insoluble tissue "fibrin." See J. V. Pickstone, *J. Hist. Med. Allied Sci.* **28**, 336 (1973).
35. G. L. Geison, *Isis* **60**, 273 (1969).
36. A. Haeckel, *Q. J. Microsc. Sci.* **9**, 27, 113, 219, and 327 (1869).
37. For example, in 1876 A. Fick (*38*, p. 59) wrote: "In the muscle and nerve fibers the protoplasmic molecules are regularly arranged, so that the propagation of the characteristic process proceeds regularly in one direction for long distances."
38. A. Fick, *Gesammelte Schriften* (Stabel, Würzburg, 1903–1905), vol. 4.
39. Valuable recent treatments of the relation between iatrochemistry and iatrophysics during the period 1600–1800 are those of T. S. Hall [*Ideas of Life and Matter* (Univ. of Chicago Press, Chicago, 1969)] and of A. B. Davis [*Circulation Physiology and Medical Chemistry in England 1650–1680* (Coronado Press, Lawrence, Kan., 1973)].
40. P. F. Cranefield, *J. Hist. Med. Allied Sci.* **12**, 407 (1957); **21**, 1 (1966); E. Mendelsohn, *Hist. Sci.* **3**, 39 (1964); C. A. Culotta, *Hist. Stud. Phys. Sci.* **4**, 3 (1974). A contemporary statement of the biophysical program of physiological research may be found in a lecture by A. Fick in 1874, and reprinted in (*38*, pp. 386–394).
41. W. His, *Unsere Körperform und das Physiologische Problem ihrer Entstehung* (Vogel, Leipzig, 1874), p. 152; E. Strasburger, *Neue Untersuchungen über den Befruchtungsvorgang bei den Phanerogamen als Grundlage für eine Theorie der Zeugung* (Fischer, Jena, Germany, 1884), p. 111. These views were in general accord with the widely accepted theory of the micellar structure of protoplasm, advocated by C. von Nägeli, *Mechanischphysiologische Theorie der Abstammungslehre* (Oldenbourg, Munich, 1884).
42. See H. J. Hamburger, *Int. Z. Phys.-Chem. Biol.* **1**, 6 (1914). For a recent account of the role of van't Hoff, Arrhenius, and Wilhelm Ostwald in the rise of physical chemistry during the last quarter of the 19th century, see W. Jost, *Annu. Rev. Phys. Chem.* **17**, 1 (1966).
43. In view of the importance attached to the colloid nature of protoplasm until about 1930, it should be noted that Graham's definition of proteins as noncrystallizable substances had already been put into doubt at the time he proposed that they be called colloids. By 1860, microscopic observation had revealed the presence of crystalline albuminoid materials in plant cells and in erythrocytes, and protein crystals had been isolated from an aqueous extract of Brazil nuts [F. Cohn, *J. Prakt. Chem.* **80**, 129 (1860)]. Because they differed from crystals of simpler organic compounds in several respects, especially their ability to imbibe water, Nägeli called them "crystalloids" and included them among the micellar aggregates he considered to be characteristic structural features of living cells. The widespread conviction that proteins could not be crystallized is indicated by Pasteur's statement in 1883: "You know that the most complex molecules of plant chemistry are the albumins. You know besides that these immediate principles have never been obtained in the crystalline state. May one not add that they probably cannot crystallize" [L. Pasteur, *Conférences Faites a la Société Chimique de Paris en 1883–1886* (Bureau des Deux Revues, Paris, 1886), p. 36]. Within 15 years, albumins had been crystallized from several sources, including egg white and blood plasma.
44. T. Graham, *Philos. Trans. R. Soc. London* **151**, 184 (1861).
45. By the 1880's, the presumed chemical homogeneity of protoplasm had been abandoned in the face of the discovery of phospholipids, nucleins, and iron compounds in the cellular substance. A widespread view was that protoplasm does not consist "of albumins and globulins, but of far more complex proteid substances . . . which represent the true protoplasmic protein, and the albumins and globulins should rather be considered partly as nutrient materials of the cell, and partly as breakdown products in the chemical transformation of protoplasm" [O. Hammarsten, *Pflügers Arch.* **36**, 449 (1885)].
46. E. Pflüger, *Pflügers Arch.* **10**, 251 and 641 (1875). For valuable discussions of the background of Pflüger's hypothesis, see C. A. Culotta [*Bull. Hist. Med.* **44**, 109 (19 → *Trans. Am. Philos. Soc.* Pt. 3 **62**, 1 (1972)].
47. O. Loew and T. Bokorny, *Die Chemische Ursache des Lebens* (Finsterlin, Munich, 1881).
48. O. Loew, *The Energy of Living Protoplasm* (Kegan Paul, London, 1896).
49. From the beginning of his career, P. Ehrlich sought to apply the findings of the new organic chemistry to biological problems. His doctoral dissertation in 1878 offered a theory of the interaction of organic dyes with animal tissues and fibers, and he opposed the physicalist notion that dyes are merely adsorbed [L. Michaelis, *Naturwissenschaften* **7**, 165 (1919)]. Later, his "side-chain" hypothesis of intracellular oxidation was based on the ability of various tissues to reduce dyes, and during the 1890's he elaborated this hypothesis to explain the neutralization of a bacterial toxin by an antitoxin or an antibody [L. Aschoff, *Ehrlichs Seitenkettentheorie* (Fischer, Jena, Germany, 1902)]. Near the end of his career, after he had embarked on chemotherapy, his theory of drug action again became one based on specific chemical combination [J. Parascandola and R. Jasensky, *Bull. Hist. Med.* **48**, 199 (1974)]. This organic-chemical mode of thought owed much to E. Fischer's lock-and-key hypothesis of enzyme-substrate combination, but was questioned by those who preferred the physical-chemical approach, as exemplified in Overton's theory of drug action or Arrhenius's treatment of the interaction of toxins and antitoxins [S. Arrhenius, *Quantitative Laws in Biological Chemistry* (Bell, London, 1915)].
50. M. Verworn, *Die Biogenhypothese* (Fischer, Jena, Germany, 1903).
51. W. Kühne, *Unters. Physiol. Inst. Heidelberg* **1**, 291 (1878).
52. E. Pflüger, *Pflügers Arch.* **18**, 249 (1878). A similar view was expressed by C. von Nägeli, [*Abh. Königl. Akad. Wiss. München* **13** (2), 86 (1879)].

53. See F. Hoppe-Seyler [*Physiologische Chemie* (Hirschwald, Berlin, 1881), p. 982] for his criticism of Pflüger, and E. Baumann [*Pflügers Arch.* **29**, 400 (1882)] for his criticism of the book by Loew and Bokorny (*47*).
54. M. Traube, *Ann. Phys.* **103**, 331 (1858). This article was reprinted in M. Traube [*Gesammelte Abhandlungen* (Mayer & Müller, Berlin, 1899)]. In it (p. 74), he called for attempts to isolate individual ferments and added that "if they could not be isolated without changed properties, [a healthy science] would only conclude that all the separation methods had exerted a deleterious effect on this substances."
55. Among the leading 19th-century physiological chemists who studied the chemical reactions effected by microorganisms were M. Nencki (*56*) and L. Brieger [*Ueber Ptomaine* (Hirschwald, Berlin, 1885–1886)]. In German universities, bacteriology became a part of "hygiene" (public health). It is noteworthy that the famous hygienist Pettenkofer had been a physiological chemist in Liebig's Munich laboratory, and that later Hoppe-Seyler and Nencki were professors of both physiological chemistry and of hygiene in Strassburg and Berne, respectively.
56. M. Nencki, *Opera Omnia* (Vieweg, Braunschweig, Germany, 1904).
57. R. E. Kohler, *J. Hist. Biol.* **4**, 35 (1971); *ibid.* **5**, 117 (1972).
58. E. Buchner, *Ber. Dtsch. Chem. Ges.* **30**, 117 (1897).
59. See F. B. Ahrens, *Samml. Chem. Chem-Tech. Vort.* **7**, 445 (1902). The attitude of leading physiological chemists toward Buchner's zymase, as well as to identification of soluble oxidases, was more cautious. A representative statement, made in 1900, is that of M. Nencki (*56*, vol. 2, p. 724): "Perhaps future researchers will determine whether living protoplasm is only a mixture of various enzymes or the protoplasm is one single molecule that can perform different functions."
60. A full account of the development of knowledge about the chemistry of muscular contraction has been prepared by D. M. Needham, *Machina Carnis* (Cambridge Univ. Press, Cambridge, 1971).
61. W. M. Bayliss, *The Nature of Enzyme Action* (Longmans Green, London, ed. 5, 1925), pp. 143–144.
62. O. Warburg, *Biochem. Z.* **214**, 2 (1929).
63. J. H. Northrop, *J. Gen. Physiol.* **13**, 739 (1930); *Biol. Rev. (Biol. Proc.) Cambridge Philos. Soc.* **10**, 263 (1935). The impact of Northrop's work is evident, for example, in the isolation of tobacco mosaic virus in an apparently crystalline form by W. M. Stanley [*Phytopathology* **26**, 305 (1936)] and in the change of Warburg's research strategy in his study of intracellular oxidations. During the 1930's and 1940's, Warburg and his associates isolated, in crystalline form, some of the enzymic components of Buchner's zymase.
64. See, for example, R. Neumeister, *Betrachtungen über das Wesen der Lebenserscheinungen* (Fischer, Jena, Germany, 1903), p. 78.
65. Such energy was thought to be used for intracellular syntheses by "activating" a metabolite so as to convert it into a more reactive or "nascent" form. This mode of language lasted well into the 20th century, as in the case of "active" acetate, which became acetyl-coenzyme A during the 1950's. Wilhelm Ostwald had suggested in 1900 that the utilization of chemical energy occurs in coupled reactions with the formation of defined labile intermediates, but the nature of such intermediates could not be predicted at that time.
66. The proponents of the enzyme theory of life noted that if an enzyme catalyzes the hydrolysis of a protein, and a particular equilibrium is reached, the same enzyme should catalyze the reverse reaction. Evidence was promptly forthcoming around 1900 that some hydrolytic enzymes could, in fact, catalyze condensation reactions; but such reversals did not need oxygen, whereas physiological biosynthesis did. Some biologists, notably Jacques Loeb [*Dynamics of Living Matter* (Columbia Univ. Press, New York, 1906), pp. 9–13], attached importance to the apparent synthetic action of hydrolytic enzymes, but the biochemists who were studying physiological biosyntheses were more skeptical. The tortuous resolution of the problem did not come until the late 1930's when ATP, discovered 10 years earlier, proved to be the chemical link between oxidation and biosynthesis. During those 10 years, the enzymic components of Buchner's zymase and the intermediates in alcoholic fermentation had been identified, and much had been learned about the transfer of electrons from metabolites to oxygen. For a recent summary of these developments, see the papers at a conference held in 1973 on the history of bioenergetics, and published in *Mol. Cell. Biochem.* **5**, 1 (1974).
67. A notable early advocate of this approach was E. Baumann, especially in his *Ueber die Synthetischen Processe im Thierkörper* (Hirschwald, Berlin, 1878). Among the earliest experiments were those of A. Ure and of Wöhler during the 1840's on the increased excretion of hippuric acid on the administration of benzoic acid to human subjects.
68. The emergence of experimental pharmacology allied to physiological chemistry has been described by G. Kuschinsky [*J. Hist. Med. Allied Sci.* **23**, 258 (1968)]. During the last quarter of the 19th century, the pharmacological institute of Schmiedeberg at Strassburg was an important center of biochemical research. Later, the ties between pharmacology and biochemistry were especially close in the United States, where J. J. Abel played a considerable role in the development of both disciplines, and in England, where H. H. Dale and G. Barger had a similar influence.
69. This approach owed its impetus to the physiological speculations of Liebig during the 1840's [see F. L. Holmes, introduction of facsimile edition of J. Liebig, *Animal Chemistry* (Johnson Reprint Corp., New York, 1964), pp. vii-cxvi]. During the 1890's, the leading investigator in this field was Max Rubner, who was able to account for the heat production of a dog by measuring its carbon and nitrogen balance in a respiration calorimeter; he found that the heat produced by the animal equaled the heat of combustion of dog fat and protein minus that of the urinary matter, thus showing that Hess's "law of heat summation" applies to a living organism. It is noteworthy that Rubner was a tireless opponent of Buchner's claim to have isolated the agent of alcoholic fermentation in the form of a soluble enzyme [M. Rubner, *Arch. Physiol. Suppl.* (1913), p. 1].
70. Another 19th-century example is the appearance of a curious black pigment in a disease (alcaptonuria) later identified by A. B. Garrod as an "inborn error of metabolism" and shown, by 1900, to arise from a metabolic failure to oxidize an intermediate (homogentisic acid) in the breakdown of the amino acid tyrosine. Of special importance was the determination of the chemical structure of homogentisic acid by M. Wolkow and E. Baumann, *Z. Physiol. Chem.* **15**, 228 (1891).
71. It is easy, through hindsight, to select chemical hypotheses of intermediary metabolism whose validity was justified by later biochemical studies. A notable early example is the suggestion by J. N. Collie [*J. Chem. Soc.* **63**, 329 (1893); *ibid.* **91**, 1806 (1907)] that two-carbon compounds may be polymerized in biological systems to form complex organic compounds. At the time such hypotheses were offered, however, their value usually could not be assessed, and they evoked understandable skepticism among biologists. Since the time of Liebig, organic chemists had produced a succession of chemical guesses about metabolic pathways, and most of them had been eventually discredited when tested in appropriate biological systems. After 1900, as a consequence of the interplay of physiological studies and chemical experiments in model systems, the proportion of frutiful chemical hypotheses increased, and many of them were validated by metabolic experiments performed with the technique of labeling with isotopes introduced during the 1930's. The importance of these hypotheses, whether they were substantiated or disproved, lay, therefore, in the fact that they offered a guide to the experimental study of metabolism.
72. F. G. Hopkins, *Nature (London)* **92**, 214 (1913).
73. For an extended appreciation of Hopkins's influence, see J. Needham, *Perspect. Biol. Med.* **6**, 2 (1962).
74. Historians of biochemistry refer frequently to Hopkins's criticism of the biogen theory, but it should also be noted that O. Meyerhof [*Pflügers Arch.* **146**, 159 (1912)] had shown that no detectable heat was released when living cells were killed. Meyerhof later wrote that "the concept of . . . an energy-rich 'biogen molecule' belongs to the realm of fantasy" [O. Meyerhof, in *Handbuch der Physik*, H. Geiger and K. Scheel, Eds. (Springer, Berlin, 1926), vol. 11, p. 244].
75. The 1913 lecture of Hopkins reflected the prevailing views about the colloidal nature of enzymes. Moreover, in 1931 he cited approvingly J. H. Quastel's theory of activation of cell metabolites at cell surfaces [F. G. Hopkins, *The Problems of Specificity in Biochemical Catalysis* (Oxford Univ. Press, London, 1931)]. This theory of the 1920's, like that of A. J. Kluyver at that time, was designed to avoid the necessity of assuming the multiplicity of separate enzymes suggested by the manifold chemical capacities of microorganisms. In his 1913 lecture Hopkins also echoed a widely held opinion about intracellular biosynthesis: "I think we are entitled to look upon assimilation and dissimilation . . . as being dependent upon changes of equilibrium alone. They are processes of condensation and hydrolysis respectively" (*72*, p. 221).
76. H. A. Krebs and K. Henseleit, *Z. Physiol. Chem.* **210**, 33 (1932).
77. R. Schoenheimer and D. Rittenberg, *Science* **82**, 156 (1953).
78. O. Chievitz and G. Hevesy, *Nature (London)* **136**, 754 (1935).
79. During the 1920's, the adsorption theory of the behavior of proteins had begun to give away, under the influence of work like that of Jacques Loeb [*Proteins and the Theory of Colloidal Behavior* (McGraw-Hill, New York, 1922)] and of Edwin J. Cohn [*Physiol. Rev.* **5**, 349 (1925)], to the concept that proteins combine with other substances in stoichiometric chemical reactions. The period during which colloid chemistry dominated biological thought has been denoted the "dark age of biocolloidology" (*3*, pp. 279–284). This disparagement is unfortunate, as it obscures important contributions of colloid chemists to the development of biochemistry. For example, T. Svedberg's demonstration during the 1920's that proteins are large molecules of defined particle weight, together with the insistence of the organic chemist H. Staudinger that macromolecules lie at one end of a continuum that includes all organic compounds, challenged biochemists to study the structure of proteins more intensively. The challenge was met after 1930 through the application of new methods, and culminated during the 1950's in F. Sanger's use of chromatography to elucidate the amino acid sequence of insulin and in J. C. Kendrew's determination of the three-dimensional structure of myoglobin by means of x-ray crystallography.
➡ J. D. Watson and F. H. C. Crick, *Nature (London)* **171**, 737, 964 (1953).
81. Such skepticism was expressed by F. Hoppe-Seyler (*82*, p. 15); by F. Miescher [*Die Histochemischen und Physiologischen Arbeiten* (Vogel, Leipzig, 1897), p. 107]; and by W. B. Hardy [*J. Physiol. (London)* **24**, 158 (1899)]. Hardy emphasized the ease with which artifacts might be produced by treatment of protoplasm with the reactive chemicals used in cytological studies, and he questioned the existence of the mitochondria described by R. Altmann during the 1890's.
82. F. Hoppe-Seyler, *Über die Entwicklung der Physiologischen Chemie* (Trübner, Strassburg, 1884).
83. F. Hofmeister, *Die Chemische Organisation der Zelle* (Vieweg, Braunschweig, Germany, 1901). This lecture has been cited by R. E. Kohler [*Isis* **64**, 185 (1973)] as the "first clear statement of the new faith" in the enzyme theory of life. It is noteworthy, however, that the examples Hofmeister offers in support of this theory are similar to those discussed by Baumann in 1878 (*67*) and by Hoppe-Seyler in 1884 (*81*), but that he omits mention of Buchner's zymase. Twelve years later, Hofmeister returned to the theme of the 1901 lecture, with special emphasis on the colloid chemistry of protoplasm [F. Hofmeister, *Z. Morphol. Anthropol.* **18**, 717 (1914)].
84. E. Olby, *The Path to the Double Helix* (Macmillan, London, 1974).
85. The recurrent tensions have produced many acerbic statements. For example, if in 1844 Liebig stated that "in chemical-physiological work, physiology is not threatened most by chemists, but by physiologists and physicians" [J. Liebig, *Reden und Abhandlungen* (Winter, Leipzig, 1874), p. 81], 4 years later duBois-Reymond wrote of Liebig that "I consider his physiological fantasies as worthless and pernicious" [E. duBois-Reymond, *Zwei Grosse Naturforscher des 19. Jahrhundert* (Barth, Leipzig, 1927), p. 19]. If in 1913 Hopkins complained that "it is a rare thing in this country to meet a professed biologist . . . who has taken the trouble so to equip himself in organic chemistry as to understand fully an important fact of metabolism in terms of structural formulae" (*72*, p. 214), only a few years ago Florkin wrote that "chemists cannot provide any proper methods to tackle the specific problem of biochemistry, the molecular structure of cells, and never showed any interest in the problem" (*3*, p. 12).
86. I am indebted for valuable criticisms and suggestions to F. L. Holmes of the University of Western Ontario, E. Mayr and E. Mendelsohn of Harvard University, E. Chargaff of Columbia University, and M. Borell of Yale University.