

MOLECULAR EVOLUTION AND THE ORIGIN OF LIFE

Revised Edition



SIDNEY W. FOX

KLAUS DOSE



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and the Origin of Life*

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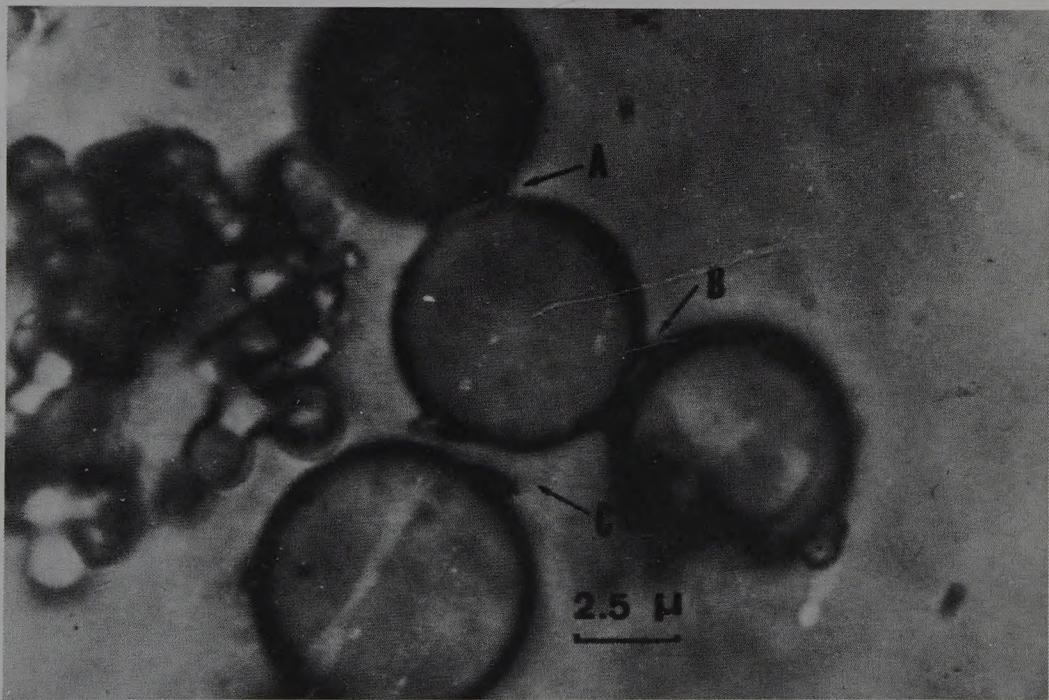
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Sidney W. Fox and Klaus Dose

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Top: Disk electrophoresis of a hemoproteinoid at pH 8.9 in polyacrylamide gel after staining with amido black. The single fraction consists of a family of closely related molecules. The experiment demonstrates the self-ordering of amino acids and hemin into selected structures.

Bottom: Proteinoid microspheres displaying junctions that are (A) intact, (B) cracked, and (C) separated.

Molecular Evolution and the Origin of Life

Revised Edition

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*To Dr. Richard S. Young, Dr. Freeman
H. Quimby, and Dr. Orr E. Reynolds of the
National Aeronautics and Space
Administration, who have labored quietly
and effectively to aid, in several ways,
development of the present knowledge of
the origin of life.*

Foreword

Until quite recently attempts to answer the question of how life originated were thought to be irresponsible speculations and not worthy of serious scientists. Now the situation has largely changed. It is generally accepted today that the development of the first forms of life on Earth was not a solitary "happy" event (as had formerly been assumed) but an event whose repetition was an integral part of the general development of matter—and thus an event that lends itself to serious scientific investigations.

Methods have recently been developed that make possible objective study of the different periods in the long history of the evolution of organic matter, as well as study of the subsequent formation of multimolecular forms—the predecessors of present-day life on Earth. The evolution of organic matter began even before the formation of the Earth—on cosmic objects such as planetesimals and particles of gas and dust. After the Earth had formed, and its lithosphere, atmosphere, and hydrosphere had developed, monomeric and polymeric matter became more complex. Then the first forms of life evolved, and the elaboration of their structures and metabolism continued. The question of how life originated can thus be answered only through the joint efforts of scientists of various specialties—astronomers, geologists, physicists, chemists, and biologists. Such extensive surveys

as J. D. Bernal's *The Origin of Life* and Melvin Calvin's *Chemical Evolution*, which occupy a prominent place beside the descriptions of individual experiments in the special literature of the field, provide a basis for the ongoing synthesis of related findings made in the various specialties.

Molecular Evolution and the Origin of Life is a book of this scope. In it the successive stages of the evolution of carbon compounds are carefully analyzed, beginning with their cosmic origin and concluding with the Darwinian evolution of primitive living systems and a critical discussion of the prefatory problem of extraterrestrial evolution.

It was by no means easy to compile such a work. Not only is profound knowledge of the literature of all of the specialties necessary in order to make the proper correlations, but the advance of knowledge is now so rapid that continual revision of early parts of a manuscript is required while later parts are being written.

The authors of this book have succeeded in meeting these requisites because, as dedicated and outstanding scientists, they are at the center of scientific events relating to discoveries about the development of life. I am convinced that *Molecular Evolution and the Origin of Life* will be welcomed with great satisfaction by all readers interested in the far-reaching concepts of our world and its origins.

A. OPARIN

Pont-à-Mousson, April 1970

Preface to the First Edition

Where we came from is a fundamental question for all of mankind. A first step in understanding the answer to this question is to rephrase it more scientifically: what did living systems come from?

Perhaps no line of investigation has had to contend with so many preconceptions as this one. For mankind as a whole, the great religions have sought to satisfy the curiosity aroused by this age-old personal question. But religious answers have not provided a disciplined scientific understanding. Science, like any other loosely organized activity of large numbers of people, tends to develop its own dogmata. In science, especially for biological phenomena, the dominant established mode of thinking is what we may call "reductionism." We may learn more and more about individual identifiable components of life, in structure and function, but such knowledge can hardly inform us of the evolutionary origins of those components. In order to construct a comprehensive theory of biology, we must not only understand how the materials and processes of the biological realm come into existence in the cell, we must especially understand how they first came into existence. The realization that this sort of approach—which we may label "constructionistic"—to the solution of this basic problem is possible has grown apace since 1950.

Three official international congresses on the origin of life have been convened—Moscow, 1957; Wakulla Springs, Florida, 1963; and Pont-à-Mousson, France, 1970. Even in the discussions at these congresses can be discerned a great tendency to continue reductionistic analyses, which have, of course, been of great value in the work of science. The constructionistic exceptions entail experimental projects that are close to traditional synthetic organic chemistry and that deal with models of the prebiotic synthesis of relatively simple organic compounds such as amino acids, pyrimidines, and purines. Topics of synthetic organic chemistry are covered within the first four chapters of this volume. One salient difference in emphasis is, however, discernible: the traditional organic chemist seeks always to reduce his study to that of a single pure organic compound; the organic chemist who studies models of the origin of life, on the other hand, derives his greatest satisfaction from experiments that produce a variety, or a family, of organic compounds. The production of a *limited variety* of organic compounds appears to be related to the mode of synthesis in contemporary organisms (twenty types of amino acid, two types of pentose). The student of the origin of life finds reality in the simultaneous synthesis of multiple products under conditions that simulate those of the primitive Earth because they can be more readily conceptualized as evolutionary precursors of present-day biological syntheses.

A theory of the origin of life faces some of the problems that have existed for other evolutionary conceptualizations, notably for the theory of the origin of species as formulated by Darwin. With models of protolife, however, superposed disciplines (geology, chemistry, biology) introduce rigor not available to other evolutionary generalizations. This new rigor has also made possible, for example, a first explanation of how enzymes, which today derive only from other enzymes, could have originated when no enzymes to make them existed, and how cells, which today derive only from other cells, could have originated when no cells to produce them existed.

Differing connotations are invoked by words such as proteinlike, enzymelike, cell-like, and lifelike depending upon whether we focus on the primitive or the contemporary. Even for the requirements of the contemporary cell, all models of a cell may be inadequate, at present and in the future. If we employ the perspective of the primitive, we will require that the model have *some* of the properties of the contemporary. We will then be poised to experiment with the acquisition by the model of further properties. A mature theory of molecular evolution and the origin of life, we believe, will have obtained its first clues from the contemporary, it will have demonstrated how the

primitive molecules and systems could have originated, and it will have provided the explanation for how those molecules and systems evolved to the contemporary. The theory will have taken into account that, while our knowledge of the contemporary is derived from taking systems apart, evolution itself proceeded by putting components together.

The authors are indebted to a number of experts, who have critically reviewed passages or chapters in this book. These include Marcel Florkin, J. Lawrence Fox, Ronald F. Fox, Thomas O. Fox, Kaoru Harada, John Jungck, James C. Lacey, Jr., George Mueller, A. I. Oparin, D. L. Rohlfsing, C. H. Townes, and R. S. Young. Of course, none of these critics, generous with their time, is responsible for any errors that might be found in the book. Whatever virtues are possessed by the various concepts within these pages are qualities that have been developed by many critics who have helped us to challenge the ideas.

Thanks are also expressed to Dorothy Butterbrodt, who typed the manuscript, and to Christel Brand and Donna Murphy for other assistance.

SIDNEY W. FOX
KLAUS DOSE

January 1972

Preface to the Revised Edition

This revised edition, which is the first edition to be published by Marcel Dekker, is partly the result of a sudden awareness that the first edition had gone out of print, due to an increasing year-by-year demand for the book. The most thorough revision is in the interpretative Chapter 7, which has been totally rewritten. In Chapter 7 and in other chapters the main data and interpretations remain unchanged. In Chapters 1-6 and 8-11, therefore, salient material has been added in some passages, and a number of references have been included to bring the volume up to date.

In the five years since the first edition of this volume appeared, it has been possible to obtain a broader evaluation of the responses of others to the subject matter as a whole, and to the proteinoid theory that was the main theme of the first edition (as it is of this revision). This new view became possible mainly through reading published critiques of the first edition by experts. These latter reviews were often as much a commentary on the proteinoid-derived theory as they were critiques of a book for purposes of reading by specialists and teaching to students.

Questions of the origin of life are at a special zonal interface between chemistry and biology. It follows that the most appropriate orientation for judgment of this area could be the bridging science of biochemistry. We believe, however, that even more was required, i.e. that a meaningful survey

required biochemists who have holistic and cellular perspectives. It was, therefore, of special interest to read the chapters on the origin of life in the second edition of *Biochemistry* by Lehninger, and Florkin's chapter in *Comprehensive Biochemistry*, Vol. 29B, both books having appeared in 1975.

Each of these authors states what we consider to have been a special value of the view presented here and enlarged here—that it is comprehensive. No other program of investigation has yielded, by experiment, sequentially compatible simulation, from postulated beginnings, of the origin of small biomolecules → biomacromolecules → the first cells → some evolution toward contemporary cells.

Progress in conveying understanding to readers appears to us to have been rapid in some aspects of the subject. Other advances have been slower, as if they represented ideas whose time had not quite yet come. For the latter, we have in mind the unique contribution to understanding of constructionism (physical model-building). The philosophy of analyzing contemporary systems (reductionism) pervades the life sciences; it is fully established. Those frontier researches that consist of disassembling contemporary systems and partially reassembling them have been held to be bold and informative, e.g., Fraenkel-Conrat's experiments with TMV virus, Nirenberg's study of the coding mechanism, and the studies of Nomura and of Spirin on reassembly of ribosomes. But the assembly, rather than the reassembly, of a cell that might then evolve to a contemporary one is one more demanding step. What we believe needs to be much more widely recognized is that there is no known substitute for the assembly of cell-like microsystems from components produced under geologically relevant conditions. Accordingly, the experiments reported within these pages have yielded knowledge of processes and characteristics of products that could not have been foreseen, and that could not have been discovered by established styles of research. In other words, the experiments have told us much about aspects of the origin of life that we logically could never have hoped to learn from either (a) analyzing, or (b) disassembling and reassembling contemporary life. In order to design and execute appropriate experiments, it has been necessary to surmount a defeatism—a defeatism that at first asserted that obtaining the answers was not within the province of science. This kind of defeatism can be self-fulfilling. A philosophy that prevents doing experiments yields no experiments, plus a condition which makes it easy to infer that nothing can be learned, a statement which is equivalent to the original negative premise.

When the problem is admitted to be a scientific one, it then becomes more essential for scientists to reexamine some popular premises. Fundamental among these premises is the belief that the prebiotic matrix for the first biomolecules was chaotic, or random. A second such premise is that the evolution from the matrix to the earliest living systems was by chance. The

experiments provide new ways of examining the questions related to those premises. In this light, an examination of the answers, or inferences, from the laboratory is simultaneously an examination of the questions and the premises that underlie the tests of them. The answer to the origin of life is, then, a larger answer—one that is not restricted to the signal event of a first cell, but an answer that extends from cosmic beginnings through that cell to an evolved human being able to examine his ultimate matrix both on the Earth and away from it.

We thank Glenda Caldwell for extensive typing of the revised edition.

SIDNEY W. FOX
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*Molecular Evolution
and the Origin of Life*

CHAPTER 1

History of Concepts

THE PERIOD BEFORE PASTEUR

For centuries, the concept that life arose spontaneously from inanimate material was a principal doctrine of how life originated. The spontaneous generation of life was visualized as beginning with either inorganic materials or with putrefying organic matter. The idea can be traced back to the ancient Greeks and even further; it is, in fact, at least as old as the history of man. Such a long history provided much opportunity for variation and ambiguities.

Oparin (1957) has discussed the history of the concept of spontaneous generation comprehensively. We will mention only a few salient details of the early history, for the main interest in this chapter is to show how the interpretations of Louis Pasteur changed man's thinking about the beginnings of life. Also discussed is what was necessary for the prospect to modulate from the view of Pasteur to a positive outlook, and thence to serious experimentation.

A remarkably anticipatory analysis was that of Democritus (460–

370 B.C.), who, working within the conceptual framework of spontaneous generation, promulgated the concept of the atom. He derived the notion that matter is basically organized of atoms. Life arose, according to Democritus, as the result of natural forces, specifically the action of atoms of fire on atoms of moist earth. The inference that life is inherent in matter had been proposed earlier by Thales and other Greek philosophers.

In the Middle Ages, a number of scholars performed experiments in which insects, worms, eels, frogs, mice, and other organisms were “produced” from decaying or putrefying materials. This sort of evidence of spontaneous generation was respected until Francesco Redi (1626–1697), an Italian physician, showed that the white maggots in rotting meat result from the laying of eggs by flies, and are simply larvae that develop into flies.

Redi’s experiments moved the subject from dogma to controversy. A particularly heated contention developed between the Englishman John Needham (1713–1781) and the Italian Lazzaro Spallanzani (1729–1799). The two men performed quite similar experiments and obtained different results, which they used to substantiate the two sides of the controversy. In the experiments, liquids containing organic matter, such as mutton gravy, were enclosed in vessels, heated, and put aside. Later the vessels were examined for putrefaction. Each experimenter closed his vessel. Spallanzani sealed his hermetically; Needham stoppered his with corks. Spallanzani boiled the contents of his vessels for long periods; Needham heated his in a bed of hot ashes. Upon being opened, Needham’s vessels were found to have microorganisms within them and to smell of putrefaction. Some of Spallanzani’s vessels remained completely free of microbes (Hardin, 1966).

Needham then concluded that spontaneous generation was an inevitable consequence of the existence of organic matter. Spallanzani concluded that growth would not be observed if proper precautions were taken in sterilization. These experiments left the question in an unsettled state, basically because the studies did not deal with spontaneous generation. Although not recognized as such, these experiments were defining the appropriate conditions for sterilization which would prevent the growth and multiplication of microorganisms.

THE PERIOD OF PASTEUR

In modern times, the most marked turn in thinking on the subject of the origin of life occurred as the result of Pasteur’s celebrated experiments. His controversy with his contemporary and countryman, F. A.

Pouchet, had much of the quality of the Needham-Spallanzani argument. Like Spallanzani, Pasteur carried out meticulous experiments. Pasteur, however, had much greater success than Spallanzani in convincing his contemporaries. His careful studies and his histrionic elegance routed Pouchet and the other proponents of *la génération spontanée*.

As we contemplate those battles decades later, however, we learn (page 1) that Pasteur had some of the vitalistic bias of Needham, even though he evidently strove to convince himself and others that his leaning toward vitalism did not interfere with the objective interpretations of his experiments. (The concept of *vitalism* is not uniform among authors; it largely connotes the necessity for a vital force in addition to physical and chemical forces operating in the living cell.)

Pasteur proved that air contains microorganisms in nonuniform distribution. To obtain microorganisms and their germs, he merely sucked air through gun cotton, dissolved the gun cotton in a mixture of alcohol and ether, and observed microorganisms under the microscope.

By heating the air before passing it into sterile broth (Figure 1-1), Pasteur destroyed microorganisms that would have otherwise multiplied in the broth. In order to answer the charge that the "vital force" had been destroyed by heat, he refined the experiment to use swan-neck flasks (Figure 1-2). These allowed unheated air to enter, but the necks so shaped trapped viable particles that would have fallen into a flask that was open on top. When he broke the neck of one of these flasks, contamination by air and proliferation of microorganisms in

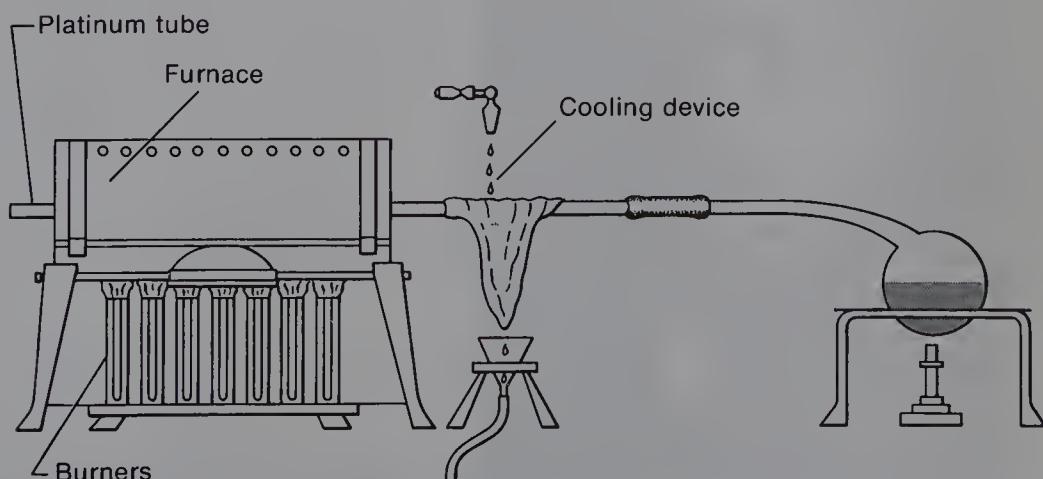
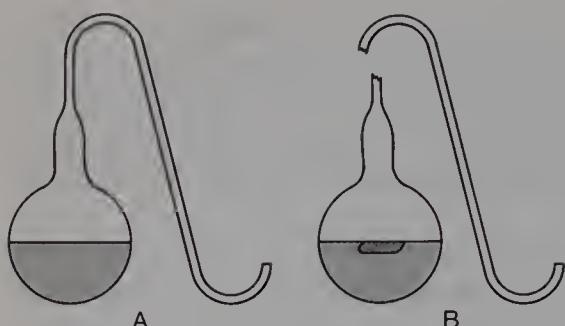


FIGURE 1-1

Pasteur's apparatus for sterilizing air. The device cools the hot air before its entry into the flask. From Keosian (1968).

**FIGURE 1-2**

Flasks with S-shaped necks: A, unbroken neck, contents of flask remained uncontaminated in Pasteur's experiments; B, flask with broken neck, contents of flask became contaminated. From Keosian (1968).

the fluid ensued. This simple and elegant experiment disproved the interpretations of his opponents.

In his triumphal lecture at the Sorbonne in 1864, Pasteur said (translated from Vallery-Radot, 1922), “Never will the doctrine of spontaneous generation recover from the mortal blow struck by this simple experiment.”

Pasteur was not thinking only of the brand of spontaneous generation that produced mice, maggots, or microbes from decaying matter. He deserves credit for recognizing that the proper adversary of vitalists of various persuasions was the force of self-organization of matter. In the same speech at the Sorbonne, he said, “There is the question of so-called spontaneous generation. Can matter organize



FIGURE 1-3
Louis Pasteur (1822–1895).

itself? In other words, are there beings that can come into the world without parents, without ancestors? That is the question to be resolved."

In equating spontaneous generation to a self-organizing act, Pasteur directed attention to the basic issue of life derived from life versus the origin of life from an appropriate material precursor or precursors. He thus recognized the nonvitalistic alternative, and he aligned his rhetoric against it with his "mortal blow" comment. The resolution of this problem in terms of self-assembly was thus deferred for nine or ten decades (Wald, 1954; Oparin, 1924, 1957; Fox, 1968d).

If the "secret of life" is a valid phrase, that secret, in the latter half of the twentieth century, appears to be the power of the forces of self-assembly (page 8).

Before leaving the discussion of Pasteur's position, we should point out some qualifications in his remark. In the lecture at the Sorbonne, Pasteur stated also, "No, today there is no circumstance known under which one could affirm that microscopical beings have come into the world without germs, without parents resembling themselves."

In saying this, Pasteur indicated that circumstances unknown to him may have permitted spontaneous generation. Also, in a statement attributed to him for 1878 (Nicolle, 1961), he stated: "Spontaneous generation? I have been looking for it for 20 years, but I have not found it, although I do not think that is an impossibility."

We thus see today that what Pasteur did was to invalidate salient experiments that had been incorrectly interpreted as proving the validity of the concept of spontaneous generation. This result is not equivalent to disproving the concept of spontaneous generation (Descour, 1922; Nicolle, 1961), although some such illogical reasoning appears to have contributed to a confusion that prevailed for decades. Also, Pasteur's experiments lacked relevance because they concerned contemporary microorganisms, rather than their primitive Darwinian ancestors.

The basic question remains as Pasteur phrased it, "Can matter organize itself?" (translated from Vallery-Radot, 1922). Today we have the evidence that permits us to say, "Yes, matter does organize itself."

THE PERIOD AFTER PASTEUR

We owe much of our modern broad understanding of the origin of life to the Russian biochemist, A. I. Oparin. He deserves credit first for providing a biochemical explanation alternative to the point of view



FIGURE 1-4
Aleksandr I. Oparin (Born 1894).

that the answer to the question of whether matter can organize itself is negative, which prevailed since Pasteur's time. Beginning in 1924, Oparin explained in outline how life could come into existence by processes familiar, for the most part, to students of organic and physical chemistry. Moreover, for this purpose he popularized the phrase *the origin of life*. A more objective phrase might be difficult to imagine. The assumption behind the phrase is that life originated, but no bias about the mechanism is indicated. This term is broad enough to allow for an act of special, or even divine, creation. The lack of bias connoted also a lack of detailed understanding, which has now been largely corrected, according to the thesis presented in this monograph.

Eighteen years after Oparin's first publication, the Mexican scientist Herrera summarized his studies of "sulphobes" (organized microstructures with the appearance of cells) in a paper titled: "A New Theory of the Origin and Nature of Life" (1942). In this article he reported obtaining sulphobes from ammonium thiocyanate and formalin, and he also reported formation of two amino acids and a



FIGURE 1-5
Alfonso L. Herrera (1868-1942).

condensation product, plus pigments. As he stated, "The particular theory offered here lacks confirmation." Herrera's studies, however, had one virtue crucially lacking in many other investigations. Herrera employed polymers from sources other than living organisms to explain the origin of organisms. Other investigators, however, employed macromolecules produced by living organisms. Starting with organisms does not permit learning with exactitude how life arose when there was no life. Herrera's experiments failed to treat adequately the question of chemical composition, they made no contribution to answering the fundamental question of how enzymes and metabolism originated, they presented no demonstration of proliferation, and they left other questions unanswered. It could not, however, be expected that Herrera should have in 1942 confronted, for example, the problems of amino acid sequence or information coding, since these problems were essentially undefined. The prospects for now doing this with his models are unpromising. A first suggestion of spontaneous generation in a twentieth-century sense can, nevertheless, be imputed to the experiments producing sulphobes. Moreover, Herrera deserves credit for historically early experiments in the field.

Herrera's studies of the reaction products of formaldehyde and ammonium thiocyanate, begun in the 1930s, were far ahead of their

time. Not until 1969 did astrophysics inform us that the reactive intermediates of ammonia and formaldehyde are present in abundance in our Galaxy.

Among the chemical experiments relevant to our subject (see Chapter 4) are those on carbon dioxide and water vapor by Groth and Suess (1938), followed by experiments of Garrison, Calvin, et al. (1951) and Miller (1953). A number of experiments performed by various workers prior to 1959 pertained to chemical synthesis in the rudimentary sense of that term. Many ways in which small organic molecules such as amino acids, purines, pyrimidines, nucleotides, and monosaccharides might have arisen on a primitive planet have now been demonstrated. Mechanisms by which macromolecules, especially proteins, might have originated have also been demonstrated, as we explain later in detail. (The term "macromolecule" is often reserved for polymers of at least 100 molecules of monomer. Polymers of 50–150 molecules, however, are treated in this volume as macromolecules.)

SPONTANEOUS GENERATION AND SELF-ASSEMBLY

About 1960, experimentation entered a new era. Emphasis in research work on self-organization, or self-assembly, of macromolecules yielding microsystems increased greatly (Wald, 1954; Lederberg, 1966; Fox, 1968a, 1968b; Lehninger, 1970). Although writers have often spoken of the "synthesis of life" (e.g., Anonymous, 1967), this new research work made the point that true synthesis, alone, would not be sufficient to yield an organized cell-like microsystem (Fox, 1968c). The process of self-assembly plays a critical role in the theory and in the total sequence of processes yielding cell-like structures in the laboratory or field. Resulting from this new research, the model of primordial events converting micromolecules (molecules of small size) to macromolecules to organized systems shows that these processes were very probably rugged, simple, and fast, and that they occurred frequently on the Earth (Fox and McCauley, 1968).

The significance of self-assembly applies both to the question of life's origin and to the appearance of structured units of contemporary living systems (Kushner, 1969; Lehninger, 1970). By inferential extrapolation from the assembly of individual organelles, and also from the model of a primordial cell, it applies to the formation of the membranes of the contemporary cell (page 213).

The concept of self-assembly was experimentally applied to a

model of the first cell a few years after the first clear-cut demonstration of self-assembly; which was provided by Schmitt (cf. 1956) from work with collagen (Figure 6-1). Wald (1954) invoked such self-organization in a general way as an explanation of the origin of the first cell.

Anfinsen et al. (1961) demonstrated phenomena related to self-assembly. In oxidizing reduced ribonuclease, Anfinsen and associates found that the recovered protein had the activity of native ribonuclease. The molecule, accordingly, reformed itself as an expression of the internal attractions. These forces are undoubtedly similar to those that cause two or more molecules to aggregate in a specific manner. Such an intermacromolecular assembly has been shown in a number of instances. Appropriate protein molecules assemble to yield complexes that are enzymically active (Reed, 1967), or in which coordinated metabolic reactions occur (Lynen, 1967). Pyruvate dehydrogenase and α -ketoglutarate dehydrogenase complexes were among the first of such reconstituted enzymes to be studied.

Another example of intermacromolecular assembly is the combination of proteins and nucleic acids, as in the tobacco mosaic virus (Fraenkel-Conrat and Ramachandran, 1959; Caspar, 1963) and in the ribosomes (Nomura and Traub, 1968; Spirin, 1968). The process of self-assembly will be described in greater detail in Chapter 6.

Another dilemma that has stood in the way of a theory of protobiogenesis is the question of the source of information (page 247). (In this volume, protobiogenesis is used as a term equivalent to the origin of life.) Experimental results from work on contemporary cells are interpreted to indicate that the flow of information is always from nucleic acid to protein. Watson (1965) has called this statement the "central dogma" of molecular biology. The model of protobiogenesis to be discussed later (page 203) suggests that information, or protoinformation, flowed from amino acids in the geochemical matrix to protein in descendants of the first organisms that separated from the environment.

The experiments based on the model of protobiogenesis (page 203) suggest that in the primordial system the information came from diverse reactant amino acids. The protocell thus did not assemble merely from macromolecules, it assembled from informational macromolecules (that is, macromolecules capable of interacting nonrandomly with other molecules or systems). This information could then have been transmitted to an evolutionary line.

The model traces a sequence from gases to amino acids to polymers to organized microsystems—the proposed evolutionary continuum from the beginning of the material cosmos to the first organisms. Information existed in a latent state in the geochemical

matrix although it did not exist as macromolecular information at the very beginning, for there were not yet any macromolecules. But order did not arise out of "chaos;" it existed at another level (traceable to cosmic origins, page 349). Between the cosmochemical matrix and the first organism there was no discontinuity in information; only new levels of assembly and a modulation of one kind of information to another.

Another context for our discussion beyond "informational macromolecules" is that of thermodynamics of nonequilibrium processes (Prigogine, 1955; A. Katchalsky and Curran, 1965). When we adopt this thermodynamic view, we can see that polyamino acid systems have evolved to more ordered and more dynamically interacting polynucleotide-polyamino acid systems. This could have occurred because the cell is an open system, capable of receiving and transforming energy.

When we examine all of the relevant experiments comparatively (page 130), we find that the compounds most common to organisms, i.e., adenine, alanine, aspartic acid, glycine, etc., are those that appear most frequently and in largest proportion in "origin" experiments. This can be understood as a result of selective assembly of the reactive intermediates formed in such experiments from the action of violent forms of energy on simulated primordial gases.

Phenomena of (selective) self-assembly or reassembly are thus seen to be operative at various levels of the hierarchically related processes of (1) formation of micromolecules (Keosian, 1968), (2) formation of polyamino acids composed of nonrandomly arranged amino acid residues (Fox et al., 1959; Fox, 1968a), (3) conformational organization within macromolecules (Anfinsen et al., 1961), (4) aggregation of macromolecules into supramolecular structures (Lynen, 1967; Reed, 1967; Fox, 1969), (5) formation of nucleoprotein(oid) (page 232; Fraenkel-Conrat and Ramachandran, 1959; Waehneldt and Fox, 1968), and (6) formation of organelles (Kushner, 1969). Accordingly, the ability of appropriate precursor macromolecules to form cells or protocells is but an extrapolation from many interdigitated phenomena (Fox, 1969). The stage of molecular evolution that concerns us most is that of the emergence of living systems. It is, however, in one view, only a salient type of self-assembly punctuating a magnificent evolutionary continuity. (In this monograph, we use molecular evolution to connote evolution at the molecular level, both before organisms and after they appeared.)

The thermal polyamino acids (page 150) have been found to have a set of properties closely similar to those of contemporary pro-

tein. These properties would have permitted further evolution (Fox, 1969). When analyzed in this manner, the (nineteenth-century) concept of spontaneous generation is seen to be embraced by the (twentieth-century) concept of self-assembly. Self-assembly, as understood by biochemists, thus applies to the origins of precellular polymers and systems as well as to contemporary biopolymers and cellular structures.

SUMMARY AND PROSPECTS

As explained above, much ambiguity has accompanied the concept of spontaneous generation during the course of its history. For example, the product of spontaneous generation was considered by some persons to be mice, or maggots, or even little men (the homunculus). Others thought of the product as being microbial. In fact, and as Oparin has pointed out, attention for development of the subject matter has been increasingly directed toward the simplest of organisms.

Another source of differences in the thinking was related to the experimental material. Many investigators of spontaneous generation worked with materials that had originated from living systems. Only a few experimenters employed minerals or other abiotic substances as starting materials.

Yet another, similar, difficulty had its roots in the fact that appropriate organic material that could be converted into living units has generally not been available. Once life had appeared *de novo*, organic material almost everywhere would inevitably have been altered, as we may now recognize (Charles Darwin recorded this thought also). The organic matter that had the capacity to undergo conversion to organisms would have quickly been consumed as food by already existing organisms. What was needed for relevant experiments was a recognition of the existence of pathways of molecular evolution. This knowledge had to wait on the development of structural organic and biological chemistry.

If a serious student of the subject had recognized in Pasteur's time that he needed to understand much more chemistry than had at that time been catalogued, that he must work with nonliving synthetic organic material, and that he should seek simple living microsystems rather than contemporary microorganisms as products, he would nevertheless have encountered a tremendous barrier. This barrier



FIGURE 1-6
Charles Darwin (1809–1882).

was the powerful influence of Pasteur, whose famous experiments had invalidated the experiments of Pouchet, a proponent of spontaneous generation and of the protoorganism. Pasteur's work showed that Pouchet had failed to support a thesis of spontaneous generation. The failure to prove the existence of facts is not equivalent, however, to proving their nonexistence. Nevertheless, for a number of years Pasteur presented his experiments as if they ruled out the concept of spontaneous generation. Great as were Pasteur's positive contributions, his drag on other fields of science was also great (Boyer et al., 1959). Moreover, the scientists of the nineteenth century did not press a search for a protocell or precursor organized systems.

We can now say that the newer development of the concept of spontaneous generation as part of a theory of the origin of life required first an awareness that the product had to be a *primitive* organism (a protocell), which could be the beginning of an evolutionary line. Second, the matter from which this protocell arose had to be understood to have had within it the power of assembling itself into a cell.

Third, this matter could be specified only by studies of organic and biological chemistry. Fourth, identification of the materials of life would not be sufficient. What had to be determined were the materials necessary to start the evolutionary sequence from even as far back as cosmic origins, but on the "right track." This required the development of a theory of molecular evolution. The requirement of the correct assembly of starting molecules precedes the requirement of the correct starting organism.

These concepts emphasize the need for viewing the problems of origins from the perspective of the primitive, in a detached manner. We draw our clues, however, from the contemporary. We can also evaluate the progress in research by judging how well some of the step-by-step changes suggest a progressive evolution from primitive to contemporary. Major evolutionary changes may, however, have yielded sudden leaps forward such as could not have been forecast from knowledge of the predecessors.

In fact, much of the special character of this kind of research rests on the awareness of the forces of self-assembly at crucial stages of evolution.

The research methods, in turn, have had to be constructionistic. Constructionism, in the sense in which we use it in this monograph, is the science of synthesis of molecules and assembly of systems from those molecules. (In a broader sense, constructionism includes intellectual synthesis, as in Darwin's formulation of the theory of the origin of species.) Our present understanding in science, especially in biology, has resulted from widespread application of reductionistic methods—that is, methods that dissect contemporary, evolved systems and their components. This approach could not tell us the truth about origins, partly because reductionism is based on the contemporary rather than on the primitive. The nature of the relationship between reductionism and constructionism has been most eloquently stated by the biologist Ernst Mayr (1964):

In principle, the evolution of new kinds of higher organisms . . . is the development of new biological systems consisting largely of new constellations and differing proportions of the same basic unit elements. In other words, biological evolution is dominated by the continuous emergence of new systems; and systems often display characteristics which one could not have predicted on the basis of the properties of the unit elements.

I pointed this out, some years ago, in a lecture in Copenhagen which was attended by Niels Bohr. In the ensuing discussion, he agreed with my conclusions, except for reminding me that an emergence of new characteristics in systems was not peculiar to living systems. . . . These

properties, Bohr said, could not have been predicted in detail on the basis of a knowledge of isolated protons, neutrons, and electrons. . . .

The systems approach . . . is in strong contrast to an approach usually referred to as reductionism. . . . As valuable as an analytical approach is, in my opinion it is only a first step and should never be considered the final goal.

The emergence of new characteristics in assembled systems, in other words, can be identified and studied constructionistically. Their very appearance partly explains the ease with which even some scientists have imputed the special effects of vitalism to the whole system.

In summary, the new thinking had to be constructionistic, it had to emphasize the primitive in a special way related to the contemporary, and it had to honor the basic principle of both evolutionary theory and facts of assembly—that operationally simple processes can rapidly develop complex products. With the overcoming of this barrier, a number of preconceptions within chemistry itself had also to be dealt with. These will be considered later. We may, however, define the objective as the formulation of a coherent, geologically relevant, and biologically disciplined theory of the origin of life.

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CHAPTER 2

Cosmology

In 1863, Charles Darwin wrote in a letter to J. D. Hooker, "It is mere rubbish thinking at present of the origin of life; one might as well think of the origin of matter."^{*} Serious thinking about the origin of matter itself, however, became possible twenty years after Darwin's death. In 1887, Heinrich Hertz in Germany initiated the discovery of the dual nature of matter—its having simultaneously wave and corpuscular properties. His experiments triggered new thoughts on the origin and evolution of matter. Hertz studied the effect of ultra-violet light on electric discharges in connection with his experiments

*See F. Darwin (1896). In an 1871 letter, however, Charles Darwin expressed the thought that he had projected backward his evolutionary concept towards the origin of the first living thing: "It is often said that all the conditions for the first production of a living organism are now present, which could ever have been present. But if (and oh! what a big if!) we could conceive in some warm little pond, with all sorts of ammonia and phosphoric salts, light, heat, electricity, etc., present, that a protein compound was chemically formed ready to undergo still more complex changes, at the present day such matter would be instantly devoured or absorbed, which could not have been the case before living creatures were formed." (C. Darwin, 1959).

to produce transverse waves, the existence of which was predicted in 1865 by J. C. Maxwell's electromagnetic theory. Hertz accidentally discovered the photoelectric effect, an effect that requires the quantum (corpuscular) theory for explanation. In 1905 Albert Einstein further developed Max Planck's earlier hypothesis that radiation is discontinuous, in order to explain Hertz' photoelectric effect.

Charles Darwin had realized that evolutionary thinking had to begin with the origin of matter. More than 100 years after he wrote the letter quoted at the beginning of this chapter, we are in a better, though not satisfactory, position to combine the lines of evolution of matter and even of energy with those of protobiogenesis.

As will be shown, the origin of organic matter can be traced back to the origin of inorganic matter. This back-extrapolation leads us to the origin of elements, stars, planets, and other celestial bodies. In this chapter, therefore, an introduction to cosmology will be presented as a prerequisite to a treatment of the origin of the raw materials for molecular evolution.

EVOLUTION OF STARS AND ELEMENTS

Equivalence of Matter and Energy

Einstein concluded that energy and mass are equivalent, that energy is absorbed in discrete steps, and that radiation is composed of discrete packets of energy, or quanta, that correspond to Newton's "multitude of swift corpuscles" of 1666. Einstein wrote the numerical relationship between the energy, E , and the mass, m , of a quantum as

$$E = mc^2 \quad (2-1)$$

c , the speed of light, being a numerical constant. Then, in the same year (1924) in which Oparin published his first book on the origin of life, Louis de Broglie postulated that not only radiant energy but matter itself possesses both wave and corpuscular properties. Thus the wavelength, λ , associated with a material particle, is given by Planck's constant, h , divided by the momentum (mass, m , times velocity, v) of the particle:

$$\lambda = \frac{h}{mv} \quad (2-2)$$

Equations 2-1 and 2-2 do not provide an answer to the question of the origin of matter; they rather describe the relationship between

matter and energy. Although we cannot at present describe the primary origin of energy or of matter, the contributions of Maxwell, Hertz, Planck, Einstein, and others have greatly advanced thinking in this area. Moreover, in this century the development of experimental discipline and critical interpretation leading to new concepts of the origin of life has passed far beyond what could possibly have been attained in the time of Darwin.

Equations 2-1 and 2-2 are prerequisites for the following discussion of the evolution of matter.

Big-bang Hypothesis

Two major hypotheses have been proposed to account for the evolution of matter in stars and to explain the apparent expansion of the Universe. One hypothesis, originated by Abbé Lemaitre (1950; see also Kuiper, 1951) and popularized by Gamow (1952), proposes that the present array of distant galaxies was preceded by a relatively small, very dense central mass, called "ylem," composed of protons, neutrons, and electrons. These authors assume that this matter had a temperature of billions of degrees and that it became unstable as it condensed under its own gravity and subsequently exploded several billion years ago. This was the "big bang." The nuclei of chemical elements, at least of the lighter ones, helium, lithium, beryllium, and boron, are supposed to have been produced in minutes, whereas the subsequent formation of galaxies and stars by gravitational aggregation presumably took longer. During their evolution, galaxies and stars continued to coast outward. Gamow and others assume that those with the highest velocities are now the farthest out. Conclusive evidence is, however, not at hand to prove this assumption. The big-bang hypothesis has been modified by Alfvén (1966, 1967) and others who assume that the primary explosion and subsequent expansion were caused by the annihilation of colliding particles of matter and anti-matter. Their hypothesis implies the existence of "anti-worlds" and perhaps "anti-life."

The big-bang hypothesis provides a simple and plausible explanation for Hubble's law (1936): the farther out a star is, the greater is the red shift of its spectral omissions. No agreement exists, however, on whether the observable red shift is to be correlated to the speed with which the star seems to retreat from the Earth. The shift may be as well due to other, though unknown, conditions that persist on the star or somewhere in the universe between the star and the terrestrial observer.

Steady-state Hypothesis

As an alternative to the big-bang hypothesis, Hoyle (1955) has put forward the steady-state hypothesis of continuous creation and conversion. According to Hoyle's original concept, the Universe is without beginning or end. Individual stars and even galaxies may develop and disappear but the main features of the Universe do not change appreciably. If the apparent outward motion of all distant galaxies is real, the observable part of the Universe is gradually being denuded of matter. To compensate for this depletion, new matter is continuously being created at a low rate. Hoyle suggested that hydrogen is the original material—the starting material for the evolution of other elements. Hoyle calculated that about 10^{32} tons of matter would have to be created per second to replace the matter that seems to be leaving our observable part of the Universe. Accordingly, the rate of creation of matter in this part of the Universe is calculated to be as low as one hydrogen atom per 10^5 cubic meters per year. The criticism has been raised that neither infinitely old nor newly created galactic material has been identified.

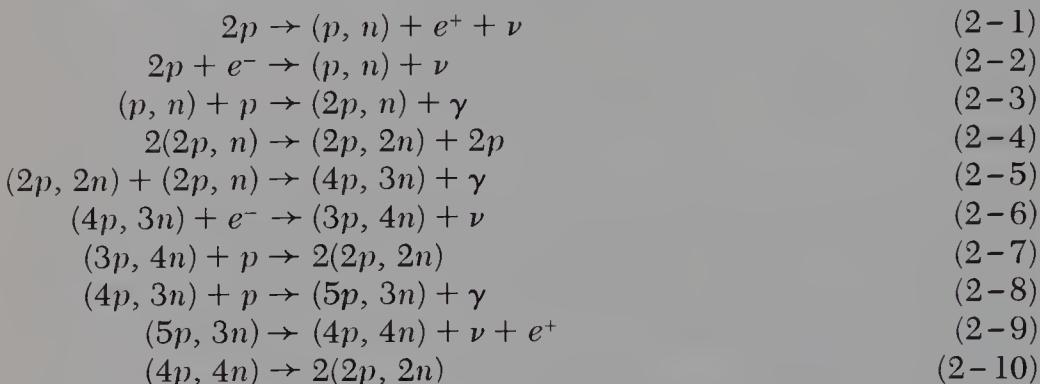
More recently Hoyle (1965) has revised the steady-state hypothesis. Evidently his idea of continuous creation of new matter cannot be correlated with astrophysical and astronomical observations and may, therefore, be discarded in favor of the big-bang hypothesis.

Evolution of Elements

Although the idea of the continuous creation of *new* matter is likely to be discarded, the concept of evolution of elements from hydrogen taking place in the interior of stars by nuclear reactions has found continued acknowledgment and extension (Burbidge et al., 1957; Fowler and Greenstein, 1956; Greenstein, 1961; Iben, 1967; Schatzman, 1965; Wallerstein, 1968), and was readily combined with the older idea of the generation of stellar energy by nuclear reactions (Atkinson and Houtermans, 1929; Bethe, 1939, 1968).

Most astrophysicists agree that stars are formed by gradual condensation of huge dark clouds of cool light elements, predominantly hydrogen. These clouds yield primarily large and diffuse protostars (Fowler et al., 1967) that continue to condense by their own gravitational pull, causing their central regions to grow hotter and hotter. As the temperature increases, more atoms become ionized and as a consequence the collisions between the bare hydrogen atoms, that is, the resulting protons, become more frequent and more violent. When

the temperature exceeds 10,000,000°C, protons begin to fuse together to form helium nuclei (α -particles) and some other light elements, while a fraction of the matter is converted into energy according to Einstein's equation, $E = mc^2$. (E means energy, m is the mass of a quantum and c is the speed of light, a numerical constant.) These reactions occur in 10 steps of which the reactions (2-1) through (2-4) have a probability of 86.5 percent compared with reactions (2-5) through (2-10).



In these equations p represents a proton, n a neutron, e^+ a positive electron (positron), ν a neutrino, and γ stands for γ -radiation. The positive electron is immediately annihilated by collision with an electron. The term (p, n) represents the nucleus of deuterium (the hydrogen isotope of mass 2), $(2p, n)$ the nucleus of helium 3, and $(2p, 2n)$ the alpha particle—the nucleus of helium 4, which is very stable and second in abundance only to hydrogen throughout the Universe. The expressions $(4p, 3n)$ and $(4p, 4n)$ stand for beryllium 7 and beryllium 8, respectively. The term $(3p, 4n)$ represents the lithium 7 nucleus, whereas $(5p, 3n)$ describes the boron 8 nucleus.

At still higher temperatures and in the presence of the nucleus of ^{12}C , another process of making helium from hydrogen is activated. The reactions are more violent and produce energy faster than the proton-proton reaction just described. This is the carbon cycle first proposed by A. Bethe (1939, 1968). The cycle moves through a relatively complicated series of six steps during which four protons are captured by a carbon nucleus, forming a helium nucleus. In the final step a carbon nucleus reappears. In this process, therefore, carbon acts only as a catalytic agent, being used repeatedly.

The proton-proton reaction presumably predominates and provides most of the energy in the less massive stars. However, no general agreement yet exists as to which of the two reactions dominate in the Sun, although the proton-proton reactions would be more favored at solar temperatures.

During the conversion of protons into α -particles, large quantities of energy are released. In the contemporary Sun 600 million tons of hydrogen fuse every second to form helium. The weight of the helium formed is 99.34 percent of that of the matter from which it had originated. The mass of the four million tons of matter that disappears every second is converted into energy that is gradually released from the Sun's surface by continuous radiation. The amount of energy produced in a star by nuclear reactions is far greater than any amount possible by gravitational condensation. In fact, whereas the source

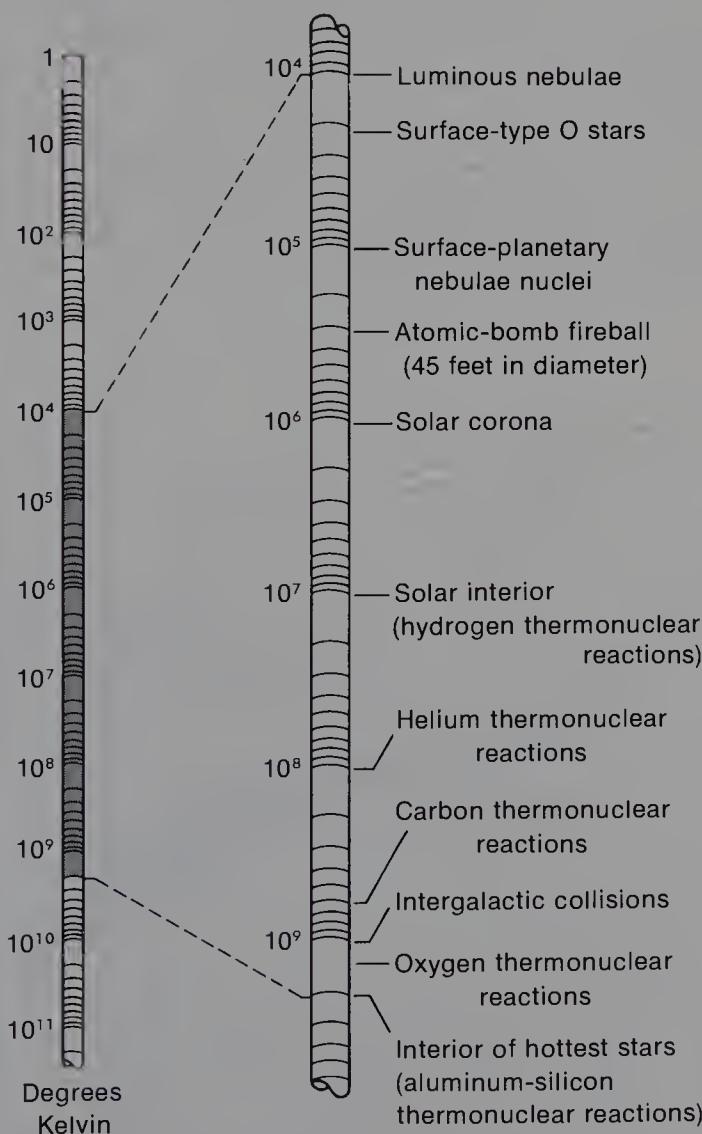


FIGURE 2-1

Temperature scale indicating the temperatures at which a number of thermonuclear reactions begin. Some temperatures on celestial bodies are presented for comparison. Source: Hurley (1959).

of nuclear energy is adequate for keeping the Sun shining for billions of years, the source of gravitational energy would be used up after 50 million years, that is, in about one percent of the Sun's present lifetime. During the process of helium production the temperature in the interior of a more massive star may rise above 100,000,000°C. At such a temperature, helium nuclei and hydrogen nuclei fuse to make new nuclei, chiefly of carbon, oxygen, and neon. At temperatures above 1,000,000,000°C, nuclei of magnesium, silicon, sulfur, argon, and calcium are formed, and at still higher temperatures nuclei of iron, nickel, and other metals appear with the release of decreasing amounts of energy. In the building of still heavier elements, energy is subtracted rather than added. The formation of heavier elements has a low probability. Their relatively low rate of formation, therefore, causes no appreciable cooling of the matter inside a star. Only some principal features of those nuclear reactions that lead to the formation of elements heavier than helium are being mentioned in this chapter. More detailed descriptions are given by Burbidge et al. (1957) and Cameron (1955, 1957, 1959).

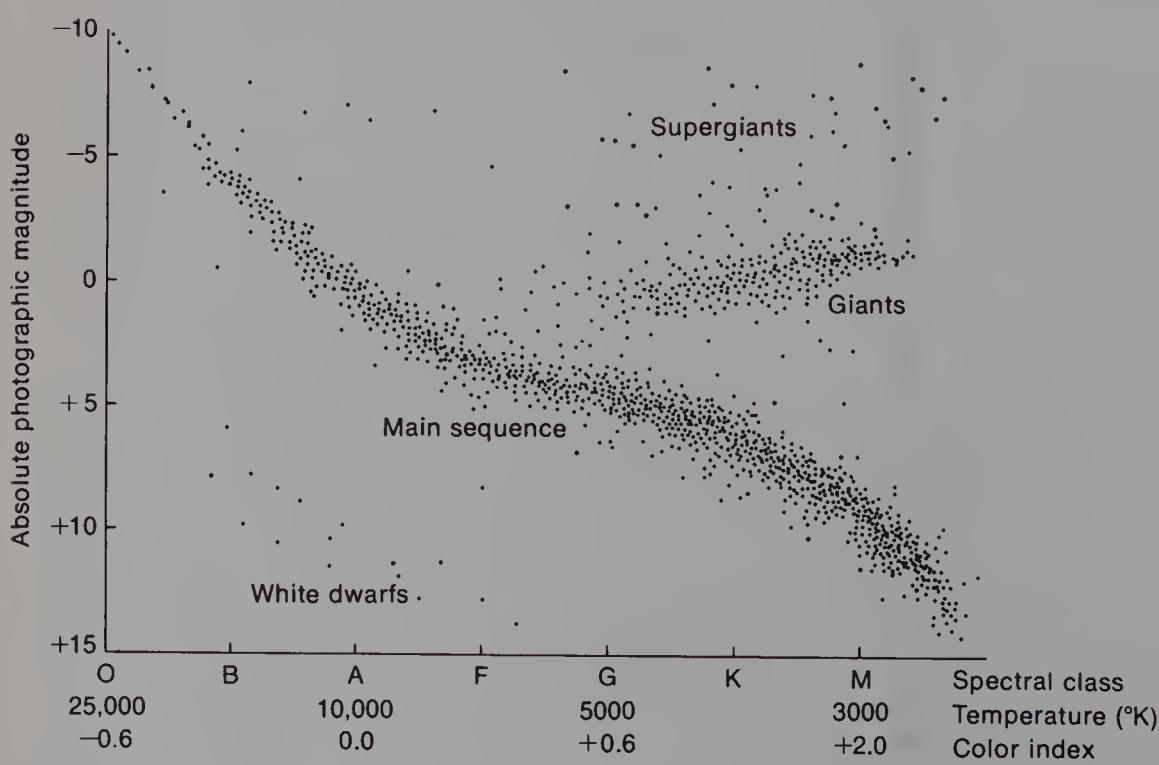


FIGURE 2-2

The Hertzsprung-Russell diagram for stars of known distance from Earth. Source: Abell (1964).

Hertzsprung-Russell Diagram

Stars have been grouped and characterized in several ways according to their various properties. Among these methods three have been widely acknowledged and discussed in the last decades. These classifications are made with the help of the Hertzsprung-Russell diagram, the mass-luminosity relation, and the elemental composition (populations or types) of the stars.

The Hertzsprung-Russell diagram (Figure 2-2) is a plot of stars' luminosities (or absolute magnitudes) against their temperatures (which are determined by spectral classes or color indices or both; for details see Abell, 1964; Jager, 1967; Unsöld, 1957; Schwarzschild, 1958; Stein, 1966). The position of a star on the Hertzsprung-Russell diagram is presumably correlated to its age and mass. Most stars cluster along a main sequence (Figure 2-2) that runs upward from the lower right of the diagram. Young, less-massive stars move from the right of the main sequence into its lower part after their internal temperatures have become high enough to convert protons into helium nuclei. More-massive stars move onto the main sequence from a position higher in the right-hand part of the diagram.

All stars spend the major portion of their lives in the main sequence. As their cores increase in density, because more and more hydrogen is being converted into helium, which is heavier, the stars' gravitational pull increases and causes condensation of the interiors. While the star is in the main sequence, however, its overall production of energy is close to being in equilibrium with its loss of energy by radiation. Accordingly, the star's position in the main sequence is virtually unchanged or it moves only a small distance towards the upper left. The temperature of the nucleus of more massive stars principally increases until conversion of helium into carbon becomes possible. The more massive the star, the earlier is this phase reached. A critical phase of contraction is reached when the nucleus of the star is burned out and the reaction zone starts to migrate towards the star's surface. In this phase of the star's life, the nucleus remains isothermal for a long period (Schonberg and Chandrasekhar, 1942). After about 12 percent of the star's mass is in the isothermal state, its interior begins again to contract. In this phase, however, the gravitational energy released from the interior causes the outer parts of the star to expand. During this process the star leaves the main sequence and moves toward the upper part of the diagram; the star is now growing to the size of a giant or supergiant (see Figures 2-2 and 3-2).

We must assume that the star finally ends as a white dwarf, appearing on the lower left of the diagram. This last phase is, however, not

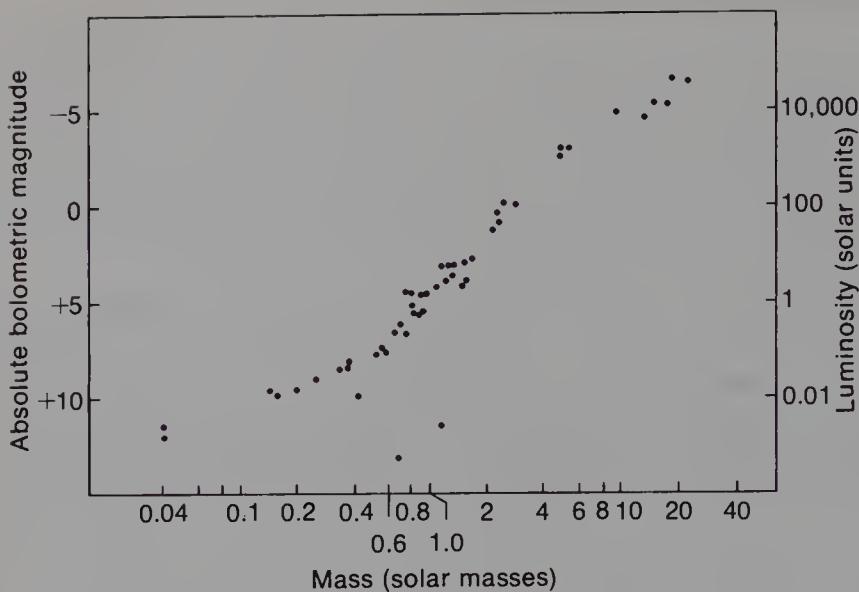


FIGURE 2-3
The mass-luminosity relation. The three points at the bottom represent white dwarf stars, which do not conform to the relation. Source: Abell (1964).

well understood. It possibly involves explosions of novae and supernovae (page 25). A more detailed discussion of processes and nuclear reactions relevant to stellar evolution is much beyond the scope of this book (cf., for detail, Meadows, 1967).

Mass-luminosity Relation

The characterization of stars by their mass-luminosity relation is illustrated in Figure 2-3. In this diagram, the absolute bolometric magnitude or the luminosity (in solar units), which is equivalent, of a star is plotted against its mass (solar masses). The stars of the main sequence of the Hertzsprung-Russell diagram, and even giants, seem to adhere to the mass-luminosity relation. White dwarfs, however, have smaller luminosities than expected from this relation. No simple explanation is available.

Population I and II Stars

The elemental composition of all stars throughout the Universe is not the same. After extensive studies of colors and spectra of stars, Walter Baade (see, e.g., Merrill, 1963) concluded that stars in various

galaxies, including those of the Milky Way system, may be classified into two general groups according to their location, motion, chemical composition, and age. These groups are called populations. Most galaxies have a spheroidal region at the center, with a flat disk surrounding it and rotating around its axis. A typical disk contains spiral arms. Stars of Population I appear in the disks of galaxies, especially in their spiral arms. They show standard galactic rotation, are relatively rich in metals, and are young. Most stars of Population II are in the central regions of galaxies or in globular star clusters. They are poorer in metallic atoms and are older.

Stars of Population II are believed to have been formed mostly from hydrogen nuclei alone, as outlined previously (page 20). When the younger stars of Population I were formed, the residual gas throughout space already contained heavier elements that had been expelled from Population-II stars during some phase of their existence. The heavier elements could have been added to the residual gas in at least two ways: first, by the continuous blowing off of matter from the surfaces of giant red stars and, second, by the explosive outbursts of novae and supernovae. Spectroscopic observations confirm the inference that gases are slowly moving into space from the surfaces of the largest cool red stars. Although the rate of this discharge is not high, huge amounts of gaseous matter can be discharged in the course of billions of years. The explosion of novae and supernovae is a well-known astronomical observation.

In a typical galaxy, about twenty-five novae appear per year; each of these indicates a stellar explosion. The stellar outbursts cannot be predicted; the duration of most of them ranges from a few days to several weeks. A small cloud, resembling a planetary nebula, appears around the star for a while after the outburst. Driven by the thrust of the explosion, the cloud expands rapidly and carries out into space a small fraction of the original mass of the star. Once or twice in a few hundred years, however, a more violent explosion may occur in one star of a galaxy. Thus appears a supernova. These explosions are so violent that a significant fraction of the star's mass is blown into space. During the days of their outburst, supernovae are the brightest objects seen. Like the ordinary novae, however, they quickly fade and become inconspicuous.

Owing to their containing heavier elements, the Population I stars have a stronger gravitational pull. Their development, therefore, is more rapid from the very beginning. They reach the high temperatures required for the various thermofusions (Figure 2-1)—that is, they enter the main sequence of the Hertzsprung-Russell diagram—relatively early in their history and exhaust their energy more quickly.

Although shining very brightly for some time, they fade after a relatively short time. More-massive Population-I stars have about 10–40 times the mass of the Sun. They disappear after only a few hundred million years. Population-I stars that are about as massive as the Sun are supposed to reach ages of about 10 billion years. Population-II stars are exclusively represented by the oldest stars of the Universe. Their life span significantly surpasses that of Population-I stars; more exact estimates are not available.

EVOLUTION OF THE SOLAR SYSTEM

The Sun must be classified as a Population-I star because of its location in the disk of our Galaxy, its standard rotation, the relative abundance of its metals, and its relative youth (approximately 5 billion years). The Sun has an estimated life expectancy of at least 5 billion more years based on its relatively small mass.

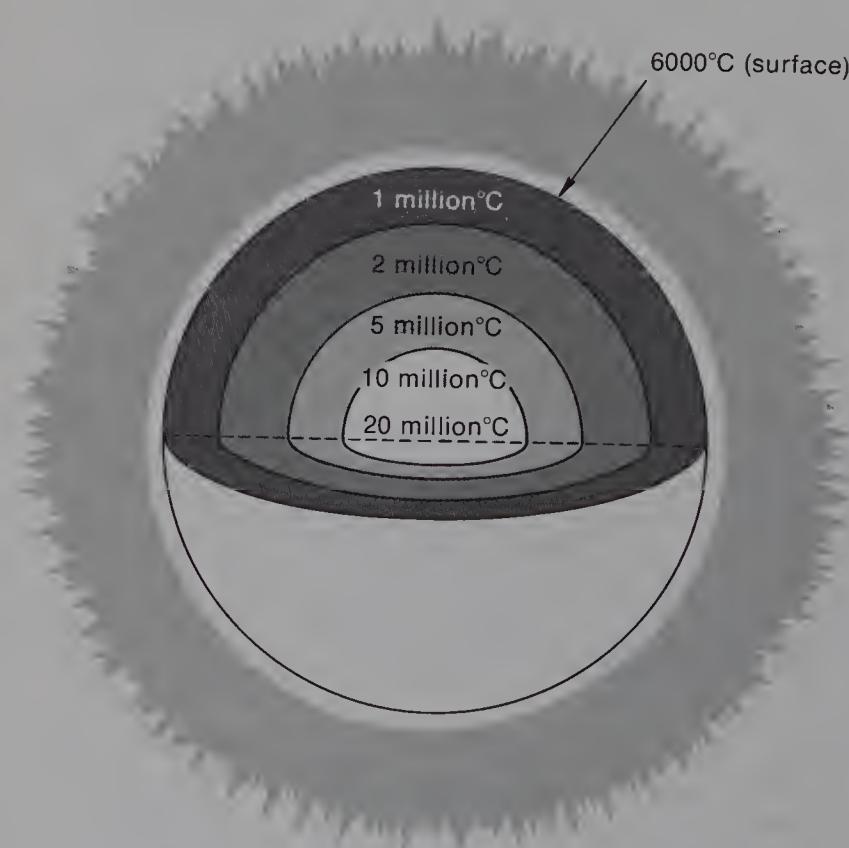


FIGURE 2-4

Cross section of the Sun, showing the temperature distribution (estimated values). Source: Hurley (1959).

The relative abundance of metals and of other heavy elements in the Solar System is significant for problems of chemical evolution. For the formation of the considerable amounts of carbon and oxygen found in the Sun, for example, temperatures above $6 \times 10^8^\circ\text{K}$ would be required (Figure 2-1). We have no evidence that the Sun ever had such a high temperature. The maximum temperature in the center of the Sun now is assumed to be about $20 \times 10^6^\circ\text{K}$, while the surface temperature is less than 6000°K (Figure 2-4). According to the temperature inferred at present for the Sun, only the thermonuclear reactions given by Equations 2-1 through 2-4 are of significance. That is, most, if not all, heavier elements that have been identified on the Sun and throughout the Solar System, including on our Earth, probably have not been formed in the Sun. The Sun and its planetary bodies, therefore, must have inherited these elements from another source. This source, presumably a cloud of hydrogen and heavier matter from older stars (page 25), may have appeared at some time in the early history of our galaxy. This cloud of gases, dust, and rocks could have been captured by the young Sun or such a cloud could have condensed to form the Sun and the other bodies of our planetary system. The question of whether the Sun and the planetary bodies of the Solar System developed from identical, or from different, sources of gas and solid matter is unsettled. Space chemists have been particularly bothered by the fact that the iron/silicon ratio in the Sun appears to be much less than in the Earth or in meteorites. As yet, a reasonable explanation for this difference is not at hand.

A detailed characterization of the evolution of the Sun by the Hertzsprung-Russell diagram is included in Chapter 3 (Figure 3-2).

Three principal hypothetical models are used to present different pictures of how the Earth and the other bodies of the Solar System may have formed. These hypotheses may shed light on the problem of the thermal history of the planets. Kuiper (1949, 1951), in particular, has argued that a part of a huge cloud of dust and gas condensed, to form primarily a proto-Sun or low density surrounded by a rotating cloud disk. The outer zones of the disk broke up into eddies of irregular size and arrangement moving at various radii from the Sun's center. The eddies in any radial zone merged into a single cluster of condensing gas and dust. These clusters have been called the protoplanets.

According to Kuiper, the proto-Earth probably was a low-temperature body of gases and solids of about 500 times the Earth's present mass. The matter of the Sun had not condensed sufficiently at an early stage to be hot enough to radiate heat to the protoplanets. The gaseous part of the proto-Earth consisted mainly of hydrogen and helium, plus

neon, methane, ammonia, and some water vapor; the solid part consisted principally of condensed water and minerals. The heavier materials were eventually spiralled into the center. During such a process of aggregation, the proto-Earth is believed to have heated very pronouncedly due to the release of gravitational energy. A second condensation cloud may have formed the Moon at that stage. So far, however, the origin and the evolution of the Moon are matters of disagreement. Some understanding has been gained through the Apollo manned flights to the Moon (Levinson, 1970).

As the Sun condensed, it became hotter and brighter by conversion of gravitational energy into heat, which was radiated into space, thus further increasing the temperature of the protoplanets. Probably huge amounts of lighter gaseous components escaped from the gravitational fields of the protoplanets at this stage. These lighter elements once amounted to about 99 percent of the protoplanetary mass, provided the portions of hydrogen and helium in the protoplanets and the proto-Sun were not much different from their portions in the contemporary Sun or Universe. Kuiper estimates that the time required to remove gases from the proto-Earth by solar heat alone was several hundred million years.

A second model of the aggregation to the primitive Earth was put forward by Shmidt (1944, 1955). According to Shmidt's hypothesis, the Earth was formed by a gradual accumulation of a low-temperature protoplanetary cloud of gases, dust, and larger particles (Levin, 1959). In an early state of its evolution this cloud already contained a multitude of asteroid-sized bodies. The collision and aggregation of these bodies would have yielded only local increases of temperature. Although the bulk of the Earth was thus made up of nonvolatile stony matter, condensed primordial gases may have been included, particularly in the asteroid bodies of cold protoplanets that were remote from the Sun. Juvenile organic compounds, which are reportedly found on the contemporary Earth (page 318), may be abiotic conversion products of simple organic molecules that were derived from the protoplanetary cloud. In this connection, the newer information on interstellar matter (page 327) may drastically revise the conceptual possibilities.

The third model, formulated by Urey (1951, 1952, 1954a, b, 1956), seems to be a compromise. Urey, as well as Shmidt, postulates the accumulation of cold solid particles, the planetesimals, to be the main process. In Urey's view, the size of these particles may have varied between the size of asteroids and that of dust. In contrast to Shmidt, Urey does not exclude the possibility of strong surface heating during the aggregation, particularly of the smaller particles.

The inner parts of the larger bodies could have remained sufficiently cool not to melt during aggregation. If this were so, in agreement with Shmidt, organic material of the protoplanetary cloud could have survived to the present time.

The thermal history of the Earth and proto-Earth evidently is correlated to the processes of condensation and aggregation. Some scientists argue that the energy released by the condensation of the protoplanet might have raised the temperature to about 2000°C. We neither know, however, the rate at which the Earth accreted nor the flux of energy into space from the proto-Earth; that is, information about the heat of aggregation once retained in the primitive Earth is not available. Today, most geologists agree with Urey's concept (1962) that the temperature of the Earth's surface during its aggregation probably was never high enough to bring about a general and complete melting of the lithosphere (<900°C). On the other hand, the temperature in the interior of the Earth is believed to have been at some thousands of degrees after the Earth was formed, because of the heat flow originating in its interior.

BIOELEMENTS IN CELESTIAL BODIES

So far we have followed the evolution of stars and elements to planetary bodies. Before we focus on the evolution of simple molecules that could have been starting material for more complex organic matter, we have to consider the elemental composition of the geochemical matrix in which this evolution of simple molecules took place.

The organic compounds of living systems consist very largely of the elements carbon, hydrogen, oxygen, nitrogen, sulfur, and phosphorus, which are referred to as organoelements, or bioelements. The presence of these elements on a celestial body was and is the first prerequisite for the evolution of biologically significant molecules. Transformations in the sequence, elements → simple compounds → simple bio-organic compounds → macromolecules → organized systems, required at each step appropriate environmental conditions. As will be shown, these bioelements are among the most abundant elements on all celestial bodies so far investigated. Differences in chemical composition between the Sun and the planets seem to be explainable on the basis of the volatility of elements and the simple compounds formed from them. However, this is not to say that all the differences in the chemical composition of celestial bodies are well understood. Much is still to be learned. Once the differences in the planetary distribution of bioelements are better under-

stood, a detailed discussion of their significance with respect to molecular evolution will be necessary.

Spectroscopic data and determinations of mass-to-volume ratios of the Sun and the major planets indicate differences between the Sun and the planets in the distribution of elements heavier than the bioelements. Also, differences among the planets themselves are indicated by the same data. Pronounced differences appear, for example, in iron/silicon ratios. Many attempts have been made to explain these differences. The possibility exists that the Sun and its planetary bodies did not originate from the same source (page 27) or that a separation of minerals and light compounds took place early in the evolution of the Solar System or that a complete explanation must combine both of these possibilities. A number of factors, such as low gravitational field and relatively high temperature at the surface, can be assigned responsibility for the disappearance of light elements from a planet like Mercury or the terrestrial Moon. High gravitational fields, on the other hand, are responsible for the retention of gases that are probably primordial on Jupiter or Saturn. The deficiency or abundance of the various elements on the planetary bodies are not completely understood. Discussion of these matters is beyond the scope of this book; various hypotheses relevant to them have been reviewed and put forward by Urey (1962) and others.

In the evolutionary context, however, attention will now be focussed on the six elements that make up most organic matter including the carbosphere of any cosmic setting.

COSMIC ABUNDANCES OF THE BIOELEMENTS

Table 2-1 shows the abundance of the six bioelements relative to carbon. Helium is also included. The body of carbonaceous complexes formed by reaction of carbon with the other elements has been called the carbosphere (Mueller, 1963). Accordingly, our biosphere is to be discussed as having emerged from a prebiotic carbosphere. The contemporary carbosphere appears to contain both prebiotic and biotic carbonaceous complexes (Chapters 10 and 11).

The abundance of lighter elements in the Universe has been determined by spectroscopic observation of the Sun, other stars, and nebulae. So far, the abundance of many heavier elements can be determined only by direct laboratory analysis, which is restricted at present to some lunar samples, meteorites, and the Earth's crust.

The bioelements carbon, hydrogen, oxygen, and nitrogen, together with the noble gases He and Ne, are the most abundant elements in

TABLE 2-1

Cosmic Abundances of He, H, C, O, N, S, and P (number of atoms per carbon or silicon atom; l.v. means low value.)

Atomic Number	Element	Si = 1		C = 1		Sun's Atmosphere
		Universe	Crust of Earth	Universe	Crust of Earth	
1	H	4×10^4	1.4×10^{-1}	1.1×10^4	5.2	5.1×10^4
2	He	3.1×10^3	7.5×10^{-8}	9×10^2	2.8×10^{-5}	1×10^4
6	C	3.5	2.7×10^{-3}	1	1	1
7	N	6.6	3.3×10^{-4}	1.9	1.2×10^{-1}	2.1
8	O	2.2	2.9	6.3	1.1×10^3	2.8×10^2
14	Si	1	1	2.9×10^{-1}	3.7×10^2	1
15	P	1×10^{-2}	3.8×10^{-3}	2.8×10^{-3}	1.4	l.v.
16	S	3.8×10^{-1}	1.6×10^{-3}	1.1×10^{-1}	6.0×10^{-1}	4.3×10^{-1}

Source: Numbers calculated from data given by Cameron (1957), Mason (1954), and Suess and Urey (1956).

the Universe. Next are magnesium (0.29 per carbon atom) and silicon (0.27 per carbon atom), the former being the most abundant metal. The abundance relative to one carbon atom of some other bioelements is: sodium (0.013), potassium (0.0017), chlorine (2.8×10^{-4}), calcium (0.018), and iron (0.14).

The data on the abundance of elements in the Universe are primarily of theoretical importance. The significance of these figures may be questioned because they reflect only the composition of the matter that can be directly determined by spectroscopic measurements. No direct data are available from the major portion of cosmic matter, that is, the interior of stars and the surface and interior of cold celestial bodies and stars too remote to be analyzed. Nevertheless, the determinations that have been made of abundance of elements do supply some valuable information (Table 2-1).

The differences in chemical composition are to be related to the different histories of the bodies investigated. Agreement exists that the lesser abundance of hydrogen, the noble gases, and all lighter elements in the Earth's crust and in meteorites, compared with their abundance in the whole Universe is owing to the diffusion of these elements, or their volatile compounds, into space at some time during the formation and evolution of the celestial bodies (page 27). No general agreement, however, is evident as to the time when these processes occurred (for more details on this and related questions, see Urey (1962), the references given with Table 2-1, and Chapter 3).

The fact that the abundance of carbon relative to silicon in the Cosmos is over a thousand times as great as that in the Earth's crust deserves special emphasis. The average C/Si ratio in the Cosmos is 3.8; in the Earth's crust it is only 2.7×10^{-3} , according to Mason (1954).

The major portion of the Earth's original carbon content, presumably in the form of CH₄, C₂H₆, H₂C=O, CO, CO₂, HCN, or their components, was probably lost with the escape of the Earth's primary atmosphere.

Nitrogen is a bioelement that is in even shorter supply in the Earth's crust relative to its abundance in the Cosmos (Table 2-1). The deficiency of nitrogen, too, is apparently a consequence of its escape with the Earth's primary atmosphere, mostly in the form of N₂ or NH₃.

The abundance of oxygen (relative to carbon) is two to three orders of magnitude greater in meteorites and in the Earth's crust than in the Universe as a whole. No striking difference, however, appears among O/Si ratios. This is not surprising because oxygen forms nonvolatile and heat-stable oxides with most metals and semi-metals, including silicon.

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CHAPTER 3

Geological Conditions on the Primitive Earth

In considering the emergence and evolution of life on the Earth, we focus attention upon three realms: the lithosphere, the hydrosphere, and the atmosphere.

THE PRIMITIVE LITHOSPHERE

The importance of the lithosphere in the study of molecular evolution derives from three facts: contemporary organisms require many of the metallic elements of the lithosphere for their metabolic functions; cell walls and other structural elements of some primitive organisms (e.g., diatoms) are built up from inorganic constituents of the lithosphere; and the lithosphere contains, like the hydrosphere and atmosphere, organic compounds (Chapter 10), the existence of which was a prerequisite for the emergence of life.

The lithosphere may be classified into the core, the mantle, and the crust. The core and the mantle seem to be of least direct interest

to molecular evolution. Based on the interpretation of seismic data and on the compositional range of meteorites, the core is believed to be predominantly iron and nickel. Within the mantle, silicates of magnesium and iron predominate; the crust contains a relatively higher percentage of the lighter silicates of aluminum, sodium, and potassium. Supposedly, the boundary between the core and the mantle is occupied by a zone that is rich in sulfides of iron and other heavy metals. The trend of gravitational segregation continues through the crust. This consists essentially of a relatively metal-rich, basaltic zone, which reaches from the outer surface of the mantle (the Mohorovičić discontinuity) to a level given by the deepest bottoms of the oceans. Also present is a discontinuous zone of silica-rich granitic rocks, of which the bulk of the continental masses is constituted. Temperatures and pressures increase towards the center of the Earth. As E. Bullard (1954) has pointed out, the temperature inside the Earth is the least well known of its physical properties. Bullard believes that at about 500 kilometers beneath the surface the mantle is only slightly below its melting point.

The chemical nature of the carbonaceous complexes of the prebiotic lithosphere, in particular the Earth's crust, is largely unknown. Currently, geological evidence does not permit significant conclusions about them. Some of the following chapters will deal with laboratory experiments simulating conditions of the early Earth. Geological and paleontological data relevant to the contemporary carbosphere and its history will be discussed in Chapter 10.

Table 3-1 summarizes some general information on the geochemistry of the lithosphere. Data relevant to the presence of carbon compounds on the Earth's surface (Schmucker, 1969), and therefore to biological and prebiological evolution, have been omitted for the reason given in the preceding paragraph.

TABLE 3-1
The Geochemical Composition of the Inorganic Lithosphere

Component	Composition	Physical State
Crust	Silicates of Al, Fe, Ca, Mg, Na, K	Mainly solid, but flows under tectonic stress
Mantle	Mainly silicates of Mg and Fe. Probable FeS margins	Solid, but flows under stress
Core	Fe-Ni alloy	Outer part liquid, central part possibly solid

THE PRIMITIVE HYDROSPHERE

Since life as we know it is dependent in crucial ways upon water, major attention must be given to the hydrosphere.

The primitive oceans, like the present oceans, were cradled in basins. These basins have been referred to by Rittmann (1962) and others as great rift valleys. No agreement exists concerning the water masses of the primitive oceans. According to data supplied by Rubey (1951, 1955), less than one-tenth of the volume of water in the contemporary oceans was originally present on the surface of the Earth, the remaining nine-tenths having been later supplied by outgassing from the interior. Urey (1952) comes to similar inferences on the basis of the current rate of outgassing of juvenile water (that is, water that came from the interior of the Earth to the surface for the first time) by volcanic activity, hot springs, and fumaroles. Less agreement exists concerning the rate of increase of the volume of the oceans during the various periods of the Earth's history (Rubey, 1964). According to Weyl (1968), the volume of the oceans increased significantly during Precambrian times and approached its present value at the beginning of the Cambrian. If the ratio of the rates of outgassing, particularly of chlorine and water, remained relatively constant during the Precambrian, the salinity of sea water would not have varied greatly during geological periods of time (Rubey, 1964).

The partial pressures of water vapor in the primitive atmosphere is closely correlated with the development of the early ocean. Rittmann (1962) suggests that, during the early history of the Earth (page 27), the surface temperatures descended through 374°C , the critical temperature of water. Above this temperature the liquid phase of water does not exist, irrespective of the pressure. When the temperature dropped below the boiling point of water (which, accordingly, must have been below 374°C at any pressure), an intense rain would have showered down upon the then relatively hot crust of the Earth. On reaching the crust, the water masses would have volatilized again, and then recondensed so that a very vigorous circulation would have been initiated. However, as atmospheric pressures and temperatures of the primitive Earth are unknown, no definite statement can be given concerning state and evolution of the hydrosphere during the first hundreds of millions of years of the Earth's existence. The displacement of large water masses from the atmosphere towards the crust, that is, towards the center, must have resulted in an acceleration of the Earth's rotation. There probably were local differences in the rate of cooling, differences, for example, between polar and equatorial

regions, and if there were, the water masses would not have condensed simultaneously.

A controversy exists regarding the *pH* of the primitive ocean. One picture is that of an initially acid ocean, produced by acid effluvia (CO_2 , H_2S , SO_2 , etc.) from volcanoes, being neutralized by cations leached from the igneous rocks of the land by rain (Rubey, 1964; Vinogradov, 1959). The second, more popular, picture is that of a slightly alkaline primitive ocean (*pH* 8–9) produced by the interaction, from the very beginning, of the acid volatiles from outgassing with the alkaline components of the predominantly basaltic crust (Abelson, 1966). On the contemporary Earth only about 28 percent of the crust is available for weathering. On the surface of the crust most of the continental rocks are sedimentary. They are not so alkaline as basaltic rocks, even though the huge amounts of silicates that have been weathered exhibit a significant buffering capacity (Sillen, 1961). Accordingly, the *pH* of the ocean probably was slightly above 8 during most of the Earth's history, excepting for a period, about which we cannot be certain, during the first hundred millions of years. Both pictures are consistent with the assumption of a nonoxidizing atmosphere containing substantial amounts of CO_2 . Aside from the primary ocean, however, acid pools and springs resulting from condensates of volcanic outgassing are known to exist even today (Sigvaldason and Elisson, 1968). These local features in the hydrosphere may have played a crucial role in protobiogenesis.

Ultraviolet light from the Sun penetrated the upper 19 meters of the ocean (Berkner and Marshall, 1965, 1966) and may have initiated photochemical conversions of organic compounds primarily formed in the atmosphere. An accumulation of different types of biochemical compound in vast parts of primitive waters was, however, extremely unlikely, for several reasons. First, amino acids, aldehydes, cyanides, and other such reactive materials are especially unstable in aqueous solution. Second, most of the resulting products are far from identical with biologically important molecules. Third, the primitive ocean was steadily irradiated with a relatively high dose of solar ultraviolet light (page 49). A steady irradiation of a rather homogeneous solution results in degradative rather than synthetic reactions; a photochemical equilibrium stage is finally approached. Accordingly, Abelson (1966), Hull (1960), Sillen (1965), and many others have criticized the hypothesis that the primitive ocean, unlike the contemporary ocean, was a "thick soup" containing all of the micromolecules required for the next stage of molecular evolution. The concept of a primitive "thick soup" or "primordial broth" is one of the most persistent ideas at the

same time that it is most strongly contraindicated by thermodynamic reasoning and by lack of experimental support. Evidently, the evolutionary process was not simple in this way. Even for such an early stage, solid-solid, solid-liquid, gas-liquid, and liquid-liquid phase interactions must be considered.

The ways in which organic matter have been thought of as concentrating in amounts useful for evolution include: absorption by minerals on the shores (Bernal, 1961); the elevation (as in the contemporary ocean) of surface-active dissolved organic matter to the surface, by rising bubbles, followed by compression into lines of convergence by the Langmuir circulation (Sutcliffe, 1963); and concentration by density gradients in the tropical thermocline, which must have existed since the depth of the terrestrial waters exceeded 300 meters (Weyl, 1968). The zone resultant from the action of this last phenomenon, being rich in inorganic and organic substances, would have favored the evolution of early photosynthesizing organisms. These organisms would have been shielded from ultraviolet radiation at that depth, whereas visible light would have reached them. Even so, this does not solve the crucial thermodynamic problem of the prebiotic condensation of micromolecules to macromolecules in the aqueous system that the ocean represents.

Other modes of concentration include recessions of the seas, drought, concentration by evaporation at elevated (page 179) or ambient temperatures in lagoons (Bernal, 1951, 1967), and solid or near-solid phase reactions. Reactions that proceed in the molten state obviate the problem of dilute aqueous solution. The postulates listed in this paragraph have been subjected to simulative conditions in the laboratory and have yielded a number of reactions, which have in turn successfully led to the plotting of evolutionary pathways.

THE PRIMITIVE ATMOSPHERE

The assumption has been widely adopted that upon its agglomeration the Earth was without an atmosphere (the gaseous constituents of the materials from which it was formed—the primary atmosphere—having mostly escaped into outer space) and that its present atmosphere (the secondary atmosphere) evolved from volcanic outgassings. The work of Goldschmidt (1937), Brown (1949, 1952), Kuiper (1951), Urey (1959, 1962), Alfvén (1954), Fesenkov (1959), Vinogradov (1959), Berkner and Marshall (1964), Rubey (1955, 1964), and many others has supported this assumption. Conclusive evidence for this assumption is available from comparison of the fractionation factors, that is, the

cosmic abundance of an inert (noble) gas divided by its terrestrial abundance. Table 3-2 shows that these fractionation factors are relatively large for the inert gases, which are lighter, and smaller for the heavier gases, although a simple correlation between atomic weight and fractionation factor does not exist. Probably the inert gases, because of their low chemical reactivity, largely escaped into space, while significant proportions of the other gaseous components were withheld owing to chemical binding by minerals. No evidence that significant proportions of the gases were occluded *per se* is at hand.

Hydrogen and helium, too (page 31), are much more abundant on the Sun than on the Earth. Their fractionation factors have not been included in Table 3-2, however, because the escape of hydrogen is influenced by its ability to form heavier molecules with other atoms and because the abundance of helium on the contemporary Earth is largely determined by its production during radioactive decay of heavy elements. For the same reason, Table 3-2 lists only argon 36; another argon isotope, ^{40}Ar , is continuously produced from decay of ^{40}K . The present accumulation of ^{40}Ar in lithospheric occlusions corresponds to the estimated age of the solid Earth of about 5 billion years. Therefore, significant amounts of ^{40}Ar were not occluded at the time the Earth's lithosphere was formed. This view is consistent with the agglomeration of the lithosphere from condensed mineral dust or from more coarse and bulky bodies, the planetesimals, the gravitational fields of which were too small to retain the primary atmosphere (page 32).

Rubey (1951, 1955), Vinogradov (1959), and others have, therefore, emphasized the volcanic origin of the atmosphere of this planet. We have no quantitative geological records of the volcanic activity on the primitive Earth. Estimations by Sapper (1927), Rittmann (1962), Verhoogen (1946), F. M. Bullard (1962), and Wilson (1954), based

TABLE 3-2
Fractionation Factors of Noble Gases

Element	Atomic Weight	Fractionation Factor
		$\left(\frac{\text{Cosmic Abundance}}{\text{Terrestrial Abundance}} \right)$
Neon	20	$\sim 10^{10}$
Argon 36	40	$\sim 10^8$
Krypton	83	2×10^6
Xenon	130	$\sim 10^6$

Source: Brown (1949, 1952).

mostly on measurements of contemporary production of lava, show that the continents are enlarged at a current average rate of about one cubic kilometer per year from volcanic effluents. Vinogradov (1959) and others, however, have argued that the volcanic activity must have been more extensive on the early Earth owing to a larger heat production by radioactive decay (page 56). On a less factual basis are other considerations of the thermal history of the Earth (page 58), which nevertheless lead to similar inferences.

Accompanying the solid-liquid effluents of volcanoes are considerable volumes of gases. On the contemporary Earth, however, the history of volcanic gases often is complicated, because the lava they accompany may derive from metamorphosed material (Verhoogen, 1946), which would likely be contaminated by the degradation products of contemporary organisms and fossils. Volcanic gases consist mainly of juvenile, that is, deuterium-poor water vapor, presumably released from water of crystallization, and variable quantities of CO₂, N₂, SO₂, H₂S, S, HCl, B₂O₃, and lesser amounts of H₂, CH₄, CO, NH₃, and HF, but no oxygen. Satisfactory analyses of volcanic gases are rather few. Some detailed data have been reported on the composition of the gases produced at the lava lake at Kilauea on the island of Hawaii (Heald et al., 1963), at the Valley of Ten Thousand Smokes, Alaska (Rittmann, 1962; F. M. Bullard, 1962), at Larderello in Tuscany (F. M. Bullard, 1962), and at the cinder cone of the Surtsey volcano in Iceland (Sigvaldason and Elisson, 1968). In all cases CO₂ is, after water vapor, the second major constituent. The other components that usually appear in relatively minute amounts vary from analysis to analysis. The quantitative composition of the solfatara-like steam spout at Larderello is given in Table 3-3. Studies emphasize that the chemical composition of volcanic gases differ significantly from place to place and from time to time at the same place. On the basis of the data in Table 3-3, if taken as representative for volcanic outgassing of the primitive Earth, a mildly reducing, or at least nonoxidizing, atmosphere rich in CO₂ would have been built up. The composition of the steam spout at Larderello is somewhat like that of the atmosphere of Venus (Table 3-6; see also Reese and Swan, 1968).

Equilibrium calculations for the interpretation of the differences in composition of volcanic gas samples collected at different times from the Hawaiian volcano Kilauea have been put forward by Heald et al. (1963). By comparing their calculations with experimental results, these authors showed that, at least in some cases, volcanic gas may be regarded as a closed system in equilibrium at temperatures around 1000°K. Changes in the composition of gases emitted from the same source are often caused by changes in pressure or tempera-

TABLE 3-3
Composition of Solfatara-like Steam Spout at Larderello in Tuscany

Component	Native Gas		Dry Gas (25°C)	
	g/kg	mole%	g/kg	mole%
H ₂ O	955.52	98.2	—	—
CO ₂	42.65	1.8	970	93.0
H ₂ S	0.88	0.05	20	2.5
H ₂ BO ₃	0.30	0.01	—	—
NH ₃	0.30	0.03	7	1.7
CH ₄	0.15	0.01	3	0.9
H ₂	0.04	0.04	1	1.9

Source: Rittmann (1962).

ture of the original magmatic gases, or both. Certain instances are complicated by interaction with gases from nonvolcanic sources, such as surface water or organic materials, or by nonequilibrium reactions with the surrounding rocks. Collecting gas samples from an active volcano imposes a number of serious problems. One of these problems is that the locality itself is changing with time. Samples collected at the same site, at different times, may actually be of different magmatic origin. In Table 3-4 are summarized data on the chemical analysis of gases from the Surtsey volcano in Iceland collected between 1964 and 1967 within a distance of 150 meters from the crater. These data show to what extent the composition of the gas samples may vary. The O₂ and the relatively large amount of N₂ of the samples of May 21, 1964, August 19, 1964, and November 25, 1964 are likely owing to contamination with air.

Some authors have reasoned that the *primordial* or *primitive* atmosphere of the Earth (i.e., the atmosphere when life began) was a "reducing atmosphere" consisting mainly of hydrogen, methane, ammonia, water vapor, and noble gases (Russell, 1935; Oparin, 1938; Urey, 1952; Miller and Urey, 1959). In fact, hydrogen makes up more than 80 percent of cosmic matter, and hydrogen and helium together, more than 99 percent. Carbon, nitrogen, and oxygen are, after hydrogen and helium, the most abundant elements in the Universe (Table 2-1). If hydrogen and helium had not escaped into space during the formation of the Earth, and if the temperature of the primordial atmosphere never significantly exceeded 25°C, the formation of a methane-ammonia-water atmosphere would have been favored, according to the following equations and equilibrium constants:

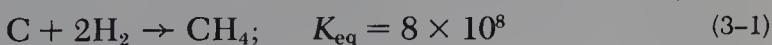
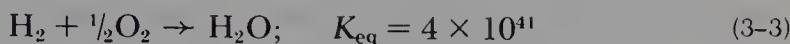


TABLE 3-4
Chemical Analysis of Gases from the Surtsey Volcano in Iceland (in mole percent, except S, which is given in milligrams of S per gram of condensate)

Date	Sample Number	H ₂ O	HCl	SO ₂	CO ₂	H ₂	CO	O ₂	N ₂ + Ar	S	T°K	Distance from Crater (m)
May 21, 1964	6	62.83	tr.	0.00	0.07	0.00	0.00	6.73	30.37	1100	100	
August 19, 1964	11	11.74	n.d.	0.00	0.44	0.00	0.00	15.09	72.73	1100		
October 15, 1964	12	79.20	0.80	5.40	9.18	4.56	0.68	0.00	0.18	1.6	1400	50
	13	79.20	0.80	4.02	9.64	4.88	0.70	0.00	0.76		1400	
November 25, 1964	14	76.45	0.49	0.90	0.99	0.00	0.00	3.69	17.48	1400	30	
	15	76.45	0.49	2.05	0.00	0.00	0.00	3.39	17.62	1400		
February 21, 1965	17	86.16	0.40	3.28	4.97	4.74	0.38	0.00	0.07	1400		
	22	86.16	0.40	1.84	6.47	4.70	0.36	0.00	0.07	18.3	1400	0
	24	86.13	0.43	2.86	5.54	4.58	0.39	0.00	0.07	12.2	1400	
September 2, 1966	25	78.10	0.40	14.60	3.14	1.59	0.09	0.00	2.08	1400	150	
March 31, 1967	29	89.25	1.15	2.46	3.29	2.67	0.11	0.00	1.07	8.0	1400	
	30	89.21	1.00	2.80	1.10	1.73	0.11	0.00	4.05	10.2	1400	20
	31	87.11	0.49	3.32	0.96	1.64	0.32	0.00	6.16	7.7	1400	20

Source: Sigvaldsson and Elisson (1968).



However, these ideas do not seem to be geologically realistic because evidence (page 39) indicates that the lighter, if not all, constituents of the primary atmosphere escaped into space early during the formation of the Earth. Since in the course of the Earth's evolution, its crust most probably attained temperatures in the range 200–1000°C, so that no general and complete melting occurred during the last 5 billion years (Urey, 1962). Before significant amounts of biochemical compounds could be formed and stored, however, the average temperature on the surface of the Earth must have dropped to about the present level, because most biochemical compounds would not have survived long at temperatures well above the present ones. Early in the evolution of the Earth most of the free hydrogen probably had disappeared into outer space and what was left of methane and ammonia was oxidized by oxygen, OH-radicals, and other oxidizing species that arose from decomposition of water by electric discharges, the various radiations, and extreme local heat, e.g., from volcanoes. Abelson (1966) has inferred that the atmosphere of the primitive Earth did not contain ammonia or methane because most of its ammonia would have been destroyed by the Sun's ultraviolet radiation within ~30,000 years. Abelson reasons that considerable amounts of methane, if present on the primitive Earth, would have been converted into higher hydrocarbons in thermal or radiation reactions. The methane would have had to survive chemical attack by water and its photolysis products: H_2O_2 , ·OH, etc. The higher hydrocarbons would have been occluded by sedimenting clays. The earliest rocks, therefore, should contain considerable proportions of C- and H-rich organic compounds. Such is not the case, as Abelson emphasizes. We therefore stress that the strongly reducing primary atmosphere, consisting of methane, ammonia, hydrogen, and water had already disappeared before a significant accumulation of organic compounds (formed from the constituents of the secondary atmosphere) took place.

In their attempts to confirm the absence of significant amounts of oxygen in the Earth's earliest atmosphere, many authors have used geological evidence. The incomplete oxidation of early sedimentary materials (up to 3.5 billion years old) as demonstrated by Rankama (1955), Ramdohr (1958), Lepp and Goldich (1959), and others, and summarized by Holland (1962), seems to prove that the early atmosphere was a reducing atmosphere. A few examples may elucidate their point of view: the South African gold-uranium deposits of the Dominion Reef and the Witwater system contain significant amounts of uranite,

UO_2 (Ramdohr, 1958), and the sulfides of iron, lead, and zinc (Holland, 1962). Thermodynamic considerations show that uranite as well as these sulfides are highly unstable in the presence of even traces of oxygen (Holland, 1962). The isotopic datings of these and similar sediments indicate absolute ages of 1.8–2.5 billion years (Rutten, 1962). Similar evidence is provided by Precambrian rocks found near Tampere, Finland, which are of about the same age. In these rocks, FeO has a preponderance over Fe_2O_3 indicating low partial pressures of oxygen during the time of sedimentation (Rankama, 1955). No agreement has been reached, however, concerning the equilibrium between FeO , Fe_3O_4 , and Fe_2O_3 as a function of the O_2 -partial pressures. Holland (1962) has pointed out that Fe_2O_3 (hematite) would be stable under extremely low O_2 pressures. Other geologists, however, tend to use the occurrence of hematite as an indication that there were significant amounts of oxygen in the Precambrian atmosphere. Krejci-Graf (1966), for example, points out that hematite was formed in the Precambrian as well as at other times. The fact that all oxidation states of iron from hematite to magnetite, Fe_3O_4 , to siderite, FeCO_3 , to pyrite, FeS_2 , have been found in sediments of all ages merely indicates that the redox state of deposits primarily depends on local conditions that do not always reflect the average conditions prevailing on the Earth at the time of deposition. Various examples may be cited. Carbon-containing sediments that were formed about 2 billion years ago already show a typical accumulation of the light carbon isotope, indicating that this isotope has passed through assimilating plants (page 295). The northern Indian Ocean is practically free of oxygen below 150 meters, because of its poor ventilation. During the saprophil periods of the Ordovician-Silurian and Lias ages, sediments were widely deposited in oxygen-free waters. Krejci-Graf (1966) infers, therefore, that it is not possible at present to make general deductions concerning the Earth's early atmosphere from geological evidence.

The Earth's primordial or primitive atmosphere is widely believed not to have contained in its early stage significant amounts of oxygen, for the following reasons: (1) even contemporary volcanic outgassings contain practically no oxygen; (2) both the primary atmosphere of the evolving proto-Earth and the original secondary atmosphere of the Earth must have been free of oxygen for thermodynamic reasons; (3) laboratory experiments show that chemical evolution, as accounted for by present models, would be largely inhibited by oxygen (Chapter 4), (4) organic compounds that, according to Oparin (1924, 1953, 1959, 1964, 1965), Haldane (1929, 1965), and others, have accumulated on the surface of the Earth in the course of chemical

evolution, are not stable over geological times in the presence of oxygen; (5) biochemical studies seem to indicate that "primitive" forms of metabolism are anaerobic (Haldane, 1929, 1965); and (6) the evolution of the contemporary oxygen atmosphere is correlated with photosynthesizing life forms, albeit less with direct photodissociation of H₂O vapor.

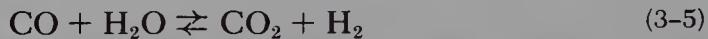
Although geological and paleontological evidence permit no unequivocal agreement about how much oxygen was in the atmosphere, on an average, more than 1.0 billion years ago, oxygen may have been present in certain places at these early times. Agreement exists, however, that the average oxygen level towards the end of the Precambrian (0.6–1.0 billion years ago) was at least a few percent of the present level, that is, high enough to sustain some primitive animal life (Fischer, 1965). Concepts about how and when the oxygen in the atmosphere began to increase significantly, however, remain controversial. Kuhn (1956), assuming that the early atmosphere consisted of hydrogen, methane, water vapor, and ammonia, calculated that photolysis of ammonia, methane, and water by solar radiation and continuous escape of hydrogen into space would have yielded our contemporary oxygen-nitrogen atmosphere after about 2 billion years. Urey (1959) has pointed out, however, that production of oxygen by photodissociation of H₂O (Nicolet and Bates, 1950, Nicolet and Mange, 1954) as summarized by Equation 3-4



would be limited at some self-regulating concentration by the shadowing effect of O₂ so produced. In fact, oxygen is distributed exponentially above the base of the stratosphere while water vapor is precipitated to very low concentrations at higher altitudes, leaving the major part of water in the shadow of oxygen. The presence of significant amounts of CO₂ could further add to the shadowing effect of oxygen and would lower the upper limit of oxygen concentration in the primitive atmosphere to less than 0.1 percent of the present level (Berkner and Marshall, 1965, 1966). The increase in the average proportion of oxygen to the present level is attributed to photosynthesis. Rabinowitch (1951) has estimated that, in the present atmosphere, all of the oxygen passes through the photosynthetic process in ~2000 years, all CO₂ in ~350 years, and that all of the water of the hydrosphere passes through it in ~2 × 10⁶ years. On the early Earth the development of the oxygen atmosphere was temporarily inhibited by the reaction of oxygen with reducing materials of the Earth's crust and the atmosphere, and by the dissolution of oxygen in

lakes and oceans. The shadowing effect of oxygen would have caused a decrease of water photolysis by ultraviolet light with increasing oxygen concentration. At the present, however, we do not have sufficient data to estimate the rates of oxygen production and oxygen consumption during the geological past.

Revelle (1965) regards both CO and CO₂ as possible constituents of a primitive atmosphere. The existence of CO in the presence of water at room temperature, however, is not favored thermodynamically because CO reacts with H₂O, according to Equations 3-5 and 3-6. At 25°C the reaction summarized by Equation 3-5 has a value of ΔG = +6.831 kcal. At such a temperature, the formation of CO is favored, but the time required to reach the equilibrium is about 10²⁰ years.



At 1200°K, however, ΔG = 0 and the reaction proceeds very rapidly. Contact with hot lava, therefore, would accelerate the reaction (3-5) from both sides. Abelson (1966) has argued that at the top of the atmosphere the equilibrium just mentioned would be governed by the thermodynamics valid for the higher temperature. Moreover, the reaction would proceed from left to right because hydrogen would more readily escape into space. On contact with the water of the primitive oceans, on the other hand, carbon monoxide would be removed according to Equation 3-6, the second principal reaction:



Subsequent dissociation of the formic acid produced would favor a practically complete conversion of carbon monoxide. An alkaline pH of 8-9 as suggested for the ocean, therefore, would drive the reaction from left to right (Abelson, 1966). The half-life of carbon monoxide with respect to Equation 3-6 at pH 8 at 25°C is 7 × 10⁴ years in the absence of catalysts. Although the order of magnitude of several times ten thousand years is relatively short on a geological time scale, the actual half-life might have been significantly shorter because of the probable catalytic activities of minerals dissolved or dispersed in primitive waters. The instability—that is, the chemical reactivity—of juvenile carbon monoxide can be used as an argument for its immediate participation in the production of prebiotic organic compounds.

Table 3-5 summarizes the ideas of a number of authors about the composition of the primitive atmosphere and hydrosphere prior to the evolution of life.

TABLE 3-5
The Primitive Atmosphere and the Primitive Hydrosphere

Composition	Author
Atmosphere CH ₄ , NH ₃ , H ₂ O, H ₂	Oparin (1924, 1953), Urey (1952)
Atmosphere CH ₄ , CO ₂ , NH ₃ , N ₂ , H ₂ O, H ₂ Hydrosphere CO ₂ , NH ₃ , H ₂ S, H ₂ O}	Bernal (1951)
Atmosphere CO ₂ , N ₂ , H ₂ S, H ₂ O Hydrosphere CO ₂ , NH ₃ , H ₂ S, H ₂ O	Rubey (1955, 1964)
Atmosphere CO, CO ₂ , N ₂ , H ₂ S, H ₂ O	Revelle (1965)

Source: Fox (1957).

Note: The possibilities of H₂CO, NH₃, H₂O from interstellar matter and conversion products therefrom should be recalled in relation to this table (page 327).

CONTEMPORARY PLANETARY ATMOSPHERES

A comparison of contemporary planetary atmospheres is of general interest. Our present knowledge of the composition of the atmospheres of Venus, Mars, Earth, and Jupiter is summarized in Table 3-6. The data on the composition of the atmosphere of Venus represent the first (and as yet only) results of measurements made *in situ* of a planet other than the Earth (Reese and Swan, 1968). The data on the composition of the atmospheres of Mars and Jupiter are largely based on spectroscopic measurements (Rasool and Jastrow, 1964). They must be understood as being probable values. The only gases detected spectroscopically in the atmosphere of Jupiter are NH₃, CH₄, and H₂. Helium is spectroscopically not observable from the Earth, but has been included owing to its cosmic abundance. The data on the atmospheres of Mars and Jupiter are set in parentheses because of their questionable accuracy. The extent to which such analyses lead to correct inferences about the Earth's early atmosphere is problematical.

Because of its great mass and the low intensity of incident solar radiation, Jupiter probably still retains much of its primary atmosphere. Mars, on the other hand, seems to have retained only rudiments of a secondary atmosphere, due to its relatively small mass and distance from the Sun. The planet Mercury, which is still smaller and is closest to the Sun, seems to be without an atmosphere at all. The explanation in each case is the effect of volatilization by solar radiation.

The question of why the atmosphere of Venus differs so significantly from the Earth's atmosphere is still under discussion. The

TABLE 3-6
*Composition of the Atmospheres of Venus, Earth, Mars, and Jupiter
at Their Surfaces (percentage by volume)*

Component	Venus	Earth	Mars	Jupiter
H ₂		0.00005		(60)
He		0.0005		(36)
CH ₄		0.002		(<1)
CO ₂	97	0.03	(90)	
NH ₃		none		(<1)
N ₂	<3	78.09	?	
O ₂	<0.4	20.95	<10 ⁻⁴	none
H ₂ O	0.1–0.2	—	very small	
A		0.93	?	
Ne		0.002		(3)
Pressure (mb) at surface	90,000	1,000	(20)	(>10 ⁶)
Mean temperature (°K) at surface	750	300	~230	(1000)

Source: Data on Venus from Reese and Swan (1968); data on Mars from Otrouchenko and Mukhin (1971); data on Jupiter from Rasool and Jastrow (1964).

hypohydrous character of Venus has been related to the possible formation of thick polar ice caps (Libby, 1968). The proportion of oxygen in the atmosphere of Venus is lower than 0.4 percent. The partial pressure of oxygen on Venus is nevertheless significant (80–160 mb) because of the high total atmospheric pressure of about 20 kg cm⁻². The partial pressure of oxygen on Venus, therefore, comes close to that in the Earth's atmosphere (209.5 mb). The origin of the atmospheric oxygen of Venus is unknown.

ENERGIES AVAILABLE ON THE PRIMITIVE EARTH

Those constituents of the primitive lithosphere, hydrosphere, and atmosphere that contained such elements as carbon, hydrogen, oxygen, nitrogen, sulfur, and phosphorus were the raw materials for the evolution of organic compounds. In order to yield molecules of biological significance, the simple starting materials required energy, in the form of free energy or activation energy. In this section we discuss questions of the quality and quantity of energies that were available for the first stages, and for the later stages, of molecular evolution on the Earth.

Although not widely recognized, the first experiments expressly designed to explain the origin of organic compounds such as are found in living things seem to have been those of Groth and Suess (1938; see page 67 of this book). These studies tested the action of ultraviolet light on two possible constituents, CO_2 and H_2O , of a primitive atmosphere. Calvin and his associates revived interest in this kind of experimentation with similar studies published in 1951 (Garrison et al., 1951). Additionally, wide attention to experimental protobiogenesis was stimulated by the experiments of Miller (1953, 1955) with electric discharges in a "primitive" atmosphere. Since then, a large number of biologically interesting compounds have been synthesized in related experiments. A common feature of this type of experiment is the production of organic compounds by the action of various forms of energy upon simple starting materials under conditions that presumably obtained on the primitive Earth.

The theories or hypotheses on which most scientists based the early experiments, however, had at least two defects. The first is use of the questionable concept, already mentioned, of a methane-ammonia-hydrogen-water atmosphere, which is without firm geologic and geophysical foundation (page 38; see also Abelson, 1966). The second defect is use of the concept that the absolute amount of energy available on the Earth, rather than its quality, is what was important for molecular evolution (page 142). No regard is given, in this approach, to the destructive quality of type of energy. Many authors have neglected the importance of local distributions of energy and have not taken into account a relevant body of information from geology and physical chemistry.

Solar Radiation

Solar radiation incident on the atmosphere and lithosphere is the most abundant type of free energy on the Earth (Nawrocki and Papa, 1961). At present, a total flux of optical solar radiation of 1,060,000 cal $\text{cm}^{-2} \text{y}^{-1}$ hits the upper boundary of the Earth's atmosphere. If this flux is projected per square-centimeter surface area of the Earth, the number obtained is 265,000 cal $\text{cm}^{-2} \text{y}^{-1}$. The spectral distribution of optical solar radiation incident on the Earth's atmosphere is summarized in Table 3-7.

Radiation of wavelengths down to about 1400 Å originates from the solar photosphere. This photospheric radiation of the contemporary Sun is very stable and probably self-regulating. As can be seen from Table 3-7, wavelengths greater than 1500 Å account for more

TABLE 3-7
Spectral Distribution of Optical Solar Radiation Incident on the Earth's Atmosphere

Wavelength Region (\AA)	Percent of Incident Energy	Cal $\text{cm}^{-2} \text{y}^{-1}$ ^a
Total	100.0	about 1,060,000
Below 1500	0.001	10
1500-2000	0.03	300
2000-2500	0.2	2,000
2500-3000	1.0	10,000
3000-3500	3.1	33,000
3500-4000	5.4	57,000
4000-7000	37.0	390,000
7000-10,000	24.5	270,000
Above 10,000	29.0	300,000

Source: Calculated from data presented in Smithsonian Physical Tables, 1959, Table 808, and after Nawrocki and Papa (1961).

^aFluxes as occurring in space at position of the Earth.

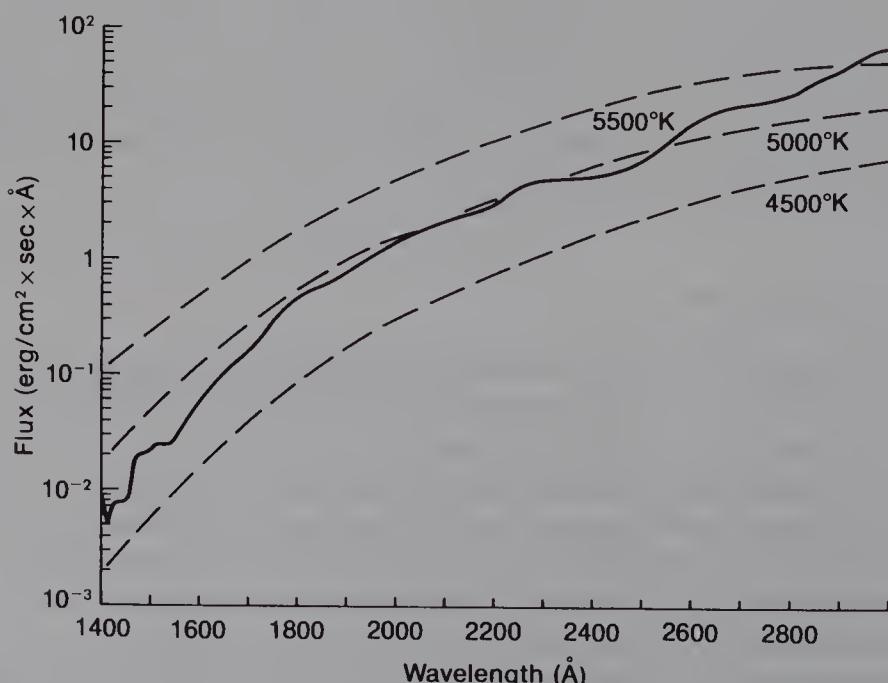


FIGURE 3-1
 Solar intensity 1400-3000 \AA . Source: Nawrocki and Papa (1961), cited by Berkner and Marshall (1964).

than 99.99 percent of the total radiation. The present flux of solar radiation is consistent with a black-body radiation of the solar photosphere of about 5000°K as shown in Figure 3-1. However, we cannot assume that the photospheric radiation of the Sun has remained virtually unchanged during the past 4.5 billion years. In fact, the change in solar radiation fluxes is closely related to solar evolution and can be described best with the help of Figure 3-2. The solar evolu-

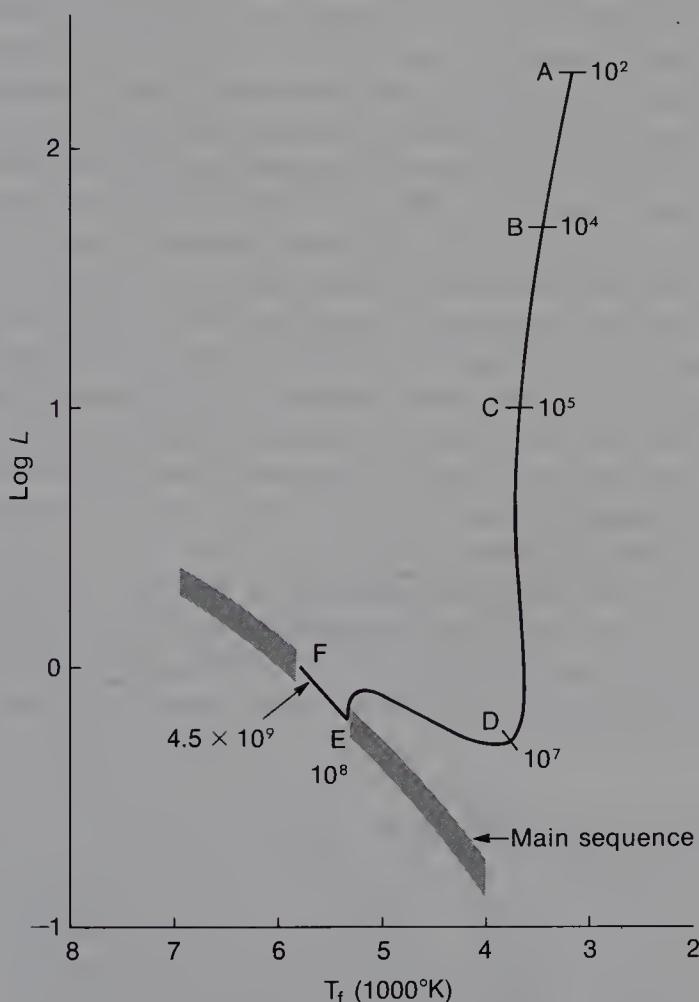


FIGURE 3-2

Hertzsprung-Russell diagram showing the position of the main sequence (hatched) and the path the Sun has passed through in the course of its evolution (thick line). Several characteristic points in the evolution are marked A,B,C,D,E,F; they are labeled with the time in years needed to reach these points from the contraction of a nonrotating, nonmagnetic proto-Sun out of infinity. Source: de Jager (1967).

tion is shown by the thick line in the Hertzsprung-Russell diagram. Some characteristic phases in the Sun's evolution are marked by letters a to f; they are labeled with the time in years required to reach these phases from the contraction of a nonrotating, nonmagnetic proto-Sun out of infinity. The early proto-Sun was initially fully convective. During the subsequent contraction, the radius and the luminosity decreased rapidly as the temperature probably increased from 3000°K to about 3700°K. The smallest luminosity, about 0.5, was reached after 10^7 years. At this time, the thermal convection stopped temporarily and the internal energy transport took place by radiation, the Sun being then in complete radiative equilibrium. After passing phase d, the temperature increased to well above 4000°K. Then, also, the luminosity showed a slight increase from 0.5 to 0.65 in phase e and the radius reached almost its present value. About 5×10^7 years before entering the main sequence, at e, nuclear reactions presumably started in the interior of the Sun. When the main sequence was reached, almost the total energy production in the Sun's interior took place by nuclear reactions. After this time solar evolution proceeded very slowly. About 4.5×10^9 years later, the present phase f was reached. Energy transport within the Sun's envelope has since been achieved mostly by convection. Some characteristic data with respect to solar evolution and heat production are summarized in Table 3-8.

This analysis of the thermal history of the Sun indicates that the intensity of solar radiation in the near-ultraviolet part of the spectrum most probably has never been higher than its present value, although the total solar radiation flux was at much higher values until the low

TABLE 3-8
Main Phases of Solar Evolution

Phase	Time (yr)	T_f (°K)	L/L_\odot	R/R_\odot	Energy Transport	T_{Earth} (°K)
a	10^2	3000	200	70	C	1250
b	10^4	3500	50	17	C	720
c	10^5	3700	10	10	C	590
d	10^7	3750	0.5	2	R	265
e	10^8	5300	0.65	0.97	R	258
f	4.5×10^9	5800	1	1	C	290

Source: Jager (1967).

Note: Phase and time, see Figure 3-2. T_f is surface temperature, L/L_\odot and R/R_\odot are relative luminosities and radii, C or R indicate energy transport by convection or radiation to the Sun's surface; T_{Earth} is assumed black-body temperature of the Earth.

at phase d was reached. As will be argued below, however, long-wavelength solar radiation was of minor importance, at least during the first phase of molecular evolution. Most probably, the ultraviolet solar radiation fluxes during the last 4.4 billion years (from phase e) attained the present values from 5 percent of the present value at $\lambda = 1500 \text{ \AA}$ and from 40 percent of the present value at $\lambda = 2500 \text{ \AA}$, according to Jager (1967).

No conclusive data are available for the history of solar radiation fluxes of wavelengths shorter than 1400 \AA . Although amounting to less than 0.001 percent of the total solar radiation energy, quanta of these wavelengths would be readily absorbed by and would be highly effective on all constituents of any planetary atmosphere. Quanta of these extremely short wavelengths are mostly emitted by the Sun's corona and chromosphere, the latter being a thin transition region between photosphere and corona. The coronal and chromospheric radiations are highly variable owing to the convective energy transport in this region. If the Sun had no convective region it would have no corona and chromosphere; the total solar spectrum could be described much better by Planck's law of the radiation of black bodies. The corona, actually a tenuous gas, has a temperature of $1.5 \times 10^6 \text{ }^\circ\text{K}$. It emits not only short-wavelength ultraviolet light, but X-rays as well. In particular, intense bursts of hard X-rays may be emitted during solar flares. As a consequence of the impact of coronal and chromospheric ultraviolet and X-radiation on the Earth's atmosphere, the Earth's ionosphere and exosphere were formed. Each consists of gases that have high kinetic temperatures. As a result of the impact of solar radiation, the components of the upper atmosphere are partly excited and ionized. The escape of light atmospheric gases such as H_2 and He is strongly increased by this effect.

In order to estimate the quality and quantity of solar ultraviolet light incident on the surface of the Earth, the absorption spectra of the atmospheric gases must be evaluated. The ultraviolet absorption spectra of H_2O , CO_2 , O_2 , and O_3 are shown in Figure 3-3. Except for ozone, these atmospheric components show no appreciable absorption above 2000 \AA . Substantial absorption, however, occurs only at wavelengths shorter than 1800 \AA . Similarly, NH_3 begins to absorb substantially below 2200 \AA , whereas CH_4 absorbs below 1500 \AA .

The absorption spectrum of ozone for longer wavelengths is shown in Figure 3-4. As described in the figure, small amounts of ozone may have been formed according to the reaction after the photolysis of



water (Equation 3-4) had raised the oxygen pressure of the primitive

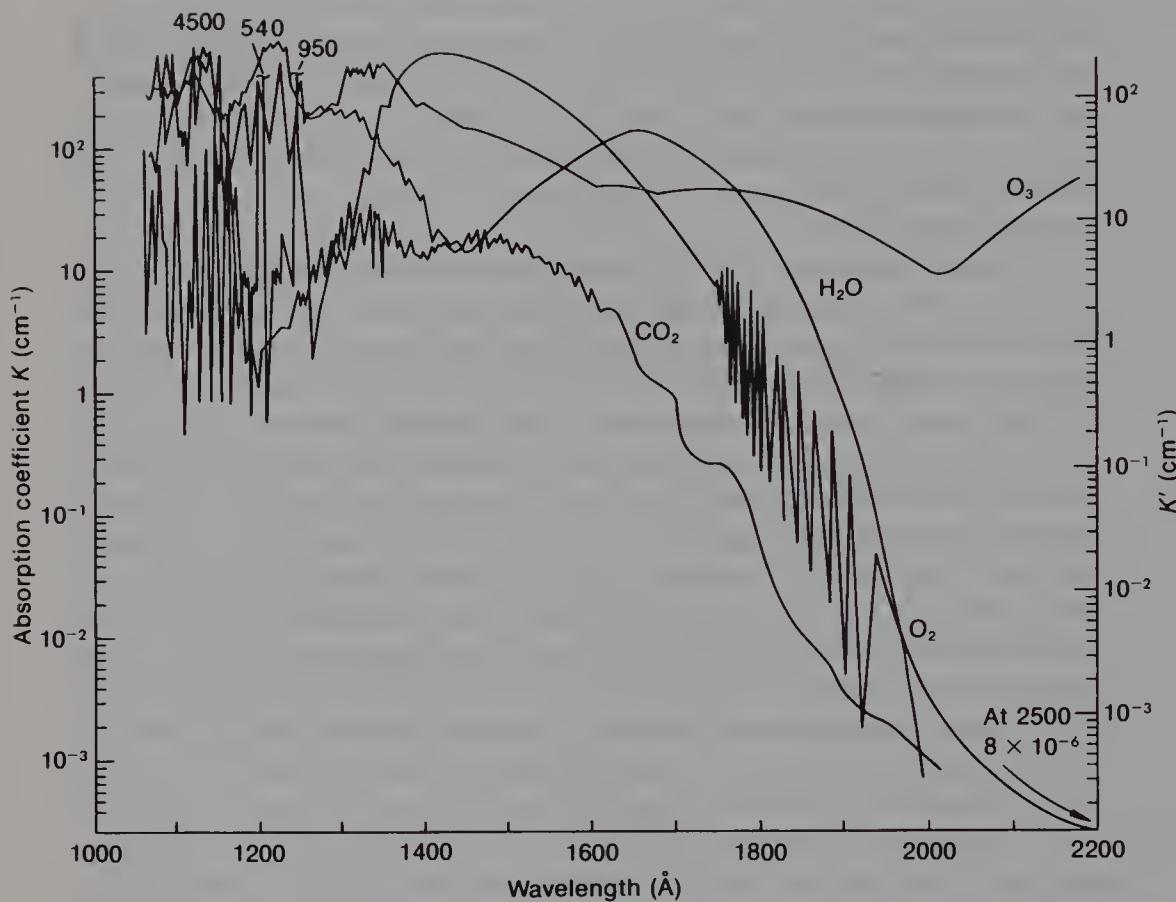


FIGURE 3-3
Composite of ultraviolet absorption in atmospheric gases. Source: Berkner and Marshall (1964, 1965).

atmosphere to a value somewhat less than 0.1 percent of its present value. Oxygen atoms are formed by photolysis of water or molecular oxygen (Nicolet and Bates, 1950; Nicolet and Mange, 1954). The ozone level of the primitive atmosphere probably was of inferior significance as long as the oxygen level remained low also.

Accordingly, the primitive ozone shield had little or no appreciable effect. Solar ultraviolet light above about 2000 Å irradiated the surface of the early Earth with the full intensity indicated in Table 3-10. Below approximately 2000 Å, the intensity of solar ultraviolet light on the surface of the early Earth decreased sharply, due to the absorption by water vapor, carbon dioxide, and the other atmospheric components. On the contemporary Earth, atmospheric ozone absorbs almost all solar ultraviolet light in the range of 2000 Å to 3000 Å. Be-

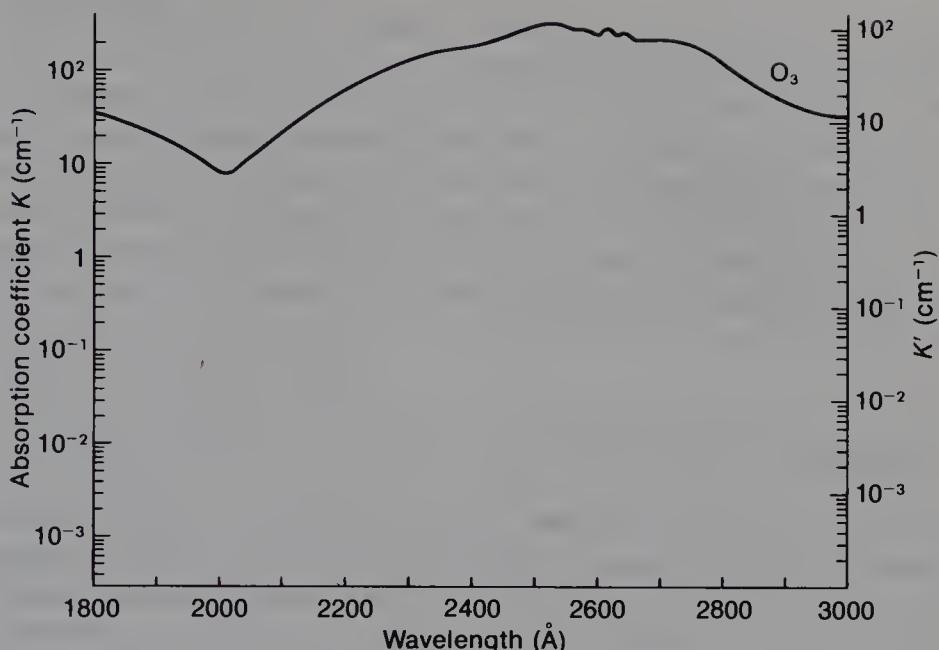


FIGURE 3-4
Ultraviolet absorption in ozone. (Data from Watanabe, 1959; and from Vigroux, 1953).

below 2000 Å, ozone absorption strongly supplements that of the other atmospheric components. Thus, no appreciable solar ultraviolet light of wavelength less than 3200 Å penetrates to the surface of the Earth.

As can be seen from Table 3-7, solar radiation below 2000 Å that reaches the upper atmosphere at the present time amounts to about 0.03 percent of the total. When we take into account the fact that the flux of ultraviolet light of this quality 4 billion years ago was roughly 40 percent of the present value, a value of about $120 \text{ cal cm}^{-2} \text{ y}^{-1}$ is obtained.*

Simple organic molecules as well as all possible constituents of suggested primitive atmospheres (page 38) do not absorb visible light. Although amounting to over 90 percent of the optical solar radiation incident on the primitive Earth, visible light, therefore, had little if any direct effect on the photochemistry of the early atmosphere. Visible light might, however, have been quite effective during a later phase of molecular evolution in which organic compounds appeared in aqueous systems on the primitive Earth. Such aqueous systems may have contained substantial proportions of minerals that absorbed visible light. These materials, dissolved or colloidally

* $30 \text{ cal cm}^{-2} \text{ y}^{-1}$ if projected upon the Earth's total surface.

dispersed, very likely contributed to further conversion of organic compounds by acting as sensitizers.

Substantial amounts of solar light incident on the atmosphere are not absorbed on the surface of the Earth because of light scattering and reflection. Appropriate values which account for these processes on the primitive Earth are not available. Moreover, lack of this information rules out estimation of the contribution to molecular evolution of the various wavelengths of solar radiation for comparison with those of other forms of energy.

High-energy Radiations

Unlike ultraviolet irradiation, most of the high-energy radiation originates from the interior of the Earth. The major sources of high-energy radiations on the Earth 4–4.5 billion years ago were probably the unstable isotopes ^{238}U , ^{235}U , ^{232}Th , and ^{40}K . On decay, the first three isotopes, which are heavier, give off α -particles as well as γ -rays and β -particles; ^{40}K gives off γ -rays and α -particles only. One gram of ^{238}U in equilibrium with its daughter products continuously liberates 0.71 calories per year. The corresponding numbers for ^{235}U and ^{232}Th are 4.3 and 0.20 cal $\text{g}^{-1} \text{y}^{-1}$. All the ^{40}K in one gram of natural potassium gives off ionizing radiation to yield 2.7×10^{-5} cal y^{-1} . In Table 3-9 are summarized the average proportions of uranium, thorium, and potassium that are found in granitic and volcanic rocks.

The typical granite produces more than 5 $\mu\text{cal g}^{-1} \text{y}^{-1}$ of high-energy radiation. Other rocks that make up the bulk of the crust above the Mohorovičić discontinuity may contain smaller amounts of radioactive elements. A probable average value for heat production in the upper lithosphere is about 2 $\mu\text{cal g}^{-1} \text{y}^{-1}$ (Birch, 1954; cf. Hurley, 1959; Mason, 1952). From these figures, the energy equivalent of radiation now being produced in the continental crust down to a depth of 35 km is calculated to be about 0.5 $\mu\text{cal cm}^{-2} \text{ sec}^{-1}$, or 15.5 cal $\text{cm}^{-2} \text{ y}^{-1}$.

TABLE 3-9
Proportions of Uranium, Thorium and Potassium in Typical Granitic and Volcanic Rocks

Rock Type	U (ppm)	Th (ppm)	K (%)
Granitic rocks	4	14	3.5
Volcanic rocks (basaltic)	0.6	2	1.0

Source: Birch (1954).

The half-life of ^{40}K is about 1.4×10^9 years. The ^{40}K content of the Earth's crust 4 billion years ago was probably, therefore, about four times what it is now. As the half-life of ^{238}U is about 4.5×10^9 years, there was probably about twice as much ^{238}U in the crust of the primitive Earth as there is now. Uranium-235 has a half-life of 7.1×10^8 years. Theoretically, its abundance in the crust 4.25 billion years ago would have been 64 times what it is now. Thorium-232 has a half-life of 1.4×10^{10} years. The abundance of this isotope in the Earth's crust 4 billion years ago, therefore, would have been only slightly more than what it is today.

The present ratio $^{238}\text{U}/^{235}\text{U}$ is about 150. Four billion years ago the same ratio was about 5. We can calculate that the radiation produced by the radioactive elements in granitic rock 4 billion years ago was about $12 \mu\text{cal g}^{-1} \text{y}^{-1}$ or about three times the present value. Correspondingly, the average value for the upper lithosphere is estimated to have been about $6 \mu\text{cal g}^{-1} \text{y}^{-1}$.

These estimates are valid only if the geological structure of the Earth and the elemental composition of its crust have remained unchanged during the last 4–4.5 billion years and if, in particular, no elements have been transported through the Mohorovičić discontinuity. Measurements of the abundance of ^{40}K , ^{235}U , and ^{238}U and their stable decay products in various geological materials, however, allow the conclusion that widespread changes in the elemental composition of the upper crust *did not* occur during the last 4 billion years.

High-energy radiation from the Sun and outer space incident on the Earth's atmosphere and lithosphere probably added little to the amount of high-energy radiation available from the crust. The present cosmic radiation near sea level at higher latitudes corresponds to an average skeletal dose of 30 mRad per year or as little as 7×10^{-2} micro-calories per gram per year. If organic material were exposed to the same radiation, it would receive an even lower dose, consistent with the lower rate of absorption by lighter elements. The level of cosmic radiation 4 billion years ago is unknown, however.

If the radiation energy produced, on the average, per unit time in the continental crust down to 35 km is projected per square centimeter of surface, a value of $15.5 \text{ cal cm}^{-2} \text{ y}^{-1}$ is obtained for the contemporary Earth. The extrapolated value for the Earth 4 billion years ago is $47 \text{ cal cm}^{-2} \text{ y}^{-1}$. This value may be compared with the average flux of ultraviolet light of wavelengths shorter than 2000 Å on the Earth 4 billion years ago, which probably was about $120 \text{ cal cm}^{-2} \text{ y}^{-1}$ at the upper atmosphere or, if projected on the total surface of the Earth, $30 \text{ cal cm}^{-2} \text{ y}^{-1}$. Little doubt exists that the major part of both forms of radiant energy dissipated as heat without directly contributing to molecular evolution.

In certain regions, particularly in regions abundant in solid-liquid and solid-gas interfaces, conditions for formation and conversion of organic molecules by high-energy radiations should have been favorable. Heat and high-energy radiations share one advantage — the capability of initiating molecular evolution anywhere on or in the Earth's crust starting with any carbon compound regardless of its optical absorption properties and its state of aggregation. Usually less densely ionizing radiations such as γ -rays, X-rays, and β -rays convert organic compounds with higher yields (Swallow, 1960). The β - and γ -rays produced by ^{40}K , therefore, may have been more effective than the α -particles given off by the uranium and thorium isotopes. Horowitz and Miller (1962) have claimed that high-energy radiation and volcanic heat were available on the primitive Earth only in small amounts. They have inferred that it is very unlikely that these forms of energy could be important in the synthesis of organic compounds on the primitive Earth. These speculations disregard a large body of geo-physical information and chemical experience, as will be shown in the next section and in Chapter 4.

Heat from the Earth's Interior

In contrast to the invariably violent forms of energy, i.e., ultraviolet light, high-energy radiation, and electric discharges, heat may produce gentle effects, as well as the most violent. Heat, or more specifically infrared radiation, is the only form of energy that occurs in such small quanta as to bring about specific interaction of molecules without undue destruction. In the elementary process of heat absorption and conversion, practically no lower limit exists for the size of quanta. Heat-induced reactions may be controlled in such a way that highly reactive species such as radicals, radical ions, or electronically excited molecules *are not* produced. On the other hand, heat may be applied in such high quantities per molecule that highly reactive intermediates result.

If a simulated primitive atmosphere is exposed to temperatures of about 500°C and higher, the effects are comparable to those produced by the other three violent forms of energy, as will be shown in Chapter 4. However, if organic compounds are exposed to lower temperatures, the chemistry of heterolytic reactions will generally prevail; this entails a considerably more specific kind of process for conversion of organic molecules. The mere availability of abundant amounts of energy is of little significance because in any synthetic experiment any excessive application of any form of energy would

destroy the product that is produced by an appropriate dose of energy. In all evolutionary experiments, in general, removal of products from the zone of reaction is of great importance. This applies with the same strength to the use of heat. The occurrence of time- or space-dependent temperature jumps, the availability of quenching zones of relatively low temperatures, of low radiation fluxes, etc., therefore, are as crucial as the availability of a form of energy. A continuous, large zone of heat is not needed, but the existence of an abundant number of zones with temperature gradients can be crucial. Temperature gradients in the range 0–300°C would be appropriate for synthesizing the more sensitive biochemical substances, including macromolecules, out of the raw materials. The raw materials such as amino acids were likely produced at least in part, however, from the constituents of the primitive atmosphere by violent forms of energy, including heat above 500°C.

On the primitive Earth, local regions of elevated temperatures, mostly of volcanic origin, probably had the same function as the Bunsen burner or other heat sources in the laboratory of the synthetic organic chemist. No geological evidence permits the estimation of volcanic activity on the primitive Earth, although many geologists assume that 4 billion years ago there was considerably more volcanic activity than at the present time.

According to estimates of Sapper (1927), between 1500 and 1914 active volcanoes produced about 64 km³ of lava and 329 km³ of fragmentary materials. This corresponds to an average value of about one km³ of lava and fragmentary materials per year. Assuming a Δt of 1000°C, a Cp of 0.25 cal g⁻¹, and a density of 3.0 g cm⁻³, the heat content of one km³ volcanic material at 1000°C is 0.75×10^{18} cal, or if projected upon the whole surface of the Earth, the heat directly released from emitted volcanic material is about 0.15 cal cm⁻² y⁻¹. Not included in this number is the amount of heat released at steam spouts, fumaroles, and similar locations. That is, the figures represent only minimum amounts. This point has been ignored by a number of authors (Horowitz and Miller, 1962; Miller and Urey, 1959).

Although the volcanic activity on the primitive Earth presumably was much greater, the volcanic activity on the contemporary Earth is not small by geological standards. With a production rate of 1 km³ of volcanic materials per year, 4.5×10^9 km³ would have been produced in 4 billion years. Provided that no losses occurred, due, for example, to erosion, this amount of volcanic material would have been sufficient to cover the total surface of the Earth to a depth of more than 8000 meters. In fact, a careful statistical analysis performed by F. W. Clarke (quoted by Rittmann, 1962) led to the conclusion that

>95 percent of the accessible part of the Earth's crust consists of crystalline rocks, the formation of which may be related to volcanic activity. However, estimates of volcanic activity in earlier times, based on geological evidence, are extremely hard to obtain because erosion has since reduced the igneous rocks, but it is not known by how much.

The amount of heat released by volcanic emissions that we have been discussing does not include, of course, the heat available on hot spots where volcanoes are not active—e.g., large areas in Yellowstone National Park, the fumaroles at Larderello, or the solfatara near Naples. Temperatures of 90–300°C at or near the surface are quite common at such steam spouts. These are temperatures that have been shown to be highly favorable for many organic condensation reactions, such as the formation of protein-like anhydropolyamino acids (page 142). Also not included in the amount of $0.15 \text{ cal cm}^{-2} \text{ y}^{-1}$ is the heat available below the surface. Organic materials of igneous origin have been found in hydrothermal veins (Sylvester-Bradley and King, 1963; Mueller, 1963). These materials actually represent mostly conversion products of biogenic compounds, but it is likely that in prebiological times organic materials, too, were reworked in the crust by volcanic heat at different pressures and pH values before the final products were deposited in hydrothermal veins. Under these conditions, temperature jumps of 100°C and more are less likely to occur. The intermediates, therefore, could have reacted until an equilibrium state was reached (Eck et al., 1966).

Sources of heat suitable for advancing molecular evolution must have been abundant on the primitive Earth. As stated earlier, the amount of heat now available from volcanic emissions represents only a minimal value. In any event, the amount of heat available from volcanoes has been and is a minute fraction of the total amount of heat available in prebiotic or biotic times.

More important than the amount is the quality of the energy, which for heat is easily controlled by the organic chemist in the laboratory (Gilman, 1924). The advantages of heat, such as effectiveness in small doses and widespread occurrence, apply also to the geological realm.

Electric Discharges

Electric discharges are actually not a separate form of energy. Electric discharges are composed of an electron beam, high local heat, and a whole spectrum of optical radiations. The electrons in electric flashes, although moving with relatively slow speeds, as com-

pared, for example, to the β -rays of ^{40}K , still have sufficient kinetic energies to ionize and excite electronically like the high-energy radiations. A large portion of the kinetic energy of the electrons and of the ions being produced in their tracks is converted into heat by collisions of the second kind. In this way, locally very high temperatures are produced to yield radicals and electronically excited states of the atmospheric constituents. Accordingly, electric discharges are a violent form of energy (Chapter 4). Electric discharges probably contributed significantly to the evolution of micromolecules. Although polymeric materials as well have been produced in electric-discharge experiments, these polymers are ill-defined. Some of them, for example, yield amino acids upon hydrolysis, but it is likely that they contain many cross-links that are not found in such abundance in biological polymers. Owing to the violent conditions under which these polymers are produced, their micromolecular entities may be linked together by any kind of chemical bond that is thermodynamically stable.

According to estimate, about 20×10^{18} cal y^{-1} are released in electric discharges on the contemporary Earth. If projected to each square centimeter of surface, the corresponding number is $4 \text{ cal cm}^{-2} \text{ y}^{-1}$. This value includes $0.9 \text{ cal cm}^{-2} \text{ y}^{-1}$ due to lightning and about $3 \text{ cal cm}^{-2} \text{ y}^{-1}$ due to corona discharges from pointed objects (Miller and Urey, 1963). Again, no rigorous statements can be made about the quantity of electric phenomena that have occurred on the primitive Earth, although a number larger than the present-day estimate appears likely.

Summary

In Table 3-10 are summarized the amounts of energies in the form of optical radiation, high-energy radiation, heat, and electric discharges, available on the contemporary and the (hypothetical) primitive Earth. The values given for heat by volcanic emissions do not include the heat available slightly below the surface (in hydrothermal veins), in zones of steam spouts, or in similar areas, all of which together would probably yield multiples of the values given. In earlier times, the amount of volcanic* emissions was probably substantially larger, but exact data are unavailable.

* Volcanic emission signifies much more than just heat, another example being plateau basalts (F. M. Bullard, 1962, p. 55). Plateau basalts are the results of processes of earlier times.

TABLE 3-10
Energies Available from Various Sources (see text for details)

Type of Energy	Contemporary Earth		Earth, 4×10^9 to 4.5×10^9 Years Ago (hypothetical values)		
	Total (cal y^{-1})	Projected per cm^2 surface (cal $cm^{-2} y^{-1}$)	Total (cal y^{-1})	Projected per cm^2 surface (cal $cm^{-2} y^{-1}$)	
Total optical solar radiation	$1,340,000 \times 10^{18}$	265,000	$850,000 \times 10^{18}$	170,000	
Solar radiation below 2000 Å	380×10^{18}	75	150×10^{18}	30	
Solar radiation between 2000–3000 Å	$15,200 \times 10^{18}$	3,000	$6,000 \times 10^{18}$	1,200	
High-energy radiation (from the crust 35 km deep)	79×10^{18}	15.5	240×10^{18}	47	
Heat from volcanic emissions (rocks and lava only)	0.75×10^{18}	0.15	$>0.75 \times 10^{18}$	>0.15	
Electric discharges	20×10^{18}	4	20×10^{18}	4	

A way in which both radioactivity and volcanic heat are distinguished from the other kinds of energy listed is that these are forms of energy emanating from the crust of the Earth. This significance may be critical because the reactions of simple solid compounds to form more complex compounds—e.g., the condensation of mononucleotides to polynucleotides—would likely not occur in the gaseous phase. Such reactions would most probably occur in the lithosphere or solid part of the Earth, or perhaps under some special conditions in the hydrosphere. In this respect, an overemphasis upon the processes in the gaseous phase, that is, on atmospheric processes, is misleading.

The mere abundance of any form of energy is of no significance if it has failed to give rise to the kind of subtly structured substances, such as proteinlike materials or polynucleotides, that are required at a salient stage of evolution. In this respect, particularly, the intensity rather than the quantity of energy available is of crucial significance. (Also, huge amounts of energy were, theoretically, not needed, inasmuch as the minimal initial requirement was for a very small number of replicating organisms that could metabolically convert organic material.)

In addition, some other forms of energy may have contributed to molecular evolution. Microwaves, supersonic shock waves (Hoch-

stim, 1963), for instance, have been used to form organic molecules. However, too few data are at hand to permit a discussion of the significance of these experiments with respect to molecular evolution.

One of the emphases that might finally be made about the primitive geochemical matrix is that it was not general nor, in many times and places, average. The change of state is normal to geochemical events; such changes (temperatures, phases, rain, light, and darkness, e.g.) would protect products that would otherwise suffer rapid decomposition. Anyone who performs research in the laboratory, or reviews that performed by others, and then carries out field trips in relation thereto, must conclude that the Earth provided an extremely varied set of reaction locales during its long history.

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CHAPTER 4

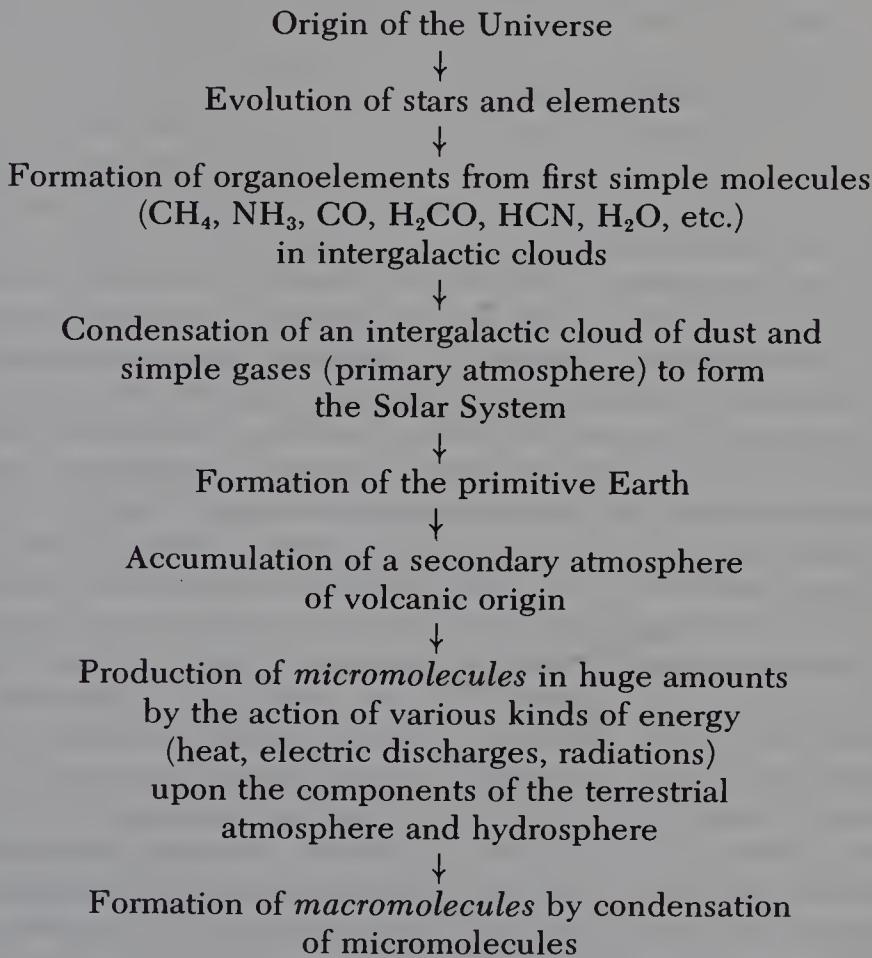
Micromolecules

In the first part of the preceding chapter the composition of the raw material for chemical evolution, that is, mainly the composition of primordial or primitive atmospheres, has been discussed. In the last part of Chapter 3 have been evaluated the various forms of energy that can initiate a large variety of chemical reactions leading to biologically significant molecules from the various atmospheric constituents (see the flowsheet).

We distinguish for practical reasons between *micromolecules* and *macromolecules*. Micromolecules in the context of this volume are small organic molecules such as amino acids, fatty acids, monosaccharides, and other metabolites or building stones of organisms. Their molecular weight is usually well below 1000 daltons. Macromolecules, e.g., protein(oid)s, nucleic acids, or polysaccharides, have a molecular weight well above 1000 daltons. They are composed of micromolecules (monomers or building stones). In some cases a clear distinction between micromolecules and macromolecules is difficult; if a molecule is composed of only a few (less than 10) monomers or building stones, it is called an oligomer (e.g., oligonucleotide or

oligopeptide). The discussion of the formation of oligomers will be taken up in the next chapter.

The ideal concept of the several evolutionary sequences on the atomic and molecular level as treated in Chapters 2-5 is summarized by the following flowsheet:



The earliest published experiment expressly designated as demonstrating the formation of organic compounds from the hypothetical constituents of an early atmosphere, suitable for the development of organic life was, to the best of our knowledge, that by Groth and Suess (1938). These authors clearly indicated that their objective was to produce carbon compounds that were prerequisite for the evolution of organic life. They irradiated a CO₂-H₂O atmosphere with ultraviolet light (the two resonance lines of xenon at 1470 Å) and identified formaldehyde and glyoxal as major products. They interpreted their experiments as "giving an explanation for the formation of certain carbon compounds that were probably the prerequisite for the evolution of organic life."

Later, Calvin and associates (Garrison et al., 1951) tested the earlier theories (Oparin, 1924, 1953; Haldane, 1929) for the prebiotic origin of organic compounds on the primitive Earth. Using the Berkeley cyclotron, they irradiated with α -particles aqueous solutions containing ferrous ion in equilibrium with an atmosphere of carbon dioxide and hydrogen. Formaldehyde, formic acid, and succinic acid were formed.

Miller (1953), while a graduate student with Urey at the University of Chicago, carried out an experiment that was the first to direct major public attention to micromolecules. Miller exposed gaseous mixtures of methane, ammonia, water, and hydrogen to electric discharges and produced many organic compounds, including amino acids. These experiments differed from those of Groth and of Calvin in that organic nitrogen was included among the ingredients. A major significance of Miller's experiments was the fact that they represented a first step toward the laboratory production of proteinlike molecules.

Experiments designed to produce nitrogen-containing organic molecules by exposing atmospheres of various composition to electric discharges date back to the nineteenth century. Berthelot (1898), for example, described experiments in which simple alcohols and ethers were induced to react with nitrogen by application of electric discharges. Loeb (1913) was the first to report formation of glycine by exposing to silent discharges various atmospheres including a mildly reducing atmosphere—e.g., one consisting of water vapor, ammonia, and carbon monoxide or carbon dioxide. The relevance to molecular evolution is not explicitly stated in his paper.

Since Woehler (1828) first described the abiological synthesis of urea, organic chemists have performed countless experiments that can be interpreted in the context of chemical evolution. Chemical evolution is, of course, part of organic chemistry. The evolution-minded scientist, however, focuses his attention on organic compounds that can be obtained under conditions likely to have prevailed on the primitive Earth, whereas the synthetic organic chemist works with a larger range of conditions.

The greatest amount of work in the simulation of prebiotic production of micromolecules has been concerned with amino acids, in part due to the fact that this class of organic compounds is readily formed and easily detected and analyzed. Amino acids are the monomers of the biologically important class of compounds, proteins. Also, as has been indicated, amino acids have been synthesized under various simulated "primitive-Earth conditions." The formation of amino acids has been studied in detail and, in many cases, quantitatively.

With respect to the production of this class of organic compounds, therefore, a body of information is available to permit a comparative discussion of the various results obtained under different conditions.

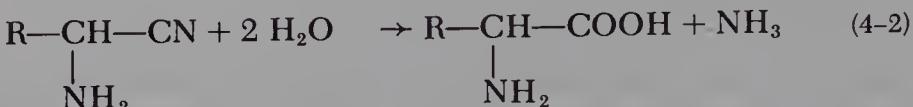
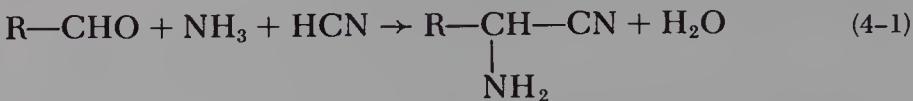
PRODUCTION OF AMINO ACIDS AND RELATED COMPOUNDS BY REACTIONS IN THE GASEOUS PHASE

Electric Discharges

Two types of electric discharge have been used to bring about reactions producing amino acids: silent discharge and spark discharge. *Silent discharges*, or brush discharges, are typically produced if a pointed anode and a cathode of appreciable size are used. In this case, electrons are only generated by collisions of positive ions with the molecules of the gas, but not with the cathode. At relatively low voltage only the positive ions which are approaching the cathode carry enough kinetic energy electronically to excite other molecules when colliding with them. Thus the gases are luminous at the cathode. These discharges are also spoken of as "corona" discharges. With increased potential gradient the "silent" discharge becomes brighter and more intense and occasionally threadlike sparks will strike across the electrodes; it becomes a spark discharge. *Spark discharges* are violent electric discharges between two electrodes. The violence and disruptiveness is ascribed to cumulative ionization by collisions between ions (generated in the gas by a strong electrostatic field). The temperatures within the spark are so high that additional ionizations are produced by thermal collisions. The shape and condition of the electrodes largely determine the actual voltage required to produce the discharge. The potentials close to the electrodes have the order of several thousand volts per cm.

By exposing gaseous mixtures of carbon dioxide, ammonia, and water vapor to silent discharges, Loeb (1913) obtained rigorous evidence that glycine was among the organic compounds produced. He also recognized that formaldehyde and other aldehydes are formed by reduction of carbon dioxide. He was probably mistaken, however, when he assumed that glycine was formed via a reductive amination of CO_2 to yield primarily formamide that would dimerize to oxamide and then, after subsequent reduction and hydrolysis, yield glycine. This pathway of glycine formation in an electric-discharge experiment cannot be ruled out. But the finding of cyanide as another

principal reaction product of the action of electric discharges on various reducing atmospheres containing CH₄ and N₂ (Glockler and Lind, 1939), or CO, N₂, and H₂ (Peters and Küster, 1929), or CO₂, CO, H₂, and NH₃ led Miller (1955) to the suggestion that most of the glycine and the other amino acids may be formed by a Strecker cyanohydrin synthesis:



Alternative mechanisms have been proposed (Reactions 4-12 through 4-16 and 4-17 through 4-19). Loeb (1913), however, was evidently not aware that cyanide was among his reaction products, nor did he discuss his experiments as being relevant to molecular evolution. He recognized that he had demonstrated an abiotic synthesis of an amino acid from the starting materials of the "natural" synthesis (in plants)—carbon dioxide, ammonia, and water—merely by exposing these materials in the gaseous state to a form of energy that (as he stated) is related to radiation.

Miller chose to simulate a strongly reducing atmosphere of hydrogen, ammonia, methane, and water vapor according to Urey's suggestions (Miller, 1953, 1955, 1957a, 1957b). The principal apparatus (referred to as apparatus 1 in Table 4-1) that he used is sketched in Figure 4-1. This spark-discharge apparatus was made of Pyrex glass, except for the electrodes, which were tungsten. The water in the small flask was boiled to promote circulation. In this way the products of the discharge were quickly removed from the reaction zone by condensation. Most of the water vapor liquefied before reaching the condenser. The aqueous solutions flowed back into the boiling flask through the U-tube, which was included in the apparatus to prevent circulation in the wrong direction.

Various modifications of this apparatus have been used in a number of laboratories, including Miller's. These modifications have been made in order to achieve a quicker removal of reaction products from the reaction zone (the apparatus referred to as apparatus 2 in Table 4-1), or to attain a continuous replacement of the reactant gases. When a silent discharge was used instead of a spark, the energy was discharged in the tube between the five-liter vessel and the condenser (the apparatus referred to as apparatus 3 in Table 4-1). In most experiments, the power input at the site of the discharge was

TABLE 4-1
Initial and Final Percentage Composition of Gases
in Electric-discharge Experiments

Reactant	Initial	Final		
		Apparatus 1	Apparatus 2	Apparatus 3
H ₂	20	74.6	76.3	50.6
CO	—	10.0	5.8	1.2
CH ₄	40	10.4	9.5	39.5
N ₂	—	5.0	8.4	8.7
NH ₃	40	8.6	10.5	3.7

Source: Miller (1955).

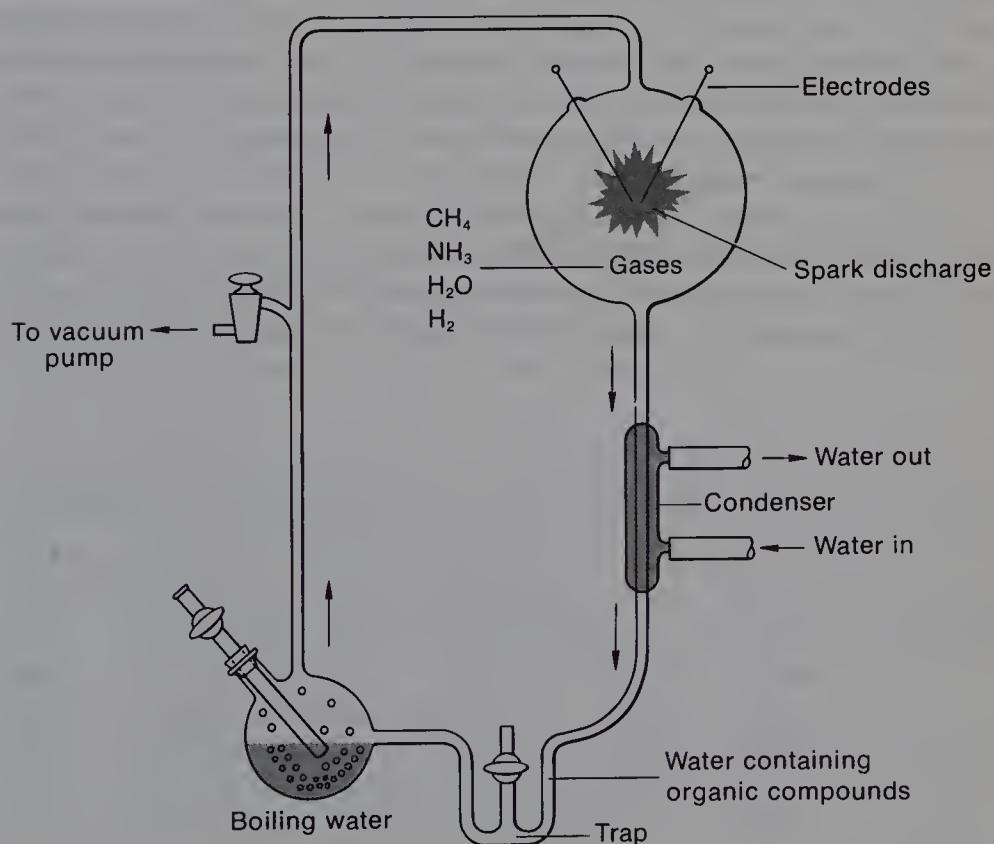


FIGURE 4-1
Apparatus for production of organic compounds from a reducing atmosphere by spark discharges as used by S. L. Miller (1955). See text for details.

between 10 and 100 watts. Since the experiments were usually conducted for a week, the gases were exposed to a total of between 1.5×10^3 and 1.5×10^4 kcal. (The higher inputs applied for the silent discharges.) The temperature of the gases close to the reaction zone was about 70–80°C; however, within the reaction zones the average temperature rose to about 900°K. The pressure in the systems varied between 80 and 100 cm. Table 4-1 shows the composition of the gas mixtures at the beginning and at the end of experiments with the different types of apparatus. Also, substantial amounts of carbon dioxide could be detected in the aqueous phase by precipitation with barium hydroxide.

In a typical experiment, the yields of organic compounds and CO₂ relative to the initial amount of CH₄ were 53 percent, 58 percent, and 22 percent in apparatus 1, 2, and 3, respectively. Since the energy input for apparatus 3 (silent discharge) was about 10 times the amount used in the other apparatuses, these results show that silent discharges are several times less effective than the spark discharges under the experimental conditions applied. During a typical one-week experiment in apparatus 1, about 200 mg of amino acids was produced from 950 mg of methane. Table 4-2 summarizes some relative yields of the amino acids produced. Experiment 1, 2, and 3 refer to use of apparatus 1, 2, and 3, respectively.

Volatile organic acids represent a second group of organic compounds that are produced in yields comparable to those of the amino acids. Some typical results are summarized in Table 4-3.

Miller presented evidence indicating that aldehydes and hydrogen

TABLE 4-2
Relative Yields of Some Identified Amino Acids in Electric-discharge Experiments

Amino Acid	Experiment No. 1		Experiment No. 2		Experiment No. 3	
	Mole ratio ^a	% ^b	Mole ratio ^a	% ^b	Mole ratio ^a	% ^b
Glycine	1	2.1	1	1.8	1	0.46
Alanine	0.54	1.7	0.65	1.8	0.11	0.08
Sarcosine	0.08	0.3	0.04	0.1	1.07	0.74
β-Alanine	0.24	0.8	0.33	1.0	0.05	0.03
α-Aminobutyric acid	0.08	0.3	0.54	0.2	0.01	0.01

Source: Miller (1955).

^aGlycine = 1.

^bYields based on carbon placed in the system as methane. In addition, substantial amounts of two unidentified ninhydrin-positive compounds and small amounts of about 25 others were produced.

TABLE 4-3
Percentage Yields of Acids in Electric-discharge Experiments

Acid	Experiment No. 1	Experiment No. 2
Formic	3.9	0.4
Acidic	0.5	0.7
Propionic	0.6	0.2
Glycolic	1.9	0.2
Lactic	1.8	0.03

Source: Miller (1955).

Note: Yields are based on carbon placed in the system as methane.

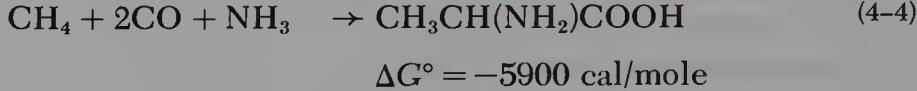
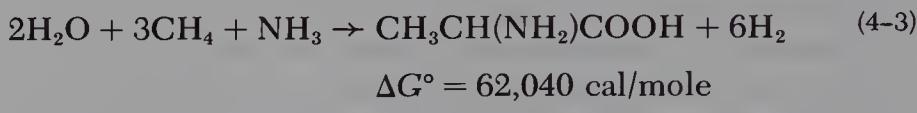
cyanide are primary products. Amino acids may be formed in a secondary reaction by a cyanohydrin synthesis (see Equations 4-1 and 4-2, page 70). According to Miller, the hydrolysis of the nitriles (Equation 4-2) took place in the gaseous phase. However, a substantial portion of the amino acids may have been formed by free radical reactions as well. For more details on amino acid formation by free radicals, see page 94. Evidence was presented that the amino acids were not synthesized by microorganisms. After the same sort of material was sterilized at 130°C for 18 hours, the same apparatus gave the same results.

Miller was notably unable to detect purines, pyrimidines, and other aromatic compounds among the reaction products. He presented evidence indicating that, besides fatty acids and their derivatives, many other compounds were formed. These included various polyhydroxy compounds of unknown composition. The failure to produce aromatic compounds was at least partly due to the fact that the gaseous mixtures that Miller used were too rich in hydrogen (see also pages 83, 86, 113).

After Miller, many other authors, using similar or different gaseous mixtures, essentially repeated his experiments and extended his experimental results. Abelson (1956, 1957) particularly studied the influence of CO₂, N₂, and O₂ on the formation of amino acids. He found that electric discharges produce amino acids also from a mixture of CO₂, N₂, H₂, and H₂O. Amino acids are obtained with higher yields, however, if the N₂ is replaced by ammonia. Relative yields, however, are not necessarily meaningful in the context of molecular evolution. The production of any organic material in such experiments is strongly inhibited by the presence of oxygen. Heyns et al. (1957), for instance, found that amino acids are produced from mixtures of methane, ammonia, water vapor, and oxygen only after the oxygen has

been exhausted by oxidation of ammonia and methane. No amino acids are produced if the atmosphere contains free oxygen or if the atmosphere consists of CO₂, N₂, and H₂O. Ammonium sulfide, when added to one of the reducing gas mixtures, had no pronounced effect on amino acid production. Thiocyanate was among the sulfur-containing compounds produced additionally; no sulfur-containing amino acid, however, could be detected. Thiocyanate is formed quite easily by reaction of sulfur or hydrogen sulfide with cyanides, the latter being abundantly produced by exposing to electric discharges any reducing atmosphere containing carbon and nitrogen.

Pavlovskaya and Pasynski (1959) theorized that amino acid synthesis from a hydrogen-rich atmosphere [reaction (4-3)] is less favored thermodynamically than synthesis from an atmosphere containing carbon monoxide [reaction (4-4)]:



They found that amino acids are produced also on exposing a mixture of CH₄, CO, and NH₃ to electric discharges. This is not very surprising, however, for another reason; CO is abundantly produced by sparking or heating mixtures containing CH₄ and H₂O (Miller, 1955; Pease and Chesebro, 1928). Pavlovskaya and Pasynski therefore, essentially confirmed the observations of Miller (1955). In addition they submitted some, albeit inconclusive, evidence that lysine is also formed under their experimental conditions. Moreover, in agreement with their thermodynamic reasoning, they found that depletion of hydrogen enhances the yields of amino acids. This result is also plausible when reaction mechanisms are considered; hydrogen competes with the other atmospheric constituents to combine with, e.g., methyl and related radicals, thus inhibiting the formation of larger molecules. Consequently, if the partial pressure of hydrogen is kept low, the formation of larger molecules is favored. Referring to Chapter 3 (page 38), we recall that the atmosphere of the primitive Earth probably contained little free hydrogen. In this respect, the simulation experiments described do not satisfactorily represent early geological reality because no provisions are made to remove hydrogen from the systems and to keep their partial pressures low. The Miller apparatuses represent one type of closed system (Prigogine, 1955). They are closed in the sense that they do not permit the escape of

hydrogen and other materials; in fact, the hydrogen becomes dominant (up to 76 percent of the gas) during the reaction (Table 4-1). Hydrogen, on the other hand, escapes under geological conditions (page 39). *Closed systems are generally not found in nature.* Only rare exceptions, as in hydrothermal veins, can be verified. Petroleum pools are a kind of closed system, but their contents have been decomposed, which also emphasizes the need for open systems.

Franck (1960) modified the carbon source in his experiments by using methanol and isoctane instead of methane. He obtained 2.73 g of amino acids from 200 ml of ammoniacal methanol. According to his results, α -aminonitriles are probably formed as the precursors of α -amino acids. In discussing the possible reaction mechanisms, he placed emphasis on the importance of radical reactions.

Oro (1963b) partly replaced the methane with ethane when repeating Miller's experiments. Among the amino acids which were produced he identified proline, valine, and leucine in addition to glycine, alanine, aspartic acid, and asparagine which had already been reported by other authors. Oro also confirmed the formation of amines, aminonitriles, and reported some material yielding amino acids after hydrolysis. The formation of amines, amides, urea, and some amino acids also was observed by Dodonova and Sidorova (1961) while reproducing Miller's experiments.

Grossenbacher and Knight (1965) reported that by sparking a methane-ammonia-water atmosphere they produced threonine, serine, isoleucine, leucine, and lysine in addition to the simpler amino acids that had previously been produced under similar conditions. More recently, Ponnampерuma and Flores (1966) exposed a mixture of methane, ammonia, and water to electric discharges and converted over 90 percent of the methane into other compounds; hydrogen cyanide accounted for 18 percent of the products. Among the amino acids that they identified were phenylalanine and most of the other proteinous aliphatic amino acids except cystine or cysteine, methionine, and the basic amino acids. These reported results resemble closely those obtained by Harada and Fox (1964, 1965) by heating the same gases. The major part of the energy released in electric discharge, in fact, is heat. By exposing an equimolar mixture of methane and ammonia to electric discharges, Ponnampерuma and Woeller (1967) produced several aliphatic nitriles, α -aminoacetonitrile, α -aminopropionitrile, and the C-methyl and N-methyl derivatives of α -aminoacetonitrile, which they detected by gas chromatography and mass spectrometry. Since these α -aminonitriles were formed in the complete absence of water, the Strecker synthesis from aldehydes and HCN would not apply here.

TABLE 4-4
Production of Amino Acids by the Action of Electric Discharges on Various Simulated Atmospheres

Investigators	Gas Mixture	Products ^a
Miller (1953, 1955)	CH ₄ , NH ₃ , H ₂ , H ₂ O	Simple aliphatic amino acids, ^b fatty acids, and related compounds
Abelson (1956)	CO ₂ (CO), N ₂ (NH ₃), H ₂ , H ₂ O	Simple amino acids
Pavlovskaya and Pasynski (1959)	CH ₄ , H ₂ O, NH ₃ or CH ₄ , CO, NH ₃	Simple amino acids
Oro (1963b)	CH ₄ , C ₂ H ₆ , NH ₃ , H ₂ O	Simple amino acids, also leucine and valine
Matthews and Moser (1966)	CH ₄ , NH ₃	Polymeric material that yields simple amino acids after hydrolysis

^a Besides the amino acids, a variety of other compounds of biological significance were formed.

^b Such as glycine, alanine, aspartic acid, and glutamic acid.

The inference that Miller's synthesis does not have a geological relevance has become increasingly widespread (Florkin, 1975). Statements that electric discharge experiments in flasks give superior yields of amino acids, e.g., Lawless and Boynton (1973) assume geological relevance and also ignore the fact that volatile reactant gases are recirculated for prolonged periods within the walls of a flask that retains them (Florkin, 1975; Fox, 1976). Some characteristic results in the formation of amino acids obtained in typical electric-discharge experiments are summarized in Table 4-4.

Kliss and Matthews (1962) have postulated the production of polypeptides and a mechanism for the formation of amino acids from methane and ammonia (page 183). Evidence permitting determination of whether this material has a genuine polypeptide structure has not, however, been presented yet (page 185).

Ultraviolet Light

Experimental difficulties occur when the use of ultraviolet light of wavelength less than 2000 Å is required (page 50). The components of "primitive atmospheres" for experiments such as those

under consideration absorb only below 1800 Å. Since quartz glass absorbs strongly in this region, all apparatuses for such experiments must have a special window to transmit short-wavelength ultraviolet light. Lithium fluoride windows are used. Unfortunately, lithium fluoride windows become cloudy on exposure to water vapor. This problem can be overcome by covering the lithium fluoride window with a monolayer of magnesium fluoride but the transmission of the window is thereby reduced by about 50 percent.

In the 1950s Groth (Groth, 1957; Groth and von Weyssenhoff, 1957, 1959, 1960) resumed the experiments initiated two decades earlier (Groth and Suess, 1938). Groth and von Weyssenhoff exposed a mixture of methane (40 cm), ammonia (15 cm), and water vapor (10 cm) at 55°C to ultraviolet light (the 1165 Å- and 1235 Å-lines of krypton and the 1295 Å- and 1470 Å-lines of xenon). Traces of glycine and alanine could be detected by paper chromatography. No amino acids were detected when the 1850 Å-line of mercury was used. When the authors replaced the methane with ethane they could identify glycine, alanine, α -aminobutyric acid, formic acid, acetic acid, and propionic acid. Details of mechanism were not presented. By addition of mercury vapor to the reactants, they sensitized the systems and thus increased the yields.

Later, Terenin (1959) essentially confirmed the results of Groth and Weyssenhoff by irradiating a methane-ammonia-water-vapor mixture with the Schumann ultraviolet radiation of a hydrogen lamp. Two years later, Dodonova and Sidorova (1961) reported that they had irradiated a mixture of methane, ammonia, water, and carbon monoxide with radiation from the region between 1450 Å and 1800 Å and demonstrated that the amino acids glycine, alanine, valine, and norleucine, as well as the amines methylamine, ethylamine, and some other interesting compounds such as hydrazine, urea, and formaldehyde were formed. More recently, Ponnampерuma and Flores (1966) irradiated with ultraviolet light an atmosphere of methane, ammonia, and water vapor. They used a helium lamp that was discharged at 10,000 volts, thereby emitting a continuous spectrum from 1000 Å to 2000 Å. The photon flux thus produced, though relatively high for a laboratory experiment, was still five orders of magnitude smaller than the present solar flux in the same spectral region. By the end of a 48-hour experiment, not more than one-half percent of methane had been converted into organic compounds.

More powerful sources for high-vacuum ultraviolet radiation need to be designed so that more significant effects on simulated primitive atmospheres can be produced experimentally. As yet, no author has presented quantum yields for the amino acids obtained by ultraviolet irradiation of simulated primitive atmospheres. Only some rough

estimates are available (Hull, 1960). The results of some typical experiments are summarized in Table 4-5. Only a few experiments have been carried out in order to accomplish syntheses of biologically significant compounds from the constituents of the primitive atmosphere by use of long-wavelength ultraviolet light. As shown in Table 3-10, the energy of the ultraviolet light from the early Sun was $6,000 \times 10^{18}$ cal y^{-1} in the spectral region 2000–3000 Å, whereas the corresponding value for the region below Å amounts only to about 150×10^{18} cal y^{-1} . The proposed constituents of the early atmosphere (H_2 , CH_4 , NH_3 , H_2O , CO_2 , N_2 , and perhaps CO) are transparent at $\lambda > 2000$ Å, except for NH_3 , which shows a very weak absorption in the region 2000–2400 Å. A photochemical conversion of the primitive atmosphere with long-wavelength ultraviolet light can therefore only be achieved in the presence of an appropriate sensitizer. Sagan and Khare (1971) and Khare and Sagan (1971) have considered hydrogen sulfide as a suitable photoacceptor. This gas is indeed also found as a constituent of volcanic effluvia (Table 3-3) and has been proposed earlier as a component of both the primitive atmosphere and the primitive hydrosphere (Table 3-5). The above authors have irradiated with the mercury line $\lambda = 2537$ Å various mixtures of methane, ethane, ammonia, water, and hydrogen sulfide and could indeed observe the formation of a number of simple amino acids, including cystine, with an overall quantum yield on the order of 5×10^{-5} . The use of long-wavelength ultraviolet light as an energy source for prebiotic experiments with simulated atmospheres is very intriguing in view of the abundance of this kind of energy on the primitive Earth. So far, however, the body of information on this branch of prebiotic chemistry is rather small. On the other hand, long-wavelength ultraviolet light has been quite widely used for the conversion of simple molecules in aqueous solution.

Ionizing Radiations

One of the difficulties that has to be overcome when ionizing radiations are used in the laboratory production of amino acids is the problem of protecting the products from subsequent radiolysis. A solution to the problem can be achieved by appropriately shielding all parts of the apparatus except the reaction vessel, as sketched in Figure 4-2. In a simpler way, protection of the products is achieved when the gases are irradiated in tall cylindrical glass vessels, such as are used for chromatography. If a beam of soft X-rays is allowed to

TABLE 4-5

Production of Amino Acids by the Action of Ultraviolet Light on Various Simulated Atmospheres

Investigators	Wavelength	Gas Mixture	Products ^a
Groth and Weyssenhoff (1957)	Various Hg lines	CH ₄ , C ₂ H ₆ , NH ₃ , H ₂ O (Hg as sensitizer)	Simple amino acids
Terenin (1959)	1000–2000 Å	CH ₄ , NH ₃ , H ₂ O	Simple amino acids
Dodonova and Sidorova (1961)	1450–1800 Å	CH ₄ , CO, NH ₃ , H ₂ O	Simple amino acids, also norleucine
Sagan and Khare (1971)	2537 Å	CH ₄ , C ₂ H ₆ , NH ₃ , H ₂ S, H ₂ O	Simple amino acids, also cystine

^a Besides the amino acids, a variety of other compounds of biological significance were formed.

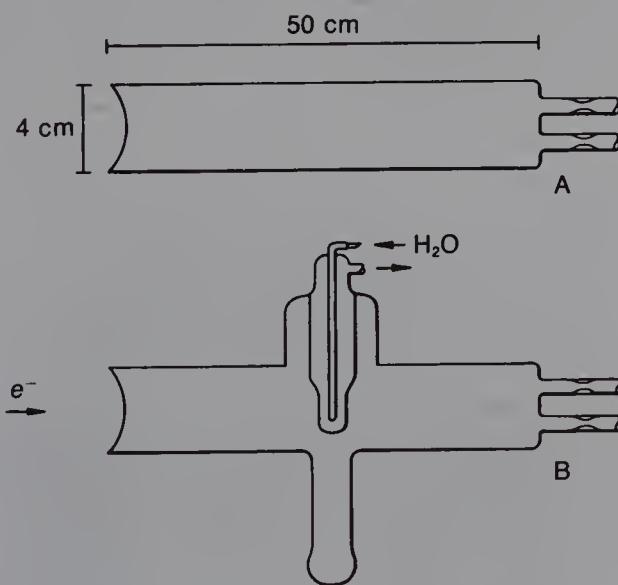


FIGURE 4-2

Two irradiation tubes (A and B) for high-energy radiations. Tube B contains a cold finger for trapping the products. Source: Palm and Calvin (1962).

enter through a thin window in the lid of such vessels, the radiation reaching the aqueous phase at the bottom may be several orders of magnitude less intense than at the top. In addition, the products are removed from the zone of high-intensity radiation by convection and subsequent dissolution or condensation, since the top is warmed by the X-ray tube while the bottom is kept cooler. The choice of the most appropriate apparatus is governed by the quality of radiation to be used. The overall conversion rates strongly depend upon the kind of apparatus. The G-values, being initial yields, should, however, be independent of the apparatus used.

Dose and Rajewsky (1957) were the first to produce evidence that amino acids and related compounds are formed when simulated atmospheres of varied composition are exposed to ionizing radiation. They X-irradiated mixtures of gases with doses of between 10^6 and 10^8 rads in large chromatography vessels containing varied proportions of methane, carbon dioxide, carbon monoxide, water vapor, ammonia, nitrogen, and hydrogen at room temperature. Formation of amino acids is favored if the atmosphere contains both CH_4 and CO_2 as sources of carbon and NH_4 as the nitrogen source. Recently Dose and Risi (1968) published the yields as G-values for the production of several aliphatic compounds obtained by X-irradiating (with 50×10^6 rads) an atmosphere consisting of 25% N_2 , 24% NH_3 , 24% CH_4 , 24% CO_2 , and 3% H_2O at normal pressure and room temperature. The results are summarized in Table 4-6. Evidence has been presented

TABLE 4-6
G-values (numbers of molecules produced per 100 ev energy absorbed) for the Formation of Some Important Aliphatic Compounds by X-irradiating a Simulated Primitive Atmosphere (see text for details)

Product	G-value
Acetic acid	0.0005
Succinic acid	0.0002
Lactic acid	0.0003
Pyruvic acid	0.0001
Methylamine	0.0020
Ethylamine	0.0002
Glycine	0.0004
Alanine	0.0001
Aspartic acid	0.0002

Source: Dose and Risi (1968).

that the aliphatic acids are mostly produced by direct carboxylation and the amino compounds result from direct amination via radical reactions, provided CO_2 and NH_3 are available. The reaction mechanisms are discussed on pages 95-97 in Equations 4-12 through 4-20. Formation of amino acids via the α -aminonitrile in a Strecker synthesis, as suggested by Miller (page 70), is of secondary importance when CO_2 is present as an electron trap and source for the —COOH groups. Berger (1961) irradiated a mixture of methane, ammonia, and water vapor with protons. Among the products he obtained were urea, acetamide, and acetone. A similar mixture was irradiated by Palm and Calvin (1962) with 5 Mev β -particles from a linear accelerator. They identified urea, lactic acid, alanine, glycine, and substantial amounts of hydrogen cyanide as being among the organic substances produced. These authors therefore suggested that hydrogen cyanide would be an important intermediate in the formation of amino acids and aromatic compounds (page 108) under the experimental conditions they were using (absence of CO_2). Direct evidence, however, for the mechanism in involving hydrogen cyanide in a Strecker-type condensation or radical reaction was not presented. In the presence of highly reduced phosphorus compounds that scavenge OH radicals, only small amounts of urea are formed. These results indicate that most urea is probably formed through the Woehler synthesis (Woehler, 1828) after OH radicals have oxidized HCN to HOCN (page 89).

Oro (1963c), while in Berkeley, used the irradiation equipment of Palm and Calvin (1962) to demonstrate that amino acids and hydroxy acids are also formed when the ammonia is replaced by nitrogen and the methane by ethane. In addition, he found that amino acids are also produced when the gaseous mixtures are irradiated in the solid state at the temperature of liquid nitrogen. These results show that chemical evolution in space (e.g., formation of comets and cosmic clouds) may proceed even at very low temperatures if the level of ionizing radiation is sufficiently high.

Later, Ponnampерuma and Mack (1965) found that besides amino acids and many other organic compounds, adenine and some sugars including pentoses and hexoses are formed when a mixture of methane, ammonia, and water vapor is irradiated with 4.5 Mev electrons from a linear accelerator (page 113). In these experiments, also, precautions were taken to shield the products from further radiolysis by employing the apparatus identical to that previously used by Palm, Calvin, Oro, and others (Figure 4-2). Choughuley and Lemmon (1966) produced evidence that the nonproteinous amino compounds taurine, cysteic acid, and cystamine are formed when a mixture of methane, ammonia, water, and hydrogen sulfide is exposed to a beta-

TABLE 4-7

Production of Amino Acids by the Action of Ionizing Radiation on Various Simulated Atmospheres

Investigators	Radiation	Gas Mixture	Products ^a
Dose and Rajewsky (1957)	X-rays	CH ₄ , CO ₂ , CO, NH ₃ , N ₂ , H ₂ , H ₂ O	Simple amino acids
Palm and Calvin (1962)	Electrons	CH ₄ , NH ₃ , H ₂ O, H ₂	Simple amino acids
Oro (1963c)	Electrons	C ₂ H ₆ , N ₂ , H ₂ O, H ₂	Simple amino acids
Choughuley and Lemmon (1966)	Electrons	CH ₄ , NH ₃ , H ₂ O, H ₂ S	Simple amino acids, also taurine, cysteic acid (and cystamine)

^a Besides the amino acids, a variety of other compounds of biological significance were formed.

beam. This is noteworthy because sparking the same gaseous reactant mixture produces only ammonium thiocyanate as the major sulfur compound (Heyns et al., 1957).

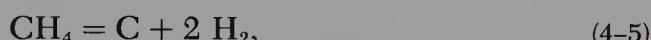
The results of some typical experiments with ionizing radiations are summarized in Table 4-7.

Thermal Energy

High temperatures were not employed for the synthesis of amino acids and other simple organic compounds from a simulated primitive atmosphere until Harada and Fox (1964) studied the effect of short exposures to high temperatures of gaseous mixtures of methane, ammonia, and water. Methane was bubbled through a solution of concentrated ammonium hydroxide and then introduced into a hot reaction tube of Vycor glass containing silica sand, silica gel, volcanic lava, or alumina, at 900–1100°C. After passing the reaction zone, the gas was absorbed in 3 N aqueous ammonia in the cold. When the end products were hydrolyzed and examined by an amino acid analyzer, twelve proteinous amino acids were identified among products of the reactions over silica: aspartic acid, glutamic acid, glycine, ala-

nine, valine, leucine, isoleucine, serine, threonine, proline, tyrosine, and phenylalanine, in addition to very small proportions of nonproteinous amino acids like β -alanine, α -aminobutyric acid, and allo-isoleucine. Some characteristic results are included in Table 4-8 (page 84).

These experiments were the first for which were recorded the synthesis of the benzenoid amino acids tyrosine and phenylalanine. This synthesis from a mixture of gases containing no free diatomic hydrogen was, in fact, to be expected on the basis of theoretical considerations already outlined (page 74). This reasoning visualized that a hydrogen-free atmosphere might yield hydrogen-poor amino acids containing aromatic rings. Furthermore, this reaction proceeded in an open system. Had substantial amounts of hydrogen been generated by decomposition of methane,



they could have escaped because they were not confined.

Another unique feature of this synthesis was the profound effect of silica, either from the beach or from the laboratory, on the kinds of amino acid obtained. When the reaction was carried out in hot empty tubes, the main products were, as found in two laboratories (Harada and Fox, 1964; Oro, 1965), a few simple amino acids like those obtained by electric-discharge experiments; in the presence of quartz sand or silica gel the yield of aromatic and other more complex amino acids increased. Sand and related materials are thus not inert at such temperatures. The widespread geological occurrence of these materials is, of course, relevant.

The mechanism of the thermal reaction is undoubtedly complex. Hydrogen cyanide plays a role in the reaction. This inference is supported by the observation of Kotake et al. (1956), who found that hydrogen cyanide is produced in high yield from methane and ammonia on an aluminum silicate contact catalyst according to the equation:



Glycine, alanine, and aspartic acid may be synthesized without the corresponding aldehyde as an intermediate from aqueous ammonium cyanide (Oro and Kamat, 1961; Lowe et al., 1963). Also, heating of

TABLE 4-8
Production of Amino Acids by the Action of Heat ($\sim 1000^{\circ}\text{C}$) on Various Simulated Primitive Atmospheres

Investigators	Temperature and Contact Materials	Gas Mixture	Products ^a
Harada and Fox (1964)	900–1100°C silica sand, ^b silica gel	CH ₄ , NH ₃ , H ₂ O	Glycine, alanine, aspartic acid, glutamic acid, valine, leucine, isoleucine, serine, threonine, proline, tyrosine, and phenylalanine. Also β -alanine, α -aminobutyric acid, and alloisoleucine
Oro (1965)	1100°C no contact material	CH ₄ , NH ₃ , H ₂ O	Simple amino acids predominate
Taube et al. (1967)	800–1100°C silica	CH ₄ , NH ₃ , H ₂ O	Same products as those reported by Harada and Fox, 1964. Lysine reported.
Friedmann et al. (1971)	1300°C tungsten wire	NH ₃ , C ₂ H ₄ , C ₂ H ₆	Phenylacetylene and indoles. From these phenylalanine, tyrosine, and tryptophan were produced in subsequent reactions at lower temperatures.

^aBesides amino acids, a variety of other compounds of biological significance was formed.

^bThe relative yields of more complex amino acids are increased in the presence of these materials.

glycine gives rise to a number of other proteinous amino acids, among other products (Heyns and Pavel, 1957). Similar results are obtained by heating ammonium formate or formamide (Harada, 1967). More recently, Samochocka et al. (1968) submitted evidence that the high-temperature synthesis of amino acids from acetylene, ammonia, and $^{14}\text{CO}_2$ does not yield ^{14}C -labeled amino acids, which adds to the existing confusion concerning reaction mechanisms.

At temperatures of about 1000°C, C—H, N—H, and O—H bonds are mostly split homolytically. In this way a huge number of radicals are produced in the reactant gas mixtures. Radical combinations, therefore, lead to the formation of larger molecules when the gas mixtures are suddenly cooled to room temperature. These larger molecules include amino acids.

Taube et al. (1967) have published many quantitative data on the formation of various organic compounds by the high-temperature synthesis over silica. They also confirmed, by paper electrophoresis, the synthesis of nine of the amino acids reported by Harada and Fox (1964). Among the amino acids that they identified were tryptophan and lysine. They also identified and analyzed by gas chromatography a number of nitrogen compounds, including hydrogen cyanide, nitriles, and amines, as well as some aliphatic and aromatic hydrocarbons, aldehydes, ketones, alcohols, acids, formamide, and carbon monoxide. During these experiments, the gases remained in the reaction zone for 0.125 second. They were heated from 100°C to 800°C within 0.28 second and cooled to 20°C within 0.4 second. The optimal temperature of the reaction zone, which was packed with silica gel, was 1000°C. At this temperature 95.4 percent of the ammonia reacted. At 1150°C the methane was converted into graphite. The data presented permit the estimate that about 7×10^6 kcal are necessary to produce one mole of glycine or alanine at 1000°C.

Reactions of HCN gave somewhat similar sets of amino acids for Ferris et al. (1973a, b) who studied their products by ion-exchange chromatography. Probably HCN is the intermediate in the Harada-Fox, Oro, and Taube syntheses also (Harada, 1967); all of these syntheses are remarkable for giving almost exclusively proteinous amino acids, except for β -alanine. Lawless and Boynton (1973) reported obtaining only glycine, alanine, and aspartic acid of the proteinous monomers, and β -alanine, in a packed tube synthesis. The amino acids they report by analysis were identified by gas chromatography, a method that tends to give results different from those of ion-exchange chromatography (page 345), especially at low levels. The different results deserve study.

These experiments that use open tubes must be distinguished from other experiments that employ a hot wire in a closed flask. Friedmann and Miller (1969) have used a tungsten wire electrically heated to approximately 1300°C in a one-liter flask to produce phenylacetylene from methane, propane, or ethylene. The conversion rates are up to 1000 times larger than those for corresponding spark experiments. Also, the formation of indoles from ammonia plus ethylene, ethane, or propane was achieved in the same type of experiment

(Friedmann et al., 1971). Phenylacetylene and indoles have been used as starting materials for the production of phenylalanine, tyrosine, and tryptophan by a chain of succeeding reactions in aqueous solution (Friedmann and Miller, 1969; Friedman et al., 1971).

The geological relevance of a closed flask containing a hot tungsten wire is not explained easily, but an experiment in which gases flow through tubes packed with silica or other minerals finds a geological counterpart in the flow of volcanic gases through fissures or pipes of hot igneous rocks of lava.

Some typical results obtained by the action of heat ($\sim 1000^{\circ}\text{C}$) upon various atmospheres are summarized in Table 4-8.

Conclusions

Comparison of thermal reactions with those energized by electric discharges, ultraviolet light, and ionizing radiation suggests that a larger variety of amino acids is produced by heat on contact with silica; in the absence of surface-active materials all forms of energy seem to favor only the formation of the simpler aliphatic amino acids. The difference in the quality and the effects of the various forms of energy is apparent rather than real. This qualification applies particularly to the electric-discharge experiments, because most of the energy released in an electric spark is actually heat. The published results of the experiments with high temperatures (Oro, 1965) and with electric discharges (Miller, 1955) in the absence of surface-active materials show a remarkable similarity. In both, glycine and alanine dominate the composition. We can infer that under suitable experimental conditions, in particular with low partial pressure of hydrogen and presence of appropriate surface-active materials, aromatic amino acids and other biologically significant, unsaturated, heterocyclic compounds will be formed by electric discharges even as they are formed by heat alone.

The obtainment of most of the amino acids common to protein, with few or none of the amino acids not found in protein, has special significance. Living systems characteristically produce only a limited multiplicity of organic compounds including amino acids; they produce many of them almost simultaneously through some common intermediates, but in different final reactions. This kind of *pansynthesis* (simultaneous synthesis of many substances) is even more attractive when it is found to occur under geologically relevant conditions, as is true for the fast, hot-vapor phase reaction at a silica surface. A reaction that is carried out in an *open* system over silica can be shown to have innumerable counterparts in nature.

With respect to the kinds of reaction mixture used, the conclusion is drawn that the elemental composition is of more significance than the kinds of molecule used. High temperatures and electric discharges initiate extremely violent reactions; they not only produce organic molecules of biological significance, they also destroy them and the original reactants as well. The composition of the gas mixtures at the end of the reaction corresponds thermodynamically to an equilibrium state that would be reached at 600–1000°C, with the exact temperature depending on experimental conditions. The following equilibria are of special importance:



Table 4-9 shows the calculated equilibrium constants of these reactions at various temperatures (Pease and Chesebro, 1928). As can be seen from this table and from reactions (4-7) to (4-9), CO, CO₂, and H₂ are formed from CH₄ and H₂O, and vice versa, in the experiments with simulated primitive atmospheres.

Palm and Calvin (1962) found that small amounts of CO and CO₂ are formed by β -irradiation of a mixture of methane, ammonia, and water. Similar results may be expected for ultraviolet irradiation of the same mixture, although constants of thermal equilibria may not be directly applied to radiation chemical and photochemical equilibria.

Some similarities may be discerned among various forms of energy with respect to their primary chemical effects on the components of the gas mixtures. In electric-discharge reactions, electrons cause ionizations, excitations, local production of heat, and, subsequently, formation of radicals. When ionizing radiations are used, secondary electrons produce essentially similar effects although the conversion of the kinetic energy of the electrons into thermal energy is more widely distributed spatially. Therefore, the events of thermal reactions are not as spectacular as those of electric-discharge experiments. High-vacuum ultraviolet light causes primarily excitations and some ionizations. However, in secondary reactions most of the electromagnetic energy is converted into thermal energy. Radical reactions dominate in all subsequent chemical reactions. If violent heat ($\Delta t \sim 1000^\circ\text{C}$) is applied directly, principal primary reactions are the homolytic cleavage of the various bonds, producing similar, if not identical, kinds of reactive species, which are produced by the other forms of energy as well.

TABLE 4-9
*Calculated Equilibrium Constants of Reactions (4-7),
(4-8), and (4-9)*

Temp. °C	Reaction		
	(4-7)	(4-8)	(4-9)
500	0.0325	0.00539	0.0989
600	0.949	0.0696	0.1983
700	15.06	11.03	0.339

Source: Pease and Chesebro (1928).

Not surprising, therefore, is the fact that for the most part the same products are formed by the action of any of these forms of energy upon any of a variety of mixtures of gases. The physical properties of the products are of importance. Amino acids are the favored species of reaction product because they contain the stabilizing inner salt structure, e.g., $^+ \text{NH}_3 - \text{CHR} - \text{COO}^-$. Accordingly, their vapor pressure is very low. Being the less volatile products, amino acids will, therefore, precipitate faster out of the reaction zone than do other materials. In geological settings, this process could result in separation of amino acids from other organic compounds. For example, an aqueous solution of amino acids and hydroxy acids falling upon hot rock would literally undergo fractional distillation. The amino acids would be concentrated into the distillation residue, since they would be the least volatile components. Moreover, the amino acids would rapidly polymerize (page 153) to large molecules that would be even less volatile.

Some typical results obtained by a number of authors who exposed various atmospheres to different types of energy are summarized in Table 4-10. These results show some similarities in relative amounts of several amino acids and other small molecules produced. Perhaps these similarities are to be related to the fact that with all four types of energy, radicals, ions, and excited states are intermediates in reactions that ultimately produce the various compounds. On the other hand, some differences (not shown in Table 4-10) must not be overlooked. The kinds and amounts of amino acids obtained in the thermal experiments depend, for example, on the temperature and on the properties of the minerals with which the reaction tubes are filled. The observation that there are some principal similarities among results from experiments with all four types of energy reported must be regarded as a first approximation. Surface-active materials like

TABLE 4-10

Relative Amounts of Some Amino Acids and Related Compounds Produced from Various Atmospheres with Electric Discharges, X-rays, β -rays, and Heat

Products ^a	Molar Ratio (Glycine = 1)			
	Electric Discharges (sparking) (CH ₄ , NH ₃ , H ₂ O, H ₂)	X-rays (CH ₄ , CO ₂ , H ₂ O, NH ₃ , N ₂ , H ₂)	β -rays (CH ₄ , NH ₃ , H ₂ O, H ₂)	Heat (CH ₄ , NH ₃ , H ₂ O)
Glycine	1.00	1.00	1.00	1.00
α -Alanine	0.54	0.25	2.0	0.17–0.83
β -Alanine	0.24	0.20		0.52
α -Aminobutyric acid	0.08			0.17
Aspartic acid	<0.01	0.50		0.13–0.63
Urea	<0.01	>100	>100	
Acetate	0.24	1.25		
Lactate	0.49	0.75		

^aA wider variety of compounds was actually obtained in all experiments.

Source: Electric-discharge data partly recalculated from Miller (1955) and Horowitz and Miller (1962).

X-ray data recalculated from Dose and Risi (1968) and unpublished results.

β -ray data recalculated from Palm and Calvin (1962).

Molar ratios of heat-produced compounds recalculated from Harada and Fox (1964) and Taube et al. (1967).

silica may favor the production of a larger variety of amino acids and other compounds when electric discharges, ionizing radiations, or ultraviolet light is used instead of heat. Results of such experiments, if any have been performed, are, however, not at hand. If in a thermal experiment with silica gel as the surface-active material the temperature is raised from 950°C to 1050°C, the proportion of glycine decreases, and the proportion of all other amino acids increases. The amount of data presently available, however, does not permit a more conclusive discussion. Quantitative differences between the amounts of a given product formed may also result when the conditions of a given radiation chemical experiment are changed. For instance, ionizing radiations form rather large amounts of urea from methane, ammonia, and water, inasmuch as they produce high concentrations of OH radicals. These OH radicals likely react with hydrogen cyanide, which is also produced abundantly. The resulting cyanate is subsequently converted into urea by a Woehler synthesis:



The concentration of OH radicals and, subsequently, the yields of urea may be reduced by the addition of OH scavengers, e.g., phosphines (Palm and Calvin, 1962). Then a larger variety of amino acids and other compounds is formed. A variation of linear energy transfer (LET), dose rates, and temperatures will also influence the yields of the products obtained by ionizing radiations. Similarly, in an electric-discharge experiment the results are expected to depend on the kind and intensity of the discharge used. For all these possible variable effects, however, no data are available for a detailed discussion.

Of special interest to the evolutionary chemist would be a quantitative comparison of the yields of the various compounds, in particular of amino acids. Unfortunately, only a few meaningful data are available.

In electric-discharge experiments (Miller, 1955), 1.5×10^3 kcal (spark discharges) or 1.4×10^4 kcal (silent discharges) had to be employed to produce 47 mg or 60 mg, respectively, of glycine. No quantitative data are presently available for the production of glycine or other compounds from a reducing atmosphere by high-vacuum ultraviolet light. Hull (1960), however, calculated that the upper limit for the quantum yield of glycine formation would be about 10^{-6} for the ultraviolet irradiation (in the wavelength range 1165–1470 Å) of a mixture of ammonia, methane, and water vapor (Groth and Weyssenhoff, 1957, 1959, 1960). The G-value (number of molecules per 100 ev absorbed energy) for the production of glycine by X-irradiation of a mixture of methane, carbon dioxide, ammonia, nitrogen, and water vapor has been found to be 4×10^{-4} (Dose and Risi, 1968). A slightly smaller yield for the production of glycine by 5 Mev β -rays in similar experiments is obtained when calculated from the data of Palm and Calvin (1962). This is in good agreement with the theoretical expectation. The LET of both forms of radiation is of the same order of magnitude. The high dose rate of the 5 Mev β -rays produced in short pulses by a linear accelerator favors the formation of high local concentrations of radicals. In this way, recombinations, or back-reactions, occur with higher probability. Thus a smaller number of radicals per unit of absorbed energy is available for synthetic processes initiated by 5 Mev β -rays as compared with the X-rays.

To produce about 10 micromoles of glycine by heat, 1 mole of methane, 0.6 mole of ammonia, and 0.4 mole of water vapor must be heated to 1050°C from room temperature (Taube et al., 1967; Oro, 1965; Harada and Fox, 1964, 1965). If an average heat capacity of 0.6 cal g⁻¹ is assumed for the gas mixture, 2×10^4 cal are required to produce 10 micromoles of glycine; many other compounds are formed simultaneously by the same expenditure of energy.

TABLE 4-11

*Approximate Expenditure of Energy for the Production of 1.0 Mole
of Glycine from Constituents of Reducing Atmospheres*

Form of Energy	Kcal per Mole $\times 10^{-6}$ of Glycine Produced	Net Amount of Energy Applied in Typical Experiment	
		Kcal	Other units
Spark discharges	2.4	1,500	—
Silent discharges	19	14,000	—
Ultraviolet light (1165–1470 Å)	220	0.4	10^{21} quanta
X-rays	5	1	50 Mrad
β -rays	10	2.4	10^{11} ergs
Heat	2	100	—

The calculated requirements of the various forms of energy for the production of 1.0 mole of glycine are summarized in Table 4-11. The data of Table 4-11 clearly demonstrate that none of the various forms of energy is particularly more efficient than the others. For the formation of glycine, spark discharges and heat show about the same efficiency and ultraviolet light seems least effective. However, the information on the efficiency of ultraviolet light must be regarded as highly tentative; because of the low output of existing sources for high-vacuum ultraviolet light, the experimenters were unable to determine exact quantum yields for amino acid formation. Reviewers (e.g., Horowitz and Miller, 1962) sometimes have not distinguished between yields and conversion rates of carbon compounds when trying to compare the efficiencies of the various forms of energy. Thus they have misinterpreted, in particular, the results of experiments in which relatively small amounts of organic material were produced with ionizing radiations or ultraviolet light. Evidently, these amounts were small because, for technical reasons, only relatively small amounts of energy could be applied. More than 1000 times larger amounts of organic compounds are produced by electric discharges relatively easily in spectacular experiments, because more than 1000 times the energy is delivered to the system. The power delivered in the electric-discharge experiments was 10–100 watts over an average period of one week. X-ray machines or high-vacuum ultraviolet lamps that would give such a tremendous output of energy continuously over several days have yet to be constructed. Sources for β -rays or other corpuscular radiations yielding a comparable output are available; for many reasons, however, the application

of high doses is of no advantage when the same scientific information can be obtained with less effort.

The problem of protecting the products from subsequent degradation by the same kinds of energy that have formed them existed on the primitive Earth as it does in the contemporary laboratory. Heat, high-energy radiation, and, to some degree, electric discharges effectively produced biologically significant compounds from the constituents of the primitive atmosphere probably at only relatively short distances from the surface of the Earth; subsequent degradation of the compounds could then have been prevented by their dissolution in water or through sorption by minerals. If material were formed in the upper atmosphere, however the chances for its escaping subsequent degradation were rather small. As Hull (1960) has calculated, the half-life of glycine in the upper atmosphere would be 30 days. This value is to be compared with the half-life of about three years for the transport of glycine from the stratosphere to the Earth's surface, as calculated from present fallout data. Therefore, about 97 percent of the glycine produced photochemically in the upper atmosphere could never have reached the Earth's surface, because of subsequent photo-decomposition. Hull estimates that the rate of photochemical formation of glycine in the primitive atmosphere would have corresponded to a value of 3×10^5 molecules cm^{-2} sec $^{-1}$. Assuming that 3 percent of the original glycine reached the surface of the sea and the half-life of glycine in the sea is 1000 years, he finds that the maximum concentration of photochemically synthesized glycine in the primitive ocean would have been as little as 10^{-2} M. These calculations, though mathematically correct, have a serious deficiency. They neglect the fact that evolution of any kind is not brought about by statistically homogeneous and steady environments, but rather by the heterogeneities and irregularities of geochemical events. Though the average conditions may have been quite unfavorable, it is possible to visualize many sets of conditions under which the evolution and accumulation of organic materials could have occurred with high probability. Bernal (1960, 1961), when criticizing Hull's overall concept, pointed out that organic compounds might show altered reactivities after adsorption and concentration by clay particles or other surface-active minerals. In this respect, we recall, e.g., that the threshold wavelength of photolysis of water or ammonia is shifted considerably toward the red when the molecules of these substances are adsorbed to the surface of silicates of aluminosilicates (Terenin, 1959).

The writers of many papers dealing with molecular evolution may be criticized either for taking into consideration only *average* geo-

chemical conditions or for assuming that an equilibrium is attained in an open system.

PRODUCTION OF AMINO ACIDS AND RELATED COMPOUNDS BY REACTIONS IN THE CONDENSED PHASE

Electric Discharges

Only a few electric-discharge experiments have been conducted in liquids or solids. Franck (1960) exposed ammoniacal methanol to electric discharges; he produced amino acids in high yields. Whether these experiments have much bearing on molecular evolution, however, is doubtful, because the geological relevance of ammoniacal methanol has not been explained.

Optical Radiations

Formation of amino acids from simple molecules by photochemical reactions was investigated long ago. Baudisch (1913) observed that glycine is formed by ultraviolet irradiation of a solution of potassium nitrite, carbon monoxide, and ferric chloride. Dhar and Mukherjee (1934) observed that glycine and other amino acids can be obtained by photochemical processes from ammonia, glycol, and other C₂ sources or glucose. However, none of the early experiments were explicitly designed as evolutionary studies. Evaluation of the likelihood that such geological conditions existed is difficult.

Abelson (1953) performed one of the earliest evolutionary experiments to test whether amino acids could be synthesized by ultraviolet light. He irradiated an aqueous solution of ammonium formate in the presence of ammonium hydroxide, sodium cyanide, and ferrous sulfate with light of the wavelength 2537 Å. A major product was α -aminoacetonitrile, which gave rise to glycine after hydrolysis. Abelson estimated that 10 percent of the formate was converted into glycine. Bahadur (1954) exposed to sunlight an aqueous solution of formaldehyde with various inorganic salts—e.g., potassium nitrate and ferric chloride. Among the products he reported were aspartic acid, serine, and lysine. The idea that nitrate was present on the primitive Earth, however, poses some difficulties. Reid (1959) irradiated a mixture of formaldehyde, ammonia, and carbon dioxide in the presence of some

inorganic salts with ultraviolet light of the wavelength 1848 Å. Evidence for the formation of glycine and alanine was presented. Pavlovskaya and Pasynski (1959) found that serine, glycine, alanine, glutamic acid, valine, and phenylalanine are formed when aqueous formaldehyde is irradiated in the presence of ammonium salts with a high-pressure mercury lamp. Abelson (1966), who had reported earlier (1956) that hydrogen cyanide is produced when various mixtures of carbon monoxide, nitrogen, and hydrogen are exposed to electric discharges, found that glycine, serine, alanine, and aspartic acid are formed when an aqueous cyanide solution is exposed to the ultraviolet light of a mercury lamp. Ellenbogen (1958) reported that he was able to produce phenylalanine, methionine, and valine among other compounds when he exposed to ultraviolet light an aqueous mixture of ferrous sulfite and ammonium chloride through which he bubbled methane and ethane (9 percent). Later, Steinman et al. (1968) claimed that methionine may be produced among other amino acids when aqueous 0.1 M ammonium thiocyanate (labeled with ^{14}C) is irradiated with a Pen-Ray Quartz ultraviolet lamp. Their evidence for the identity of the material produced is weak, however, because it is based only on a comparison of R_f values obtained by paper chromatography. The yield was so small as to raise the question of contamination, which was not ruled out in the experiment. Deschreider (1958) produced various amino acids, e.g., aspartic acid, alanine, and glycine, by ultraviolet irradiation (2537 Å) of aqueous mixtures of succinic acid, maleic acid, propionic acid, and acetic acid in the presence of ammonium carbonate and thiocyanate. Ferrari and associates demonstrated that amino acids may be produced when hydroxy-acids, ketoacids, and dicarboxylic acids are ultraviolet irradiated in the presence of ammonia or other nitrogen sources (Ferrari, 1959; Cultera and Ferrari, 1959; Ferrari and Cultera, 1960). Unfortunately, most of the reports describing photochemical syntheses lack quantitative details and rigorous evidence for the identity of the materials produced. Discussions of reaction mechanisms, which might be helpful, are also missing.

Characteristic results obtained by a number of investigators who tried to simulate the prebiotic formation of amino acids by the action of visible and ultraviolet light upon aqueous systems are summarized in Table 4-12.

Ionizing Radiations

By exposing a dilute aqueous solution of ammonium acetate to β -rays, Hasselstrom et al. (1957) were able to produce glycine and

TABLE 4-12

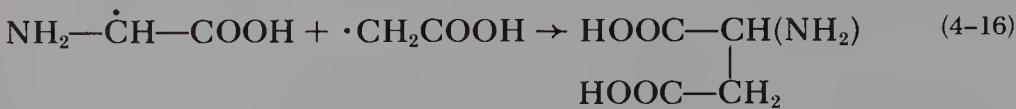
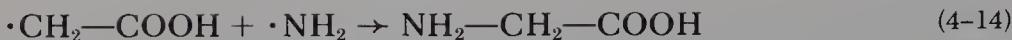
Production of Amino Acids by the Action of Ultraviolet Light upon Aqueous Systems under Simulated Prebiotic Conditions

Investigators	Light (Wavelength or Source)	Reactants (Aqueous Solution)	Products ^a
Abelson (1953)	2537 Å	HCOONH ₄ , NH ₄ OH, NaCN, FeSO ₄	Simple amino nitriles (e.g., aminoacetonitrile), yielding amino acids after hydrolysis
Bahadur (1954)	Sunlight	H ₂ CO, various inorganic salts (nitrates)	Simple amino acids, also serine and lysine
Reid (1959)	1848 Å	H ₂ CO, NH ₄ , HCO ₃ ⁻ , inorganic salts	Glycine and alanine
Ellenbogen (1958)	Mercury lamps	FeSO ₃ , NH ₄ Cl under CH ₄ and C ₂ H ₆	Various amino acids including phenylalanine, methionine
Pavlovskaya and Pasynski (1959)	High-pressure Hg lamp	H ₂ CO, ammonium salts	Simple amino acids, also serine, valine, phenylalanine
Steinman et al. (1968)	Pen-ray Hg lamp	NH ₄ SCN	Various amino acids including methionine
Ferrari (1959), Culteria and Ferrari (1959), Ferrari and Culteria (1960)	Hg lamp	Various hydroxy- acids, ketoacids, dicarboxylic acids, NH ₄ OH or other sources of N	Mostly the analogous amino acids

^a Besides the amino acids, a variety of other compounds of biological significance was formed.

aspartic acid. These two α -amino acids are probably produced by the following reactions, which largely depend on the production of OH radicals by the radiolysis of water:

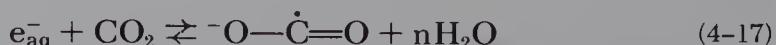




When the same solutions are X-irradiated, substantial amounts of β -alanine are formed, possibly due to the decarboxylation of the intermediate aspartic acid (Dose and Ettre, 1958). A variety of amino acids is obtained when the ammonium salts of fatty acids are irradiated in aqueous solution. The overall *G*-value of the substitution of hydrogen by an amino group has the order of 0.1, when 0.5 M solutions are irradiated. The *G*-value decreases proportionally to the concentration; the production of α -amino acids is not favored (Dose and Ettre, 1958; Dose and Risi, 1968).

Paschke et al. (1957) notably produced glycine, and probably alanine, by ^{60}Co γ -irradiation of aqueous ammonium carbonate. For the reduction of carbonate by ionizing radiation, attention must again be paid to the work of Garrison et al. (1951), who were able to reduce aqueous carbonic acid to formaldehyde and other compounds, and to the work of Hasselstrom and Henry (1956), who obtained oxalic acid when they irradiated aqueous solutions of calcium carbonate and ammonium carbonate.

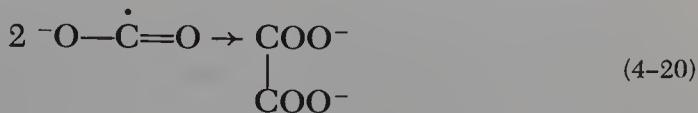
The radiation chemical reduction of carbonic acid plays an important role in the production of organic acids in studies with ionizing radiations. The radiation chemically active form of carbonic acid in aqueous systems is the carboxylate radical $\cdot\text{CO}_2^-$. It can be formed by the addition of a solvated electron to a $-\text{C=O}$ double bond of carbon dioxide according to Equation 4-17 (Garrison et al., 1951, 1952; Hart, 1952; Weiss, 1960; Gordon et al., 1963). The solvated electron, e_{aq}^- , results from thermalized electrons that are trapped between water dipoles (Dainton and Jones, 1962; Hart and Boag, 1962; Schulte-Frohlinde and Eiben, 1962; Hart, 1955):



The $\cdot\text{CO}_2^-$ may combine with any other carbon radical to yield a carboxylic acid:



When two carboxylate radical ions dimerize, oxalic acid is produced:



Aliphatic amines yield amino acids. The initial G-values for the overall carboxylation of aliphatic amines in an 0.5 M solution has the order of 0.1. This value decreases proportionally with decreasing concentration of the amines. However, many side reactions occur whenever organic compounds are irradiated. Dose and Ponnamperuma (1967) exposed aqueous solutions of *N*-acetylglycine and ammonia to γ -rays and β -rays. Besides the amination of the acetyl residue, yielding glycylglycine (page 146), the formation of at least fifteen derivatives of various amino acids was observed.

A summary of characteristic results on the formation of amino acids by the action of ionizing radiation upon various aqueous reactants under simulated prebiotic conditions is given in Table 4-13.

TABLE 4-13

Production of Amino Acids by the Action of Ionizing Radiation upon Aqueous Reactants under Simulated Prebiotic Conditions

Investigators	Type of Radiation	Reactants (Aqueous Solution)	Products ^a
Hasselstrom et al. (1957)	β -rays	Ammonium acetate	Glycine, aspartic acids
Paschke et al. (1957)	γ -rays	$(\text{NH}_4)_2\text{CO}_3$	Glycine and alanine (?)
Dose and Ettre (1958)	X-rays	Ammonium acetate	Glycine, β -alanine, aspartic acid
Dose and Ettre (1958)	X-rays	Ammonium salts of fatty acids	Homologous amino acids; α -amino acids not favored
Dose and Risi (1968)	X-rays	Carbonates of alkylamines	Homologous amino acids

^a Besides the amino acids, a variety of other compounds of biological significance was formed.

Thermal Energy

Our discussion of the use of thermal energy for the production of micromolecules focuses upon those experiments that have been designed as experiments simulating prebiotic conditions.

Fox et al. (1955) observed that aspartic acid and alanine are formed among other products when solid ammonium fumarate and ammonium malate are heated to 200°C for 3 hours. Similarly, Heyns and Pavel (1957) prepared alanine and asparagine, as well as glycylglycine, oxalic acid, fumaric acid, and many other compounds, by heating solid glycine.

Related experiments have been carried out to investigate the possible evolution of organic compounds in comets. Spectroscopic evidence for the presence of $C_2N_2^+$, CH, CH^+ , C≡N, CO^+ , NH, and OH in comets has been obtained (Mueller, 1963; Oro, 1961a). If allowed to react at moderate temperatures, these radicals and radical ions can be expected to yield hydrogen cyanide, acetylene, carbon monoxide, formaldehyde, hydrazine, hydroxylamine, and other reactive products as primary intermediates (Oro, 1965). Oro et al. (1959) were able to demonstrate that in aqueous solution these reactive intermediates readily produce a large number of organic molecules of biological interest. Several biologically significant amino acids, including glycine, alanine, β -alanine, serine, threonine, and aspartic acid, are obtained, for example, if aqueous formaldehyde in the presence of hydroxylamine hydrochloride is kept between 100°C and room temperature for various lengths of time. Oro and Kamat (1961) later demonstrated that glycine, alanine, aspartic acid, and glycineamide are produced by heating aqueous ammonium cyanide to 70°C for 25 days. These results have been essentially confirmed by Lowe and his associates (Lowe and Rees, 1963; Lowe et al., 1963).

Fox and Windsor (1970) demonstrated that the heating of aqueous formaldehyde and ammonia (at 185°C for 8 hours under nitrogen) drives off water and yields, on further heating, a product that is hydrolytically converted into amino acids. Six to ten amino acids have been identified in repetitions of the experiment. They include proline, valine, the two leucines, and phenylalanine. Glycine is the predominant amino acid. Formaldehyde, ammonia, and water have been identified in galactic clouds (page 330). These experimental results, therefore, pertain to a cosmochemical as well as a geochemical context.

The relevance of such results to terrestrial occurrences is of course analogical rather than substantive (Sagan, 1972), but the mention of such compounds in association with prebiotic terrestrial evolution evidently implies a direct relationship for some (Hulett, 1971).

TABLE 4-14

Production of Amino Acids from Aqueous or Solid or Liquid Reactants under the Influence of Thermal Energy in Experiments Simulating Prebiotic Conditions

Investigators	Duration and Temperature	Reactants	Products ^a
Fox et al. (1955)	3 hours at 200°C	Solid ammonium fumarate and ammonium malate	Alanine, aspartic acid
Heyns and Pavel (1957)	A few hours at about 200°C	Solid glycine	Alanine, asparagine, diglycine identified
Oro et al. (1959), Oro and Kamat (1961)	Hours or days at 70–100°C	Simple aqueous reactants, e.g., HCN, NH ₃ , or H ₂ CO, NH ₂ OH	A large variety of amino acids (12 identified), including the leucines, arginine, no aromatic amino acids
Fox and Windsor (1970)	185°C for 8 hours	Aqueous H ₂ CO, and NH ₃	Up to 10 amino acids identified after hydrolysis, including proline, valine, the leucines, and phenylalanine

^aBesides the amino acids, a variety of other compounds of biological significance was formed.

Wolman et al. (1971) essentially confirmed the formation of a set of amino acids by hydrolysis of the reaction product of formaldehyde and ammonia, and by hydrolysis of the nearly equivalent compound hexamethylenetetramine. They interpret the results differently than do Fox and Windsor (1970, 1971), who discussed their results in relationship to the occurrence of ammonia and formaldehyde in interstellar clouds. Comments have been exchanged (Wolman et al., 1971; Fox and Windsor, 1971).

A number of characteristic results in the formation of amino acids from solid and liquid reactants under the influence of thermal energy in experiments employing simulated prebiotic conditions are given in Table 4-14.

Glow Discharge Electrolysis

Harada has introduced glow discharge electrolysis as a newer means of prebiotic formation of amino acids in a series of papers

(Harada, 1974; Harada and Iwasaki, 1974, 1975). The reactions occur in a liquid phase. These papers present also a recent overview of methods for the synthesis of amino acids.

Comments

This section on formation of amino acids in liquids and solids will not close with a section of conclusions quite like that of the section on formation of amino acids from gaseous constituents of simulated atmospheres. The absence of such treatment here is a consequence of the fact that experimentation with liquids and solids to produce amino acids has been much less extensive. Experimenters may have simply preferred to study atmospheres; nature may have preferred liquids or solids. Nature undoubtedly employed liquids and solids for the evolution of more complex molecules.

HYDROCARBONS AND FATTY ACIDS

Electric Discharges

While studying the effects of different kinds of electric discharge on pure methane, Ponnamperuma and Woeller (1964) found that a high-intensity arc yields a clear yellow fluid that is easily fractionated by gas chromatography. Benzene was the most abundant product; toluene was next in abundance. Some aliphatic fractions such as 2,2-dimethylbutane, 2-methylpentane, 3-methylpentane, 2,4-dimethylhexane, and 3,4-dimethylhexane were identified by gas chromatography. When methane was exposed to semi-corona discharges, a colorless distillate was obtained. This was poorly resolved by gas chromatography and contained virtually no benzene or toluene, but did contain relatively large amounts of cycloaliphatic hydrocarbons instead. No isoprenoid-type molecules were discovered. The predominance of aromatic compounds in the experiments with high-intensity arcs may be related to the formation of benzene from methane at high temperatures and low partial pressures of hydrogen.

Miller (1955) demonstrated that formic acid, acetic acid, propionic acid, and certain oxygen derivatives, e.g., glycolic acid and lactic acid, are formed when a gaseous mixture of methane, ammonia, water vapor, and hydrogen is exposed to a spark discharge. These results have already been referred to (page 73).

After a mixture of methane and water vapor has been exposed to a semi-corona discharge, several monocarboxylic acids from C₂ to C₁₂ could be detected by gas-liquid chromatography and mass spectrometry (Allen and Ponnamperuma, 1967).

Optical Radiations

Groth and Suess (1938) early demonstrated that formaldehyde and glyoxal are formed when a mixture of carbon dioxide and water vapor is exposed to high-vacuum ultraviolet light. When resuming the experimental program two decades later, Groth and Weyssenhoff (1957) found that besides amino acids, formic acid, acetic acid, and propionic acid are formed in detectable amounts by ultraviolet-irradiating atmospheres containing methane, ethane, ammonia, and water vapor (page 77).

Ionizing Radiations

By exposing an aqueous solution of carbon dioxide and ferrous ions in the presence of hydrogen to a beam of α -particles, Garrison et al. (1951) demonstrated that formaldehyde, formic acid, and succinic acid are formed by the reduction of carbonic acid. They concluded that the first fatty acids and related compounds might have been formed in a similar way on the primitive Earth. Later evidence was accumulated to demonstrate that simple fatty acids and their derivatives are formed, in addition to other compounds, when various mixtures of methane, ammonia, water vapor, or other gases are exposed to ionizing radiations (Dose, 1962; Dose and Risi, 1968). The radiation chemical carboxylation of hydrocarbons, as a preparative method, has been studied in many laboratories (McKusick et al., 1960; Guetlbauer and Getoff, 1966). Several different mechanisms have been discussed for the radiation chemical formation of carboxylic acids. The $-\dot{\text{O}}-\dot{\text{C}}=\text{O}$ radical ion as a reactive intermediate [see reactions (18) to (20)] has drawn special attention in this respect. Carboxylic acids may also be produced by successive oxidation of hydrocarbons, e.g., by means of OH radicals, according to the following overall reaction (Phung and Burton, 1957; Fricke et al., 1938):



Another way to produce carboxylic acids is by the hydrolysis of aliphatic nitriles, which may be formed from direct combination of alkyl and CN radicals, to mention only one principal pathway.

Thermal Energy

Eck et al. (1966) have calculated equilibrium concentrations of hydrocarbons formed from gaseous mixtures (e.g., of the composition corresponding to C:H:O = 30:40:30, that is, a gas mixture with a carbon dioxide:methane ratio of 1:6) for various temperatures. In Table 4-15 are given the equilibrium concentrations for the gas mixture, C:H:O = 30:40:30, at 500°K and one atmosphere pressure. The column "aromatics deleted" has been included because in the Fischer-Tropsch synthesis the temperature can be kept low enough that aromatic hydrocarbons do not form rapidly. This presumably pertains because the activation energies of the reactions leading to aromatic hydrocarbons are too high. The formation of aromatic compounds may be disregarded, therefore, if the gas mixture is exposed to the elevated temperature for a limited time only.

If we are thinking of gases kept at 500°K through the course of geological time and in the presence of surface-active materials, however, the formation of aromatic compounds must not be disregarded. As can be seen from Table 4-15, the methane/ethane ratio becomes about 100 if the aromatic compounds are deleted. Similar values are reached for the cyclopentane/methane and cyclohexane/methane ratios. Hydrocarbons with one double bond are slightly less favored than the saturated ones. Considerably less favored are those compounds having a triple bond. Of the keto compounds, acetone is most favored; of the carboxylic acids, acetic acid is more favored than formic acid or any other fatty acid. Temperatures of 500°K may have occurred widely on the primitive Earth, in particular within the crust (page 58). Simple hydrocarbons, fatty acids, and some ketones or aldehydes may, therefore, have been readily formed in igneous processes on the primitive Earth. When the reactions are, however, allowed to proceed over longer periods, benzene and the other aromatics have to be included in the equilibrium because they become the major products. When the methane/asphalt ratio is only 35, igneous asphalt can be formed abiogenically from methane, carbon dioxide, and water under geological conditions.

In model prebiotic experiments, equilibrium states are usually not reached. This applies also to the experiments of Oro and Han (1966) in which they passed a stream of methane through a contact of silica gel kept at about 1000°C. By this procedure they produced various higher hydrocarbons including aromatic hydrocarbons. This experiment and its results are analogous to the action of electric discharges on gaseous methane which has already been discussed (Ponnamperuma and Woeller, 1964); cf. Harada and Fox (1965).

TABLE 4-15

Mole Fraction Composition of Gas Mixture, C:H:O = 30:40:30 at One Atmosphere Pressure and 500°K

Component	Aromatics Included	Aromatics Deleted
H ₂	0.94×10^{-4}	0.96×10^{-6}
O ₂	$< 10^{-38}$	$< 10^{-38}$
H ₂ O	0.11×10^{-3}	0.95×10^{-8}
CO	0.41×10^{-2}	0.29
CO ₂	0.61	0.38
Methane	0.38	0.32
Ethane	0.66×10^{-4}	0.46×10^{-2}
Propane	0.80×10^{-7}	0.46×10^{-3}
Butane	0.21×10^{-9}	0.99×10^{-4}
Pentane	0.29×10^{-12}	0.11×10^{-4}
Hexane	0.47×10^{-15}	0.15×10^{-5}
Heptane	0.73×10^{-18}	0.19×10^{-6}
Octadecane	$< 10^{-38}$	0.15×10^{-16}
Cyclopropane	0.76×10^{-13}	0.42×10^{-7}
Cyclobutane	0.23×10^{-11}	0.11×10^{-3}
Cyclopentane	0.13×10^{-11}	0.48×10^{-2}
Cyclohexane	0.15×10^{-13}	0.46×10^{-2}
Ethylene	0.88×10^{-8}	0.59×10^{-4}
Propene	0.53×10^{-9}	0.29×10^{-3}
Butene	0.92×10^{-12}	0.42×10^{-4}
Octene	0.21×10^{-23}	0.43×10^{-8}
Acetylene	0.55×10^{-16}	0.36×10^{-10}
Propyne	0.29×10^{-16}	0.16×10^{-8}
Octyne	0.15×10^{-30}	0.31×10^{-13}
Allene	0.35×10^{-17}	0.19×10^{-9}
Ketene	0.86×10^{-13}	0.50×10^{-9}
Formaldehyde	0.20×10^{-11}	0.14×10^{-11}
Acetaldehyde	0.75×10^{-11}	0.44×10^{-9}
Formic acid	0.13×10^{-10}	0.81×10^{-13}
Acetic acid	0.20×10^{-8}	0.10×10^{-8}
Butyric acid	0.30×10^{-14}	0.10×10^{-10}
Octanoic acid	0.14×10^{-25}	0.23×10^{-14}
Nonanoic acid	0.83×10^{-29}	0.11×10^{-15}
Palmitic acid	$< 10^{-38}$	0.54×10^{-22}
Methanol	0.21×10^{-12}	0.15×10^{-14}
Ethanol	0.86×10^{-14}	0.52×10^{-14}
Octanol	0.15×10^{-31}	0.29×10^{-20}
Acetone	0.51×10^{-11}	0.25×10^{-7}
Dimethyl ether	0.51×10^{-18}	0.31×10^{-18}
Lactic acid	0.87×10^{-22}	0.33×10^{-22}
Oxalic acid	0.65×10^{-13}	0.26×10^{-15}
Pyruvic acid	0.71×10^{-28}	0.95×10^{-26}
Glycerol	0.71×10^{-38}	$< 10^{-38}$
Carbon suboxide	0.24×10^{-27}	0.95×10^{-20}
Benzene	0.96×10^{-4}	
Naphthalene	0.73×10^{-4}	
Asphalt	0.11×10^{-1}	
1,3-Butadiene	0.80×10^{-14}	0.36×10^{-4}
Isoprene	0.26×10^{-15}	0.95×10^{-4}

Source: Eck et al. (1966).

Note: Graphite was omitted from the equilibrium in column 2. Graphite and all aromatic compounds were omitted from the equilibrium in column 3.

Aliphatic carboxylic acids may be obtained by less drastic conditions, e.g., at lower temperatures, as well. Oro et al. (1959), for example, reported that among other acids, formic acid and some hydroxy acids are produced from formaldehyde and hydroxylamine in aqueous solution at moderate temperatures.

Although much information on the formation of hydrocarbons and fatty acids under conditions thought to have prevailed on the primitive Earth has been published, much is left to be accumulated. The data available in this field do not permit a conclusive discussion.

SACCHARIDES AND RELATED COMPOUNDS

Thermal processes at moderate temperatures have been applied to produce sugars under simulated prebiotic conditions. Since 1861, formaldehyde has been known to condense readily to sugars in the presence of alkaline catalysts in aqueous solution (Butlerow, 1861a, 1861b; Loew, 1886, 1887, 1889; Fischer and Passmore, 1889; Langenbeck, 1954). Fischer and Passmore (1889) and later H. Euler and A. Euler (1906) and Schmitz (1913) identified carbohydrates and related compounds such as fructose, cellobiose, sorbose, xylulose, and glycolaldehyde among the condensation products of formaldehyde or glyceraldehyde. They also reconfirmed Loew's finding that the products are optically inactive. Later Mariani and Torraca (1953) demonstrated that about thirty different monosaccharides can be formed by alkali-induced condensation of formaldehyde; these monosaccharides have been detected by paper chromatography (Mariani and Torraca, 1953; Mayer and Jaeschke, 1960; Pfeil and Rueckert, 1961). Pentoses and hexoses are produced in preference to smaller and larger monosaccharides. This is understood on the basis of their stabilizing ring structures. The evidence does not permit the conclusion that the formation of biologically significant isomers is favored. Resuming the earlier work of Orthner and Gerisch (1933), Pfeil and Rueckert (1961) studied in detail the mechanism of the formation of glycolaldehyde, glyceraldehyde, hydroxyacetone, and some higher monosaccharides (Figure 4-3). The reactions probably start with the relatively slow condensation of two molecules of formaldehyde according to the equation that follows (phase of induction or lag phase, see also H. Euler and A. Euler, 1906):



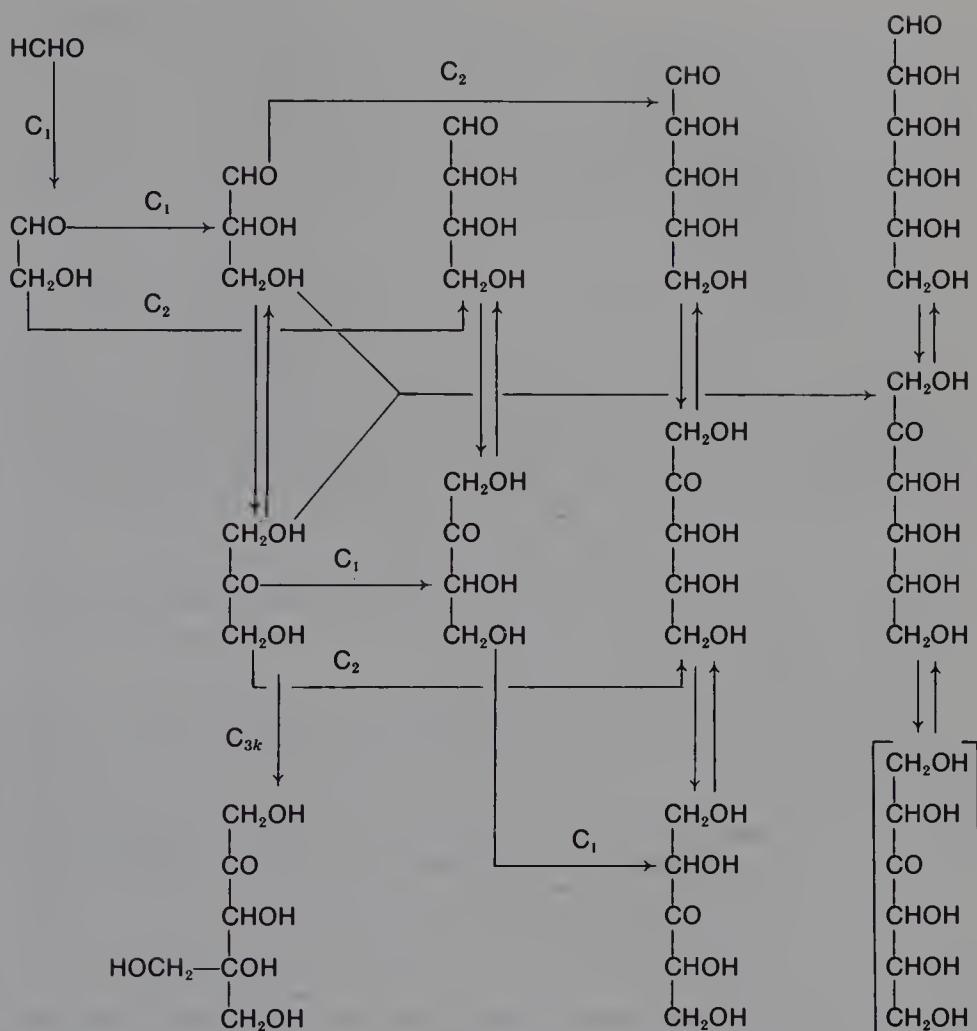
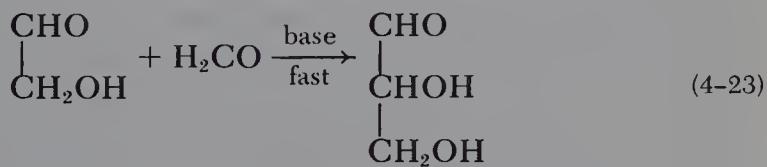


FIGURE 4-3

Formation of monosaccharides from formaldehyde by base catalyses. Source: Pfeil and Rueckert (1961).

The reactions then proceed more rapidly as a base-catalyzed aldol condensation:



In the course of these reactions first trioses, then tetroses, pentoses, hexoses, etc., are formed (see also Langenbeck, 1942, 1954; Mayer and Jaeschke, 1960).

The alkali-catalyzed condensation of formaldehyde to sugars proceeds best in the presence of alkaline earth hydroxides or weakly basic hydroxides of lead and tin, as already indicated by Loew's experiments of 1886-1889. However, tetramethylammonium hydroxide, a strong base, does not catalyze the condensation, unless the salt of an alkaline earth metal is added (Akerlof and Mitchell, 1963). Aldol condensations generally are not dependent on the presence of any polyvalent cation. The presence of the bivalent cations could therefore be required to induce the condensation of formaldehyde to glycolaldehyde, according to Wanzlick (1962) and Breslow (1959). These suggestions cannot be fully generalized, however, because Oro and Cox (1962) found that the aldol-type condensation of acetaldehyde and glyceraldehyde (or formaldehyde) yielding 2-deoxyribose and other sugars is accelerated best by bivalent cations (e.g., Ca^{2+}). A scheme to explain the formation of various monosaccharides from formaldehyde is presented in Figure 4-3. The mechanisms for monosaccharide synthesis have to be elucidated more carefully before pertinent conclusions may be drawn.

Formaldehyde was identified among the compounds formed in the first molecular-evolution experiments of modern times (Groth and Suess, 1938; Garrison et al., 1951). Formaldehyde is abundantly produced when gaseous mixtures consisting in part of methane and water vapor are subjected to an electric discharge (Miller, 1955), to γ -irradiation (Palm and Calvin, 1962), or to β -irradiation (Ponnamperuma and Flores, 1965). Ponnamperuma (1965) claimed that sugars (ribose and deoxyribose) are directly formed by electron irradiation of methane, ammonia, and water. Even in the contemporary atmosphere, photochemical formation of formaldehyde is significant. As much as one milligram per milliliter of formaldehyde has been found in rain water (Dhar and Ram, 1933), but it must be noted that all the formaldehyde found in rain water is not necessarily of abiotic origin. Formaldehyde may now be formed as a product of degradation of organic matter, e.g., by forest fires.

Because of the importance of nucleic acids and their constituents, experiments have recently focussed on the origin of pentoses.

Ultraviolet irradiation or γ -irradiation (^{60}Co) of aqueous formaldehyde induces the formation of ribose, deoxyribose, and other sugars (Ponnamperuma and Mariner, 1963; Ponnamperuma, 1965). The condensation of formaldehyde to various sugars by ultraviolet light was first described in 1924 (Irvine and Francis, 1924). Oro and Cox (1962) have found that 2-deoxyribose and its isomer, 2-deoxxylose, are formed in yields as high as 15 percent by heating glyceraldehyde and

acetaldehyde in aqueous solutions. The synthesis of 2-deoxyribose from these two aldehydes is catalyzed with particular effectiveness by bivalent cations (Table 4-16).

Recently, Gabel and Ponnampерuma (1967) refluxed aqueous solutions of formaldehyde of various concentrations over kaolinite, in an attempt to simulate the conditions of a hot spring on the primitive Earth. The products were separated into trioses, tetroses, pentoses, and hexoses. At a formaldehyde concentration of 0.5 M, only trioses, tetroses, and pentoses were formed. When the concentration of formaldehyde was reduced to 10^{-2} M, hexoses were formed as well. Ribose was found in the end products. Postulation of a primitive hot spring in an environment rich in kaolinite would eliminate the need for postulating a primitive basic ocean (page 37) containing huge amounts of formaldehyde and its products of condensation.

Abelson (1966) has pointed out that destructive processes would have operated on any formaldehyde in the atmosphere or in the ocean of the primitive Earth. Since springs are usually more shortlived than oceans anyway, the Abelson thesis does not necessarily affect the validity of the Gabel-Ponnampерuma experiments. Formaldehyde produced in the atmosphere would have tended to react with hydrogen cyanide or would have been destroyed slowly by heat or ultraviolet light according to the equation



before reaching the hydrosphere or lithosphere. Only small amounts of formaldehyde could have reached the primitive ocean, to which Abelson assigns a pH 8-9. A fraction of this formaldehyde might have

TABLE 4-16
Bases that Catalyze the Synthesis of 2-Deoxyribose

High Activity	Moderate Activity
MgO	NH ₄ OH
Ca(OH) ₂	N(Me) ₄ OH
Ba(OH) ₂	N(Et) ₄ OH
	LiOH
	NaOH
	KOH

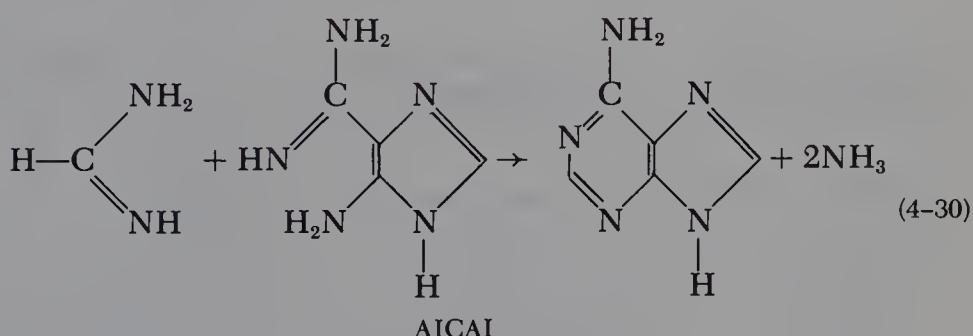
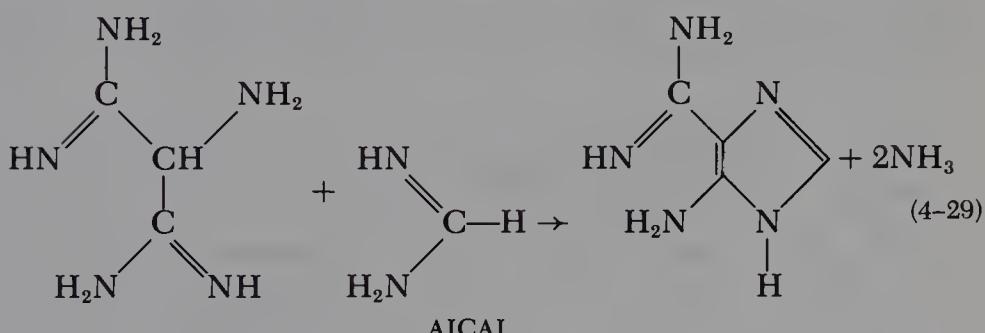
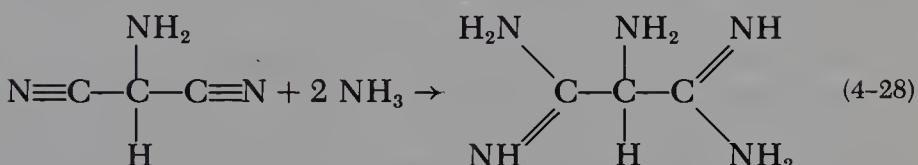
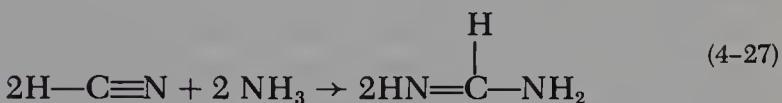
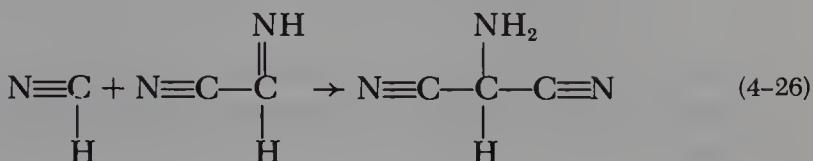
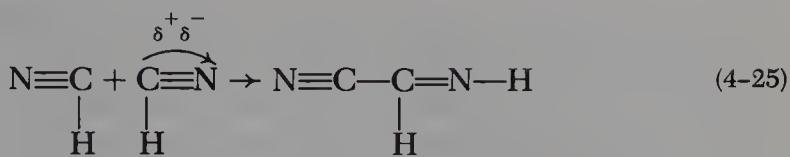
Note: Reaction conditions: 0.1% glyceraldehyde + 0.1% acetaldehyde; 0.1 M base; 50°C; one hour.

Source: Oro (1965).

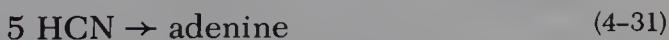
reacted to yield sugars. The formation of sugars presumably competed with the formation of paraformaldehyde or hexamethylenetetramine in the presence of ammonia. Neither sugars nor their precursors would have been stable in the presence of amino acids in the primitive hydrosphere, because amino groups readily react with carbonyl groups (Maillard reaction, formation of Schiff bases; Banu and Jivu, 1968; Cram and Hammond, 1967). We need, however, to bear in mind the possibility that the primitive ocean or hydrosphere was acidic (page 37).

PURINES, PYRIMIDINES, AND RELATED COMPOUNDS

Purines and pyrimidines are essential constituents of nucleic acids, and many derivatives of adenine are important coenzymes. Adenosine triphosphate (ATP), nicotinamide adenine dinucleotide (NAD), and coenzyme A are a few examples. Much attention has been focussed on the origins of purines and pyrimidines because of developments in molecular biology. Since 1960, Oro and co-workers (Oro, 1960; Oro and Kimball, 1961a, b, 1962; Oro, 1965) have synthesized various compounds by heating solutions of hydrogen cyanide (1–15 M) in aqueous ammonia for one or several days at moderate temperatures. In these experiments one major product from hydrogen cyanide is an insoluble black polymer that yields certain amino acids on hydrolysis (page 182). After this black residue is removed by centrifugation or filtration, adenine can be isolated from the red-brown supernatant by chromatographic methods. Adenine is the main ultraviolet-absorbing compound among the products and has been identified by at least eight different procedures. Among these are ultraviolet spectrophotometry, determination of the melting point of its picrate, and the specific method of Gerlach and Doering (1955). From a 11.1 M hydrogen cyanide reaction mixture, a yield of 110 mg of adenine per liter of original mixture was obtained. This synthesis has been confirmed by Lowe et al. (1963). In detailed studies, Oro and Kimball (1961b, 1962) have shown that 4-aminoimidazole-5-carboxamidine and formamidine are probable intermediates in this synthesis. On the basis of their experimental results and by reviewing the relevant literature (Voelker, 1957, 1960), Oro and Kimball proposed the following reaction scheme:



According to this scheme, the overall reaction is



A more complete discussion of this mechanism may be found elsewhere (Oro, 1961b, 1963a; Oro and Kimball, 1962).

Besides reacting with formamidine according to Equation (4-30), 4-aminoimidazole-5-carboxamidine (AICAI) may also undergo partial hydrolysis to yield 4-aminoimidazole-5-carboxamide (AICA). AICA may react with guanidine or formamidine (Oro and Kimball, 1962) to yield guanine, xanthine, or hypoxanthine. These reactions are summarized in Figure 4-4.

Sanchez et al. (1966a) confirmed that aminomalonitrile is, in fact, one important intermediate in adenine formation. Their suggestions and experimental results are in line with the work of Oro and Kimball (1961a, b, 1962).

Hypoxanthine has been detected among the products formed from hydrogen cyanide, ammonia, and water (Lowe et al., 1963). Guanine is also obtained by condensation of 4-aminoimidazole-5-carboxamide with cyanogen (Sanchez et al., 1966a, b).

Some alternatives to Oro's reaction schemes have been proposed by other authors. According to Calvin (1961, 1962, 1969), the unstable trimer of hydrogen cyanide, aminomalonitrile, would be formed first (Equations 4-25 and 4-26). The aminomalonitrile would subsequently react with a fourth hydrogen cyanide molecule to produce 4-amino-5-cyanoimidazole:

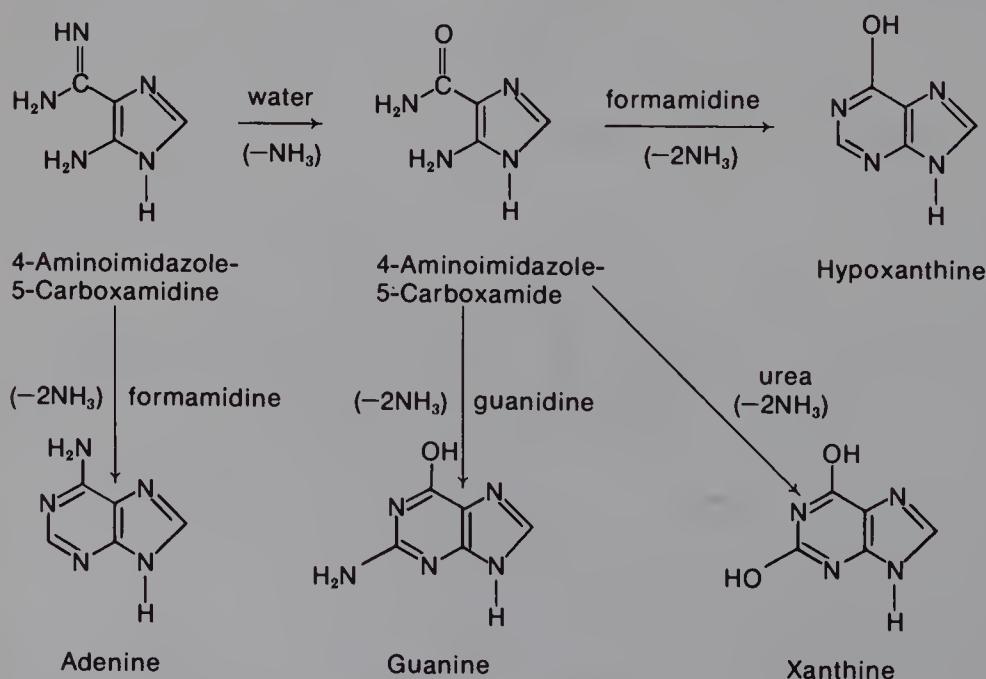
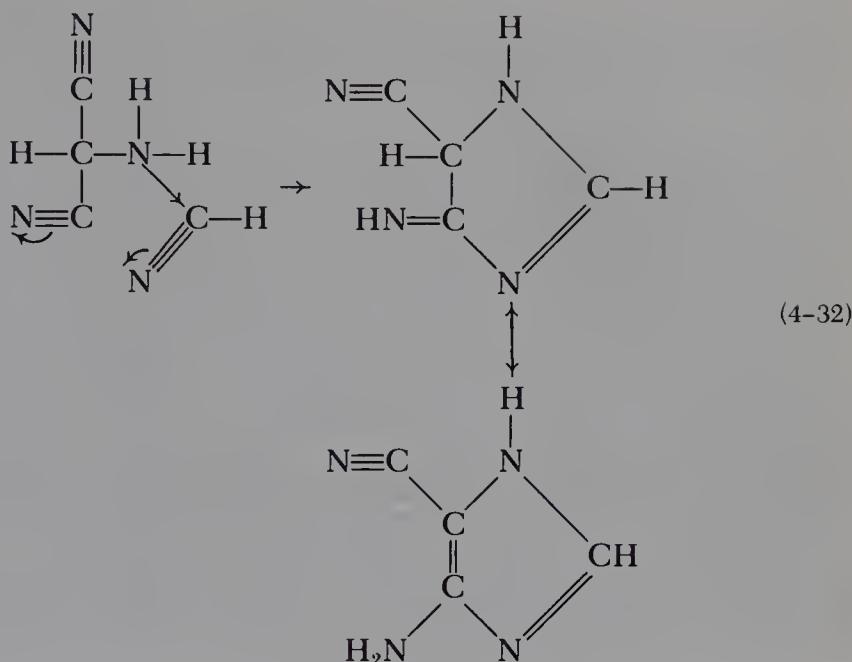
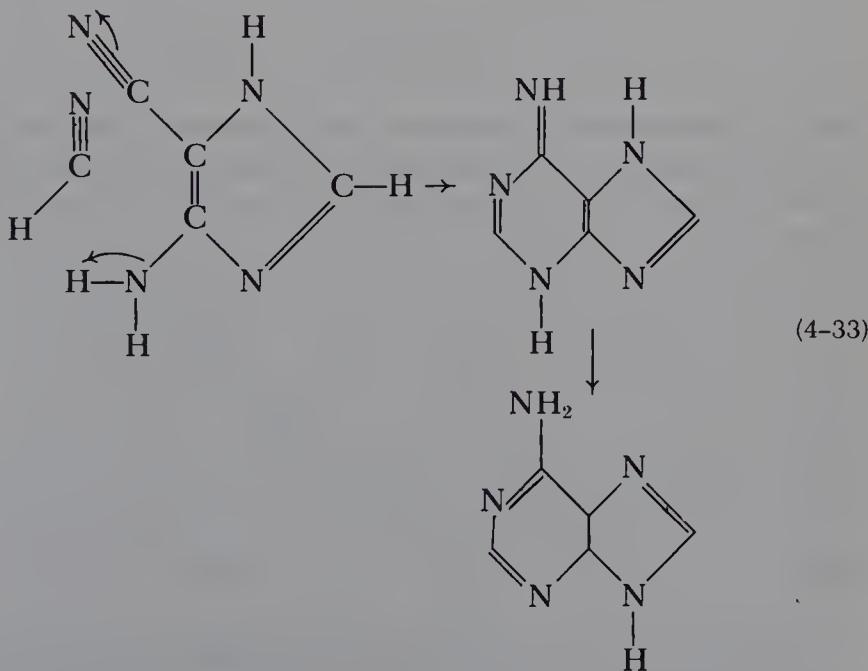


FIGURE 4-4
Proposed mechanism for the synthesis of purines on the primitive Earth. Source: Oro (1961b).

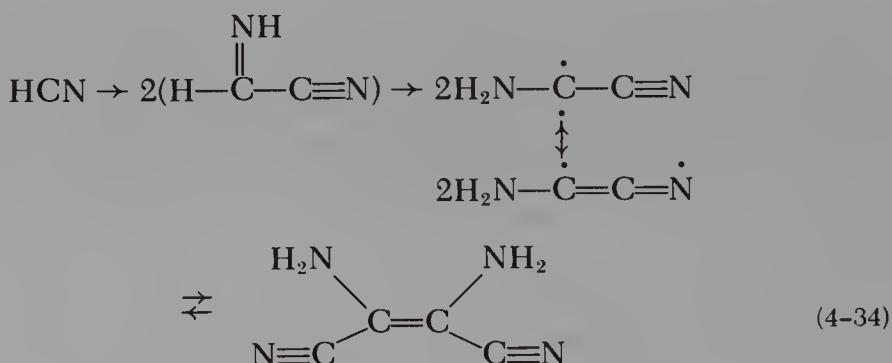


Aminomalonitrile analogously yields 4-aminoimidazole-5-carboxamidine when the reactant hydrogen cyanide is replaced by formamide, as shown by Ferris and Orgel (1965). In this reaction one mole of ammonia is released. The synthesis of adenine by addition of hydrogen cyanide to 4-aminoimidazole-5-carbonitrile, and subsequent ring closure [reaction (4-33)], was confirmed by the same authors.

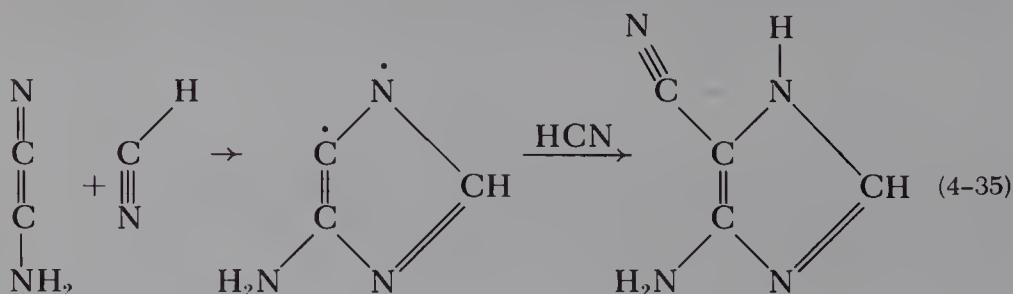


The same intermediate, 4-aminoimidazole-5-carbonitrile, is also obtained by photochemical isomerization of diaminomaleonitrile, the HCN tetramer (Sanchez et al., 1968; Ferris et al., 1969). Ferris et al. (1969) also showed a pathway for a prebiotic formation of 6-aminonicotinonitrile, a potential nicotinamide-like hydrogen transfer system. The starting material for this synthesis is cyanoacetylene, which is first hydrolyzed to cyanoacetaldehyde, which in turn condenses to 1,3-dicyano-4-hydroxy-1,3-butadiene. The latter compound is converted into 1,3-dicyano-4-amino-1,3-butadiene in the presence of ammonia. Then a subsequent cyclization to 6-aminonicotinonitrile is achieved by ultraviolet irradiation. This step has been verified by the same authors.

Kliss and Matthews (1962) have suggested that two molecules of hydrogen cyanide interact to produce iminoacetonitrile or amino-cyanomethylene, which would react like a carbene or a diradical. Either of the latter might then give rise to the tetramer, diaminomaleidinitrile, or to polyaminomalonitrilimine:



These reactions would compete with reaction (4-35), which yields a radical derivative of aminoimidazole. Upon subsequent addition of another hydrogen cyanide molecule, 4-aminoimidazole-5-carbonitrile is formed:



A radical mechanism, as outlined by reactions (4-34) and (4-35), may also apply if cyanide is exposed to various forms of radiation.

Ponnamperuma (1965) has reported the formation in very small yields of adenine and guanine by exposing a dilute solution of hydrogen cyanide to ultraviolet light.

Although hydrogen cyanide is abundantly produced by exposing a reducing atmosphere to electric discharges, Miller (1955) failed to detect purines, pyrimidines, or any other defined aromatic compound among the products. Despite many efforts to trace purines and pyrimidines, none were definitely identified among the end products of the electric-discharge experiments. An explanation of why even small amounts of these compounds are not found deserves some analysis. Oro's synthesis of adenine, which is itself an unsaturated compound, begins with the highly unsaturated $\text{HC}\equiv\text{N}$ as the primary reactant. Also, free hydrogen cyanide was a byproduct in Miller's experiment. Moreover, the atmosphere in Miller's experiment was overladen with H_2 . Accordingly, we can understand that from such a hydrogen-rich atmosphere (page 74) Miller did not detect adenine or other aromatic compounds of biological significance.

Palm and Calvin (1962) β -irradiated similar hydrogen-rich atmospheres of methane, ammonia, hydrogen, and water vapor and they presented evidence that 4-aminoimidazole-5-carboxamide and adenine may have been formed. The formation of adenine was better established when Ponnamperuma, Lemmon et al. (1963), in the same laboratory, repeated these experiments more systematically. The latter authors found that adenine is produced in highest yields, about 0.01 percent of the starting methane, when the starting material does not contain free hydrogen. When discussing the mechanism of synthesis of adenine from the constituents of a primitive atmosphere, these authors referred to the scheme proposed by Oro and Kimball (see Figure 4-4). In fact, hydrogen cyanide is produced in significant amounts by exposing "reducing atmospheres" to β -rays (Palm and Calvin, 1962).

The selectivity with which adenine is formed in various evolutionary experiments seems to be correlated with its particularly high resonance energy (Pullman and Pullman, 1962), which likely contributes also to its increased stability against ultraviolet and ionizing radiations. The yields in the thermal formation of adenine from hydrogen cyanide were greatly increased by use of the anhydrous reactant in liquid ammonia; this latter conversion has been developed into an industrial process (Wakamatsu et al., 1966). The known biologically important pyrimidines have a relatively low resonance energy compared to adenine. This in turn is probably related to the relative sensitivity of pyrimidines to radiations and to the low yields with which they are formed in most evolutionary experiments. Pyrimidines are formed in substantial yields under less violent conditions.

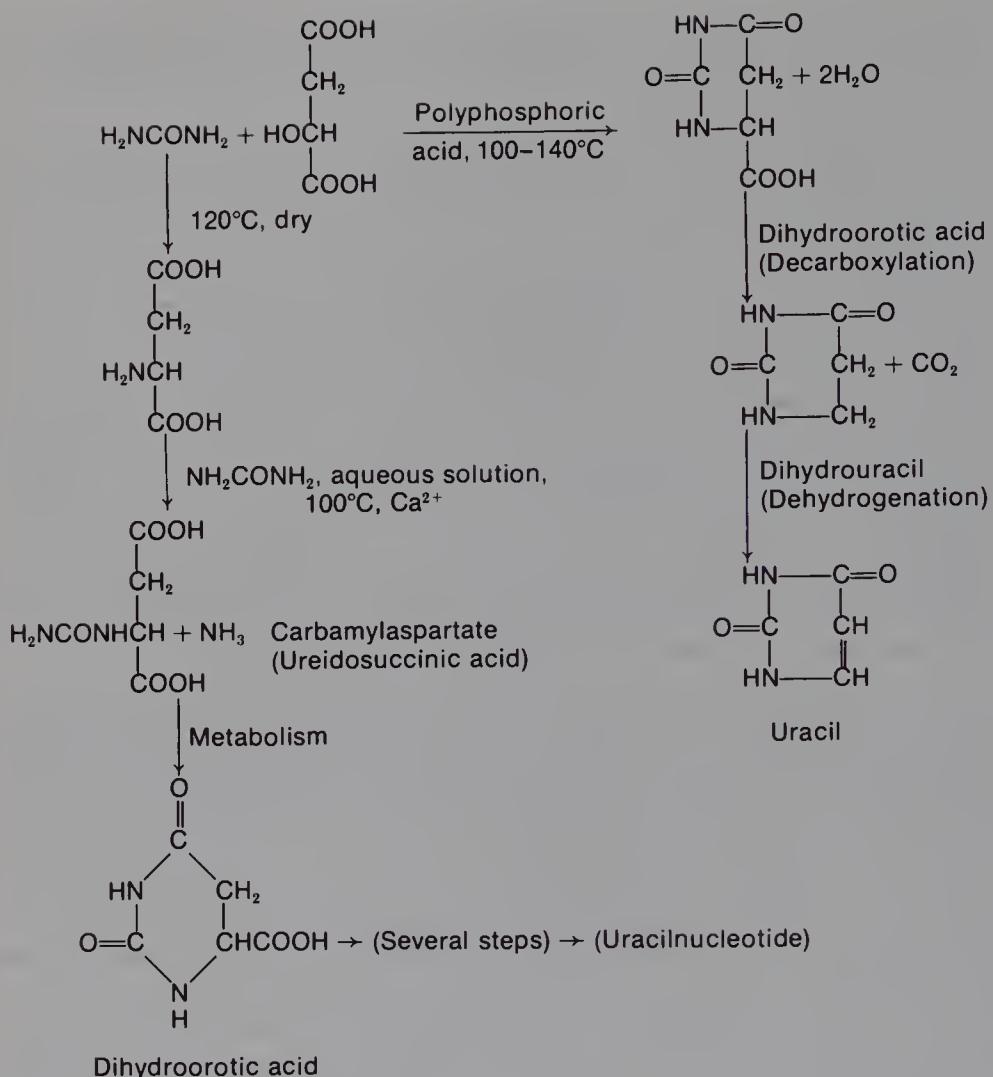


FIGURE 4-5

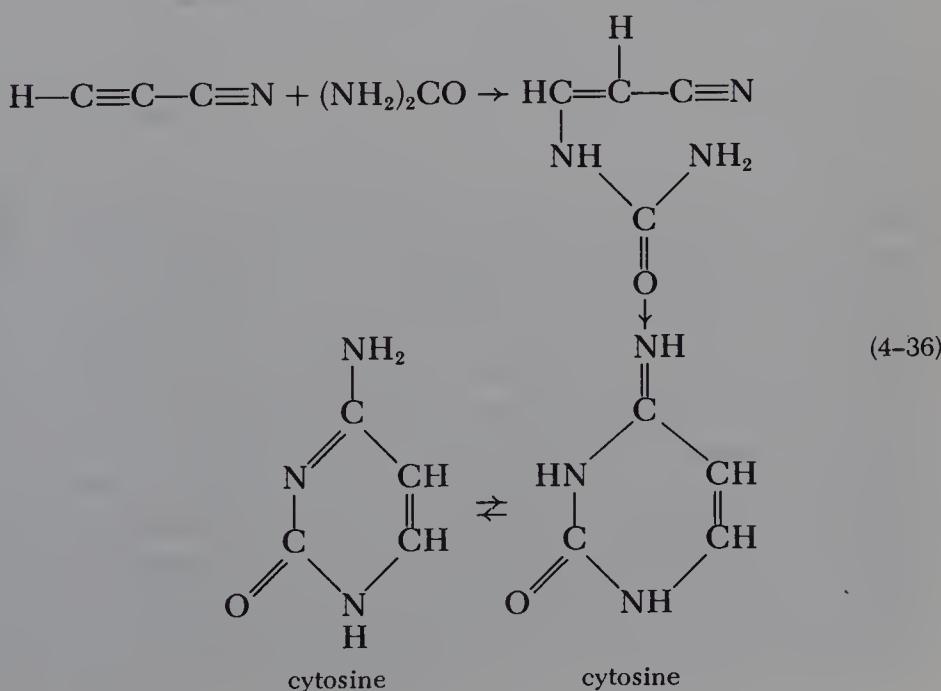
Biotic, and model of prebiotic, pathways to uracil. Source: Fox et al. (1956).

Pyrimidines, as well as the key biological intermediate, ureidosuccinic acid, can be prepared under a variety of simple, geological conditions. Lippich (1908) published a report on the production of ureidosuccinic acid from aspartic acid and urea in the presence of barium hydroxide in aqueous solution. Fox et al. (1956) showed that the reaction could proceed in the presence of either calcium hydroxide or magnesium hydroxide under conditions essentially like those of hot springs. The starting compound, aspartic acid, has been formed earlier from malic acid and urea (Fox et al., 1955). In 1961 the pyrimidine, uracil, was reported as synthesized under presumably prebiotic conditions (Fox and Harada, 1961). This synthesis of uracil was demonstrated by heating of malic acid and urea for 15–120 minutes at

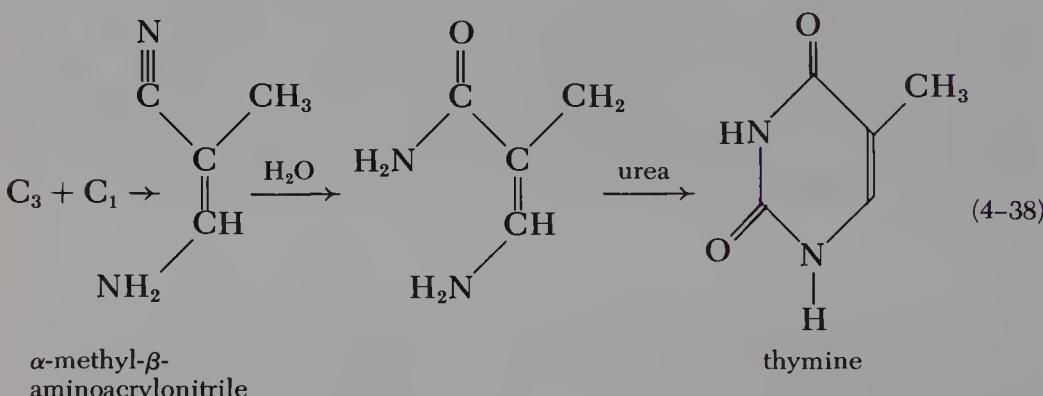
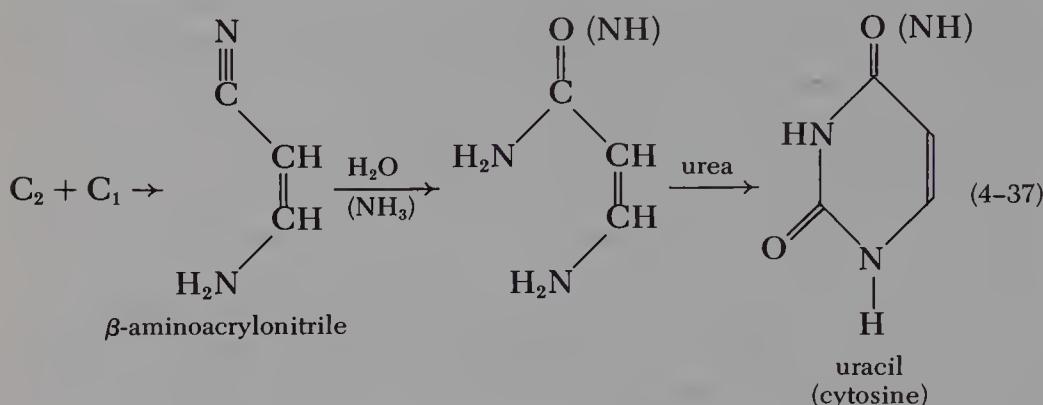
100–140°C in the presence of polyphosphoric acid. From 10 micromoles of malic acid, 1.4 micromoles of uracil was obtained. A detailed reaction mechanism was not offered, although the similarity between the thermal synthesis and the biological synthesis was pointed out. Malic acid, one of the precursors in the synthesis of uracil, can be visualized as producible in the evolutionary context from acetic acid by ionizing radiation (Garrison et al., 1953), which could have arisen directly from a primitive atmosphere exposed to electric discharges (Miller, 1955) or to ionizing radiation (Dose, 1962; Dose and Risi, 1968). Urea, which figures in each of the steps, is abundantly produced under the same experimental conditions. The origin of acidic polyphosphates will be explained (page 122).

The reactions in the prebiotic and biotic syntheses of uracil mentioned are shown in Figure 4–5. The similarity of the two pathways is evident. How the dehydrogenation steps might occur is not clear. Dihydroorotic acid, however, is easily oxidized in metabolism.

In another approach, Sanchez et al. (1966b) demonstrated that cyanoacetylene might be an important intermediate in the synthesis of a number of pyrimidines. Cyanoacetylene is a major nitrogen-containing product obtained by the action of electric discharges on a mixture of methane and nitrogen. By fusing cyanoacetylene with urea, for example, cytosine can be produced. In this reaction, β -ureidoacrylonitrile may be formed as an intermediate. This could yield cytosine upon cyclization and tautomerization according to the following equation:



Oro (1963d) found that acrylonitrile, β -aminopropionitrile, or β -amino-propionamide may condense with urea in aqueous systems at 135°C to yield uracil. He further theorized that the C₁, C₂, and C₃ fragments detected spectroscopically in comets would give rise to β -amino-acrylonitrile, α -methyl- β -aminoacrylnitrile, or related compounds. The following mechanisms for the synthesis on the primitive Earth of pyrimidines from β -aminoacrylonitrile or α -methyl- β -aminoacrylonitrile have been proposed (Ferris et al., 1968):



Carbon suboxide, C₃O₂, which is found in cosmic bodies, may also prove to be a suitable intermediate for the synthesis of pyrimidines and other compounds (Oro, 1961a). So far, however, experimental evidence has yet to be presented to support the theoretical reaction schemes. Pyrimidine derivatives are produced in relatively high yields by X-irradiating aqueous solutions of β -alanine, ammonia, and urea or cyanide. One component was identified as barbituric acid; significant amounts of the well-known pyrimidine bases were not among the reaction products (Dose et al., 1964; Dose, unpublished).

The formation of adenine upon exposing a gaseous mixture of methane, ammonia, and water vapor to ionizing radiation (Ponnamperuma, Lemmon et al., 1963) is well established. However, most ioniz-

ing radiations originate in the lithosphere and do not affect the atmosphere very much. But adenine and guanine are also produced in model prebiotic experiments from dilute solutions of hydrogen cyanide or its oligomers and ammonia under various experimental conditions (Oro, 1965). Pyrimidines seem not to be formed directly by exposing presumably primitive, H₂-poor atmospheres to the various forms of energy. Pyrimidines and related compounds are obtained, however, by heating or irradiating aqueous solutions of various C₃ molecular species, such as β -aminoacrylamide, in presence of urea, hydrogen cyanide, or ammonia, to mention only those experiments that most likely are pertinent to an evolutionary context. Even for these results, however, more evidence has to be presented about the yields and the identities of most of the products. Justification for relating most of the liquid-phase experiments, especially those employing high concentrations of volatile reagents, to the context of molecular evolution is lacking also (page 128).

NUCLEOSIDES, NUCLEOTIDES, AND "ENERGY-RICH" PHOSPHATES

Many investigators have adopted the assumption that the prebiotic formation of nucleic acid-type materials was a crucial step in the emergence of life (page 241). Accordingly, the origin of nucleic acids and of their constituents deserves special attention. Pentoses, purines, and pyrimidines have been obtained in evolutionary experiments. This section will show that, to some extent, nucleosides can be phosphorylated under plausible prebiotic conditions to yield nucleotides. Moreover, nucleotides may be condensed to polynucleotides, though to only a relatively low degree of polymerization, according to evidence so far presented (page 185).

This section reemphasizes the probable contributions of phosphate to prebiotic chemistry (Fox and Harada, 1961). The uncertainties that apply to prebiotic chemistry apply with special force to compounds of phosphorus; this problem has been reviewed and debated by Gulick (1955). As is well known, the phosphates play a very large role in biochemical reactions. Especially, they permit energy-requiring reactions to occur in an aqueous milieu (not in aqueous solution). Their service in the metabolism of organisms suggested their initial function in polymerization of amino acids in hypohydrous media (Fox and Harada, 1958; Fox, 1960). The general utility of phosphoric media (as anhydritizing agents, as activating agents, as phosphorylating agents, and in some cases as acid catalysts) has suggested that the

first biochemical pathways evolved in a predominantly phosphoric medium (Fox and Harada, 1961). Amino acids have been condensed, mononucleotides have been condensed, uracil has been formed, nucleosides have been phosphorylated [including adenosine to adenosine triphosphate (ATP)], and other reactions have been achieved by the use of phosphoric acid or polyphosphoric acid and by their salts, and sometimes by polyphosphoric acid derivatives (e.g., ethyl metaphosphate). A number of these reactions have not been demonstrated in dilute aqueous solution with the same results, despite many attempts. We can visualize that the function of phosphates in prebiochemical reactions might have evolved to very similar functions in biochemical reactions in particulates not in aqueous solution.

Nucleosides and Related Compounds

Early attempts (Ponnamperuma, Mariner et al., 1963) to demonstrate a simple prebiotic synthesis of adenosine entailed heating deoxyribose or ribose with adenine, cytosine, or guanine in the dry state for a few minutes at temperatures in the range 130–170°C. Several adducts that were obtained could be characterized by chromatography and by the kinetics of their hydrolysis. The two major products obtained from deoxyribose and adenine, for instance, were tentatively identified as two diastereoisomeric forms of 2,3-dideoxy-(9-purinyl)-pentose (Reid et al., 1967). Earlier reports on the formation of adenosine and other nucleosides under similar experimental conditions (Ponnamperuma and Kirk, 1964) were not confirmed.

The failure to confirm the results claimed earlier was reported in joint work in which two of the authors were from the laboratory of Orgel (Reid et al., 1967). This paper referred to the production of adducts of deoxyribose and ribose with adenine, cytosine, or guanine by heating the *dry* components at 130–170°. This result was presented in more detail later (Fuller et al., 1972a, b). The addition of small amounts of water inhibited the condensation. The various attempts to achieve nucleoside synthesis under plausibly prebiotic conditions have been quite unsuccessful so far. The problems involved are now more widely recognized (Miller and Orgel, 1974) although they are rooted simply in the rather complex stereochemistry of biologically relevant nucleosides. Many investigators who sought to synthesize adenosine or related compounds from adenosine and ribose obviously did not become aware of the many isomers (including optical antipodes) which can be produced in these experiments. In the ab-

sence of a stereospecific control during the synthesis, the yields for adenosine and related nucleosides are expected to remain very low.

So far, the various fruitless attempts to produce adenosine and other nucleosides and deoxynucleosides under simulated conditions of the primitive Earth suggest that these compounds gained their important places in biological systems in a later phase of evolution. Failure to produce such compounds, however, may simply reflect the fact that appropriate experiments have not been performed. The suggestion has been made that in prebiological and the first biological systems the place of nucleosides or deoxynucleosides as coenzymes or constituents of genetic material was taken by other compounds.

It is increasingly apparent that anhydropolymerizations (page 139) can explain key prebiotic chemical events that yielded condensation polymers.

Mono- and Oligo-phosphates of Nucleosides and Related Compounds

The above results are different when phosphoric acid is introduced into the reaction. Ponnamperuma (1965) reported that adenosine (yield of about 0.01 percent), but no nucleotide, is produced by heating dilute aqueous solutions of adenine, ribose, and phosphoric acid. According to Ponnamperuma, ribose is first phosphorylated to ribose-1-phosphate and then the phosphate residue is replaced by the purine residue to yield adenosine. As stated, however, the criticism that has been raised for the formation of adenosine in the absence of phosphoric acid (Reid et al., 1967) may also apply to its formation in the presence of phosphoric acid. Adenosine is not necessarily an intermediate in the formation of ATP, because ribose is phosphorylated quite easily to ribose phosphates (Halmann et al., 1969), which might in turn give direct rise to adeninepentose phosphates. Adenosine monophosphate would probably be among the products thus obtained. Since any HO-group of the pentose may condense with phosphoric acid or react with adenine, the prebiotic production of a variety of unusual adenylpentose phosphates or isomers of adenosine can be visualized.

Of questionable prebiotic relevance are the attempts to produce AMP, ADP, ATP, and other nucleotides with ethyl metaphosphate as phosphorylating agent (Ponnamperuma, 1965; Schramm et al., 1962). The identification of the compounds produced, moreover, was mostly based on single chromatographic constants. Only for ATP was

more rigorous evidence presented by performing a successful luminescence test with dehydrated firefly tails (Strehler and Totter, 1952). Ethyl metaphosphate or related organic phosphates, however, have so far not been produced in experiments simulating primitive Earth conditions.

Although Ponnampерuma (1965) did not achieve a phosphorylation of adenosine by phosphoric acid in aqueous solution by ultraviolet light, he and his associate later produced AMP and other nucleotides by heating moist mixtures of nucleosides and inorganic phosphates such as disodium monohydrogen, trisodium, sodium ammonium monohydrogen, ammonium dihydrogen, diammonium monohydrogen, monocalcium, and tricalcium monophosphates in sealed tubes (Ponnampерuma and Mack, 1965). For the prebiotic formation of these materials the source of heat would have had to have been volcanic. The experimental conditions must be regarded as hypohydrous because of the relative absence of water; small amounts of water do not hinder the phosphorylation processes. Among the phosphoric acid esters that were formed, about equal amounts of the 2'-, 3'-, 5'-, and cyclic monophosphates of adenosine were identified, mostly by paper chromatography. The best yields were obtained at about 160°C. The yields were quite small at 50°C, however. The yields were also influenced by the source of inorganic phosphate. They were highest when orthophosphoric acid or its monobasic salts were used. A marked decline of yields with the dibasic salts and a more pronounced decrease with the tribasic salts of orthophosphoric acid was observed. These results indicate that the formation of nucleoside phosphates takes place more easily with the higher states of protonation of the phosphate residues (cf. Waehneldt and Fox, 1967; Rabinowitz et al., 1968).

Waehneldt and Fox (1967) demonstrated that the nucleosides—adenosine, cytidine, guanosine, uridine, and thymidine—are phosphorylated with polyphosphoric acid at temperatures in the range 0–22°C. All biologically significant mono-, di-, and triphosphates of nucleosides have been obtained by this procedure. With adenosine as starting material, e.g., 5'-AMP, 2'-AMP, 3'-AMP, 5'-ADP, 5'-ATP, and at least three other unidentified phosphates were found by chromatography, electrophoresis, and ultraviolet spectroscopy. ATP was also identified by a firefly lantern test according to McElroy and Green (1956). The total yields of conversion ranged from 25 percent to 45 percent. Although these yields are very high relatively, the occurrence of acidic polyphosphates in other than restricted locales on the primitive Earth has been debated. The possibility of the occurrence of polyphosphoric acid as a conversion product of ammonium phosphate is discussed on pages 121 and 189. Deoxyadenosine

and deoxyguanosine do not yield defined nucleotides under the experimental conditions stated above, although pyrimidine deoxyribonucleosides are successfully phosphorylated. In the presence of polyphosphoric acid, the phosphorylation of the nucleosides and the polymerization of the resultant nucleotides occur in the same reaction (page 185).

So far, the various fruitless attempts to produce adenosine and other nucleosides and deoxynucleosides under simulated conditions of the primitive Earth suggest that these compounds gained their important places in biological systems in a later phase of evolution. Failure to produce such compounds, however, may simply reflect the fact that appropriate experiments have not been performed. The suggestion has been made that in prebiological and the first biological systems the place of nucleosides or deoxynucleosides as coenzymes or constituents of genetic material was taken by other compounds.

Inorganic Oligo- and Poly-phosphates and Simple Organic Derivatives

Polyphosphates other than ATP, ADP, and related compounds may very well have been the first source of active phosphates. Inorganic pyrophosphate has been considered to be a primitive group carrier in biological systems (Baltscheffsky, 1974; Kulaev, 1974). Inorganic polyphosphates, specifically, were very likely formed by heat on the primitive Earth from various phosphate minerals (page 122). Today the only significant source of phosphorus on the surface of the Earth appears to be apatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{F}, \text{Cl}, \text{OH})_2$. Before the major portion of phosphates of the primitive Earth was precipitated in the form of insoluble and relatively heat-stable calcium phosphates, soluble phosphate minerals were probably more abundant than they are today. Among these minerals were probably microcosmic salt or stercorite ($\text{NH}_4\text{NaHPO} \times 4\text{H}_2\text{O}$), struvite ($\text{Mg}(\text{NH}_4)\text{PO}_4 \times 6\text{H}_2\text{O}$), and $(\text{NH}_4)_2\text{HPO}_4$ (if the primitive hydrosphere contained significant amounts of ammonia). On the contemporary Earth these minerals are found mostly in guano and in other decomposing organic matter. When exposed to the contemporary atmosphere, these minerals decompose slowly, although they are stable in an ammonia-containing environment such as that postulated for the primitive Earth. If stercorite is gradually heated to about 300°C, water and ammonia are released and the resulting glass-like material consists of metaphosphates and pyrophosphates of sodium called Graham's salt (Tammann, 1892). Graham's salt is hygroscopic and yields a viscous fluid on con-

tact with water. The *pH* (1–5) depends on the origin of stercorite and the degree of its contamination with ammonium phosphate. Amino acids are readily polycondensed at 100°C during three days in the presence of acidic Graham's salt, which, like polyphosphoric acid, acts as solvent and dehydrating agent (Dose and Brand, unpublished). On heating aqueous solutions of Graham's salt and adenosine at 100°C in 0.75 N NH₄OH, adenosine 2'-, 3'-, and 5'-phosphates are produced in significant yields (Schwartz and Ponnampерuma, 1968).

One of the strongest arguments for the existence of polyphosphoric acid or polyphosphates on the primitive Earth is the long known behavior of diammonium hydrogen phosphate, (NH₄)₂HPO₄ (Knorre, 1900). If the primitive hydrosphere contained ammonia, some of the phosphate would have been present as this mineral. When heated to 155°C, as Knorre showed, the water would have evaporated and the mineral would have decomposed to polyphosphates including pyrophosphates, metaphosphates, and orthophosphates. These phosphates would have assumed the form of acidic ammonium salts. Osterberg and Orgel (1972) have demonstrated the plausibility of this synthesis (page 154).

The examples given demonstrate that dimers or polymers of phosphoric acid are readily obtained by the heating of some phosphate minerals, which, in high likelihood, existed, at least at some locales, on the primitive Earth. Even extremely acidic materials are produced by the heating of ammonium phosphates or alkali ammonium hydrogen phosphates. The concentration of phosphate ions in the primitive waters was probably not as low as it is now. Insoluble calcium phosphates could be formed only after weathering and leaching had released sufficient amounts of calcium from the lithosphere.

Gulick (1955) has suggested that hypophosphites, which are more soluble than orthophosphates, would have been in redox equilibrium with the latter under the reducing conditions that prevailed on the primitive Earth. However, all lower oxidation states of phosphorus are unstable "under any pressures of hydrogen that are reasonable," according to objections of Horowitz and Miller (1962). No definite answer to these and other questions can be given because no evidence permits calculations of the partial pressure of hydrogen in the primitive atmosphere (page 39) or of the abundance of the kind of phosphorus-containing minerals in the primitive lithosphere.

Ferris (1968) has suggested cyanovinyl phosphate as a potential prebiological phosphorylating agent. In their search for energy-rich phosphates that might be considered to have been simple precursors of ATP, ADP, and other "active" phosphates, Miller and Parris (1964)

found that pyrophosphate may be produced from hydroxyapatite and cyanate salts at pH 6.5 in yields of 27 percent relative to the cyanate salt added. Since the resulting calcium pyrophosphate is insoluble, as is apatite or hydroxyapatite, the reactions yielding pyrophosphate must take place on the surface of the apatite. So far, these authors have presented no experimental evidence to demonstrate the use of calcium pyrophosphate as a source of active phosphate in evolutionary or biochemical experiments. The production of soluble inorganic polyphosphates by heating the appropriate minerals offers a simpler and more direct route in this context. Although Miller and Parris proposed the spontaneous synthesis of pyrophosphate from cyanate and hydroxyapatite in order to obviate the need for elevated temperatures (300°C), they failed to state that the production of the cyanate itself requires reactions at high temperature.

Referring to apatite as the most abundant form of phosphorus on the contemporary Earth, Neumann and Neumann (1964) and Burton et al. (1969) have suggested that apatite may play a role in condensation reactions leading to nucleosides and nucleotides. According to other opinions, phosphoarginine, phosphoguanidine (Gulick, 1955), phosphoimidazole (Brinigar et al., 1967), and similar compounds may be regarded as forms of energy-rich phosphates that might have been used in vital systems, though evidence for this use is not at hand.

Evidently, the formation of soluble phosphorus compounds, inorganic or organic, as a crucial requisite for the evolution of nucleosides is so far not well established. The evolutionary appearance of nucleotides, as simulated by experiments, finds some support, although many gaps remain to be closed.

PORPHYRINS

Most of the authors who theorize on the evolution of living systems have come to the conclusion that porphyrins have been required in at least two stages (Oparin, 1953, 1962; Gaffron, 1960; Calvin, 1959, 1962; Sagan, 1961; Evstigneev, 1975). One stage was the evolution of porphyrin-dependent photosynthesis. This must have come into existence early in the evolution of living systems, according to evidence obtained from fossil vanadium-porphyrin complexes dated to be at least 2×10^9 years old (Chapter 10). The second stage was the evolution of porphyrin-dependent respiration, which must have developed when the atmospheric oxygen level exceeded the Pasteur point or, better, the Pasteur range (Mahler and Cordes, 1967). There is general

agreement on when a biologically significant level of atmospheric oxygen was reached, though, from the appearance of animals in the fossil record, it must be dated back at least 6×10^8 years.

A number of reported experiments bear on the question of abiotic (chemical) synthesis of porphyrins. These experiments indicate that prebiotic synthesis of the porphines, the skeleton structure of the porphyrins, occurred readily. Pyrroles, the probable precursors of porphyrins, were likely formed at an early stage of chemical evolution. In the context of preparative organic chemistry (not simulated prebiotic chemistry), Meyer (1913) reported synthesizing pyrroles from acetylene and hydrogen cyanide, and Chichibabin (1915) showed that pyrroles are formed when acetylene and ammonia are passed through a heated tube. Later, Rothmund (1936) heated benzaldehyde with pyrroles to produce some porphyrin-like material. Calvin et al. (1943) added zinc salts to the mixture of the above reactants to achieve a more effective condensation. These experiments were not, however, carried out to simulate prebiotic reactions in the laboratory.

Since 1959, Szutka et al. have performed several experiments to produce porphyrin-like materials and have discussed their results as having direct bearing on the formation of porphyrin-like substances under conditions that prevailed on the primitive Earth (Szutka et al., 1959; Szutka, 1965). In some experiments, they successfully promoted the condensation by various forms of radiation. In a typical experiment the condensation of 3 ml of freshly distilled pyrrole with 6 ml of benzaldehyde in 4 ml of water was promoted by ultraviolet irradiation. The porphyrin nature of some of the products was established by optical absorption spectra of the materials and their zinc chelates. Szutka et al. found, however, that the formation of porphyrins proceeds as well, though apparently more slowly, in the dark. The γ -irradiation of mixtures of pyrrole, benzaldehyde, pyridine, and zinc acetate also gives rise to porphyrin-like material. Evidence that α , β , γ , δ -tetraphenylporphine is a major product was obtained.

Hodgson and Baker (1967), while reproducing earlier experiments of Krasnovski and Umrikhana (1965), demonstrated that small amounts of porphyrins are formed even from aqueous pyrrole and formaldehyde and in the presence of minerals, that is, under geologically relevant conditions. Hodgson and Baker characterized the porphyrins, which they had obtained, by absorption spectra. Although the spectra of the materials obtained resemble those of porphyrins, the absorption maxima and minima are displaced about 100 Å toward shorter wavelengths. This shift has not yet received an acceptable interpretation. The same authors found that the synthesis is facilitated by bivalent cations (particularly nickel and copper) and

by crushed rock and suspensions of previously ignited rock. The synthesis is more effective in the absence of water, for thermodynamic reasons (page 142).

Aqueous δ -aminolevulinic acid, the important intermediate in the biosynthesis of porphyrins, has been condensed under alkaline and anaerobic conditions to porphobilinogen, a biological precursor of porphyrin (Scott, 1956). A direct photochemical conversion of δ -aminolevulinic acid into porphyrin pigments, though in small yields, was reported by Szutka (1966). The formation of α -aminolevulinic acid on the exposure of a reducing atmosphere to β -rays was reported by Choughuley (1966).

Hodgson and Ponnamperuma (1968) resumed earlier attempts of Miller (1955) to identify porphyrins among the products formed by the action of electric discharges on reducing atmospheres. An atmosphere rich in hydrogen is, however, not favorable for the production of aromatic compounds, as explained earlier (page 73). Accordingly, Hodgson and Ponnamperuma (1968) successfully generated porphyrin-like compounds in microgram quantities from a mixture of methane, ammonia, and water vapor in the absence of initial diatomic hydrogen. The mechanism of such formation is not clear. The authors suggest that both formaldehyde and pyrrole are precursors. The primary formation of formaldehyde is well established (Miller, 1955), but the participation of pyrrole is only inferred—from the addition of pyrrole augmenting the yields. Pyrrole can be formed from sugars and ammonia. An important intermediate of this synthesis is mucic acid. Under pyrolytic conditions, this compound readily condenses with ammonia to yield the pyrrole ring.

More data have to be collected in order firmly to establish a mechanism for the prebiological formation of porphyrins.

GENERAL DISCUSSION

The experimental results reviewed in this chapter indicate that a large number of simple organic compounds of biological significance could have been formed spontaneously on the primitive Earth. Among the compounds found in these experiments are amino acids and other simple derivatives of aliphatic hydrocarbons, including the saccharides. Not among these aliphatic compounds are those having an isoprenoid skeleton. Furthermore, none of the compounds synthesized in the experiments simulating prebiotic conditions have been reported to be optically active. Optical activity would mostly be expected to have evolved at a later stage of molecular evolution (page 268).

Although attempts have been directed at producing representatives of each of the major classes of bio-organic compound, there is no reason to believe that such an extensive variety was necessary at an early stage. The availability of a limited number of key compounds, in a context of some autotrophic abilities, could have permitted biochemical evolution, of which we see only the latest stage (Fox, 1974; Hartman, 1975).

Among the aromatic compounds that have been identified as products synthesized under appropriate conditions are amino acids such as phenylalanine, tyrosine, and tryptophan and also purines, pyrimidines, and porphyrins. Most of these aromatic compounds are relatively stable. The benzene and indole rings as well as the purines and porphyrins are well known for their resistance to heat or other radiation. Porphyrins, mostly as vanadyl complexes, are among the organic materials that can be found in sediments more than 2 billion years old (page 291). More labile aromatic compounds, such as flavins, nicotinamide, or other compounds related to vitamins or prosthetic groups of enzymes have not been found in significant amounts among the materials produced in evolutionary experiments. Failure to find these compounds may be simply owing to the fact that the appropriate experiments have not yet been carried out. These compounds may, however, have appeared at a later stage of molecular evolution, perhaps after living systems had evolved. This matter will be referred to again in Chapters 6 and 7.

Most experiments on the evolution of micromolecules have dealt with the spontaneous formation of amino acids. Virtually all proteinous amino acids have been identified in simulated prebiotic syntheses. In experiments with electric discharges, ionizing radiations, or optical radiations, mostly simple amino acids (such as glycine, alanine, α -aminobutyric acid, aspartic acid, and glutamic acid) are produced preferentially. In radiation-induced carboxylation and amination reactions, α -amino acids are not formed with particular preference (Dose and Risi, 1968). Diamino acids other than lysine and ornithine are formed in comparable yields (Dose and Risi, 1968). Aspartic acid and glutamic acid are perhaps favored because they easily undergo condensation to polymers or to rings. This conversion simultaneously leads to a more effective protection of the amino group by acylation. The high temperature synthesis of amino acids, however, leads to significant yields of more complex, but mostly proteinous, amino acids (Harada and Fox, 1964; Taube et al., 1967). A simple explanation for the obvious predominance of the proteinous α -amino acids is not at hand, though the results may signify that the thermal mode is the one that was actually followed in evolution. The methods of quantitative amino acid assay by ion exchange and gas-liquid chro-

matography allow in most cases a clear distinction between proteinous and nonproteinous amino acids. Without much question, all amino acids so far produced in such simulated prebiotic syntheses are present in racemic mixtures; the low yields have seldom permitted an experimental check on this feature of the syntheses. The apparent identification in "prebiotic syntheses" of virtually all proteinous amino acids might lead to the conclusion that the problem of the evolution of amino acids has been solved already. However, in most cases the investigators failed to present quantitative information on the yields in relation to both the quantity of materials entering into the reactions and the amount of energy being supplied. In some experiments the yields of certain amino acids have been so low that the results are of doubtful significance. Questions of contamination from the atmosphere, or by infection with organisms, arise for experiments with very low yields unless strictly aseptic precautions have been observed throughout. Fingerprints on glassware have been shown to yield misleading analyses. In some experiments, amino acids have been identified only by compacting chromatographic R_f values. The assumption made, then, is that the amino acid formed is identical with one of the common types. Many of these results need to be confirmed, because inferences drawn on the basis of a single R_f value have in a number of those instances that have been investigated proven to be erroneous.

Arbitrarily, a yield as low as 1 percent or as high as 15 percent might be regarded as significant evidence in this subject matter, providing requirements of geological relevance are met. Very low yields such as 0.01–0.1 percent raise the question of whether the experimental preparations were free from contamination, and also the question of whether such a yield would have any significance in a geochemical matrix of competing reactions. The yields of individual compounds may be increased by modifying an experiment, e.g., by adding a suitable catalyst or providing a protective system, such as a cold finger or ion exchanger. As long, however, as such attempts have not been successful, low yields are to be interpreted with caution.

In many experiments, however, yields have been so low that they were not determined. The investigators often report merely the identification of a single component (for which they had been looking) in a potpourri of other products. To establish an identification, even when substantial amounts cannot be found, sophisticated tracer techniques (mostly with the help of radioisotopes) are applied. Molecular evolution, however, poses some requirements of quantification. In this review many results that have been reported indicate only minute amounts of a biologically important compound produced in a given experiment. Those results are included here even when their signifi-

cance is doubtful, because they may lead to more successful experiments.

Experiments employing plausible prebiotic conditions with volatile reactants (see Florkin, 1975) generally require the use of low concentrations. If higher concentrations, that is, higher than 10^{-3} — 10^{-2} M, are used, the results may be regarded only as having at most some bearing on molecular evolution, unless evidence is presented to establish why such concentrations might have occurred on the primitive Earth. Objections have been raised, for example, that concentrations of 1—15 M solutions of ammonium cyanide, which Oro used to produce adenine, are drastically high in light of assumptions about the prebiotic environment (Ponnampерuma and Gabel, 1968). Also, free phosphoric or polyphosphoric acid has not so far been reported on the contemporary Earth, although plausible mechanisms by which acidic polyphosphates could have arisen from various ammonium phosphate minerals on the primitive Earth (page 122) are known. The question of whether any given experiments might have occurred on the Earth, however, cannot be answered easily because extreme and even seemingly improbable conditions may persist locally on the Earth. For example, although acidic pools on the contemporary Earth are not common, they do occur and they may well have occurred on the primitive Earth. In any event, the most rigorously defensible conditions of a simulation experiment are those that are found on the contemporary Earth. Present geological conditions can generally be inferred to have prevailed on the primitive Earth, unless contradictory evidence can be established. This restriction applies, for example, to those geological conditions that depend on the presence or absence of O₂, NH₃, CH₄, CO, and CO₂.

Many experimental conditions applied to simulate prebiotic processes in the laboratory may be criticized with respect to their geological relevance, because they represent thermodynamically closed systems, such as systems in closed flasks. (Keosian, 1974; Florkin, 1975). Most geological systems, however, are open systems. Many experiments with "reducing" atmospheres may be questioned, therefore, because hydrogen partial pressures were building up to too high a value when, for example, electric discharges interacted with the gases. In most of these experiments the investigators therefore failed to detect hydrogen-poor, that is, aromatic compounds, among the reaction products (pages 73, 83, 85, and 113).

Some of the experiments may be criticized because their relationship to chemical evolution is not clear. Any experimental condition, however, that has a finite probability of occurring or having occurred on the Earth deserves to be considered for its contribution to the

theory of molecular evolution. So many factors enter into the question of relevance to geological conditions that we are at present unable to evaluate adequately the meaning of many of the experiments. The primitive Earth bore such a variety of conditions that a great number of chemical reactions were possible. Experimentation to determine more about this subject requires at the outset meticulous freedom from chemical or microbial contamination, fully reproduced experiments, quantitative evaluations, careful assessment of the quality of the evidence, confirmation and extension of the results, and pluralistic thinking in interpretation. An emphasis on the possibilities in a heterogeneous geochemical realm, and even occasionally on low yields, is appropriate as long as we entertain the thought that life need have begun no more than once, and that the probability of a particular set of events is more than zero. At the same time, adequate challenging of results helps to guide the interpretations.

One truly salient consequence of the experiments is the fact that the amino acids are readily formed in the laboratory and that they are preferentially the same types of α -amino acid that are found in organisms; the configurations are racemic, however (page 268). Moreover, those amino acids that are generally dominant among the products of the experiments, such as glycine, alanine, aspartic acid, and glutamic acid, are the same as those that are dominant in the composition of contemporary proteins, or in the free state in physiological fluids. The small molecules favored by thermodynamic parameters are those favored in either a chemical context or in biochemical mechanisms.

This kind of chemical experiment, moreover, has validated the approach of chemical heterosynthesis, which is a departure from traditional chemical synthesis. The traditional approach is to make each amino acid or each hexose, one at a time. The experiments in molecular evolution derive their basic meaning and their relative significance from the degree to which a family of molecules, for example, six proteinous amino acids, arises in a single experiment. Many of the products of biosynthesis are common intermediates for two or more pathways. Examples that are frequently studied of such intermediates are glutamate, glycerophosphate, *acetate*, pyruvate, and ADP (Lehninger, 1975). The reactions of the organism are thus a metabolic network through almost simultaneously available intermediates.

Experiments that yield families of molecules thus appear to be those that have the greatest evolutionary plausibility. Successful experiments of this kind testify best to their unique quality as model experiments for molecular evolution.

TABLE 4-17
Representative Model Experiments Demonstrating Prebiotic Synthesis of Micromolecules

Reactants	Reaction Phase	Energy	Products ²	Authors
<i>Aliphatic Compounds</i>				
CO ₂ , H ₂ O	Gaseous	UV (1470 Å)	Formaldehyde, glyoxal	Groth and Suess (1938)
CO ₂ , H ₂ O, (Fe ²⁺)	Gaseous/ aqueous	α-rays	Formaldehyde, formic acid, succinic acid	Garrison et al. (1951)
CH ₄ , NH ₃ , H ₂ , H ₂ O	Gaseous	Electric discharges	Various amino acids, hydroxy acids, HCN, urea	Miller (1953, 1955)
Ammonium fumarate CO ₂ , NH ₃ , H ₂ , H ₂ O	Dry solid Gaseous	Heat Electric discharges	Aspartic acid, alanine Amino acids	Fox et al. (1955) Abelson (1956)
CH ₄ , NH ₃ , H ₂ O, H ₂ , CO ₂ , CO, N ₂	Gaseous	X-rays	Amino acids	Dose and Rajewsky (1957)
Ammonium acetate	Aqueous	β-rays	Glycine, aspartic acid, diaminosuccinic acid	Hasselstrom et al. (1957)
Ammonium carbonate CH ₄ , NH ₃ , H ₂ O	Aqueous Gaseous	γ-rays UV (1165-1850 Å)	Glycine Simple amino acids and fatty acids	Paschke et al. (1957) Groth and Weyssenhoff (1957)
NH ₃ , HCN, H ₂ O CH ₄ , NH ₃ , H ₂ O	Aqueous Gaseous	Heat (70°C) β-rays	Amino acids Simple aliphatic compounds including amino acids	Oro and Kamat (1961) Palm and Calvin (1962)
HCHO, CH ₃ CHO CH ₂ OH—CHOH—CHO Ca(OH) ₂	Aqueous	Heat (50°C)	Various sugars including 2-deoxyribose, 2-deoxyxylose	Oro and Cox (1962)
HCHO	Aqueous	UV (2537 Å)	Various sugars including ribose and deoxyribose	Ponnampерuma (1965)
CH ₄ , NH ₃ , H ₂ O (silica contact)	Gaseous	Heat (850°C)	Amino acids	Harada and Fox (1964)
CH ₄	Gaseous	Electric discharges	Higher hydrocarbons (including aromatic hydrocarbons)	Ponnampерuma and Woeller (1964)

Aromatic Compounds				
CH ₄ (silica contact)	Gaseous	Heat (100°C)	Higher hydrocarbons (mostly aromatic hydrocarbons)	Oro and Han (1966)
HC≡C—CN, HCN, NH ₃ , H ₂ O	Aqueous	Heat (100°C)	Aspartic acid	Sanchez et al. (1966)
HCN, NH ₃ , H ₂ O	Aqueous	Heat (70°C)	Adenine	Oro and Kimball (1961a, b, 1962)
Malic acid, urea, polyphosphoric acid	Liquid-anhydrous	Heat (130°C)	Uracil	Fox and Harada (1961)
CH ₄ , NH ₃ , H ₂ O	Gaseous	β-rays	Adenine	Ponnampерuma, Lemmon, et al. (1963)
Adenosine, polyphosphate ester	Liquid-anhydrous	UV (2537 Å)	AMP, ADP, ATP	Ponnampерuma, Sagan, et al. (1963)
CH ₄	Gaseous	Electric discharge	Mostly aromatic hydrocarbons	Ponnampерuma and Woeller (1964)
Nucleoside, phosphate	Solid, anhydrous, hypophydrous	Heat (160°C)	Nucleotides	Ponnampерuma and Mack (1965)
CH ₄ (silica-gel contact)	Gaseous	Heat (100°C)	Mostly aromatic hydrocarbons	Oro and Han (1966)
Nucleosides, polyphosphoric acid	Liquid anhydrous	Heat (22°C)	Nucleotides, nucleoside tri-phosphates	Waehneldt and Fox (1967)
CH ₄ , NH ₃ , H ₂ O	Gaseous	Electric discharges	Porphyrins	Hodgson and Ponnampерuma (1968)

^aIdentified products only. The variety of compounds actually produced is usually much greater.

SUMMARY

A large body of information on possible prebiotic pathways for the formation of biologically significant micromolecules is now supplied by a great number of laboratory experiments. By tabulating systematically all these experiments we would not serve those readers who are not specialists in the field. We restrict the summary, therefore, to tabulating only those experiments that in our opinion must be regarded as representative, or key, experiments. Table 4-17 is divided into two parts. The first part concerns the aliphatic, the second part the aromatic compounds. A strict separation is generally not possible, however, particularly because aliphatic and aromatic compounds are often produced in the same experiment. The material referred to as product in many instances represents only a small fraction of the variety of materials actually produced.

A review of Table 4-17 suggests that a large variety of small organic molecules of biological significance might have arisen spontaneously on the primitive Earth. Future research on the chemical abilities of initial cell-like microsystems (Keosian, 1974) should indicate which reactants were obligatory. The photochemically active protocell suggested by experiments (page 252) might not have required a "soup" of intermediates, since it could theoretically have made many of its own.

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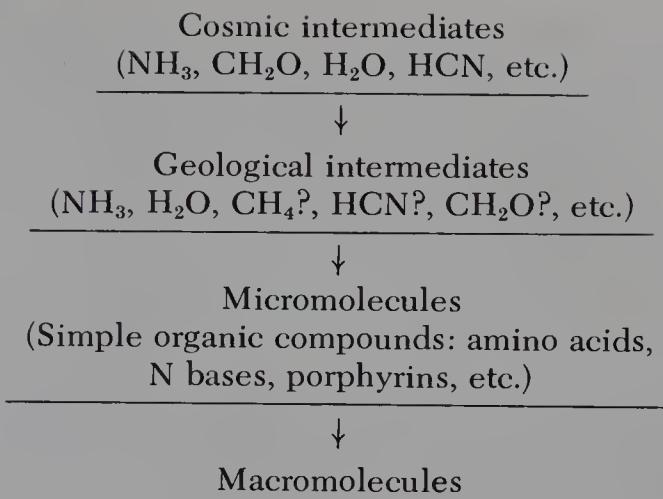
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CHAPTER 5

Macromolecules

As we approach the questions of the origins of macromolecules, we leave the subject matter of small, relatively simple molecules (micro-molecules) to deal with the large, hence very complex, molecules (flowsheet, page 139). The requisite conceptual leap is large because of the formidable knowledge of molecular architecture of the proteins and nucleic acids. The proteins can, however, be discerned as having been at the center of bioevolutionary processes. Their outstanding variety and versatility can provide the molecular basis for the numerous subtly shaded specificities that have made the appearance of a richly varied biota possible.

A fundamental fact is that noted by many biochemists (Vegotsky and Fox, 1962) that the same twenty amino acids are common to virtually all proteins. Both the qualitative and the quantitative amino acid contents are manifestations of the “unity of biochemistry.” Without this principle having been discernible, it is doubtful that experiments in the production of protein-like polymers (speaking in terms of composition and molecular weight) would have, or could have successfully, been attempted.



When we turn our attention to the nucleic acids, we find also molecules large enough to permit great variegation and variation. We find here some rigidity, relatively, and we can easily understand such a property in DNA, which must serve to store information while much biochemical activity occurs outside the threshold of its administrative office.

The degree of complexity with which we are now confronted, and the minute fraction that variation represents of all the conceptual possibilities, can also be understood. In this chapter we will begin to examine the thesis that evolutionary reality is the product of constraints that have been exercised along the path of the evolutionary development, particularly in the geologically facilitated assembly of polymers from monomers. We can hope in this way to understand the leap over the large gap from the simple, small molecules to the large, complex molecules.

Many features of the living cell are best understood as being rooted in its macromolecular constituents (Needham, 1965; Jirgensons, 1962; Fox, 1968a), especially the proteins and the nucleic acids. Compared to cellulose, chitin, lipids (Needham, 1965), lignin (Nord and Schubert, 1962), and cell-wall compounds (Salton, 1960), proteins and nucleic acids are among the most complex substances found in organisms. This very complexity has in the past discouraged the development of a theory of protobiogenesis. The undeniable fact that such polymers have great complexity, however, does not require that the processes by which they or their evolutionary precursors arose were also complex. In fact, we can logically reason that processes that

occurred spontaneously on the Earth, in the absence of a chemist, were in reaction—and in reaction sequence—operationally simple at the same time that they were mechanistically complex (Fox, Harada et al., 1970). The type of process that is generally simplest operationally, and that yields large, complex molecules is that of traditional polymerization, “zipper chemistry.”

Considerations of the possibility of polymerizing amino acids have been influenced by the historical developments in the industrial production of the nylons (Mark and Whitby, 1940). In such contexts, the site of the amino group in the carboxylic acid is crucial. The artificial polymers have been made from ω -amino acids and the like, whereas biochemists have in mind α -amino acids when they employ the term amino acid without the prefix α .

W. H. Carothers (1936), who was responsible for the development of nylon from ω -amino acids, considered the possibility of polycondensation of α -amino acids. He stated that α -amino acids would undergo either chain formation or ring formation. He hypothesized that in organisms interfacial binding of one of the two functional groups of the first α -amino acid to couple would favor chain formation.

Carothers' explanation of nonpolymerizability was essentially correct for α -amino acids in general. Recognition of the special nature of amino acids such as aspartic acid, glutamic acid, or lysine could have altered the outlook, on the basis that nonneutral amino acids do not form only rings and that they can function as chain initiators. Moreover, protein biosynthesis has been interpreted as relying on chain initiators (Lengyel and Soell, 1969). As Harada (1961) has explained, condensation of amino acids in solvents would, also, favor intramolecular reactions, whereas condensation in a melt would favor intermolecular reactions. Of special significance is the fact that α -amino acids that individually undergo decomposition will cocondense to mainly linear polymers with glutamic acid or aspartic acid (Fox, Wang, et al., 1970).

The widespread feeling of hopelessness for accomplishing thermal polymerization of α -amino acids (see also E. Katchalsky, 1951) precluded studying the origin of life-related macromolecules and the microsystems that might arise therefrom. The scope of new possibilities, revealed by experiments more than two decades after Carothers' work, was such as to permit incorporation within an initiated chain of all of the amino acids found in protein. The molecular logic of the primordial synthesis of peptide bonds has been discussed by Oparin (1957) and a geological locale for such synthesis has been

described by Ehrensvärd (1962). Ehrensvärd suggested locales near, but not too near, to volcanic regions (page 60).

Polyamino acids that have been produced in the laboratory under simulated geological conditions have incorporated all types of amino acid commonly found in protein. Most of the complexity in mechanism of formation and structure of protein is traceable to the variety of monomers. The first proteins to arise prebiotically or biotically did not necessarily require in their composition as many kinds of amino acid as contemporary proteins. However, the fact that almost all kinds of amino acid found in contemporary proteins consist of this same roster strongly suggests that the proteins in the first organisms were in fact similarly constituted. Jukes (1966) has proposed that there were thirteen to fifteen primordial amino acids on the basis of rather elaborate processes that indicate an evolutionary history. Moreover, some of the experiments yielding amino acids have produced most of those common to protein (page 130). All α -amino acids that formed in any primordial situation would thus presumably have reacted under conditions effective for any two of them (page 140).

One outstanding evolutionary advantage to such highly heteropolymeric, or heterotonic (having many kinds of monomer), macromolecules can be discerned. The virtue of heteropolyamino acids such as proteins is that they provide a much richer variety of chemically functional loci for enzymes, through the interactions of twenty types of amino acid. Conceptually, this in turn made possible evolution to a complex metabolism and also to a very large array of precisely and finely shaded "specificities" (Rohlfing and Fox, 1969). The heterotonicity made possible varieties of other qualities such as structural propensities (page 201).

Whatever the number of types of amino acid in primordial protein, further complexity was introduced into the evolutionary stream when polynucleotides appeared and interacted with proteins. The possibility of prebiotic polynucleotides has been investigated (Schramm, 1965; Schwartz et al., 1965). Such studies have largely relied on phosphate-condensing agents. The fact that polymerization of amino acids is also enhanced by inclusion of phosphates in the condensation mixture (Vegotsky, 1961; Harada and Fox, 1960) is consistent with the belief that the two kinds of macromolecule could have originated simultaneously (Calvin, 1962).

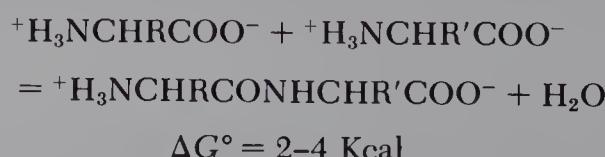
The condensation of monosaccharides has been accomplished in the laboratory, as described in a number of publications (Mora et al., 1960). Here, too, some of the conditions are similar to those for the condensation of amino acids. A typical temperature employed in the presence of hypophosphorous acid, a reducing agent, is 170°C.

POLYMERS OF AMINO ACIDS

Theory of Polycondensation of Amino Acids

The ease with which a large variety of micromolecules has been produced from reactive gases in laboratory experiments under a variety of conditions suggests that their production on the primitive Earth was abundant (page 130). The production of macromolecules is a problem for which more special restrictions and more special possibilities can be visualized on the basis of repeatedly discussed theory (Borsook and Huffman, 1944; Fox et al., 1957). Violent forms of energy—for example, high-energy radiation, short-wavelength ultraviolet light, electric discharges, and geologically high temperatures (e.g., >800°C)—produce free radicals and other reactive species from methane, ammonia, and water; these reactants recombine to yield amino acids, nitrogen bases, etc. The same forms of violent energy can cause predominant decomposition of polyanhydro- α -amino acids. For example, the same dose of γ -irradiation that leaves an appreciable fraction of a population of amino acids unchanged may be sufficient to alter chemically practically all of a population of enzymic macromolecules (A. J. Swallow, 1960; Bacq and Alexander, 1960). Accordingly, the production of specific macromolecules should proceed more smoothly if the monomers were combined in less violent reactions. The simplest way to bring this about would be to initiate thermal condensation reactions at moderate temperatures (page 153) under hypohydrous or anhydrous conditions. The relative absence of water during such reactions would provide additional protection, since organic compounds generally are more unstable in the presence of water. Absence of water is achieved by beginning with dry amino acids, by using nonhydrous intermediates, or by thermal distillation.

A first analysis of the energetics of peptide-bond formation was published by Borsook and Huffman (1944; Borsook, 1953). The data and interpretations were obtained from heats of combustion and heat-capacity measurements of amino acids and peptides. The essential reaction is depicted by the equation:



In order for the synthesis to proceed in dilute aqueous solution, 2-4

Kcal must be imparted to the reaction. When viewed as an equilibrium through the equation

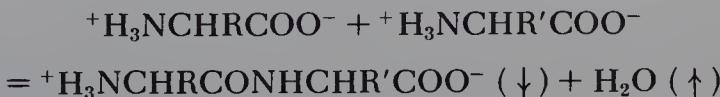
$$\Delta G^\circ = -RT \ln K$$

the maximum amount of peptide allowed by an approach to equilibrium is found to be far over on the side of hydrolysis. This thermodynamic barrier would be much greater for the formation of a protein molecule in aqueous solution. In fact, Dixon and Webb in their book on enzymes (1958) point out that solutions 1 M in each amino acid would yield at equilibrium a 10^{-99} molar concentration of protein (M. W. = 12,000). To yield one molecule of this protein, the volume of the solution would have to be 10^{50} times the volume of the Earth! The requirement for energy is diminished somewhat by location of peptide bonds in the interior of the polymers (Fruton and Simmonds, 1958), but under the most favorable circumstances the unlikelihood is still astronomically large.

A first, and presumably simplest, manner in which this thermodynamic barrier was visualized as surmountable (Fox et al., 1956; Fox et al., 1957) was by removal of water formed as a byproduct when peptide bonds were synthesized. With the water removed by volatilization, the equilibrium considerations of closed system thermodynamics no longer apply.

Other modes of removal of water relevant to the evolutionary context can be proposed. One of them is the strong binding by the polyamino acid molecules themselves of water formed (this binding has been demonstrated in hydration in the solid state [Vegotsky et al., 1960]). Another is the conduct of the reaction in the presence of anhydrous agents such as polyphosphoric acid and other polyphosphates (Harada and Fox, 1965a) or cyanamide and dicyandiamide (page 147).

According to the law of mass action, the reaction can be favored in the synthetic direction by removal of the other product, the peptide. In equational form:

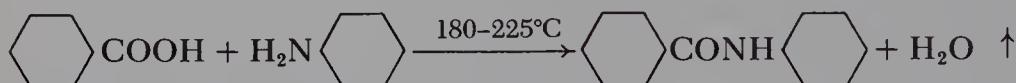


In the prebiotic realm, clays (Bernal, 1951, 1960) or other surfaces might have served to separate a peptide product and water. In primitive organisms, the predecessors of ribosomes might have had a similar function. The ribosomal type of particle would have been more selective than inorganic materials, and their function would have supported evolution of independence from the environment (page

260). The origin of protoribosomes might have been simple (page 232; Waehneldt and Fox, 1968; Fox, Harada et al., 1970). The removal of formed peptides, to aid the synthesis, could also have been aided by membranes (Fox, 1968b). As polyamino acid synthesis proceeded, at some point molecules sufficiently large to be retained would have been synthesized within a membranous cell. Contemporary ribosomal mechanisms employ phosphate energy, and also utilize the benefits of being out of solution.

The use of clays to provide nonsolution conditions, as suggested by Bernal, has been advanced by the use of montmorillonite with aminoacyl adenylates (Paecht-Horowitz et al., 1970). A similar model has been the use of basalt for thermal polymerization of amino acids (Fox, 1964). Basalt was appropriate to primordial events whereas clay is the product of metamorphic processes.

In addition to thermodynamic theory, which indicated that amino acids would be condensed by heating to a temperature above the boiling point of water (Fox et al., 1956), descriptive organic chemistry has pointed also in the same direction. Musselius (1900), for example, had shown that benzoic acid and aniline yielded a peptide-like bond when the reactants were heated to a temperature above the boiling point of water:

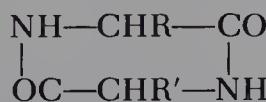


Some questions have been raised about the theory and regarding the geological relevance of the positive results that have emerged from the experiments employing heat on dry amino acids. Two related arguments are that organisms are aqueous and that, since organisms cannot tolerate temperatures such as 150°C, precursor molecules are unstable. The fact that organisms are aqueous has been used to imply that models for prebiological synthesis of peptide bonds should be studied in aqueous solution (Kenyon and Steinman, 1969), in which indeed they have been (Bahadur and Ranganayaki, 1958; Lowe et al., 1963; Oro and Guidry, 1961; Ponnamperuma and Peterson, 1965; Steinman et al., 1964; Steinman et al., 1965a, b; Steinman and Cole, 1966; Steinman, 1967; Paecht-Horowitz and A. Katchalsky, 1967; Krampitz and Fox, 1969). Although organisms are mainly aqueous, they do not carry out peptide-bond synthesis simply in dilute aqueous solution, as stated earlier. The dominant site of protein synthesis in the cell is the ribosome (Zamecnik et al., 1958); the ribosome is a ribonucleoprotein particle far from being or containing dilute aqueous solution (Dibble and Dintzis, 1960). Many zones in enzymes are

known to be hydrophobic (Kauzmann, 1959) and the biochemically significant cellular membranes are likewise substantially lipid rather than aqueous in character (Christensen, 1962). Also, the fact that organisms contain much water does not require that the macromolecules which were precursors to the first living systems had to exist in dilute aqueous solution. Moreover, in accord with a principal model of the first cell, which indicates that water acted on the appropriate precursor macromolecule (page 201), the precursor macromolecule must have been relatively free of water.

The second viewpoint that prebiotic organic molecules cannot tolerate temperatures such as 150°C because organisms cannot do so is, similarly, without solid foundation. Cells, containing water, are unable to survive being heated to 150°C, but many dry organic compounds can withstand much higher temperatures, such as 300°C. Indeed, the salient organic compounds are the most stable ones; many contain aromatic rings and polyanhydro structures. Imidazoles, thiazoles, pyrimidines, etc., are hydrogen-poor ring compounds that are, formally, dehydrogenation and dehydration products. All such molecular structures suggest a primordial origin from a nonoxidizing atmosphere at temperatures above the boiling point of water, or from reaction media containing phosphate in a water-withdrawing state (Fox and Harada, 1961).

In this chapter, a distinction is made between small peptides and higher polymers of amino acids, although all peptides are, in a wider sense, polymers of amino acids. A difficulty arises in those cases in which a given journal article does not provide sufficient information to permit an assessment of molecular size. If, for example, the inference that amino acids are combined is based solely on the fact that the products of reaction yield amino acids upon hydrolysis, we are unable to draw conclusions about the products prior to hydrolysis. The products might, for example, be diketopiperazines



as they have been shown to be in some cases. They might be hetero-adducts of aminonitriles.

The salient information that has appeared comprises (1) the experimental demonstration of the production of heteropolymers of high molecular weight and other meaningful properties under geologically relevant conditions, and (2) the contemporary existence of ribosomes, which use phosphate energy instead of thermal energy, and which also act hypohydrously in that they are nonsolution particulates.

These two firm facts suggest that the organismic synthesis of peptide bonds evolved from a process that was activated by thermal energy, perhaps involving energy-rich phosphates. Neither the model for prebiotic synthesis nor our concept of the contemporary organism is consistent with the postulation of a dilute aqueous environment.

Peptides

As already indicated, the production of relatively small yields of small peptides from amino acids in aqueous solution has been recorded from a number of laboratories. Some of the peptide syntheses have been performed in the context of prebiotic synthesis; others may be interpreted in that framework.

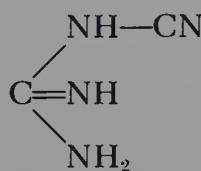
Perhaps the first synthesis of small peptides in aqueous solution in this context was that described by Bahadur and Ranganayaki (1958). These authors sterilized in an autoclave an aqueous solution containing 2 percent sucrose and 0.1 percent glycine. This solution was exposed to sunlight for one month while a control flask was protected from sunlight. The product was examined by paper chromatography. In the control flask, only glycine was observed. In the irradiated flask were found materials having the R_f values of glycine, glycylglycine, glycylalanine, norleucine, and glycylnorleucine. Explanations were offered for the presence of alanine and/or norleucine. In similar experiments in which sucrose was not included, Dose et al. (Dose and Ettre, 1958; Dose and Risi, 1968) were not able to detect any defined peptide after exposing aqueous or dry amino acids to ultraviolet light or X-rays, although some isolated material yielded free amino acids on acid hydrolysis. These latter authors found that the radiolysis of peptides in aqueous solution is at least one order of magnitude more rapid than their radiation chemical formation, even at high concentrations of the reactants (Dose and Ettre, 1958; Dose and Risi, 1968).

The yields of dipeptides claimed by Bahadur and Ranganayaki (1958) were very low as indicated by the statement that "no quantitative estimation of the peptides formed could be done." Bahadur and Ranganayaki argue that ultraviolet light is sufficiently energetic to overcome the barrier to formation of peptide bonds in water. Again, the thermodynamic calculations (page 142) do not imply that no peptide bonds can be formed, but that only small yields of small peptides can be expected unless energy is somehow coupled to the reaction. These authors do not show that they have done more than accelerate an approach to an unfavorable equilibrium. They may

merely have reaffirmed the second law of thermodynamics. They infer that protoplasm arose recently, and fail to note the great conceptual distance between minute yields of three dipeptides and the origin of the first protein.

Our introductory remarks about lack of information that would permit assignment of molecular size are applicable to studies of Lowe et al. (1963). These authors heated aqueous solutions of ammonium cyanide, in an extension of the work of Oro (page 108). Upon hydrolysis of the reaction products, Lowe and co-workers found 75 ninhydrin-reacting compounds. The behavior of one fraction eluted from Sephadex G-25 suggests that the molecular weights were in the range of 100–5000. No evidence that the products were much above 200 in molecular weight was offered. The principal amino acid found as a result of total hydrolysis was glycine. This dominance of glycine is observed in many other pansyntheses (simultaneous syntheses of many substances) of amino acids, including those reported by Oro and Guidry (1961; see also page 130).

In 1965, reports of formation of small peptides in aqueous solution appeared in two papers in the same issue of *Science*. Ponnamperuma and Peterson (1965) exposed aqueous solutions of glycine and leucine to ultraviolet light in the presence of cyanamide, $\text{H}_2\text{NC}\equiv\text{N}$. The peptides obtained were glycylglycine, glycylleucine, leucylglycine, and leucylleucine, in a total yield of 1 percent, and diglycylglycine, in a yield of 0.1 percent. Dipeptides were formed also by heating solutions of amino acids with ATP and Mg^{++} . Steinman and co-workers (Steinman et al., 1964, 1965a; Steinman and Cole, 1966; Steinman, 1967) carried out similar experiments in the dark, and used dicyandiamide



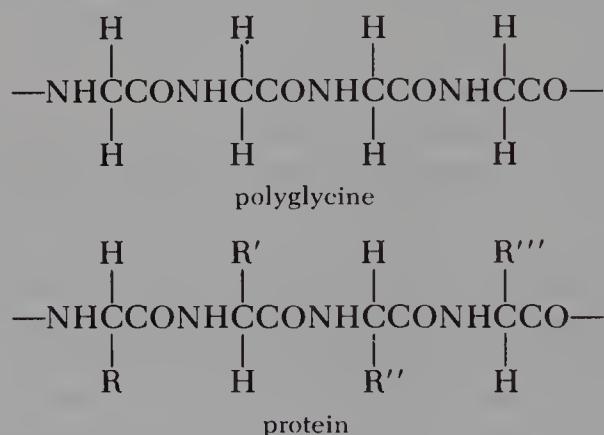
instead of cyanamide. Alanine was used as the only amino acid in Ponnamperuma's first experiments. A 1.2 percent yield of alanylalanine plus a trace of dialanylalanine were obtained.

Ponnamperuma and Peterson (1965) showed that their reaction would proceed without the cyano compound; Steinman, Lemmon, and Calvin (1964) obtained their results without radiation. Since the total yield was less than 2 percent in each study, these results leave unanswered thermodynamic questions raised previously (page 142). Yields of this magnitude are little, or no, more than those expected at equilibrium from calculations of reversible thermodynamic reactions.

Also, cyanamide is itself typically obtained by high-temperature anhydrous reactions.* Such conditions are necessary to produce reagents useable for the polycondensation of amino acids—by any indirect route in aqueous solution. The direct route (page 153), however, requires only moderate heat, relative dryness, and amino acids without a special reagent, but it does yield high polymers.

Polyglycine and Substituted Polyglycine

Any polyanhydro- α -amino acid and, therefore, any protein is formally a polysubstituted polyglycine:



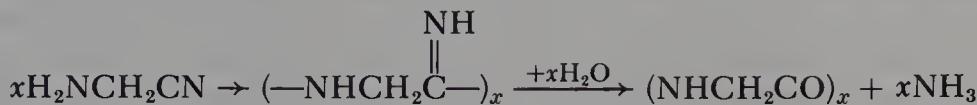
The synthesis of protein might be visualized as occurring through progressive substitution of preformed polyglycine. In fact, Akabori has developed this approach, partly with experiments, and partly with postulations of the later stages of substitution of polyglycine. He referred to the substituted polyglycine as fore-protein. The experiments and theory are described in several papers (Akabori, 1955; Okawa, 1954; Akabori, 1959; Hanabusa and Akabori, 1959); a principal paper is found in the record of the I.U.B. Symposium Series Volume I (Akabori, 1959).

Akabori proposed as a first step the spontaneous synthesis of polyglycine through aminoacetonitrile:

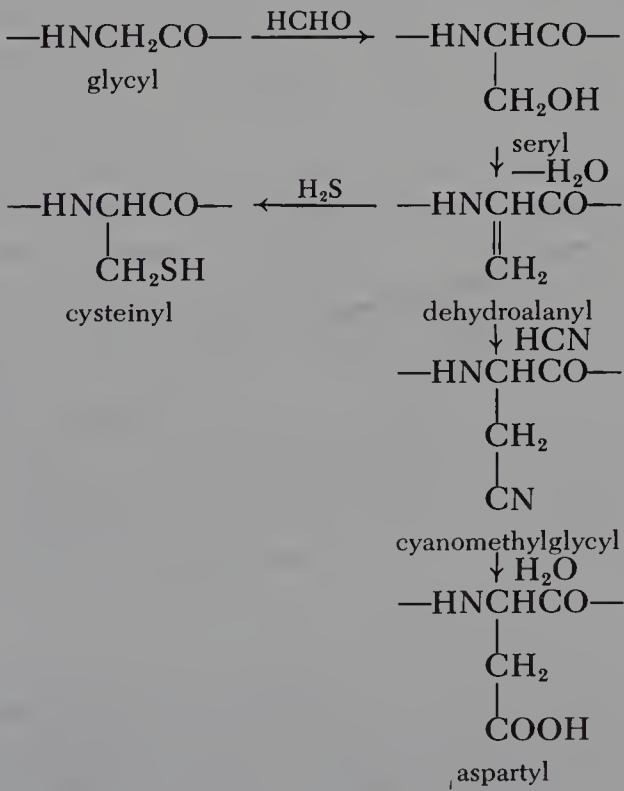


* Similarly, the synthesis of pyrophosphate, as described by Miller and Parris (1964), does not solve the problem of source of energy inasmuch as cyanate was used in the laboratory to prepare the pyrophosphate. The cyanate, also, had to be prepared by synthesis at high temperature, or by electric discharges, which are accompanied, of course, by high temperatures (page 86); the problem is thus merely complicated. See, also, critical comments by Ferris et al. (1973b).

The second step is polymerization of the nitrile on a solid surface:



The first step is a well-known laboratory operation (Anslow and King, 1932). The second step was demonstrated in Akabori's laboratory by mixing aminoacetonitrile with kaolinite and heating to 130–135°C. However, the products isolated were only glycylglycine and glycylglycylglycine. For the third step, Akabori used polyglycine of high molecular weight made from the Leuchs' anhydride or from glycine ester. The polyglycine was then reacted successively to convert glycyl and seryl residues to seryl and cysteinyl residues, respectively, as shown in the top part of the following diagram:



Conclusive evidence of the formation of seryl and threonyl residues, in yields of up to 3 percent of the glycyl residues, was obtained. The formation of cysteinyl residues was not fully confirmed. Evidence for the formation of a small proportion of leucine or isoleucine by a reaction employing butene was obtained. Akabori presented also speculations on how aspartyl, alanyl, phenylalanyl, tyrosyl, tryptophanyl, histidyl, valyl, isoleucyl, glutamyl, glutaminyl, arginyl, and lysyl

residues would arise. The postulated mechanism yielding aspartyl is shown in the diagram just given (Sakakibara, 1961). The two solidly established experimental results, however, were obtained by the formation of the seryl and threonyl residues. Sakakibara (1961) reported the production of glycine, alanine, serine, threonine, aspartic acid, and glutamic acid in polydehydroalanine and related these findings to the work of Akabori.

Somewhat related to polyglycine and substituted polyglycine are polymers that have been produced from hydrogen cyanide in a number of laboratories (Lange, 1873; Oro and Kimball, 1961; Lowe et al., 1963; Harada and Fox, 1964; Matthews and Moser, 1966a, b; Harada, 1967). Uncertainty exists about the nature of these polymers. What is clear is that mineral acid hydrolysis results in amino acids that can be, and have been, identified. These "HCN polymers" are discussed in greater detail on page 182.

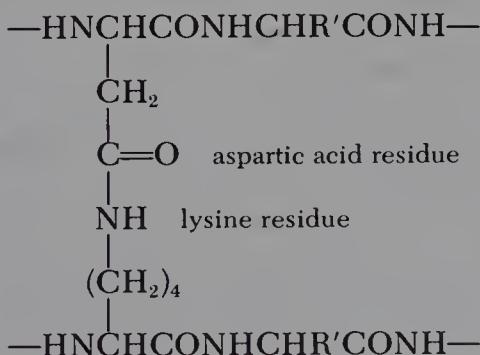
Polyamino Acids Including Proteinoids

Only the thermal method has as yet yielded, under simulated geological conditions, polymers of amino acids that (1) contain all the amino acids common to contemporary protein, (2) have molecular weights of many thousands, (3) possess an array of catalytic or rate-enhancing activities of the kinds from which enzymes and metabolism could evolve, (4) exhibit limited heterogeneity comparable to that found in contemporary populations of organic protein, and (5) yield on contact with water organized units with many of the properties of contemporary cells. The process of heating amino acids is of the utmost simplicity, and can properly be imputed to spontaneous reactions on the Earth. The formation and properties of the products invite discussion. Those questions of primary concern to protobiogeneticists are discussed here.

After the thermodynamic considerations that have been covered (page 142), a second issue concerns decomposition. The point of view generally accepted for a long time stresses the tendency of amino acids to yield unwanted products on being heated to temperatures above the boiling point of water (E. Katchalsky, 1951). This difficulty was initially overcome by the inclusion of a relatively high proportion of aspartic acid, glutamic acid, or lysine, presumably by acid catalysis or base catalysis (Fox, 1969). The use of sufficient proportions of non-neutral amino acids had been suggested by evolutionary studies of protein molecules (Fox, 1956). By heating amino acids at 170°C for 6 hours, for example, substantial yields (10–40 percent) of thermal

polymers of amino acids are obtained. The first heteropolyamino acids obtained by heat were quite high in aspartic acid, or in lysine. The mixtures employed in early experiments contained at least one-third aspartic acid. More recently, lower proportions have been shown to be adequate. By heating at 190°C for 6 hours (Fox and Waehneldt, 1968), for example, equimolar mixtures of all 18 amino acids common to protein have been found to condense to yield proteinoids possessing proportions of amino acids closely resembling those of a wide variety of contemporary proteins. The amino acids not represented in the proteinoids in the proportions usually found in proteins were cystine, serine, and threonine. These latter were largely, although not entirely, decomposed. With these exceptions, the amino acids have been found, by analysis of unfractionated products, to be stable to heat at the temperatures employed for heating (Dose and Rauchfuss, 1972; Wood and Fox, 1967). Oshima (1968) has produced neutral proteinoids by heating mixtures containing more than 80 percent nonneutral amino acids. Rohlfing (1967a) has produced similar proteinoids thermally.

A third kind of question was that of crosslinks such as have not been reported for protein. Reactions might be postulated, for example, between side chains of such amino acids as lysine and aspartic acid (D. L. Swallow and Abraham, 1958):



A fact that bears on the possibility of crosslinks is that amino acids have been recovered quantitatively from a number of purified acidic or neutral polymers. For some of these complete recoveries, hydrolysis had to be continued for 72 hours. In other experiments, prolonged hydrolysis failed to give complete recovery. Perhaps such crosslinks as are illustrated above are present; they are known to be difficultly hydrolyzable. From lysine-rich proteinoids, recoveries from hydrolysis have been much lower, 40–70 percent. Moreover, linkage through the ϵ -amino groups of some of the lysine residues has been shown in a number of studies (Harada, 1959; Harada and Fox, 1965a;

Suzuki, 1966; Heinrich et al., 1969). With these exceptions the amino acids are largely joined in proteinoids by hydrolyzable linkages.

A fourth kind of question that was visualized stems from the known effect of heat upon the conformation of protein molecules. The general tendency of protein molecules to denature when heated is widely recognized (Neurath et al., 1944). Accordingly, when proteinoids were first produced, the question arose of whether the heat employed in the condensation might "denature" the macromolecules formed. The thinking was that biological activity would be destroyed by heat denaturation, as in contemporary proteins.

The generalization that proteins are rapidly denatured in aqueous solution by heat may not be valid for all proteins. The vast majority of proteins with catalytic or other activity are, however, inactivated by heat. For proteins heated in the dry state instead of in aqueous solution, denaturation is many times as slow (Altman and Benson, 1960). Proteinoids having specific rate-enhancing activities are formed by heating in the relative absence of water. The only water present at temperatures above the boiling point is that formed as the byproduct of peptide bonds. This is largely or entirely held as bound water; it is not solvent water such as catalyzes denaturation. The essential feature is that dry proteins, unlike those in aqueous solution, do not become denatured rapidly.

Discussion of "denaturation" of proteinoids requires some further qualification. Thermal proteinoids are capable of undergoing change in conformation as the result of being heated in aqueous solution, but this is not a process identical to the transformation from helical state to "random" conformation that characterizes denaturation of contemporary proteins (Schellman and Schellman, 1964). The process has some similarities to denaturation, and some differences. What is known about the process has been related to a destruction of catalytic activities in aqueous solution. Catalytic activities arise by polymerization, and are found in the products tested in aqueous solution, where boiling destroys activity for some substrates (Rohlfing and Fox, 1969). [In at least one case such treatment has increased the activity (Dose and Zaki, 1971).]

A more fundamental structural consideration involves the fifth question, the primary structure of the anhydroamino acid sequence. This question of order in the primordial sequence of amino acid residues was discussed by, for example, Oparin (1957) before a method for the production of a model of prebiotic protein was available—that is, before any facts could be accumulated. The assumption of Oparin and of others was that any protein-like polymer that might be produced would lack internal order. This question is, in fact, re-

lated to the thinking that preceded the first experiments on proteinoids (Fox, 1956); the experiments were not performed until a rational basis for the spontaneous appearance of order in such polymers could be visualized. When an answer appeared to the effect that the reacting amino acids might themselves determine the order in which they would be coupled in such a polymer (Fox et al., 1953), the experiments were performed. The polymers have been found, by various kinds of evidence, to be indeed internally ordered to a relatively high degree. This evidence and some quantitative assessment of the order within these polymers will be described later (page 158).

The discussion of questions such as have been considered in the preceding paragraphs is complicated. The experiments and the flow of the processes are, however, simple and the interpretations are both simple and straightforward.

PRODUCTION OF THERMAL PROTEINOIDs. The synthesis of proteinoids is both simple and quite rapid. Students in elementary classes have done many of the experiments (Vegotsky, 1972; Rhodes et al., 1975). Ground or amorphous mixtures of dry amino acids containing at least a small proportion of nonneutral amino acids are, usually, heated at temperatures in the range of 120–200°C. Typically one week of heating is used for the temperature of 120°C, and 6–10 hours for 170 or 180°C. Proteinoids have been made at 80°C (Snyder and Fox, 1975) and Rohlfing (1976) has studied such syntheses at 65°C and 85°C, in both yield and molecular weight ratio at 20, 48, and 81 days.

Lehninger (1975) has pointed out that simulation experiments are often conducted at temperatures higher than was necessary on the primitive Earth, in order to conserve working time in the laboratory, and that the reactions would be expected to occur more slowly at lower temperatures. Some experiments have been done with sets of amino acids in dilute aqueous solution; a constant temperature first volatilizes the water, and then promotes the polymerization of the amino acids in the dried residue.

By variation of the proportion of the kinds of amino acid in the mixture, the products may be made acidic, neutral, or basic, or otherwise varied. The proportions of individual amino acid in the polymers are also controllable with high precision; the latter are a function of proportions in the reaction mixture (Table 5-1). The polymers may include such added substances as iron or hemin (Dose and Zaki, 1971). The reactions are very rugged; they have been carried out in the presence of a wide variety and varied amounts of added terrestrial materials. The condensations are not easily disturbed. The amber

colored products are either (1) a molten mass of polymer, with unreacted amino acids, and an inevitable proportion of decomposition products or (2) a pasty mass. The molten polymer does not dissolve significant proportions of other kinds of substance. Many minerals are not very soluble in such reaction mixtures. Also, since amino acids pass from a solid state to a molten mixture of polymer with oligomers and monomers (e.g., pyroglutamic acid or the lactam of lysine), no question of an act of concentrating amino acids from a solvent need arise. Also, as stated earlier, an aqueous solution may be heated until the dry amino acids left by evaporation condense. The amino acids are at infinite concentration in the dry state. An infinitesimal amount of amino acid can polymerize on heating as indicated, providing the small but sufficient proportion of nonneutral amino acid is present.

One kind of added substance, phosphoric acid or polyphosphoric acid, has effects beneficial to the condensation. These acids are also miscible with the reaction mixture. The temperature of condensation may be lowered by adding 2–3 parts of phosphoric acid or polyphosphoric acid. This facilitates condensation in substantial yields (5–35 percent), and at temperatures such as 60°C or lower. Reactions at 60°C ordinarily produce workable yields over periods such as 150 hours. The availability of acidic polyphosphate on the primitive Earth can be more easily visualized through the studies of Osterberg and Orgel (1972) and of Rabinowitz et al. (1968), at relatively low temperatures.

PROPERTIES OF THERMAL PROTEINOIDS. A definition of proteinoids, slightly altered from Hayakawa et al. (1967), is “macromolecular preparations of mean molecular weights in the thousands containing most of the twenty amino acids found in protein hydrolyzates. Although these polymers have other properties of contemporary protein as well, identity with the latter is not a necessary inference.” Proteinoids are a kind of artificial protein in the sense that they are produced by the chemist; they are natural in the sense that the conditions necessary for thermal polymerization of amino acids are found on the Earth. According to the definitions in Webster’s dictionary neither “synthetic” nor “artificial” is a rigorously correct adjective for proteinoids (Neilson et al., 1968); they are closer to being artificial proteins than to being synthetic proteins.

Qualitative Composition. The typical polymeric product of a thermal reaction of amino acids includes each of the amino acids contained in the original mixture, whether there were, for example, 2, 3, 5, 6, 8, or 18 types of amino acid. The observation of the thermal condensa-

bility of α -amino acids has been confirmed in a number of laboratories (e.g., Germain et al., 1963; Ussdin et al., 1967; Hardebeck et al., 1968; Oshima, 1968). The fact that all of the amino acids common to contemporary protein are thermally condensable does not signify that all of those amino acids were available for condensation to prebiotic proteins. Some amino acids may have entered the evolutionary stream later through organismic synthesis. The fact, however, that the experiments show that virtually all amino acids can be condensed in large part rather than being destroyed by a thermal reaction indicates that a wide variety of mixtures of amino acids, which might form in natural experiments, would be condensed. On the basis of the premise that evolution could not proceed unless enzymelike molecules covering a range of specificities could come into existence to produce more molecules subsequently (page 171), reaction of a fairly wide variety of the monomeric amino acids would have been necessary to permit such diversity in the macromolecules. Studies of composition and activity in thermal proteinoids indicate that their range is wide enough to provide a variety of side-chain chemistry, even before the appearance of a coding mechanism.

Inclusion in the polymeric product of several percent of serine, threonine, cysteine, or cystine can be accomplished by carrying out the condensation at lower temperatures, such as 130°C, and by including polyphosphoric acid to conserve cysteine or cystine (Genaux et al., 1967) or sodium polyphosphate (Dose and Rauchfuss, 1972). These amino acids are otherwise almost completely destroyed.

Quantitative Composition. Except for serine, threonine, and cystine, the composition of almost any contemporary protein can be simulated quite closely by a thermal condensation product. The simplicity of the condensation makes feasible the production of varied series of proteinoids. Table 5-1 lists such a set of proteinoids. These were produced within seven days from materials and glassware at hand. If the proteinoids are by choice closely related in composition, they can be studied precisely for their relationship to function. One of the functions that has been studied with the proteinoids listed in Table 5-1 is that of the tendency to form, by binding, particles of a uniform microscopic size with various polynucleotides (Waehneldt and Fox, 1968). This tendency has permitted the determination of the quantitative contribution of basic amino acid content to this function. In subsequent studies with other series from which individual amino acids were systematically omitted, selective relationships with polynucleotides have been observed. This kind of study can be carried out also with Leuchs' polymers, which have been shown also to

TABLE 5-1

Amino Acid Compositions in Molar Percentages of Hydrolyzates of a Systematically Altered Series of Proteinoids

Amino Acid	Proteinoid Number										
	1	2	3	4	5	6	7	8	9	10	11
Alanine	4.4	5.2	5.6	6.3	7.0	7.0	5.6	5.8	5.0	4.6	4.2
Arginine	3.9	4.5	4.9	5.3	4.8	5.2	3.9	4.0	4.0	3.8	3.5
Aspartic acid	40.3	30.1	20.3	13.7	7.3	6.3	5.7	4.9	4.1	3.6	3.7
Glutamic acid	13.9	13.8	12.1	10.5	8.6	7.8	8.8	8.9	8.1	7.8	7.5
Glycine	5.9	7.3	8.3	9.4	11.0	10.7	9.7	9.1	8.0	7.4	7.0
Histidine	3.9	4.3	5.0	5.3	4.8	5.2	4.3	4.8	4.6	4.4	4.0
Isoleucine	2.1	2.6	3.1	4.0	6.2	6.9	4.8	3.9	2.9	2.4	2.3
Leucine	4.8	5.3	6.7	7.9	11.0	11.2	9.5	8.1	6.5	5.8	5.3
Lysine	6.7	9.7	12.7	14.3	14.0	15.2	26.4	36.3	39.6	44.4	47.1
Proline	1.9	2.3	3.0	3.3	3.8	3.6	3.4	3.7	3.7	2.9	2.6
Valine	4.8	5.4	6.4	7.7	10.0	9.8	8.2	7.4	5.4	5.1	4.8
Alloisoleucine	2.1	2.5	3.1	3.5	5.1	5.0	3.9	3.2	2.5	2.1	2.0
Ammonia	5.8	6.9	8.6	8.3	6.2	7.1	5.2	5.8	5.4	4.6	5.5

Source: Fox and Waehneldt (1968).

be adaptable to the cocondensation of as many as 18 amino acids (Hayakawa et al., 1967). That process, however, is more demanding in time and it is devoid of any possibility of being a valid model of an evolutionary intermediate. The assembly with polynucleotides is an example of one possible kind of study, in addition to investigation of catalyses, of the effects of variation in composition and function (page 232).

Analyses of quantitative compositions of various proteinoids have shown a high degree of reproducibility from one laboratory to another (Fox, 1968a). Variation in composition is brought about not solely by variation in the proportions of amino acids in the reaction mixture, but also by the proportions of such added materials as phosphoric acid (Harada and Fox, 1960). Of special interest is the fact that the tendency of any one amino acid to be incorporated into a proteinoid is a function of the kinds and amounts of other amino acids present in the reaction mixture (Table 5-1; Fox and Waehneldt, 1968).

A fundamental question that applies to the quantitative compositions is whether one can observe evidence for or against randomness. A first test performed by the polymer chemist (Billmeyer, 1962) for randomness in copolymerization is the comparison of the composition of the reaction mixture with that of the polymer. If the composition is the same, the polymer may be said to be random. If it is not the same, the polymer is nonrandom, except for extenuating circumstances such as partial decomposition of one monomer. The results

in Table 5-2 (Fox, Harada, et al., 1963) demonstrate that the composition is different for the reaction mixture and for the polymer; note especially the 2:2:3 proteinoid. Nor can this result be explained as due to varied rates of decomposition of various amino acids. This kind of result has been obtained repeatedly in other syntheses from other reaction mixtures. Accordingly, the composition of a polymer is partially ordered, rather than being random, which is understood as being due to the proportion of each amino acid being determined by its own reactivity.

Arrangements of Amino Acid Residues. Studies of the arrangements of amino acid residues have given an additional answer to the question of randomness treated in the previous section. If the distribution of the amino acid residues in the polymers were completely random, the analysis for any one position in the chain should be the same as for the total polymer. As Table 5-3 shows, such analytical results are not

TABLE 5-2

Amino Acid Compositions in Molar Percentages of Two Proteinoids Compared to the Reaction Mixtures Polymerized

Amino Acid	2:2:1 Proteinoid		2:2:3 Proteinoid	
	Mixture (%)	Product (%)	Mixture (%)	Product (%)
Aspartic acid	42.0	66.0	30.0	51.1
Glutamic acid	38.0	15.8	27.0	12.0
Alanine	1.25	2.36	2.72	5.46
Lysine	1.25	1.64	2.72	5.38
Semi-cystine	1.25	0.94	2.72	3.37
Glycine	1.25	1.32	2.72	2.79
Arginine	1.25	1.32	2.72	2.44
Histidine	1.25	0.95	2.72	2.03
Methionine	1.25	0.94	2.72	1.73
Tyrosine	1.25	0.94	2.72	1.66
Phenylalanine	1.25	1.84	2.72	1.48
Valine	1.25	0.85	2.72	1.16
Leucine	1.25	0.88	2.72	1.06
Isoleucine	1.25	0.86	2.72	0.90
Proline	1.25	0.28	2.72	0.59
Serine	1.25	0.6	0.0	0.0
Threonine	1.25	0.1	0.0	0.0

Source: Fox, Harada, et al. (1963).

Note: Tryptophan was present in the 2:2:1 proteinoid, but was omitted, along with serine and threonine, from the 2:2:3 proteinoid.

found. The analyses were performed for those positions that are most feasible to assay in unfractionated polymers of amino acids, the *N*-terminal and the *C*-terminal. From these data, and from those presented earlier, we must infer that neither the compositions nor the sequences are random. The composition and sequence of amino acid residues in a polymer are a function of the reacting amino acids, as is shown in Tables 5-1 and 5-2. The individual proportions in the proteinoids in Table 5-2 can be seen to depend not only upon the proportions of the corresponding amino acids entering into the reaction but also upon the remainder of the composition.

Heterogeneity. The degree of heterogeneity found for thermal polyanhydroamino acids, particularly thermal proteinoids, has been studied by a number of methods based on different properties. These include electrophoretic techniques, sedimentation in the ultracentrifuge, repeated precipitation through cooling hot aqueous solutions, and by fractionation on various kinds of column (e.g., DEAE-cellulose and Sephadex). All of these methods yield concordant results indicating that the heterogeneity, or number of types of molecule, is very sharply limited.

The first study of heterogeneity by electrophoresis was performed by Vestling (in Fox and Harada, 1960) who found only two major peaks in an acidic 2:2:1 proteinoid fractionated in a moving boundary

TABLE 5-3
Distribution in Molar Percentages of Aspartic Acid, Glutamic Acid, and Basic-neutral Amino Acids in Two Proteinoids

	N-Terminal	Total Proteinoid	C-Terminal
<i>2:2:1 Proteinoid</i>			
Aspartic acid	6	71	1
Glutamic acid	46	11	8
Basic-neutral amino acids	48	17	91
<i>2:2:3 Proteinoid</i>			
Aspartic acid	n.d.	50	28
Glutamic acid	n.d.	12	1
Basic-neutral amino acids	n.d.	38	70

Source: Fox and Harada (1960; 1963).

Note: n.d. means not determined.

apparatus. Only a single peak was obtained in the ultracentrifuge by Vegotsky (1961) for a similar preparation.

A 1:1:1 proteinoid (from 1 part each of aspartic acid, glutamic acid, and an equimolar mixture of the 16 other amino acids) was first amidated to convert it to a neutral polymer. When the material was fractionated on DEAE-cellulose, quite discrete fractions were obtained, as is seen in Figure 5-1. When three of the fractions, nos. 3, 4, and 5 were hydrolyzed (a) totally, and (b) partially and the fragments were chromatographed, the results of Fig. 5-2 were obtained.

The patterns of the total hydrolyzates of the materials of fractions 3, 4, and 5 are seen in the top three chromatograms of Figure 5-2. The pattern for the total hydrolyzate of the proteinoid, not shown, is similar. All of these indicate that the proteinoid is highly uniform in composition.

The partial hydrolyzates of the three fractions of polymer eluted from DEAE-cellulose were first examined by conventional two-dimensional chromatography. The three patterns were, by eye, indistinguishable from each other. These three fractions were then

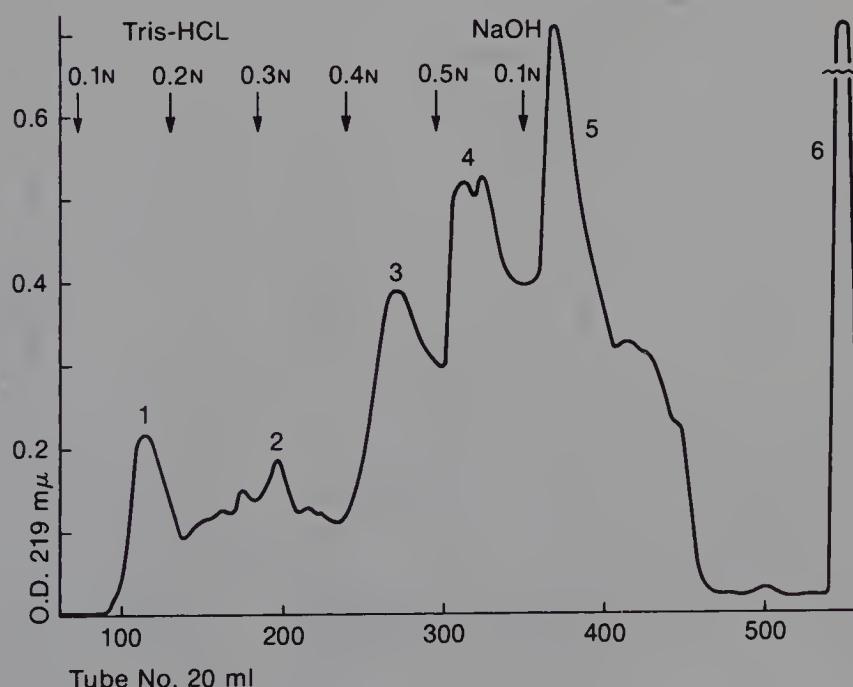
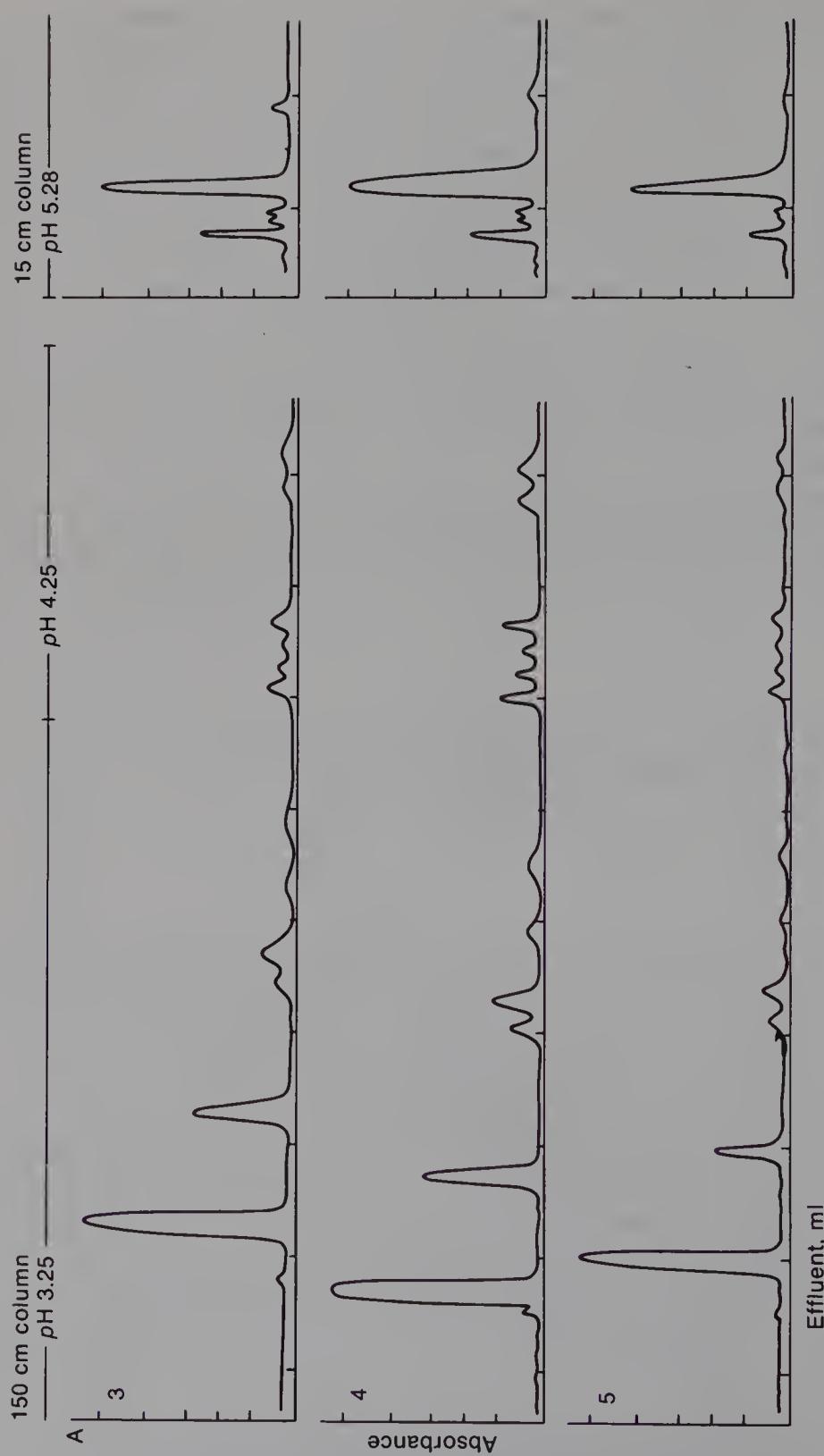


FIGURE 5-1

Elution pattern of 1:1:1 proteinoidamide, at 219 m μ , from DEAE-cellulose column with tris buffer. Similar patterns were obtained by continuous gradient elution. Fraction numbers appear on the graph.



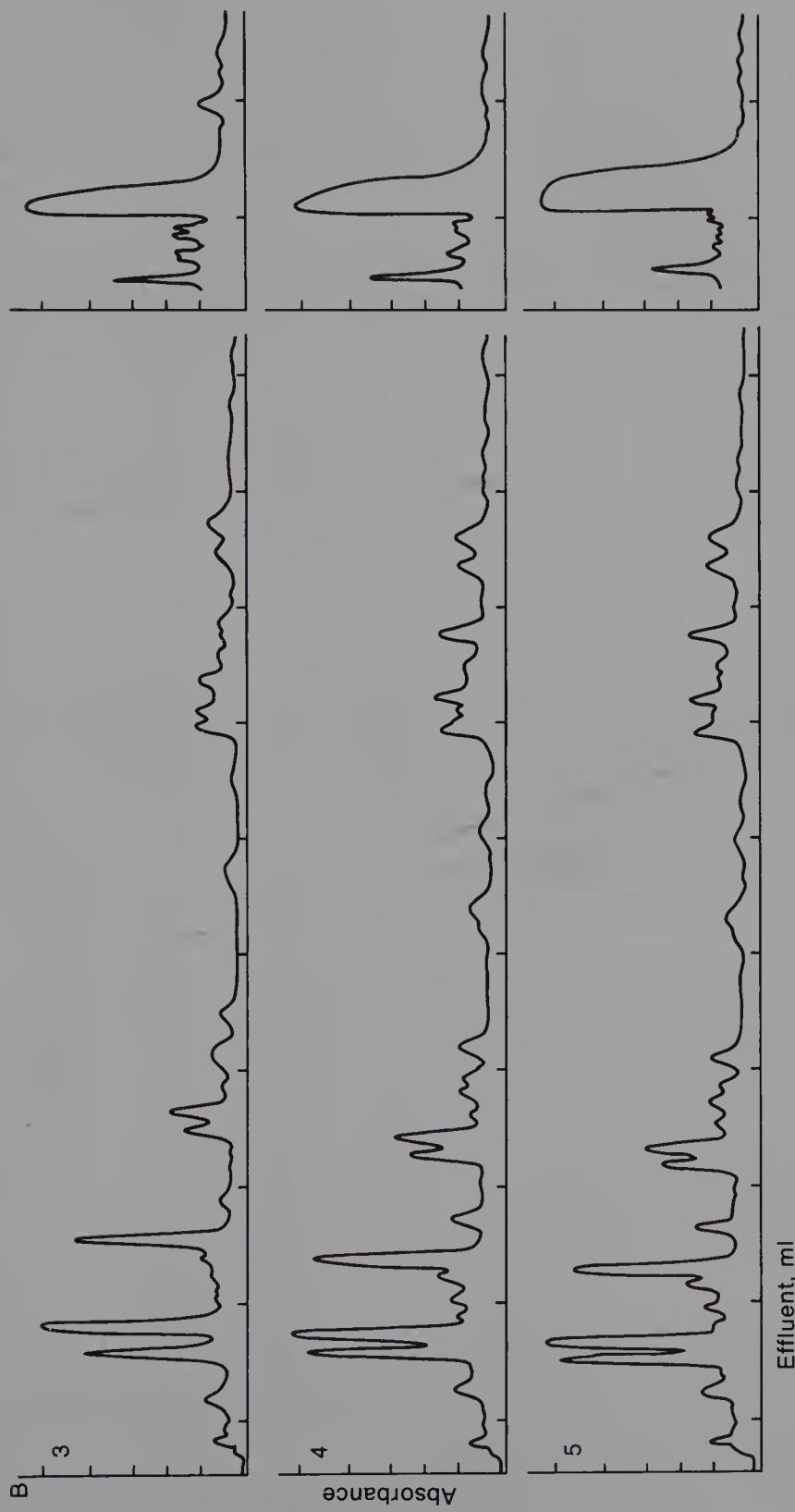


FIGURE 5-2
Top three chromatograms (p. 160) are total hydrolysates of fractions 3, 4, and 5 from the distribution of proteinoid hydrolysate. Bottom three chromatograms (p. 161) are partial hydrolysates, which equal peptide maps.

distributed on the column of the automatic amino acid analyzer to yield the three profiles at the bottom of Figure 5-2. In each analyzate are forty peaks. Fifteen are amino acids; the others are peptides as verified by alkaline hydrolysis, which increased the ninhydrin color in each case. These "fingerprints," or "peptide maps," are so similar as to lead inexorably to the interpretation that the sequence is highly uniform throughout the proteinoid.

Another polymer, a 2:2:3 proteinoid, was purified from water by dissolving to a saturated solution by heating and then allowing the solution to cool. Proteinoid precipitated from the cooled solutions. Successive purifications of this sort gave fractions for which the amino acid analyses are remarkably similar (Table 5-4).

Like many proteins, thermal polymers of α -amino acids often tend to be adsorbed by the carrier material during electrophoresis. Good results, however, with many different kinds of thermal polymer have

TABLE 5-4

*Composition of Hydrolyzates (110°C, 4 days) of 2:2:3 Proteinoid
Following One and Two Purifications (the percentage values given are
gram residues of amino acid per 100 residues in polyamino acid)*

Amino Acid or Ammonia	Unpurified	Purified	Repurified
Lysine	5.1	5.4	5.4
Histidine	1.8	2.0	2.0
Ammonia	8.6	8.1	6.9
Arginine	2.0	2.3	2.4
Aspartic acid	51.7	50.2	51.1
Glutamic acid	10.7	11.6	12.0
Proline	0.7	0.6	0.6
Glycine	2.7	3.1	2.8
Alanine	4.0	4.3	5.5
Half-cystine ^a	4.5	3.5	3.4
Valine	1.2	1.2	1.2
Methionine	1.8	1.9	1.7
Isoleucine ^b	1.2	1.3	0.9
Leucine	1.3	1.2	1.1
Tyrosine	2.0	1.9	1.7
Phenylalanine	1.8	1.7	1.5
Total recovery ^c	84.8	97.5	100.0

Source: Fox, Harada, et al. (1963).

^aHalf-cystine may be partly other material.

^bIsoleucine includes alloisoleucine.

^cTotal recovery is total residues of amino acid per 100 residues in polyamino acid.

been obtained by gel electrophoresis (acrylamide gel). In Figure 5-3 is shown the migration of a lysine-rich hemoproteinoid (molecular weight, 18,000) after electrophoresis at pH 8.3, and subsequent staining with Amido Black 10B. Hemoproteinoids have been synthesized from various mixtures of amino acids containing also 0.25–2.0% hemin. The heme is tightly, perhaps covalently, bound. Hemoproteinoids prepared from amino acid mixtures having predominant proportions of lysine possess a pronounced peroxidase activity. This hemoproteinoid is a remarkable example of the limited heterogeneity observed for thermal polyamino acids (Dose and Zaki, 1971). The same hemoproteinoid was also eluted from a Sephadex G-75 column in a single fraction. Chromatography on a DEAE-cellulose column, however, revealed two fractions. This result also shows that the separation of proteinoids by ion exchange chromatography is superior to the separation by gel electrophoresis and gel chromatography.

The C-terminal amino acid analyses of Table 5-3 have been reported for all amino acids in two proteinoids (Harada and Fox, 1975); the results indicate significant proportions of all amino acids except the dicarboxylic type. Phillips and Melius (1974) have obtained comparable results for a thermal polymer from eight amino acids inclusive of aspartic acid and glutamic acid. However, when Melius and Sheng (1975) cocondensed six amino acids inclusive of glutamic acid, with aspartic acid omitted, they found three major fractions on paper chromatography. Each fraction displayed a single C-terminal amino acid! These were respectively glycine, alanine, or leucine. This kind of result is consistent with the findings of Fox and Nakashima (1967) as partly shown on pages 160–161.

In general, each thermal polyamino acid can be easily fractionated into several individual fractions. Each of these is closer to homogeneity than the original unfractionated polymer. The results of Melius especially suggest sterically controlled mechanisms in which, once any single peptide chain begins to form, it determines with relatively high specificity subsequent sequence at each stage. This effect relates to the nucleic acid-free contemporary polypeptide syntheses studied by Lipmann (1972, 1974) and to enzymic peptide bond synthesis controlled by the reactant amino acid derivatives (Fox et al., 1953; page 153 of this book).

All of these results point to the same conclusion—that the reactions of amino acids with a growing chain during thermal condensation are sufficiently selective to yield nonrandom arrangements in individual molecules (Fox, Harada, et al., 1963; Fox, 1965). These effects are widespread among the molecules in the whole preparation, so that the polymeric preparation is far from random in its total constitution.



FIGURE 5-3
Disk gel electrophoretogram of hemoproteinoid. A single band is seen.

Certain compositions and certain sequences are favored, to a truly remarkable degree. The favored structures appear as clusters, or families, of molecules, which themselves are limited in diversity. These results have been interpreted to indicate that prior nucleic acids were not in any fashion required to specify sequences in preproteins (page 247).

Especially relevant, perhaps, is the fact that the polymerization is conducted at temperatures high enough to permit transpeptidation within the chains to a few most thermodynamically stable sequences, but not so high as to cause extensive decomposition of the polymers. This point assumes added significance when we consider the origin of the first informational macromolecules (page 247). Polyamino acids are the only such polymers known to be capable of withstanding temperatures that will result in nonrandom sequences.

The evidence for nonrandomness in proteinoids is summarized in Table 5-5.

Molecular Weight. Mean molecular weights of the proteinoids have been determined mainly by end-group assay and by sedimentation-velocity analyses in the ultracentrifuge (Fox and Harada, 1960; Fox and Nakashima, 1967). For estimation, a number of molecular-weight ranges have been approximated by the use of gel exclusion techniques. Average molecular weights of proteinoids made under comparable conditions tend to increase from the acidic types, which are the lowest, through the neutral, which are of intermediate weight, to the basic, which are the highest. Fractions of some of the thermal

TABLE 5-5
Evidence for Limited Heterogeneity in Thermal Polymers of Amino Acids

Evidence	Authors and Date
Nonrandom sequences by disparity between <i>N</i> -terminal and total analyses in thermal polymers	Fox and Harada (1958)
%s in reaction mixture ≠ %s in polymer	Fox and Harada (1960)
Limited number of fractions on electrophoresis	Vestling (1960)
Limited heterogeneity on ultracentrifugation	Vegotsky (1961)
Constant composition on repurification from water	Fox, Harada, Woods, Windsor (1963)
Single band on gel electrophoresis of acidic proteinoidamide	Fox and Nakashima (1966)
Nonrandom elution pattern from DEAE-cellulose	Fox and Nakashima (1967)
Symmetrical peaks from DEAE-cellulose	
Almost uniform amino acid compositions in various fractions	
Stoichiometric amino acid compositions	
Uniform ultracentrifugal patterns of various fractions	
Almost uniform peptide maps in all fractions	
Single spots on high voltage electrophoresis of fractions	
Single species of "active site" proteinoid	Usdin, Mitz, and Killos (1968)
Single band for gel electrophoresis of basic hemoproteinoid	Dose and Zaki (1971)

copolylsines have been found to have mean molecular weights much above 10,000 by sedimentation-equilibrium analysis (Genaux and Fox, 1961; Hennon et al., 1971). The mean molecular weights of fractions of 1:1:1 proteinoid converted to an amide and then separated on DEAE-cellulose columns are given in Table 5-6. The results

presented are those determined by ultracentrifugation together with values calculated from amino acid content. These values are quite similar to those obtained by end-group assay on comparable preparations. The acidic type of proteinoid has seldom exceeded 10,000 in molecular weight.

The mean molecular weights observed for the proteinoids fall within the lower end of the molecular-weight range of contemporary proteins.

Table 5-7 shows the effect of variation in temperature of formation on the molecular weights of two kinds of proteinoid. In Table 5-8 is presented the effect of time of heating on the yield of proteinoid. The progress curve for the formation of the 2:2:1 proteinoid is sigmoid, which suggests that the synthesis is autocatalytic.

TABLE 5-6
Mean Molecular Weights of Amide Fractions of 1:1:1 Proteinoid

Fraction ^a	By Sedimentation Analysis	By Amino Acid Composition
3	4100	5600
4	5200	5400
5	5800	8300

Source: Fox and Nakashima (1967).

^aFrom fractionation on DEAE-cellulose.

TABLE 5-7
Molecular Weights of Proteinoids Formed by Heating for Six Hours at Different Temperatures

°C	2:2:1 Proteinoid	1:1:1 Proteinoid
160	4600	3600
170	4500	3800
180	5500	4100
190	7200	8600

Source: Fox and Harada (1960).

Note: Molecular weights were determined by N-terminal assay; an average amino acid residue weight of 100 is assumed.

Studies of the rate of formation of proteinoid show a tendency of the aspartic acid content to decrease and of other amino acids to increase as the time of heating is lengthened.

TABLE 5-8
Yield of Solid Proteinoid at 170°C as a Function of Time of Heating

Time (hr)	Yield ^a of 2:2:1 Proteinoid (g)	Yield ^b of 1:1:1 Proteinoid (g)
2	2.4	1.4
4	4.0	1.4
6	9.4	1.7

Source: Fox and Harada (1960).

^aFrom 10 g of DL-aspartic acid, 10 g of DL-glutamic acid, and 5 g of an equimolar mixture of the 16 other proteinous amino acids.

^bFrom 5 g each of the same three components.

Solubility, Precipitability, and Ionic Behavior. Depending upon which amino acids are used in the reaction mixture, a proteinoid to correspond to each of the various classes of protein may be obtained. Some have solubility properties like those of albumins, others like globulins, others like histones, etc. The solubility has proved to be a function of the composition of the proteinoid. For example, polymers rich in amino acids containing hydrocarbon side chains are less soluble in water.

Proteinoids can be precipitated from aqueous solution by reagents that precipitate proteins. Included among these reagents are trichloroacetic acid, picric acid, and phosphotungstic acid.

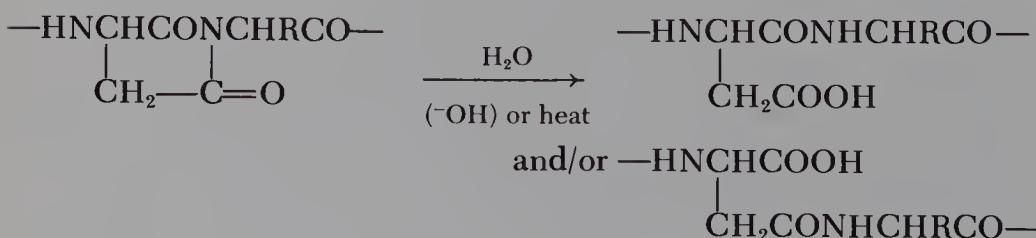
Proteinoids respond to salts in various concentrations and solutions as do proteins. They may be salted in by dilute concentrations of salt, as are globulins. They may be salted out by high concentrations of salts such as ammonium sulfate. Proteinoids behave in electrophoresis in the way that would be expected for amphoteric polymers.

Configuration of Residues. Many proteinoids have been made from mixtures of DL amino acids. When some of the reactant amino acids are of the L configuration, much racemization is observed. This racemization, although substantial, is, however, incomplete under conditions ordinarily used for the production of proteinoids. A typical proteinoid made from eighteen amino acids had $[\alpha]_D^{25} = -5.6^\circ$. Under the usual conditions of pyrocondensation, L-aspartic acid is entirely racemized, while L-glutamic acid, L-leucine, and L-isoleucine are only partly racemized (Fox and Harada, 1960; Fox et al., 1962; Rohlfing, 1967a).

These results bear on questions of the origin and evolution of optical activity (page 268).

Linkages. The evidence for peptide linkages in the thermal poly-amino acids is, in a number of respects, similar to that which was accumulated over many years for peptide linkages in proteins. A positive biuret test first indicated a polypeptide structure in proteinoids (Fox and Harada, 1958; Krampitz, 1959). Another kind of evidence for the presence of the peptide bond in proteinoids is that of the infrared spectra, which imply a polypeptide structure (page 169). The proteinoids are split by proteases in varying degrees. Here the specificity agrees with the formal relationships that have been established (Fox and Harada, 1960); for instance, acidic proteinoids are split more rapidly by pepsin than by other proteinases. The rate of splitting of proteinoids by proteolytic enzymes is usually considerably less than that for the corresponding types of protein. In the acidic proteinoids, the lower rate of splitting may be caused by masking of peptide bonds by imide linkages or cross linkages. The various proteinoids tested have not been hydrolyzed with equal rapidity. A fraction of thermal poly(glutamyl, glycyl, tyrosyl), which is glu-gly-tyr-glu-tyr-gly, is split more rapidly by aminopeptidase, carboxypeptidase, and pronase than by chymotrypsin (Fox and Nakashima, 1969). In general, the susceptibility of proteinoids to proteases is limited, and is of the expected specificity. This subject deserves further investigation. Peptides obtained by partial hydrolysis of proteinoids are split with considerable rapidity by peptidases. The relatively limited degree of hydrolysis is not consistent with the extended nutritional use of proteinoids by such bacteria as *Lactobacillus plantarum* (page 170). Perhaps bacterial proteases are more effective on substrates containing D amino acid residues than are the mammalian proteases used for tests of proteolysis. This question, also, invites further investigation.

The imide linkage is found in thermal copolymers of aspartic acid, including proteinoids. Depending upon neighboring residues, this linkage is more-or-less readily converted to a true peptide bond by treatment with dilute alkali or by warming in aqueous solution,



as shown with substituted phthalimide (Hoagland and Fox, 1967). The imide linkage has become implicated as a transitory structure in enzymes (Bernhard et al., 1962). Although it is easily formed by heating, the imide linkage in proteinoids is not stable in aqueous solution

(Fox and Harada, 1960) and cannot be expected to have endured geologically, except in hypohydrous settings. The imide linkage precludes much branching in proteinoids (Fox, 1968a). The neutral proteinoids that have been prepared more recently (Fox and Waehneldt, 1968) contain less than 5 percent aspartic acid. As a consequence, they can be only slightly branched.

Kovacs et al. (1961) have carried out an extensive study of the proportions of α - and β -linkage in thermal polyaspartic acids; they estimated the proportions by various methods of chemical conversion. They typically found a ratio of $1.0\alpha:1.3\beta$. This result has been disputed by Andini et al. (1975) and Temussi et al. (1976) by NMR (the latter two sets of authors being from one laboratory). Each of the two laboratory groups, however, prepared polyaspartic acid under its own set of conditions, and then examined only a small fraction of the polyaspartic acid which they prepared (Fox, 1976).

Glutamic acid residues tend to be linked through the α -carboxyl in thermal polyamino acids (Phillips and Melius, 1974; Melius and Sheng, 1975). This can be most easily understood on the basis that, on heating, glutamic acid forms the lactam (pyroglutamic acid) structure, in which only the α -carboxyl is free. While probably not within a direct evolutionary relationship, it is of interest that a number of *N*-terminal pyroglutamyl residues have been found in proteins (Doolittle and Armentrout, 1968).

Proportions of α and ϵ linkages in thermal polymers of lysine have been determined in a few instances (Harada, 1959; Harada and Fox, 1965a; Suzuki, 1966; Heinrich et al., 1969, Fox and Suzuki, 1976). In the polymer of alanine and lysine more than half of the lysine has been found to be linked through the ϵ -amino group, also a linkage not reported as existing in collagen (Mechanic and Levy, 1959).

Color Tests. Those color tests that are positive for proteins are also positive for proteinoids. In addition, the ninhydrin test for free α -amino acids in acidic proteinoids is negative, and becomes positive after the proteinoid is hydrolyzed. Lysine-rich proteinoids are somewhat ninhydrin-positive. The reaction increases upon hydrolysis. Other positive color tests have been recorded for the biuret test, Millon's reaction, the xanthoproteic test, and a number of standard precipitating agents such as sulfosalicylic acid, phosphotungstic acid, etc. (Fox and Harada, 1958; Krampitz, 1959).

Infrared Absorption Spectra. The absorption bands found in acidic and basic proteinoid are listed in Table 5-9. The absorption bands of acidic proteinoid at 1720 and 1780 cm^{-1} are replaced by bands of

lower wave number as the proteinoid is converted to its alkali salt. The bands mentioned are due to imide linkages, and are absent from the lysine-rich proteinoid (Fox et al., 1962).

Hydrolytic Behavior. Proteinoids are hydrolyzed by the same reagents that are ordinarily used on proteins—that is, concentrated hydrochloric acid and, occasionally, concentrated alkali for the determination of tryptophan. The extent of hydrolysis is dependent upon time and temperature of heating and upon the concentration of hydrochloric acid, as it is for proteins. The extent of hydrolysis is also dependent upon the particular proteinoid or polyamino acid. Acidic proteinoids that have undergone a moderate degree of purification (Fox et al., 1963) yield constituent amino acids quantitatively (Table 5-10). Recoveries upon acid hydrolysis of lysine-rich proteinoid are typically 60–85 percent (Fox et al., 1962). These impaired recoveries are tentatively explained on the basis of difficultly hydrolyzed cross linkages involving ϵ -amino groups and β -carboxyl groups of aspartic acid.

Proteinoids are hydrolyzed also by proteolytic enzymes (Fox and Harada, 1960), although the degree of hydrolysis appears in most cases to be considerably less than that of proteins (Table 5-11).

Nutritive Quality. Various bacteria, such as *Lactobacillus plantarum*, that are known to have requirements for preformed amino acids are able to utilize thermal proteinoids nutritionally (Fox and Harada, 1960). Proteinoids fed to rats in experiments over many months have been found not to be toxic (Krampitz and Knappen, 1964). In general, their nutritive quality is inferior to that of a protein such as casein, but a substantial fraction of casein can be replaced by proteinoids without impairment of the nutritive quality of the total nitrogen fraction of the rats' diet.

TABLE 5-9
Infrared Absorption Bands of Proteinoid

Bands in Acidic Proteinoid, cm^{-1}	Bands in Lysine Proteinoid, cm^{-1}	Structural Indication
3300	3300	—NH—
3080	3080	—NH—
1650	1650	amide I
1550	1550	amide II
1720		imide, —COOH
1780		imide

Source: Fox and Harada (1960).

TABLE 5-10

Analyses of Purified 2:2:1 Proteinoid after the Indicated Number of Days of Hydrolysis at 105°C (The percentage values given are gram residues of amino acid per total gram residues.)

Amino Acid	1	2	3	4	6
Lysine	1.58	1.64	1.98	1.79	1.85
Histidine	1.17	0.95	0.95	0.77	0.89
Ammonia	4.05	3.60	3.90	5.75	3.98
Arginine	1.06	0.94	0.93	0.90	0.98
Aspartic acid	59.8	66.0	65.6	63.6	64.3
Glutamic acid	15.5	15.8	15.9	15.2	15.8
Proline	^a	0.28	0.36	0.28	0.31
Glycine	1.44	1.32	1.38	1.36	1.33
Alanine	2.60	2.30	2.56	2.43	2.51
Half-cystine	^b	1.32	1.41	1.29	1.42
Valine	0.98	0.85	0.94	0.89	0.98
Methionine	1.05	0.94	0.99	1.04	1.08
Isoleucine	0.97 ^c	0.86	1.00	1.27	1.05
Leucine	0.95	0.88	0.96	0.93	0.96
Tyrosine	1.15	0.94	1.02	1.02	1.06
Phenylalanine	2.05	1.84	1.92	1.80	1.93
Total recovery	85 ^d	103	101	103	97

Source: Fox et al. (1963).

^aIn this chromatogram, the peak for proline was not distinct.

^bHalf-cystine may be partly other material.

^cIsoleucine includes alloisoleucine.

^dTotal recovery is total gram residues of amino acid/per weight of polymer.

Enzymelike Activities. Proteinoids may be examined for activities resembling those of enzymes. In a protobiogenic context, whichever polymers had activities sufficient to advance evolution, even if largely by metathesis, would serve as primordial enzymes. Some of the proteinoids have been shown to meet the rigorous requirements set for catalysts (Rohlfing and Fox, 1969); others have not.

Of the various activities of proteinoid, the catalytic, or rate-enhancing activities, and the tendency to form structures visible through the microscope are undoubtedly the most fundamental. These are activities that would have the most significance in a prebiotic, primordial, and primitive context. Indeed, from one point of view, the structure and ultrastructure of the cell may be viewed as contributing to an efficient deployment of the catalytic activities that make up the whole of cellular metabolism (page 211).

Among all published articles about proteinoids from various laboratories, the largest number are concerned with their enzymelike activities. The significance of these activities has been expressed in principle by Dixon and Webb (1958) in their treatise on enzymes:

TABLE 5-11

Hydrolysis of Proteinoids and Casein by Two Proteases (measured by optical density following dinitrophenylation)

Day	Pepsin on		Chymotrypsin on	
	1:1:1 Proteinoid	Casein	2:2:1 Proteinoid	Casein
0	0.6	0.4	0.2	0.2
1			0.3	0.5
2	1.5	2.3		
3			0.4	1.1
6	2.3	3.6		
7			0.5	1.9

Source: Fox and Harada (1960).

"Given pre-existing enzymes the formation of other enzymes is understandable; but the difficulty arises: If enzymes are formed only by enzymes, how were the first enzymes formed?" The reader is referred to the book by Dixon and Webb for a thorough and careful analysis of this question and the pertinent problems.

The catalytic, or rate-enhancing, activities that have been found in thermal proteinoids (Rohlfing and Fox, 1969) are listed in Table 5-12. These activities relate to five types of reaction: hydrolysis, decarboxylation, amination, deamination, and peroxidation. Some activities are so weak, relatively, that radioactivity has been employed in some experiments to detect changes in substrates. For almost all studies in the various laboratories, key experiments have been run under thoroughly aseptic conditions; absence of micro-organisms has been proved by testing and other precautions have been observed from the outset.

Conjugated proteinoids may have activities comparable to those of the important class of conjugated proteins. This relationship was first indicated by Dose and Zaki (1971) who found that the peroxidatic activity of free hemin is increased up to 50 times if the heme group is incorporated into thermal polymers of α -amino acids. The highest peroxidatic activities were found for hemopolymers containing up to 90 percent lysine in their polypeptidic part. The isoelectric point of these polymers is 8.5. Their relatively broad pH optimum (in the guaiacol test) is in the neutral pH range. The most active preparations have a molar ratio of heme to proteinoid of 1. The substrate specificity appears as broad as that of biogenous peroxidases, e.g., horseradish peroxidase. Also, NADH is oxidized. Because the affinity of the hemoproteinoids for H_2O_2 is very high, H_2O_2 is readily attacked

TABLE 5-12
Catalytic Activities in Thermal Polyanhydro- α -amino Acids Including Proteinoids

Reaction and Substrate	Remarks	Authors and Year
<i>Hydrolysis</i>		
<i>p</i> -Nitrophenyl acetate	Activity of proteinoid-bound histidine greater than that of equivalent free histidine	Fox, Harada, and Rohlfing (1962)
<i>p</i> -Nitrophenyl acetate	Thermal polymers most active	Noguchi and Saito (1962)
<i>p</i> -Nitrophenyl acetate	Inhibition by organic phosphates; reversal	Usdin, Mitz, and Killos (1967)
<i>p</i> -Nitrophenyl acetate	General description	Rohlfing and Fox (1967a)
<i>p</i> -Nitrophenyl acetate	Reactive site, and inactivation in hot water	Rohlfing and Fox (1967b)
ATP <i>p</i> -Nitrophenyl phosphate	Through Zn salt A second phosphate hydrolysis	Fox and Joseph (1965) Oshima (1968, 1971)
<i>Decarboxylation</i>		
Glucuronic acid	From glucose, CO ₂	Fox and Krampitz (1964)
Pyruvic acid	→ acetic acid + CO ₂ ; Michaelis-Menten kinetics	Krampitz and Hardebeck (1966)
Oxaloacetic acid	Rapid, requires basic polymers	Hardebeck, Krampitz, and Wulf (1968) Rohlfing (1967b)
<i>Amination</i>		
α -Ketoglutaric acid	Requires both Cu ⁺⁺ and proteinoid	Krampitz, Diehl, and Nakashima (1967)
<i>Deamination</i>		
Glutamic acid	Requires both Cu ⁺ and proteinoid	Krampitz, Haas, and Baars-Diehl (1968)
<i>Oxidoreduction</i>		
H ₂ O ₂ (catalase reaction)	Activity of hemin lowered when incorporated into proteinoids	Dose and Zaki (1971)
H ₂ O ₂ and hydrogen donors (guaiacol, hydroquinone, NADH, and others) (peroxidase reaction)	Activity of hemin increased up to 50 times in lysine-rich hemoproteinoids partly by heating in water	Dose and Zaki (1971)

even at concentrations below 10^{-6} M. This oxidation of substrates, including hydrogenated coenzymes, is probably the most effective oxidation process that can be visualized for the anaerobic conditions of the primitive Earth. The catalytic activity of hemin is rather decreased if it is incorporated into hemoproteinoids. This can be interpreted on an evolutionary basis. Hydrogen peroxide was widely formed in the primitive hydrosphere by the action of ultraviolet light and high-energy radiation. The steady state concentration of H_2O_2 was probably very low due to the Urey process and simultaneous destruction of the peroxide by the same energies. A catalytic activity would have merely contributed to a further decrease in H_2O_2 , but the peroxidatic process would have permitted the use of H_2O_2 to oxidize a variety of organic substrates. The results on the catalytic and peroxidatic activities of hemoproteinoids are summarized in Table 5-12.

The proteinoid-enhanced reactions displayed $p\text{H}$ -activity curves typical of those seen with enzymes (Figure 5-4). For each of the types of reaction recorded, Michaelis-Menten kinetics have been recorded (Figure 5-5; Rohlfing and Fox, 1969).

The synthetic nature of the proteinoids, as models for enzymes, makes feasible highly controlled studies of the relationship of molecular weight to activity. The results indicate, in each of several studies, that specific activity increases approximately in proportion to molecular weight. This, plus the fact that larger molecules were more likely to be retained by cellular membranes, meant that high molecular weight in association with catalytic structure would have been advantageous for evolution.

Rohlfing (1970) has reported that the activities of many dry proteinoids were retained, or slightly increased, after storage for years. This result indicates that preenzymes, once produced on the primitive Earth, could have remained available for prolonged periods during which evolution to organized microsystems (Chapter 6) could have occurred.

An Origin of Metabolism. Some of the individual reactions listed in Table 5-12 provide a basis for conceptualizing the origin of a small part of metabolic pathways. In Figure 5-6 is depicted a flow from oxaloacetic acid to pyruvic acid to acetic acid and side reaction from pyruvic acid to alanine, which is reversible. Each of these reactions is catalyzed by a different type of proteinoid or metal-proteinoid complex, as the figure demonstrates. On this basis, specificities are discernible. Reaction (1) is catalyzed by basic proteinoids but not by acidic proteinoids. Reaction (2) is catalyzed more effectively by acidic proteinoids than by basic proteinoids. Reaction (3) requires the

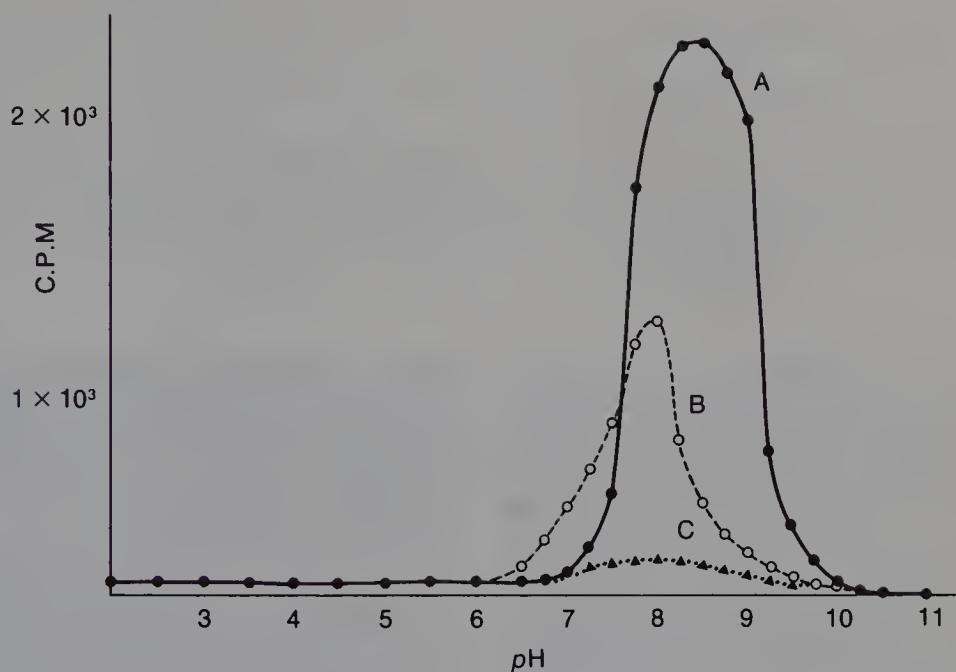


FIGURE 5-4

Curve of pH-activity for decarboxylation of pyruvic acid: (A) in presence of proteinoid, (B) in presence of amino acids, (C) in absence of amino acids or proteinoid. Source: Hardebeck, Kraimpitz, and Wulf (1968).

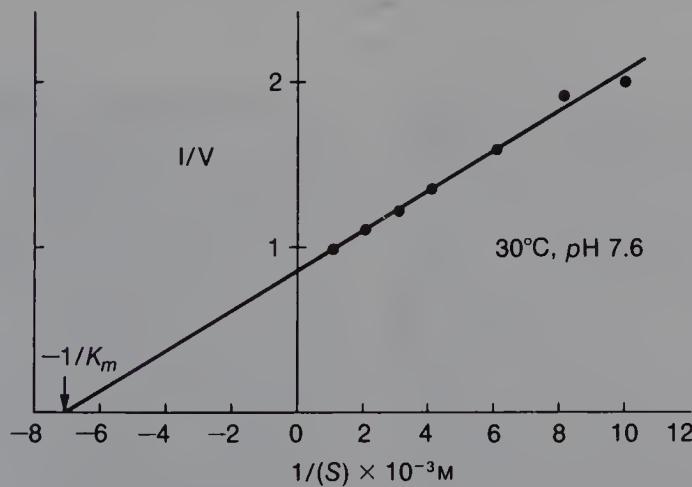


FIGURE 5-5

Lineweaver-Burk plot for hydrolysis of *p*-nitrophenyl phosphate in solution in presence of proteinoid. Source: Oshima (1968).

simultaneous presence of basic proteinoids and cupric ion. The reverse reaction (4) requires the simultaneous presence of basic proteinoids and cuprous ion. Each of these examples demonstrates a selec-

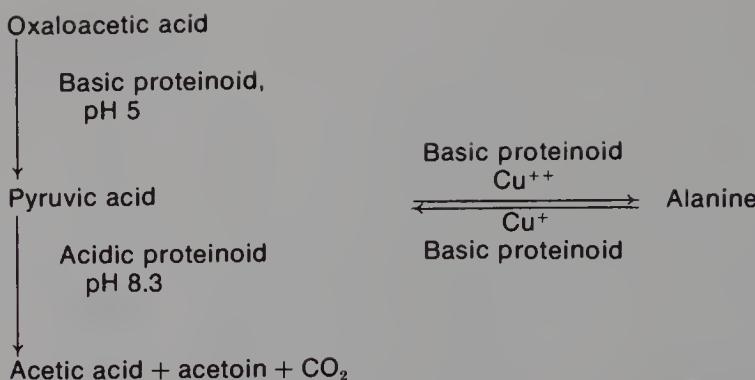


FIGURE 5-6

A model of the origins of metabolic pathways from sequential actions of proteinoids and copper-proteinoids.

tivity for the substrate by a given proteinoid. For example, the decarboxylation of oxaloacetic acid is favored by basic proteinoids, whereas acidic proteinoids favor the decarboxylation of another keto-acid, pyruvic acid.

All of these observations indicate again that proteinoid is sufficiently like protein in a general sense that it could have served as the raw material from which the powerful and highly specific contemporary enzymes evolved. This view has been criticized (Zuckerkandl and Pauling, 1965); the criticism was justified especially in terms of the relatively weak activities of the proteinoids. An alternative kind of explanation is that proteinoids yielded structures in which coding and more powerful enzymes could have developed (page 255). The similarity in composition between proteinoids and proteins, which has been recorded and analyzed (Mikelsaar, 1975), is more likely an expression of similar steric factors operating in the synthesis of each type of copolyamino acid. Quite probably, the existence of arrays of enzymelike and enzymic activities, in proteinoids and proteins respectively, is a manifestation of the fact that both proteinoids and proteins are copolyamino acids. A question for future research is how many enzymelike activities were necessary in proteinoids for the evolutionary development of a protocell that organized itself from such polymers.

Hormonal Activity. When amino acids identified with the active site of melanocyte-stimulating hormone (MSH) were polymerized by heating, the activity of the hormone was observed (Fox and Wang, 1968). The contributory amino acids are glutamic acid, glycine,

histidine, arginine, phenylalanine, and tryptophan. These amino acids were identified in studies by Li and others (Papkoff and Li, 1966) by the finding of activity in small peptides that were fragments of the hormonal protein.

The activity of the hormone itself is in the range of 10^9 - 10^{10} units per gram (Figure 5-7), and peptides obtained by fragmentation displayed activity in the range 10^4 - 10^5 units per gram.

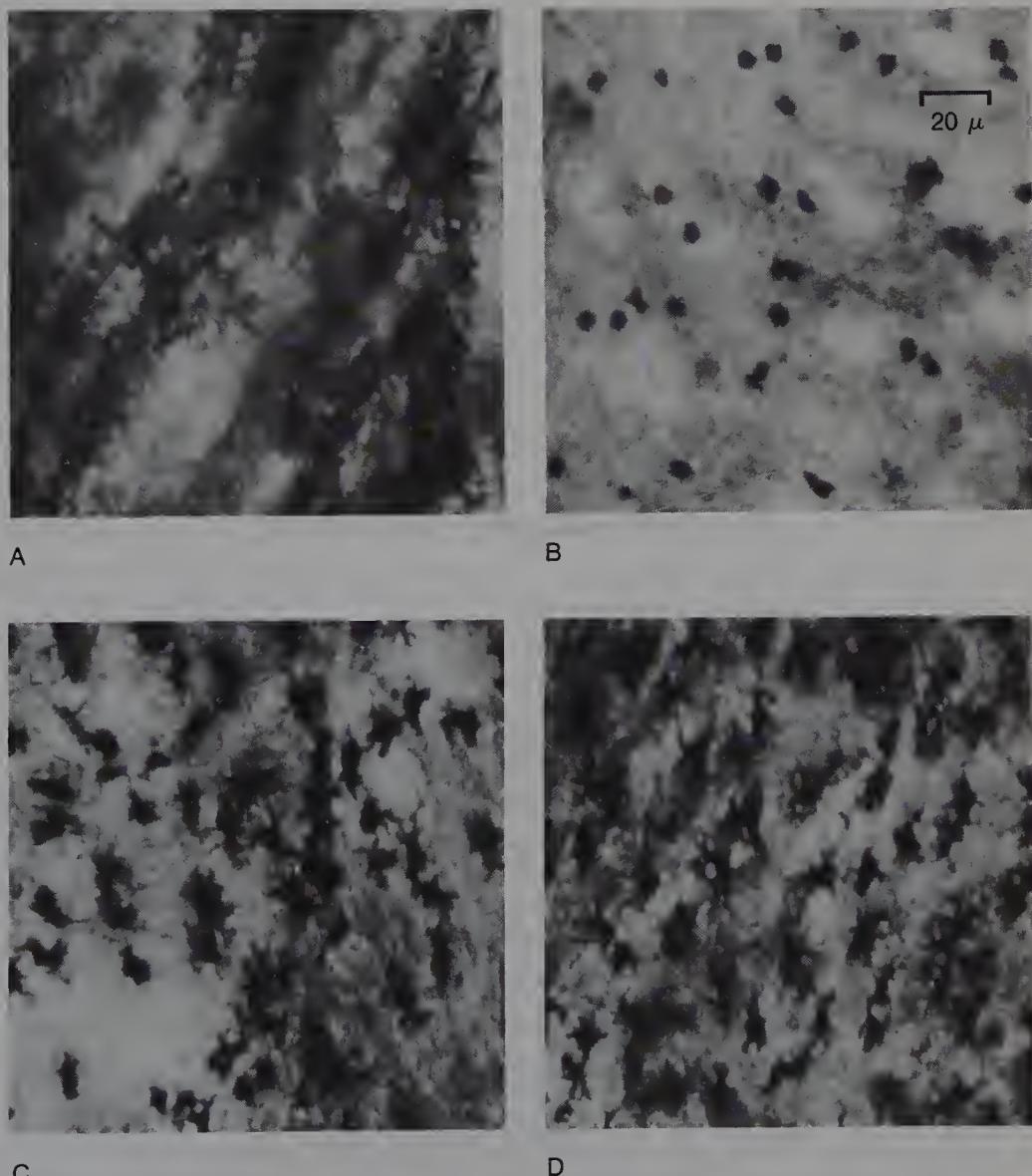


FIGURE 5-7

Melanocyte expansion. A: In normal frog. B: In hypophysectomized frog. C: In hypophysectomized frog treated with MSH. D: In hypophysectomized frog treated with thermal polymers of six amino acids.

The synthetic polymer showed activity in the range 10^3 – 10^5 units per gram. This activity was specific in that other polymers lacking one or more of the amino acids, e.g., arginine, were inactive. When fractionated, the active polymer showed approximately the same levels of activity in each fraction. Bagnara and Hadley (1970) have observed MSH activity in lizards for such polymers.

Thermal polymers of amino acids and derivatives of the polymers have elicited interest as pharmaceutical agents (Mende, 1970; Neri et al., 1973).

Summary of Properties of Proteinoids. The proteinoids are seen to have the properties of enzymes, hormones, and foods (Table 5-13). (They have not been shown to have antigenicity or helicity.) When this complex of properties is viewed in conjunction with the properties of microsystems produced on contact with water (page 201), the origin of biological systems is more easily understood. The activities of the proteinoids are carried into the microsystems that assemble from them.

TABLE 5-13

Properties Common to Thermal Polyamino Acids and to Contemporary Protein

Qualitative composition
Range of quantitative composition (except serine and threonine)
Limited heterogeneity
Range of molecular weights (4000–10,000)
Reaction in color tests (including biuret)
Inclusion of nonamino acid groups (iron, heme)
Range of solubilities
Lipid quality
Salting-in and salting-out properties
Precipitability by protein reagents
Some optical activity (for polymers of L amino acids)
Hypochromicity
Infrared absorption patterns
Recoverability of amino acids on mineral acid hydrolysis (quantitatively for acidic proteinoids)
Susceptibility to proteolytic enzymes (variable with polymer)
A number of "enzymelike" activities
Inactivatability of catalytic power by heating in aqueous buffer
Nutritive quality
Hormonal activity (melanocyte stimulation)
Tendency to assemble into microparticulate systems (Chap. 6)
Tendency to assemble selectively into nucleoproteinoid microparticles (basic proteinoids; Chap. 6)

Geological Relevance. The geological relevance of the formation of proteinoids in the laboratory is treated on pages 142 and 153. Temperatures above the boiling point of water are common on the contemporary Earth (page 60), and are believed by geologists to have been even more common on the primitive Earth. At this temperature, amino acids present are automatically dry or nearly so (page 142). Lower temperatures are, however, suitable for condensation (page 153).

PROTEINOIDS FROM AMINOACYL ADENYLATES. Three kinds of intermediate in the production of proteinoids have been studied (Fox, Wang et al., 1970): the free amino acid, the *N*-carboxyamino acid anhydride (the Leuchs' anhydride), and the amino acyl adenylate. The first of these, which yields thermal proteinoids, has now been described at some length. The second, which yields Leuchs' proteinoids (Hayakawa et al., 1967), has been of interest for comparison with thermal proteinoids, and to demonstrate that simultaneous cocondensation of all proteinous amino acids is feasible through their Leuchs' anhydrides. The product of the third kind, the adenylate proteinoid, is of interest as an evolutionary precursor to protein (Fox, 1969). Adenylate proteinoid can serve as a model for a later evolutionary stage of synthesis of proteinlike molecules than that represented by the thermal condensation. How adenyllic acid and the derivative acid anhydride might have originated in the course of molecular evolution is less completely understood than is the origin of simple amino acids (Krampitz and Fox, 1969). Another limitation to interpretation of studies of aminoacyl adenylates is the likelihood that they are not, and perhaps never were, direct intermediates in protein biosynthesis (Lengyel and Soell, 1969). More probably, their participation in pathways is through the reaction of their products with enzymes (Moldave et al., 1959). Some understanding of the behavior of the aminoacyl adenylate and the products of its reaction with polynucleotides has, however, been obtained (page 232).

Purely chemical studies of aminoacyl adenylates had, until 1969, concerned the adenyllic acid anhydride of only one type of amino acid in each experiment (Berg, 1958; Moldave et al., 1959; A. Katchalsky and Ailam, 1967; Paecht-Horowitz and A. Katchalsky, 1967; Lewinsohn et al., 1967). Some difficulties are posed in these studies because the polymers are not easily freed of AMP or its conversion products. Direct hydrolysis of AMP-containing impurities produces glycine that was not present in the polyamino acid.

A stimulating study of polymerization of alanyl adenylate is that of Paecht-Horowitz et al. (1970). These authors polymerized alanine-AMP by heterogeneous catalysis on clay particles (montmorillonite).

TABLE 5-14
*Peptides and Polymers Obtained by Polycondensation
 of One Gram of Alanyl adenylate on Montmorillonite*

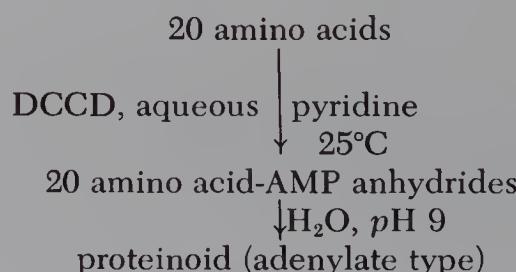
Molecular Weight	Degree of Polymerization	Weight (mg)
640	0	10
1120	16	35
1900	27	20
2130	30	8
2310	32	5
3020	42	11
4000	56	17
Adenylic acid	1	731

Source: Condensed from two tables in Paecht-Horowitz et al. (1970).

The polymers of molecular weight up to 4000 are listed in Table 5-14. This heterogeneous catalysis depends upon, of course, a kind of hypohydrous state (page 142).

Paecht-Horowitz and Katchalsky (1973) subsequently reported that the adenylate could be formed from ATP and amino acids in the presence of a zeolite. Workers in Calvin's laboratory (Warden, McCullough, et al., 1974) reported failure to confirm those results.

The condensation of mixtures of aminoacyl adenylates has been studied and found to yield proteinoids. This kind of experiment was carried out to investigate evolutionary possibilities. For this purpose, a supplemented mixture containing the twenty amino acids common to protein was reacted with DCCD (dicyclohexylcarbodiimide) in a purely chemical step to form simultaneously the amino acid-adenylic acid anhydrides of all twenty proteinous amino acids.*



* Incidentally, Lemmon (1970) has presented this reaction incorrectly as being due to condensation of the amino acids by DCCD. A reported control experiment (Krampitz and Fox, 1969), in fact, records no measurable reaction of amino acids and DCCD unless the amino acid-adenylic acid anhydrides are made first.

A number of analyses (Krampitz and Fox, 1969; Nakashima et al., 1970) of the amino acid part of the condensation products of adenylates of equimolar mixtures of amino acids (Table 5-15) reveals values remarkably like those of average contemporary protein (Vegotsky and Fox, 1962). This kind of result has been obtained in a number of variations of the experiment (Nakashima et al., 1970). Also studied have been effects of formylmethionine and related compounds, magnesium ion concentration, and effects of added polynucleotides. A principal inference from extended investigation has been the degree of rapidity of condensation of mixed aminoacyl adenylates and the corresponding difficulty of altering the internal processes in this condensation. However, condensation of mixed anhydrides has yielded results not obtained with attempts at homopolymerization. Glutamic acid, aspartic acid, and histidine are copolymerized smoothly (Table 5-15), but Berg (1958) reported difficulties with polymerization of these individual aminoacyl adenylates. Molecular weights of proteinoids condensed with mixed aminoacyl adenylates tend to run to 30,000 and higher (Krampitz and Fox, 1969).

TABLE 5-15
Composition of Hydrolyzate of a Fraction of Proteinoid from Amino Acid Adenylates Alone Compared with an Average Protein (calculated without ammonia)

Amino Acid	Composition of Adenylate Polymer, in Molar Percentages	Composition of an Average Protein, in Molar Percentages
Lysine	6.5	5.9
Histidine	2.4	1.8
Arginine	4.2	4.9
Aspartic acid	10.3	9.7
Threonine	4.9	4.8
Serine	4.2	6.0
Glutamic acid	9.7	12.7
Proline	5.1	6.2
Glycine	11.1	12.6
Alanine	14.3	9.6
Valine	7.3	5.9
Methionine	0.7	1.8
Isoleucine	4.5	6.0
Leucine	9.6	6.0
Tyrosine	0.1	2.3
Phenylalanine	4.5	3.7

Source: Krampitz and Fox (1969); data in last column from Vegotsky and Fox (1962).

Again, we observe special results from cocondensation (page 140). Other surprising results, however, emerged from comparative studies of amino acyl adenylate condensation in microparticles formed from lysine-rich thermal proteinoid and homopolynucleotides (page 235).

POLYMERS OF HYDROGEN CYANIDE

Hydrogen cyanide is readily produced by the action of electric discharges, high temperatures, and ionizing radiations upon reducing or nonoxidizing atmospheres. The yields are particularly high if electric discharges are applied. The kind of primitive atmosphere used as source for H, C, and N is of little moment. Mixtures of CH_4 , NH_3 , H_2O , and H_2 have yielded HCN (Miller, 1955) as have CO, N_2 , and H_2 (Abelson, 1966). These experiments only reevaluate a number of experiments done in the nineteenth century. The early experiments already showed that HCN is easily formed whenever the three constituent elements or compounds containing them can be brought to reaction at high temperatures or by electric discharges (see, e.g., Prager and Jacobsen, 1920).

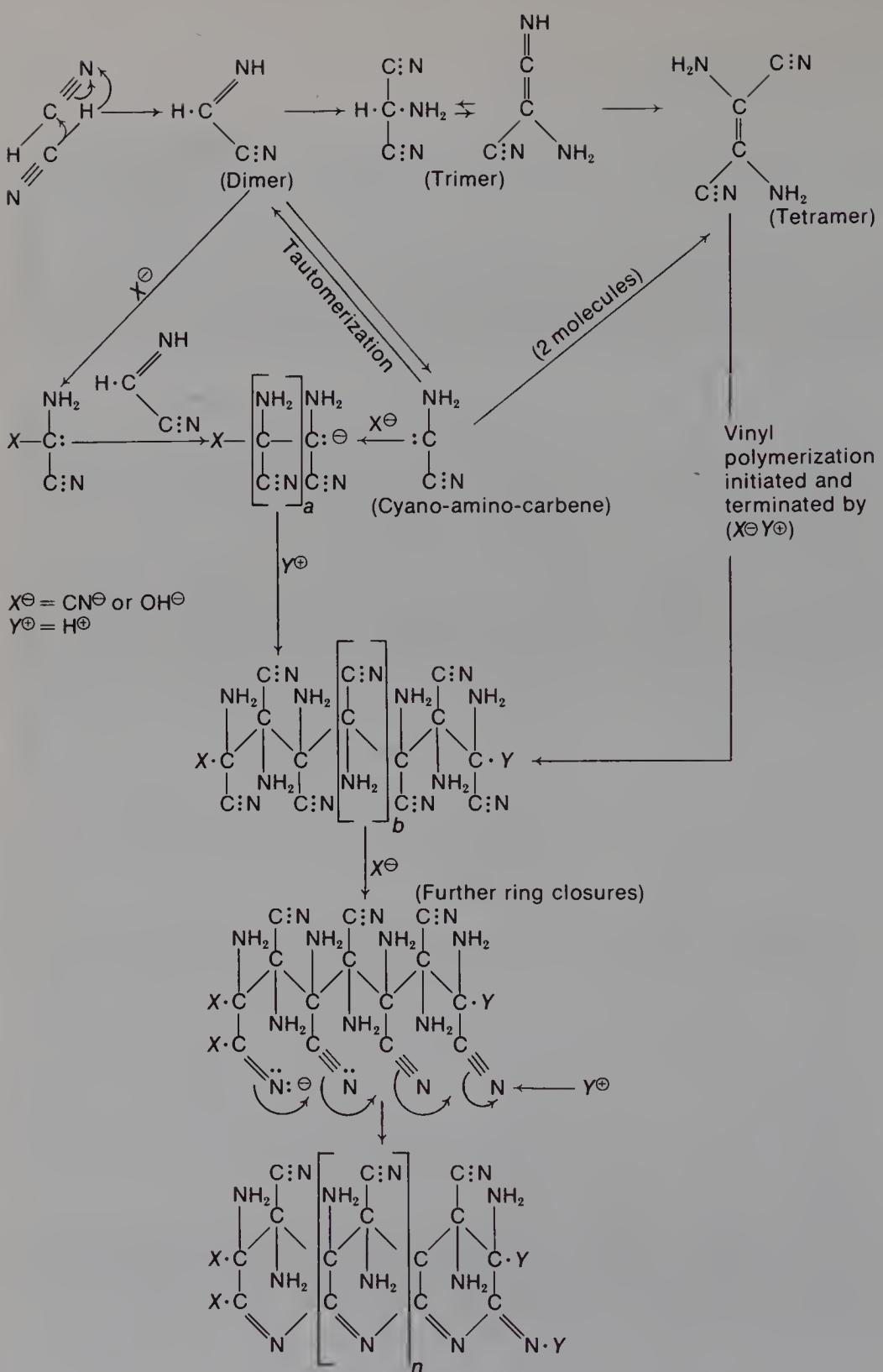
The chemistry of hydrogen cyanide oligomers and polymers is extremely complex and, so far, not well understood. It has been known for more than 140 years that hydrogen cyanide polymerizes in the presence of water, ammonia, or other bases (Boullay, 1830). The polymerization can be induced both thermally and photochemically. Since about 1960, the polymerization of hydrogen cyanide has re-excited interest among polymer scientists. A considerable number of investigators have studied the various polymers of hydrogen cyanide and drawn inferences about the mechanism of the reaction and the structures of the resulting polymers (Voelker, 1957, 1960; Wadsten and Anderson, 1959; Labadie et al., 1968a, b). Particular interest has been evinced regarding the formation of the black, ill-defined polymer, azulmic acid, first described by Boullay in 1830. The structure of azulmic acid is as yet uncertain. Only two oligomers of hydrogen cyanide can be regarded as well characterized: the trimer and the tetramer. The dimer is more difficult to characterize, probably because of its many different tautomeric and mesomeric structures (Figure 5-8). These two oligomers (Sanchez et al., 1967; Ferris et al., 1969; Oro and Kimball, 1962) as well as some polymers including azulmic acid (Lowe et al., 1963; Matthews and Moser, 1966a, b, 1967; Labadie et al., 1968a, b) and certain reaction products have drawn considerable attention in relation to prebiotic synthesis of biologically significant

materials (pages 108 and 112). A large variety of compounds is, in fact, formed in the course of polymerization of hydrogen cyanide. Some of the numerous compounds separated from the reaction mixture have been identified. Among these compounds are adenine and other purine bases plus their precursors, 4-aminoimidazole-5-carboxamide, and the corresponding 5-nitrile and 5-amidine (Oro, 1963a, b, 1965; Oro and Kimball, 1961, 1962; page 110). Hydrolysis of various oligomers and polymers gives rise to about 75 compounds showing ninhydrin reaction (Lowe et al., 1963). Among the products are ammonia, urea, and small amounts of a number of amino acids including aspartic acid, β -alanine, α,β -diaminopropionic acid, and glutamic acid (Bedel, 1924; Grysiewicz-Trochimowski, 1928; Oro and Kamat, 1961; Lowe et al., 1963; Labadie et al., 1968b).

Even peptides have been claimed to be among the products obtained by partial hydrolysis of the oligomers or polymers (Matthews and Moser, 1966a, b, 1967), but adequate evidence to confirm this has not been presented. The inference is based only on the inconclusive evidence that some of the materials can be separated on chromatographic columns designed for fractionation and analysis of peptides (Catravas, 1964), and that some of the fractions yield amino acids upon acidic or alkaline hydrolysis. When the biuret test is negative, as it is for these materials, appearance of amino acids upon hydrolysis of an HCN polymer is not evidence for peptides. Other tests to prove that these materials are polypeptidic, such as infrared profiles, have not been reported.

The black polymer (azulmic acid), however, has been shown by Labadie et al. (1968a, b) not to be attacked by pepsin, trypsin, papain, or pronase. This polymer also exhibits some catalytic activity with respect to decarboxylation of oxaloacetic acid and, to a lesser degree, to hydrolysis of ATP or *p*-nitrophenyl acetate (Labadie et al., 1968b). The black polymer may, however, possess a different type of chemical structure than the material analyzed by Matthews and Moser (1966a, b, 1967); see Figure 5-8 for suggested structures of the different oligomers and polymers.

The polymerization of hydrogen cyanide to azulmic acid is summarized (Figure 5-8) on the basis of the work of Voelker (1957, 1960), Wadsten and Anderson (1959), Matthews and Moser (1966a, b), Lowe et al. (1963), Sanchez et al. (1967), Ferris et al. (1968). The polymerization reactions are hypothetical, as are the structures of the resulting polymers. A number of related structural formulas have been proposed for azulmic acid (Voelker, 1957, 1960). Only one of these structures is shown. The materials are heterogeneous. Some of the fractions



N, the number of tetramers, seems to vary between 2 and 6, i.e. $(HCN)_n$ and $(HCN)_{24}$

FIGURE 5-8
Postulated mechanism of polymerization of HCN.

show molecular weights of about 1000, while some of the higher polymers have not been reported yet as being analyzed. An interesting feature of the polymerization reaction is that amino acids (^{14}C glycine), if added to the reactants, are incorporated into the polymer (Lowe et al., 1963). This incorporation is probably due to the reaction of the amino group of the amino acid with the nitrilo or imino group of the oligomers or polymers. There is no evidence to demonstrate that the amino acids are linked to each other or to the polymer by peptidic bonds.

An alternative to the polymerization of the 1,4 dipolar ion or the 1,3 diradical to a polymer containing C—C—N links is visualized in the polymerization of aminoacetonitrile (Akabori, 1959). Aminoacetonitrile has been produced in the laboratory from aminomalononitrile, the trimer (page 109), by partial hydrolysis and subsequent decarboxylation (Grysiewicz-Trochimowski, 1928). Aminoacetonitrile readily gives rise to glycine after complete hydrolysis, but the attempts to polymerize aminoacetonitrile to yield first polyglycimide and then polyglycine have had only limited success so far (page 145). The active hydrogen and polyglycine could, theoretically, react with other compounds such as aldehydes to introduce side chains and so construct a variety of polypeptides. The complete sequence of reactions has not been verified in the laboratory, however (Akabori, 1959).

Flores and Ponnampерuma (1972) also studied the "polymer" of HCN. They found their product, incubated with leucine aminopeptidase, released glycine. From this result, they inferred the presence of a peptide bond. However, the specificity of leucine aminopeptidase (Smith and Bergmann, 1944) is such that a carboxyl-coupled amino acid can be split by the enzyme, without need for a peptide bond in the structure.

This point was further examined by Ferris and coworkers (Ferris et al.; 1973a, b). They fractionated the HCN oligomer. They found that pronase, which splits peptide bonds between two amino acid residues, released no amino acids. They found that carboxypeptidase also released no amino acids. Definitive evidence for peptide bonds in the polymer is, thus, still wanting. The data collected by Ferris et al. also indicate that the HCN polymer is truly an oligomer, mainly tetramer. The yields of amino acids on hydrolysis were small. Quantitatively, typically over 90 percent of the amino acids are glycine.

POLYMERS OF NUCLEOTIDES

Polynucleotides are characterized in the strict sense by phosphate ester links between the 3' position of the ribose or deoxyribose of one

nucleotide and the corresponding 5' position of the next nucleotide. Since some of the prebiotic model experiments fail to yield such linkages but do yield similar ones, we use the slightly broader term polymers of nucleotides in this discussion in order to include these products of the model experiments.

Polymetaphosphate ethyl ester (PMP) has been successfully applied to achieve a polycondensation of nucleoside and nucleotides to polymers that show some relationship to the corresponding biopolymers (Schramm et al., 1961, 1962; Schramm, 1965). However, this type of condensing agent lacks geological relevance. PMP was first synthesized by Langheld (1910). According to Schramm et al. (1962a, b), PMP is produced by refluxing 150 gm of phosphorus pentoxide in 150 ml of chloroform and 300 ml of ether for 12 hours. The solvent is removed by evaporation, and a viscous colorless syrup, the PMP, results. Syntheses with this reagent are generally carried out in organic solvents under anhydrous conditions. The reagent has been used for the synthesis of polypeptides from peptides and amino acids (Schramm and Wissmann, 1958), for the synthesis of polyglycosides from simple sugars, of nucleosides from sugars and bases, and for the condensation of nucleotides or nucleosides to polynucleotides (Schramm et al., 1961, 1962a, b).

Nucleosides can be obtained by condensation of sugars and bases; nucleotides are produced either by condensing simultaneously bases, sugars, and phosphates or by stepwise phosphorylation of nucleosides (page 117). All these types of condensation reaction can be achieved also with polymetaphosphate esters (Schramm, 1965). Schramm has argued that polymetaphosphates could have been formed on the primitive Earth in the following way:

1. At temperatures higher than 300°C inorganic orthophosphates would yield polyphosphates, which could have been retained in large deposits at water-free locations after the Earth had cooled.
2. These condensed phosphates might have been esterified by reaction with organic compounds such as esters or alcohols.
3. The resulting polymetaphosphates would have aided the polycondensation of amino acids, sugars, or nucleotides at the water-free locations; the polycondensation may have taken place inside coacervate-type microbodies, as proposed by Oparin (1961), or in other aggregates protected by a hydrophobic membrane.

So far, however, only the first step has been proved to be geologically feasible (page 121).

Either nucleosides or nucleotides are polycondensed in the absence of a solvent and in the presence of an excess of polymetaphosphate ethyl ester. Better results are obtained with nucleotides as reactants. In a typical experiment, Schramm et al. (1961, 1962a, b) heated 350 mg of adenylic acid for 18 hours in the presence of 8 gm of polymetaphosphate at 55°C. The reaction mixture was dialyzed for 4 days. After subsequent lyophilization, about 70 mg (20 percent yield) of a white polymer was obtained. Similar polymers were synthesized from deoxynucleoside-5'-phosphates, 2', 3', and other 5'-nucleoside phosphates; 2', 3'-cyclic monophosphates were also used. The typical yields were about 10 percent. The concentration of the reactants had to be as high as possible when the formation of high polymeric material was desired. Dilution by a solvent is to be avoided in such cases. A homogeneous phase is needed at least at the end of the reaction.

The raw products show a broad distribution of molecular weights. The oligonucleotides have been separated on Sephadex or DEAE-cellulose. The nondialyzable fraction contains material with sedimentation constants between 1.4 and 2.4 S, corresponding to molecular weights of about 10,000. Some fractions with higher molecular weights were isolated. Schramm (1965) has claimed that the polycondensates of nucleotides prepared in his laboratory were largely linked together by phosphate bridges between the C-3' and C-5' position; in the polymerization of deoxynucleotides, pyrophosphate linkages were formed. The formation of pyrophosphate linkages with deoxynucleotides may be related to the fact that the C-2' carries no hydroxyl. In the normal nucleotides the free hydroxyl adjacent to the phosphate group splits the pyrophosphate linkages, forming 2', 3'-cyclic phosphates as intermediates. Pyrophosphate links, therefore, were not found in the polymer obtained from 2'(3')-uridylic acid, which was studied in more detail. The additional free hydroxyls lead, however, to unwanted 2'-2' and 3'-3' phosphate linkages and may become branching points. The synthetic polyuridylic acid was degraded with snake venom phosphodiesterase, which specifically splits the phosphate groups in the C-5' position. The results showed that a considerable number of linkages are resistant to this enzyme, that is, a number of phosphate linkages are not located at the C-5' position. The proportion of groups in the C-3' position has also been measured. This determination was carried out specifically by degradation with ribonuclease. This enzyme splits only polynucleotides with a free hydroxyl group in C-2' position and a phosphate bridge in C-3' position. Polyuridylic acid prepared from commercial 3'(2')-uridylic acid was only slightly degraded by ribonuclease. Polymers obtained from purified 3'-uridylic acid, however, were largely attacked by

ribonuclease. On the basis of the results he obtained with his associates, Schramm (1965) draws the conclusion that the polymers obtained from 3'-nucleotides are linear polynucleotides containing the biological 3'-5' phosphate bridges. Kochetkov and his associates (Kochetkov et al., 1964), Agarwal and Dahr (1963), Jacob and Khorana (1964), as well as Gottikh and Slutsky (1964), however, were not able to confirm these results. According to these authors, Schramm's polymers show a predominance of unnatural linkages as well as branching and cross-linking between chains. In a related context the polymerization of 5'-thymidylic acid by PMP was investigated by Hayes and Hansbury (1964). Their analyses of the condensation product showed that the number of phosphoryl groups incorporated was 5–15 times higher than the number of nucleoside residues. The polymeric fraction (nondialyzable or slowly dialyzing) could be degraded with acetic acid anhydride. This kind of degradation is due to the cleavage of unnatural pyrophosphate links. The material so degraded offered no indication for the presence of significant amounts of phosphodiester bonds such as the treatment with phosphodiesterase revealed. Also the presence of ether bonds further adds to the conclusion that polymers of this kind must not be classified as polynucleotides.

Although the structure of these polymers appears to be quite different from that of nucleic acids, the materials show some interesting similarities to biogenous polynucleotides. According to Schramm (1965), his synthetic polymer of uridylic acid shows about the same coding ability (incorporation of phenylalanine in a cell-free system of *Escherichia coli*) as polyuridylic acid prepared by phosphorylase. Furthermore, if synthetic polymers of uridylic acid are added to analogous polymers of adenylic acid, hypochromicity is observed, indicating the same phenomenon of base pairing and stacking known for DNA (Mahler and Cordes, 1967). The initial rate of polycondensation of uridylic acid with PMP is increased ten-fold upon addition of the complementary polyadenylic acid, but the reaction rate is slowed down after 20–30 percent of the uridylic acid is polymerized.

All of these biochemically unfavorable results might still have meaning in an evolutionary context. The first polymers of nucleotides did not necessarily have the same structure as biogenous nucleic acids but they could have resembled the contemporary types functionally. As stated before, however, the geological relevance of PMP is unsupportable; therefore, these interesting results can be put into an evolutionary context only with serious reservations.

Polyphosphoric acid or its salts, as condensing agents, are more defensible materials in this context than PMP. The general importance of phosphoric media in prebiological chemistry has been em-

phasized (Fox and Harada, 1961). Polyphosphoric acid and its salts result from heating of minerals such as diammonium hydrogen phosphate or sodium ammonium hydrogen phosphate at temperatures above 155°C (Knorre, 1900; page 121). The procedure for synthesizing the polymer is equally simple. Mononucleotides are mixed with polyphosphoric acid, with care to limit water uptake from the air, and the mixture is heated at 65°C for one or two hours. In Figure 5-9 (from Schwartz et al., 1965) is summarized the whole procedure for the preparation of the polymeric product from 3'(2')-cytidylic acid and polyphosphoric acid. The yield of the polymer is about one percent. Cytidylic acid appears to be the only one of the usual nucleotides that is able to self-condense (Schwartz et al., 1965; Schwartz and Fox, 1967).

Cytidylic acid has been successfully cocondensed with adenylic acid or uridylic acid, but only to give unnaturally linked adenylic acid residues (Joseph, 1968). The absorption spectrum of each of these products is free from anomalies. Evidence is presented that the free amino group of cytosine is not affected by the condensation. The increase in the extinction coefficient at the absorption maximum in basic solution, upon incubation at 37°C for 48 hours, is referred to as a hyperchromic shift, and is generally reported as alkaline hyperchromicity in terms of percentage increase. Michelson (1959) has shown that such a shift is caused by the cleavage of hydrogen bonds between linked nucleotides. This cleavage results in the "unstacking of the bases." The magnitude of the effect increases with the increasing chain length of the oligonucleotides originally present. The alkaline hyperchromicities of the synthetic polymers of cytidylic acid are 16 percent and higher. They suggest a chain length of at least four to ten residues for these products. Degradation experiments with *Escherichia coli* alkaline phosphatase, which hydrolyzes specifically (termi-

3' (2')—CMP (5 gm) + PPA (10 gm)
↓ 65°C, 2 hours
↓ Dissolve in aqueous NH₃ solution
↓ Dialyze 3 days
↓ Lyophilize
↓ Fractionate (Dowex 1 × 4)
↓ Dialyze 3 days
↓ Lyophilize
Product
(about 1% yield)

FIGURE 5-9
Preparation of cytidylic acid polymer by heating.

nal) phosphomonoester groups, showed that only a single 2'(3')-phosphomonoester was present per chain. The presence of a high proportion of phosphodiester linkages (about one per nucleotide) was found by specific degradation with ribonuclease. These results indicate that at least a significant fraction of the material produced is closely related to, if not identical with, di-, tri-, and tetra-cytidylic acid. A large fraction of the material appears to have a higher molecular weight than expected for oligonucleotides. The chemical nature of this polynucleotide-like fraction appears to be still somewhat more complex than that of simple oligo- or poly-nucleotides although this fraction contains significant regions of phosphodiester linkages. The possibility of chain branching, for example, must still be investigated.

Radiation has also been used to polymerize nucleotides. What has been said about the thermal formation of unbiological crosslinks, however, applies with the same strength to the radiation-induced links between the nucleosides or what is left of them after photolysis or radiolysis. Besides unnatural C—C links between the sugar moieties (Barker et al., 1962a, b), links between two aromatic bases, particularly pyrimidine bases (Beukers and Berends, 1960), occur with significant probability, in addition to those unnatural links formed by the thermal syntheses. Remarkable is the report, however, that a polymerization can be achieved in a dilute aqueous solution of nucleotides. Contreras et al. (1962) have reported the polymerization of nucleotides by high levels of γ -rays. In a cyclic process they steadily removed the resulting polymer from an aqueous solution by an ion exchanger in order to protect the product against further radiolysis. These authors report that the polymeric material that they obtained could be partly degraded by various phosphatases and that it even showed some coding ability. No evolutionary context is stated in their paper.

Oro et al. (1969) have polymerized nucleotides in aqueous solution by ultraviolet irradiation (2537 Å). The authors tentatively classified their product as a polynucleotide. Such a nomenclature is misleading in that a polymer is expected to contain unusual crosslinks and photolytically altered bases, although the authors present evidence for the presence of a significant percentage of natural 3'-5' linkages. As stated at the beginning of this section, the fact that these polymers are not identical with contemporary nucleic acids does not denigrate their prebiotic significance provided the polymers show some coding ability, interaction with proteins or proteinoids, or other properties that at least exhibit their functional relationship to contemporary nucleic acids.

An interesting nonenzymic, though specific, polymerization of adenosine-5'-phosphate or guanosine-5'-phosphate in the presence

of the complementary templet (polyuridylic or polycytidylic acid, respectively) has been reported (Sulston et al., 1969; Schneider-Bernloehr et al., 1968; Orgel, 1969). The energy for this condensation can be supplied by the addition of a variety of condensing agents. Predominantly 2'-5' internucleotide linkages are formed. This kind of formation of polynucleotides, however, is rather a model for contemporary polynucleotide synthesis than an approach to prebiotic polycondensation inasmuch as the preexistence of nucleic acids or polynucleotides is required.

Moravek et al. (1968) prepared oligomers of uridylic acid by heating in the dry state respective mixtures of uridine and phosphate, uridine and uridylic acid, and uridylic acid alone to about 160°C. The largest oligomer obtained and identified was UpUpU. Of the six possible types of internucleotide bond, UpU and UpUpU contain only 3' → 5' links besides 2' → 5' links.

POLYMERS OF MONOSACCHARIDES

The term "polysaccharides" is properly restricted to those polymers of monosaccharides that are linked together by glycosidic bonds; for example, saccharose (cane sugar) is 2- β -D-fructofuranosyl- α -D-glucopyranoside or lactose is 4-O- β -D-galactosidopyranoside-D-glucopyranose. Analogous to our use of "polymers of nucleotides" in the previous section, we use "polymers of monosaccharides" here. The monomers in biogenous polysaccharides are usually not linked together by typical ether bonds or C—C crosslinks. Crosslinks of these types occur, however, when monosaccharides are polymerized in prebiotic model experiments with the help of radiation or heat.

Barker et al. (1959, 1962a, 1962b) studied in detail reaction mechanisms and the nature of the materials produced by irradiating glucose and other simple aliphatic compounds in dilute aqueous solution. If aqueous solutions (0.1 percent) of carbohydrates, hydroxy acids, and amino acids are γ -irradiated in the absence of oxygen, polymeric material is finally produced. When oxygen is introduced, polymers are not detected because dimerization of radicals is inhibited. Polymers of different structures and in different yields are obtained if aqueous glucose is γ -irradiated in the presence, for example, of barium carbonate, carbon monoxide, or hydrogen. These polymers contain many atypical C—C crosslinks. The original carbohydrate monomer is gradually destroyed by the action of OH radicals and solvated electrons produced by the radiolysis of the aqueous solution. Thus, new carbonyl functions appear. The inclusion of carboxylic groups and γ -

lactones has also been established for these polymers. The polymeric character even persists after acid hydrolysis, periodate oxidation, and other degradative reactions.

Ultraviolet light has also been used to polymerize monosaccharides in aqueous solution (Steinman, 1965; Goda, 1962). The condensation is aided by kaolin, for which the role is uncertain. Among other compounds, disaccharides are formed, according to the claims. Reaction mechanisms that would be helpful in making this kind of photochemical condensation plausible were not offered, however.

As is the case for other models of prebiotic condensation reactions, polymers of monosaccharides resembling more closely the contemporary types seem to be produced best in thermal reactions.

Polyglycosides, polymers of fructose and ribose, have been produced by Schramm et al. (1962; also Schramm, 1965; Schwartz, 1962), usually by heating the monosaccharides at 50–60°C with polymetaphosphate esters in organic solvents. Under mild conditions and with dimethylformamide as a solvent, rather specific polycondensation—mainly to a 1,6-polyglucose—is obtained. This method lacks geological relevance because of the ungeochemical solvent and condensing agent.

More relevant to the geological context of the prebiotic realm have been the attempts to polycondense monosaccharides at temperatures above the boiling point of water, occasionally in the presence of acidic catalysts. The water may be also removed *in vacuo* (Mora and Wood, 1958) or by a water-binding reagent such as phosphorus pentoxide in ether (Mora, 1965). When high concentrations of monomers are present in a melt, the carbonium ion (resulting from the carbonyl function of the monosaccharide) reacts easily with any hydroxyl group of another monomer. In a hexose, such as glucose, the C-6 hydroxyl, being a primary hydroxyl, is more reactive than the secondary hydroxyls C-2, C-3, and C-5. The reactivity of the C-1 (glycosidic) hydroxyl, on the other hand, is significantly greater than that of any of the others. For these reasons, typical glycosidic bonds, mostly of the 1,6 type, predominate in the linkage of the monomers if the temperature is kept so low that the activation-energy level of the other hydroxyls is not reached. According to Mora (1965), this occurs when the temperature is kept "just above the melting point" (α -D-glucose melts under decomposition at 200°C; the melting point for α -D-glucose is about 141–143°C). At higher temperatures a considerable amount of an insoluble gel is produced. This material has a large proportion of unusual ether cross-links. It, too, can be quantitatively hydrolyzed back to glucose. This is a proof that no other cross-links (e.g., C—C cross-links) have been formed. According to Mora (1965), ether cross-

links do not appear in significant quantities if the temperature is kept at about 155°C. The molecular weight of the polymers increases with the reaction temperature. At 155°C, two fractions with average molecular weights of 16,200 and 8,250 were isolated; at 175°C the corresponding fractions had molecular weights of 32,800 and 20,000. A reaction mechanism for some of the principal reactions of the polycondensation of glucose at 155°–157°C is given in Figure 5–10 (from Mora and Wood, 1958). This mechanism explains also mutarotation and competing hydrolysis.

The results of periodate oxidation of various polymers are in agreement with the general principle that the type and frequency of a given kind of link appears according to the relative reactivity of the given hydroxyls at the given temperature.

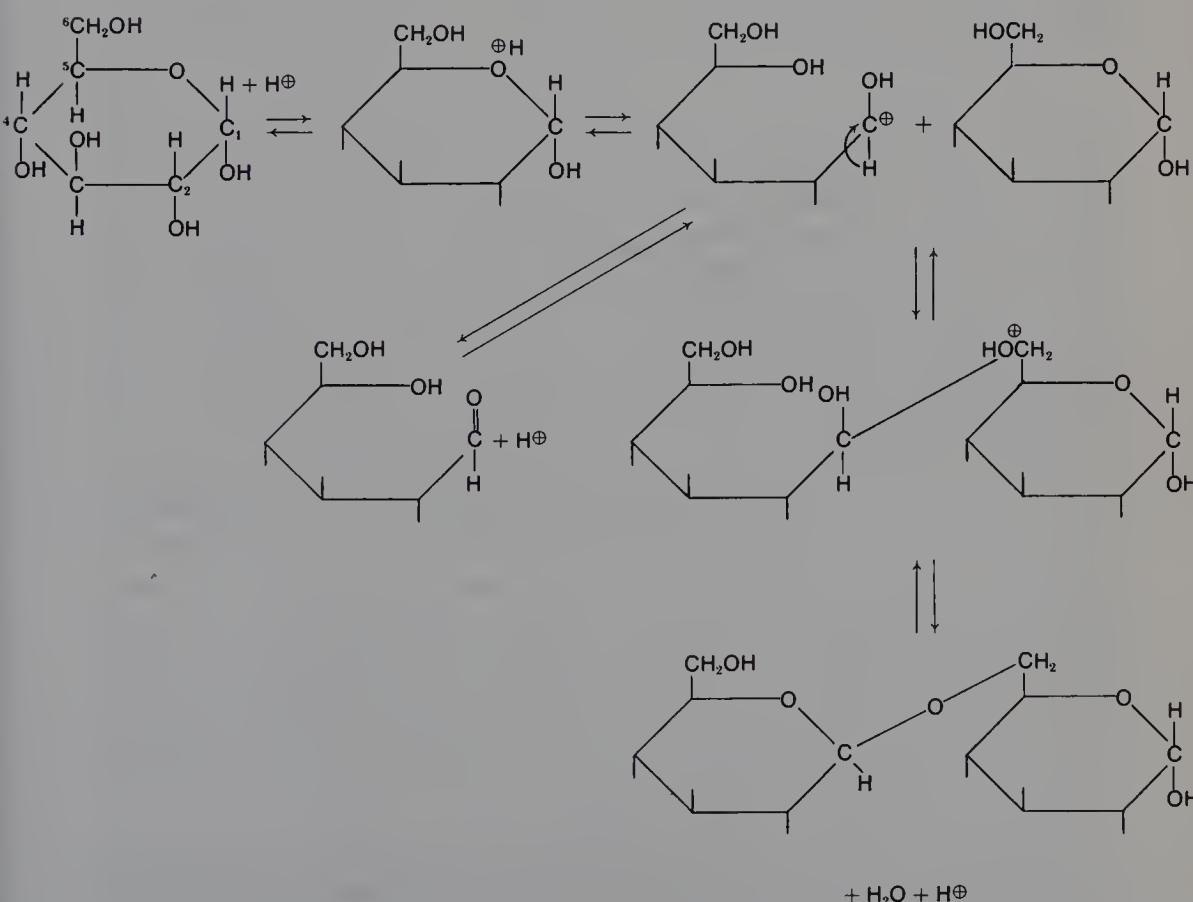


FIGURE 5-10
Polycondensation of glucose, mutarotation, and hydrolysis.

OVERVIEW

By far the most meaningful of polymers produced in attempts to simulate prebiotic reactions, are the proteinlike type. Salient characteristics include high molecular weight; qualitative and quantitative compositions closely resembling those of proteins; sharply limited heterogeneity in physical properties, composition, and sequence; diverse arrays of enzymelike activities; ability to react selectively with polynucleotides; and tendency to assemble easily into microsystems having a number of properties of contemporary cells. The remarkable similarity found has some of its roots also in the ease with which these polymers come into existence, an ease which is fully imputable to spontaneous geological processes.

But proteinoids are not proteins. Their role is that of mother substance, or "urprotein" (Alcock, 1936). The evolutionary relationship is one to be considered in detail later (page 256). It was probably a relationship interrupted by additional act(s) of assembly of supramolecular structure.

Among the unanswered questions about proteinoids is which amino acids were necessarily included in the first copolyamino acids on Earth. Extensive study of the scope of the thermal copolymerizability of amino acids indicates that the twenty contemporary amino acids, or more, can be simultaneously copolymerized. Large numbers of amino acid types are often included in thermal copolymerization in the laboratory to test the versatility of the reaction. This does not signify that all of these types were present on the primeval Earth for condensation, nor does it signify that all of these types were essential to further evolution.

Similar ignorance applies to the properties of the proteinoid. Although many properties are known (Table 5-13), three stand out. The three are: internal molecular order, arrays of enzymelike activity, and morphogenicity. Of these three, the last, i.e., the tendency of the polymer to aggregate to cell-like structures (Chapter 6) is the most fundamental. Undoubtedly some molecular order was necessary for this purpose, and some enzymelike activities were required for further evolution of the assembled microsphere. But how much order, and which activities? Those are questions to which the answers are forming, and which invite further research.

The HCN polymers, which have been proposed as evolutionary precursors of protein, are discussed in this chapter. The constitution of these polymers is unknown; what evidence has been presented indicates that they are not polypeptides.

Polymers of nucleotides much smaller than nucleic acids have been reported from thermal polymerization. Except for oligomers of cytidylic acid, evidence for contemporary 3'-5' linkages is controversial or missing.

Polymers of monosaccharides have been produced by heating monosaccharides. The evidence available places these in a position of promise for further investigation. Although more evidence is desirable, ideas about the prebiotic origin of evolutionary precursors of starches, glycogen, etc., can be seriously entertained.

One of the classes of substance that may not have been needed was lipid. The hydrophobic sidechains of amino acids in proteinoid possess lipid quality such that materials like lecithin need have appeared only later as an evolutionary refinement. A yet unsettled question is that of whether a kind of lipid material is also produced in the thermal condensation of amino acids. Solubility of fractions of some proteinoids in alcohol and in acetone suggest the presence in the raw polymer of such materials. The significance of the lipid quality in proteinoids relates especially to questions to be treated in Chapter 6.

The totality of the properties observed in proteinoid are listed in Table 5-13. Of properties common to many proteins and missing from those described for proteinoids are antigenicity and helicity. Evidence for antigenicity has not been sought more assiduously because little or no evolutionary advantage has been visualized for this property. Perhaps proteinoids of higher molecular weights than those produced thus far would have antigenicity. Helicity has been sought even less; helical structure may well have originated in conjunction with base stacking of nitrogen bases in polynucleotides.

The ease of obtaining proteinoids as compared to the difficulty of obtaining polynucleotides has been used as an argument that proteins appeared first on the Earth (Lacey and Pruitt, 1969).

From the broadest point of view, the greatest significance in this chapter is the description of the one plausible kind of material that could have given rise to the first cells on Earth. The ways in which such material, the thermal proteinoid, arises from amino acids, some characteristics, molecular structure, and other properties of the polymers have been described. Despite eighteen years of research, no polymers of comparable molecular size, ease of formation, versatility, or information content have been reported.

The next chapter will review the simple process by which such polymers would have yielded the first cells on Earth. Many of the catalogued cell-like properties of the resultant microscopic units will be described.

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CHAPTER 6

*Self-assembly of
Polyamino Acids
and Other Substances
into Microsystems*

No other concept in the theory of the origin of life seems to have posed the difficulties that have attended the ideas of the origin of the cell. As Wald pointed out (1954), the question of how molecules could have come to be arranged into an entity as complex as an organism seemed, for a time, an almost insuperable obstacle to imagining a spontaneous origin of life. Although conceptualization of the solution of the problem has posed great difficulty, a first answer from the laboratory for the question of the origin of the primordial cell has proved to be exceedingly simple.

The possibility that appropriate molecules could assemble themselves into a cell was pointed out by Wald (1954), on the basis of the then unpublished reports of experiments by Schmitt (1956; see also Pauling, 1953). These experiments were viewed in the context of self-organization, or as it has come to be called, self-assembly. Schmitt's experiments employed collagen (Figure 6-1). Since the time of those experiments, many self-assembly experiments have been reported (cf. Kushner, 1969; Lehninger, 1970).

**FIGURE 6-1**

Self-assembly of collagen. These microstructures viewed under the electron microscope show uniform diameter, cross-striations, and a regular period for the cross-striations. The collagen has assembled itself upon precipitation from solution. Photomicrograph courtesy of Jerome Gross.

Self-assembly in two protocell models—the coacervate droplet (page 222) or the proteinoid microsphere (page 203)—is complete within minutes. The process is simple as well as rapid. The products in each case are compositionally and structurally complex. The proteinoid microspheres illustrate the fact that complexity emerges directly from the interactions of a few simple components under familiar conditions. The fact that the resultant microstructures are largely open systems is critical. Such systems can theoretically take up energy during evolution to increase in complexity.

The flow in the contexts of our considerations to this point now becomes the sequence: amino acids → proteinoid → protocell; the treatment of the origin of the genetic code and its employment of nucleic acids will be taken up later (Chapter 7). Although the sequence designated is not the only conceivable primordial sequence, it is the only one for which an experimental model consistent with the geological environment has been reported.

Understanding about primitive cells cannot be attained without superimposition of many different perspectives. We cannot hope, for example, to understand protein molecules through the study of amino acid sequences alone, or through study of total conformation, enzymic activities, color tests, or susceptibility to proteases, taken alone. Each of these kinds of information and many more must be viewed and the views disciplined before an integrated understanding of proteins can emerge. Similarly, in understanding contemporary cells one must

understand enzymes, membranes, organelles, diffusion, cytokinesis, inheritance, and so one. Likewise, an understanding of proteinoid microspheres requires a balanced integration of information from studies of properties and association of properties. Of the two main models for protocells, more of the requisite kinds of information is available for the proteinoid microspheres; accordingly, more space in this discussion is given to them. A more basic reason for paying particular attention to this model, however, is that the proteinoid microsphere arises from monomers, rather than from polymers obtained from organisms already in the biota, as is true for the usual experiments with coacervate droplets (Lehninger, 1975). The origin from monomers comports with the direction of evolution itself.

PROTEINOID MICROSPHERES

Preparation

All that is necessary in preparation of this model of the protocell is interaction of appropriate polyamino acids with water or salt solution (Fromer, 1970). Many thermal polyamino acids are suitable (Table 6-1). Other polyamino acids also interact with water in the same way; the attributes of the resultant microparticles have, however, been investigated only for proteinoid microparticles. The water brought into contact with the polymer may be cold or hot. The self-

TABLE 6-1
Thermal Polyamino Acids Yielding Regular Microparticles

Polymer	Form of Unit
Thermal proteinoid	Spherule
Thermal poly(aspartic acid, glutamic acid)	Spherule
Thermal poly(aspartic acid, lysine)	Spherule
Thermal poly(aspartic acid, leucine)	Spherule
Thermal poly(aspartic acid, methionine)	Spherule
Thermal poly(aspartic acid, glutamic acid, leucine)	Spherule
Thermal poly(glutamic acid, glycine)	Oblate spherule
Thermal poly(alanine, aspartic acid, glutamic acid, glycine, diaminopimelic acid, glucosamine)	Nonuniform spherule
Thermal polyglycine	No spherule

Source: Fox (1960).

assembly is more immediate and gives more definitely formed particles if the water or salt solution is first heated and the clear decanted solution is allowed to cool without disturbance (page 355).

Microparticles may also be made by chilling solutions saturated at room temperature (Figure 6-2, Young, 1965). Contaminating organisms are seldom seen, except when the pH of suspensions is adjusted to near neutrality. In those cases, growth of contaminating microorganisms occurs within hours.

Physical Properties

The proteinoid microspheres are spherical, microscopic, and they tend to be uniform in diameter (Figure 6-3). Shapes other than simple

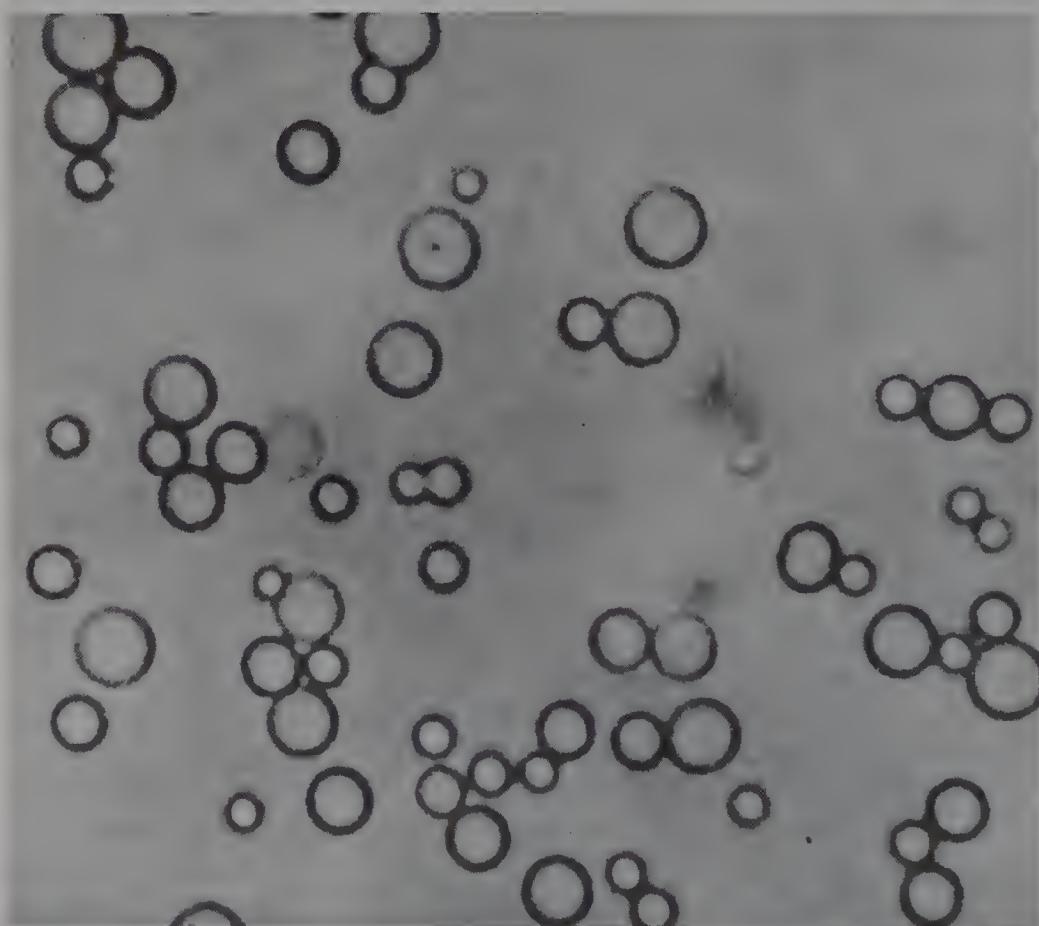


FIGURE 6-2

Proteinoid microspheres produced by chilling a solution saturated at room temperature. From Young (1965).

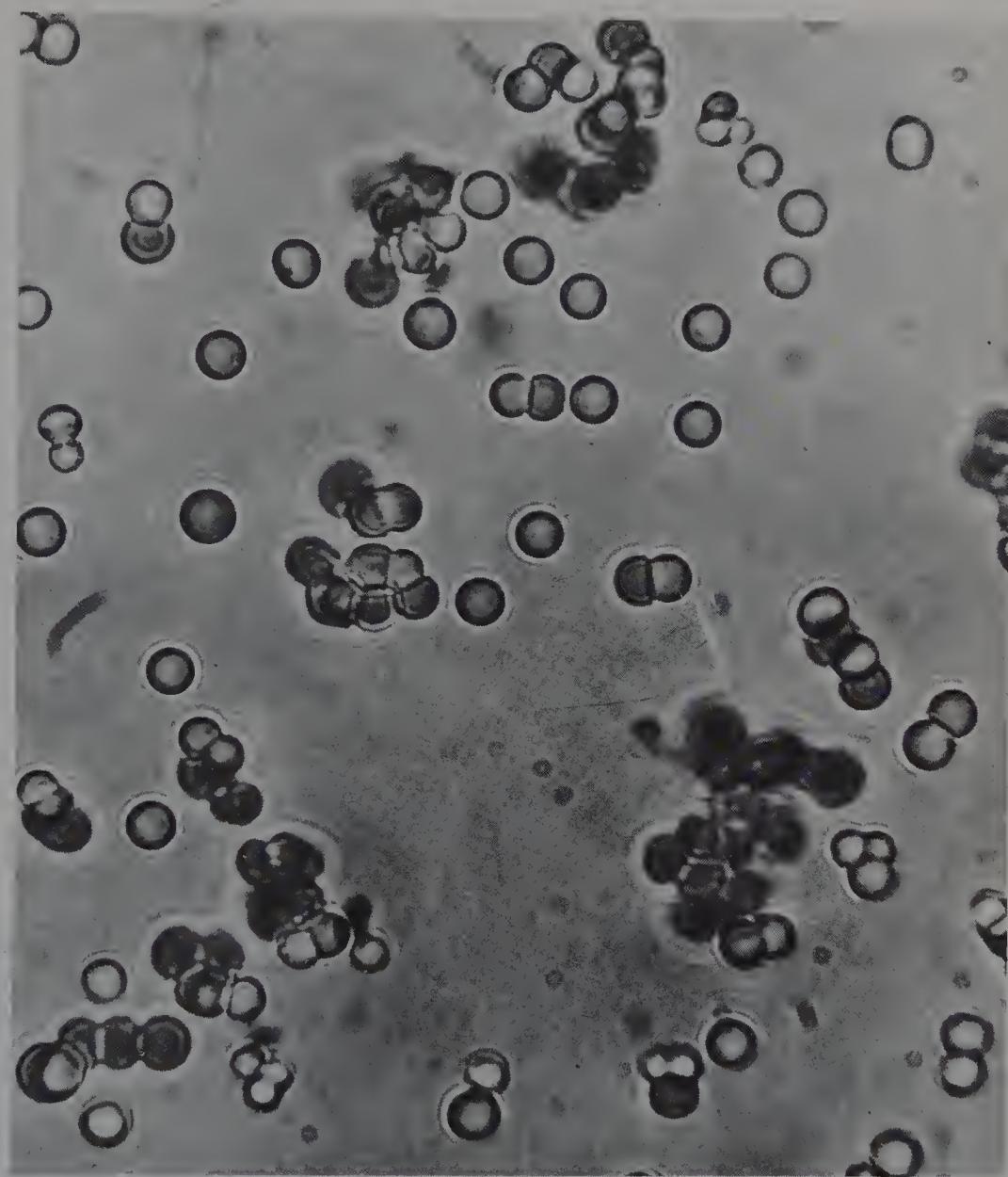


FIGURE 6-3
Proteinoid microspheres made by cooling a hot, clear solution (light microscope).

spherules occur (Table 6-1), at times as the consequence of the presence of substances other than proteinoid.

The size of the microspheres tends to fall in the range 0.5–7.0 μm diameter, although smaller or much larger particles can be made. The shape most usually found is spherical (Figure 6-4), but associations to yield units that are streptococcal-like in appearance are obtained at times (Figure 6-5). In size, shape, and mode of association the



FIGURE 6-4
Structured proteinoid microsphere. Described in Fox (1965a).

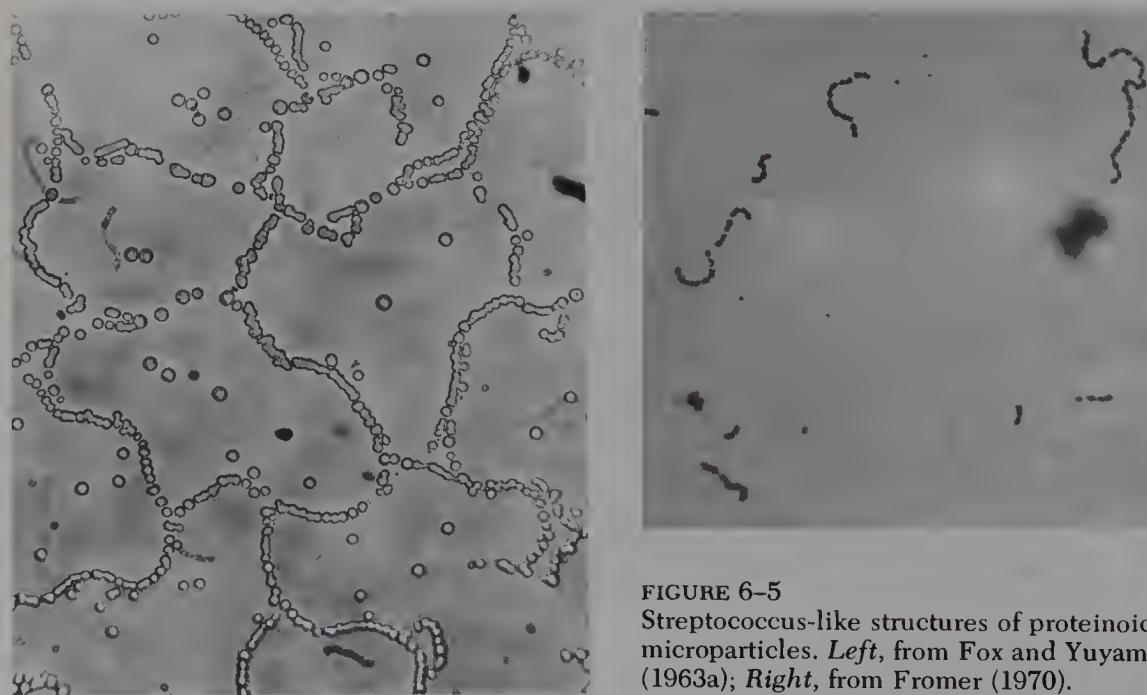


FIGURE 6-5
Streptococcus-like structures of proteinoid microparticles. *Left*, from Fox and Yuyama (1963a); *Right*, from Fromer (1970).

proteinoid microsphere has a number of properties in common with coccoid bacteria (Fox, 1969).

Some minor deviations in shape from the purely spherical are of interest in their resemblance to either the "organized elements" found in some meteorites (page 335), or the microfossils found in association with some ancient geological strata (Figure 10-5, page 304; Fox, 1969).

In Table 6-1, the results of formation of microparticles from various thermal polyamino acids can be seen. Consistent with the data in the table, many copolymers of amino acids, especially when one of the monomers is aspartic acid, yield spherules when they assemble in the presence of water. Copolymers of glutamic acid and glycine, however, produce erythrocyte-shaped particles.

The uniformity in size of the microparticles represents one similarity to cells. The uniformity (Figure 6-3) suggests also a precisely controlled equilibrium state between processes of aggregation and disaggregation, cohesion and spreading.

The operational factors controlling size of the microparticles appear to be many: kind of polymer, added substances, ratio of solid to liquid, concentration of electrolyte in solution, temperature of heated solution, rate of cooling, etc. In Figure 6-6 is seen the effect of concentration of sodium chloride on size of particle. The maximal size is found in solutions of about 1.0 percent sodium chloride. Other factors, such as ratio of solid to liquid, are also significant in determining size.

The number of microspheres obtained from a given weight of polymer is very large. One gram of polymer can typically yield 10^8 – 10^9 microspheres.* This number of similar, but not identical, units is

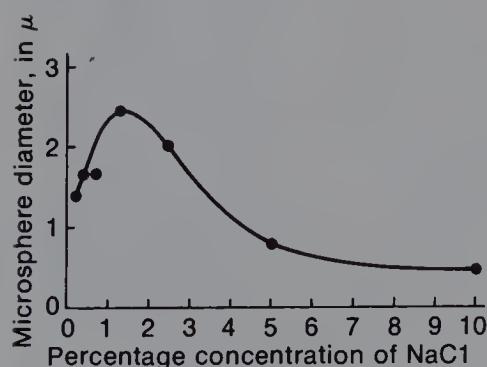


FIGURE 6-6
Effect of sodium chloride concentration on size of proteinoid microspheres made in a standard manner. Source: Fox et al. (1959a).

* Each microsphere contains approximately 10^{10} molecules of proteinoid.

an appropriate finding to support ideas of spontaneous evolution. Presence of a large number of similar units would have most efficiently set the stage for Darwinian selection.

The stability of the proteinoid microsphere distinguishes it from other models of the protocell. It can be centrifuged without being disrupted, and it has greater stability in solutions of salt than many preparations of coacervate droplets. The uniformity of the microspheres, in fact, is probably closely related to their stability, both characteristics being related to strong internal interactions. The stability is demonstrated also by the fact that embedded microspheres, or only their membranes, can be sectioned for electron microscopy (page 209).

The stability of the microsphere to extreme *pH* varies, however. This is a quality, moreover, that is profoundly influenced by combinations of acidic proteinoids with other types of proteinoid and with other substances. Microspheres produced from acidic proteinoid tend to dissolve at *pH* values such as 6 and higher. When acidic proteinoid and basic proteinoid are allowed to form particles, they do so under conditions in which each component alone is soluble. The combination is not soluble at *pH* values of 8–9 (Fox and Yuyama, 1963b). Very complex structures appear when acidic proteinoids are treated with either basic proteinoids or contemporary histones (Miquel et al., 1970). Heating of mixed amino acids with seawater yields basic and acidic proteinoids simultaneously. The microspheres produced therefrom are stable in alkaline solutions (Snyder and Fox, 1975).

Even more stable at higher *pH* values, and also in suspensions at the boiling point of water, are those microspheres produced from appropriate proteinoids and polynucleotides. This fact may be related to the known occurrence of RNA in contemporary cell membranes (Dowben, 1969) and to its known stabilizing influence on proteins (Greenstein and Hoyer, 1950; Hamer, 1954). These complexes of proteinoids and polynucleotides, however, deserve discussion in a context that includes more highly ramified particles (page 232). Another component that contributes substantially to stability is calcium (Miquel et al., 1970), as it does in contemporary cells (Wiener, 1965).

Structural Properties

Many optical micrographs of proteinoid microparticles do not show much detail. However, Figure 6–7 indicates concentric boundaries such that optical artifacts would seem to be ruled out. Electron micrographs reveal ultrastructure that is comparable to that of some of the simple bacteria similarly prepared for electron microscopy (Figure 6–8; Murray, 1957).



FIGURE 6-7
Proteinoid microparticles showing concentric boundaries. Source: Fox and Yuyama (1963a).



FIGURE 6-8
Electron micrograph of section of proteinoid microsphere prepared by fixing with osmium tetroxide, embeddment in methacrylate resin, and sectioning. Source: Fox and Fukushima, 1964.

When the pH of a suspension of proteinoid microspheres made from acidic proteinoid is raised by 1–2 units, several phenomena are observed. As in the electron micrograph of Figure 6–9, one of these phenomena is that of diffusion of material from the interior to the exterior; this is described in greater detail later (page 213).



FIGURE 6–9

Electron micrograph of a section of osmium tetroxide-stained proteinoid microsphere after pH has been elevated. Double layer in boundary is prominent.

Another is the fission into two particles, which is also modelled in more detail later (page 214). The third phenomenon, a structural one, is the appearance of a double layer. This may result from either the effect of the increase in *pH* or the fact that the diffusion outward of polymer of the interior makes the visualization of the ultrastructure in the boundary easier. A double layer may be seen at that stage. This arrangement is highly reminiscent of a structure in contemporary cells, although the layers are typically 2–4 times as thick as those in the boundaries of contemporary cells (Sjostrand, 1953). Unpublished experiments by Yuyama (1964) have shown that the outer layer in the artificial particle is negatively birefringent under polarized light, while the inner layer is positively birefringent. Such a coupling of optically opposite signs is not seen in the microparticles prior to the elevation of *pH*.

Chemical Properties

GRAM STAIN. The first proteinoid microspheres were made from 2:2:1 proteinoid. These had the size, shape, and general tendency to associate found in the coccoid bacteria (Lamanna and Mallette, 1959; Fox and Yuyama, 1963b). Tests indicate that microspheres made solely from 2:2:1 proteinoid are Gram-negative. Gram-positive microspheres, however, form from a mixture of acidic 2:2:1 proteinoid and lysine-rich proteinoid when the proportion of lysine-rich proteinoid is between 25 and 50 percent (Figure 6–10).

This result does not necessarily resolve the controversies about the sign of the Gram stain in bacteria (Bartholomew and Mittwer, 1952). More than one set of factors may influence the Gram stain. The results do demonstrate the utility of proteinoid microspheres as models in that the preparations are certainly free of compounds suspected of contributing to the Gram stain, such as magnesium ribonucleate (Fox and Yuyama, 1963b).

The effects observed with this stain are not simply explained. The more acidic particles do not bind crystal violet, one of the dyes used in the Gram stain, and which is itself basic. Only when the basic proteinoid is incorporated do the particles retain this basic dye.

Other properties of acidic microspheres parallel those of Gram-negative bacteria; some properties of the more basic particles resemble those of Gram-positive bacteria (Table 6–2).

ENZYME-LIKE ACTIVITIES. The catalytic activities that are found in the proteinoid are incorporated into the proteinoid microspheres. In some

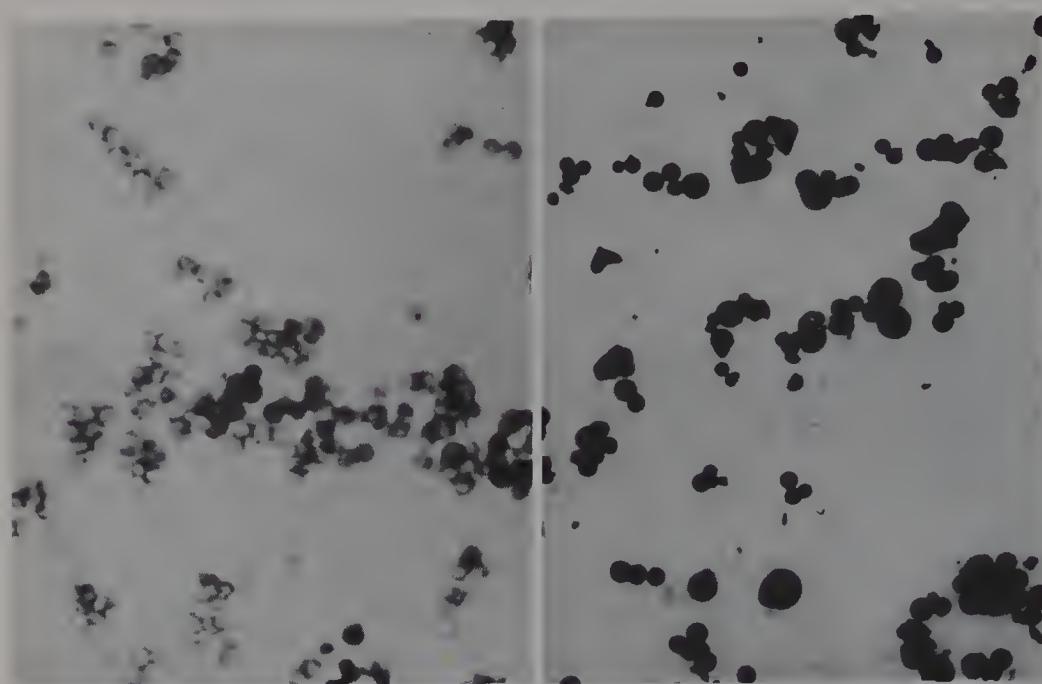


FIGURE 6-10

Left: Microspheres of acidic proteinoid stain Gram-negative *Right:* Microspheres of acidic proteinoid mixed with appropriate proportions of basic proteinoid stain Gram-positive.

TABLE 6-2

Properties Gram-positive and Gram-negative Bacteria Have in Common with Gram-positive and Gram-negative Proteinoid Microspheres

Property	Gram-positive Bacteria and Microspheres	Gram-negative Bacteria and Microspheres
Cell-wall thickness	Greater	Less
Cell walls ruptured by heat	No	Yes
Permeability to dye	More permeable	Less permeable
Rupture by sudden changes in pressure	More resistant	Less resistant

Source: Bacterial properties paraphrased from Lamanna and Mallette (1959). Some properties of microspheres determined by S. Brooke, unpublished experiments, others from Fox and Yuyama (1963b).

cases, however, such as the degradation of glucose (page 173), part of the activity is lost in the brief period of heating. The heating can be bypassed since microspheres may also be produced by chilling of solutions saturated at room temperature (Young, 1965). These questions require further investigation (Figure 6-2).

In the evolution of catalytic activities in microspheres, the tendency of the units to compartmentalize would have been beneficial. Zones permitting different pH values and ion concentrations, according to this kind of inference, would have helped to differentiate enzyme activities.

Morphodynamic Properties

SIMULATION OF OSMOSIS. On transfer to hypertonic or to hypotonic solutions, proteinoid microspheres shrink or swell, respectively (Table 6-3). This effect is observed without any lipid being added. The proteinoid, however, has lipid quality due to the hydrocarbon sidechains of amino acid residues, such as leucine and phenylalanine (Fox et al., 1969). By entertaining the proteinoid microsphere as a model of the protocell, we can visualize that later evolution would have incorporated discrete lipids into cells, to provide greater efficiency in the functions of a membrane. The osmosis-like effect is one that ought not be considered *per se* but in association with other factors, such as the double layer structure.

SELECTIVITY IN DIFFUSION. Two kinds of experiment have demonstrated that the microspheres contain selective barriers (Fox et al., 1969; Table 6-4). Some experiments show that polysaccharides are

TABLE 6-3
Effect of Hypertonic and Hypotonic Sodium Chloride Solutions on Microspheres

Solution in which Microspheres Were Prepared	Initial Range of Diameters	Solution to which Microspheres Were Transferred	Final Range of Diameters
2.0 N NaCl	2.8-3.2 μm	0.2 N NaCl	3.4-3.9 μm
0.2 N NaCl	3.4-3.6 μm	2.0 N NaCl	2.0-2.1 μm

Source: Fox et al. (1969).

Note: The solution to which particles were transferred in each experiment was saturated with proteinoid.

TABLE 6-4
Content of ^{14}C -Carbohydrates in Proteinoid Microspheres after Four Standard Washings with Saturated Proteinoid Solution

Carbohydrate	Amount Retained in Microspheres d.p.m.
^{14}C -Glucose	68
^{14}C -Glycogen	1860
^{14}C -Starch	636

Source: Fox *et al.* (1969).

Note: The microspheres were produced in 2.0% solutions of ^{14}C -carbohydrate.

retained selectively under conditions in which monosaccharides diffuse out freely.

Another result already touched on by Figure 6-9, is that proteinoid diffuses from the interior of the microsphere to the exterior following elevation of pH from approximately 2.5 to 4.0. This phenomenon has been examined rigorously. Diffusion was observed in a quartz optics microscope by use of ultraviolet light; proteinoid absorbs in the ultraviolet range. The polymer in the interior proved to be almost indistinguishable in amino acid composition from the polymer in the boundary. One possible explanation for the distinction in behavior is that more densely assembled polymer in the boundary permits diffusion through itself without being disrupted. Also, however, more amide is present in the proteinoid of the boundary than in that which diffuses (8.2% ammonia hydrolysis versus 5.4%). The phenomenological fact, in any case, is the selectivity of the boundary.

CLEAVAGE. A kind of binary fission has been observed in acidic proteinoid microspheres, although not documented photographically to the stage of separation into daughter particles (Figure 6-11; Fox, 1968). One difficulty in experiments of this type is that elevated pH leads also to gradual or rapid dissolution of the proteinoid. More recent experiments (Brooke and Fox, 1970) demonstrate that binary fission, which is attributable to surface tension, can occur without substantial dissolution by use of pure water or heat on calcium-containing proteinoid microspheres.

The tendency of variously composed particles in the range of size greater than the colloid range is to divide into two. This tendency is

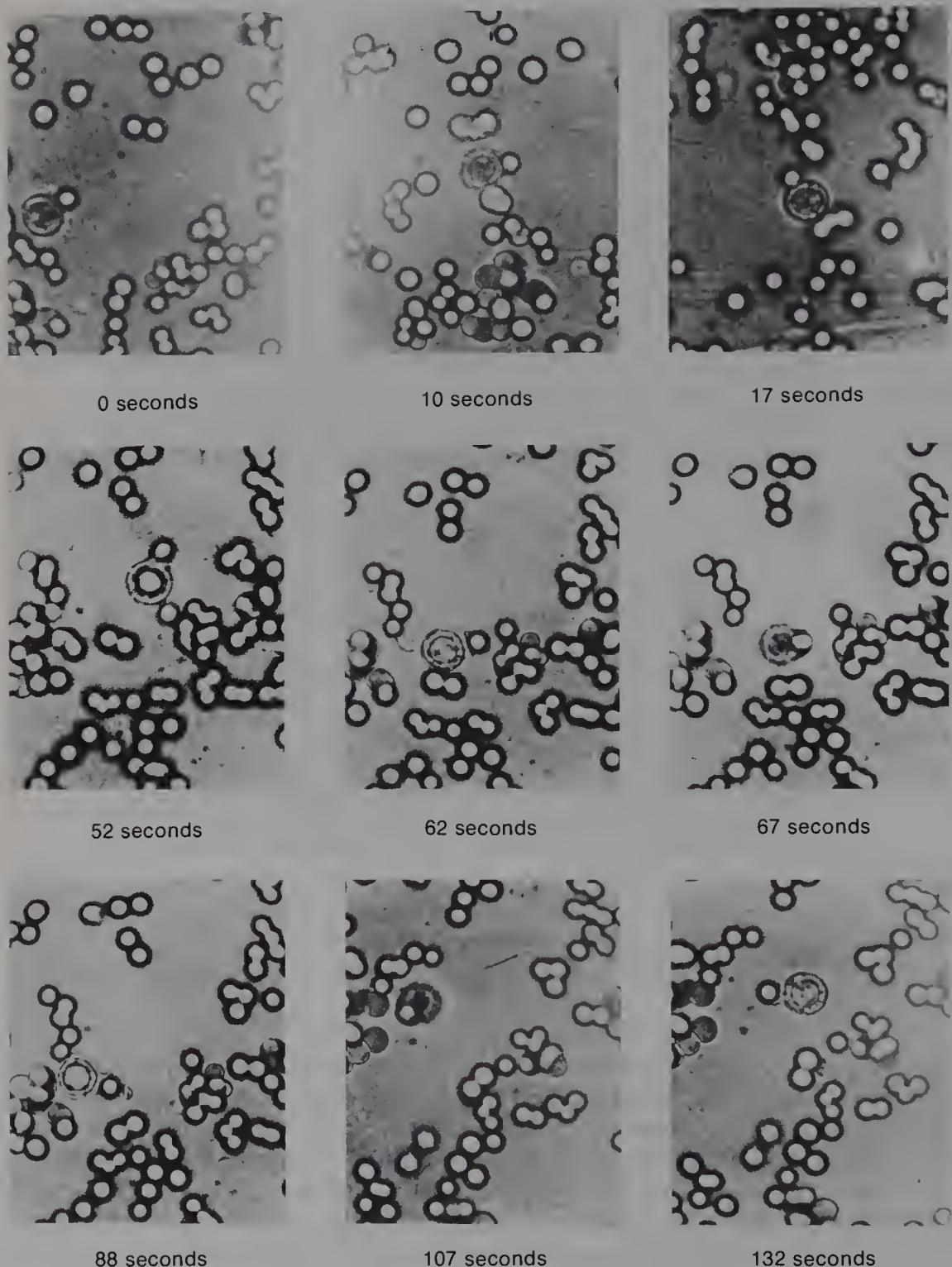


FIGURE 6-11

A model of incipient binary fission of cells seen in proteinoid microspheres. This is not an actual sequence. Various stages are modelled.

observed in soap droplets, mercury droplets, oil droplets, and it occurs in molten glass droplets on the Moon (Fox et al., 1970) as well as in proteinoid microspheres and in contemporary cells. What crucially differentiates dividing protein- or proteinoid-containing particles from the others are their other associated properties. One of these other properties is the array of catalytic potentials. These have some specificity and weak power that can be understood as occurring only in polyfunctional macromolecules, which can in turn compose morphological units in a biologically familiar range of size.

MOTILITY. This property is illustrated in Figure 6-12. This activity

**FIGURE 6-12**

Time-lapse illustration of nonrandom motility in an asymmetric proteinoid micro-particle.

may be observed many times while viewing a field of microspheres. The sequence of Figure 6-12 proceeded under experimental conditions that are most favorable for activity. Most of the particles are regular spherules; in this series the one that is markedly asymmetric described nonrandom movement and rotation. The particles were made from proteinoid complexed with zinc (Fox et al., 1966). Adenosine triphosphate (ATP) was added to the suspension of particles, because Zn-proteinoid (page 173) is known to liberate phosphate from ATP, and thereby to liberate energy. The motility is enhanced by these additions.

BUDDING. Buds appear spontaneously on proteinoid microspheres allowed to stand in their mother liquor (Figure 6-13). These buds have the appearance and consistency of those found on yeast and bacteria, but they are, of course, lacking in the total essential metabolic properties of yeasts (Fox et al., 1967). They can be released from their "parent" microspheres when a suspension of the joint particles is subjected to mild warming, sparking with a Tesla coil, or mechanical shock (Figure 6-14). The temperature attained by the application of electrical shock or by direct warming is typically 42°C.

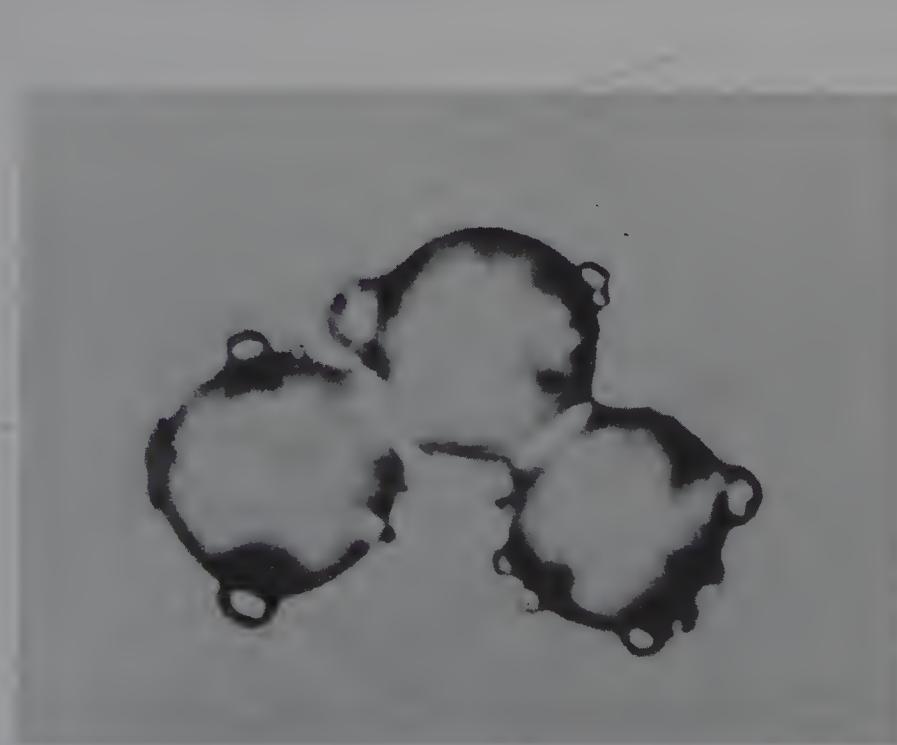


FIGURE 6-13

Buds on proteinoid microspheres. See Lehninger (1970) for related discussion.

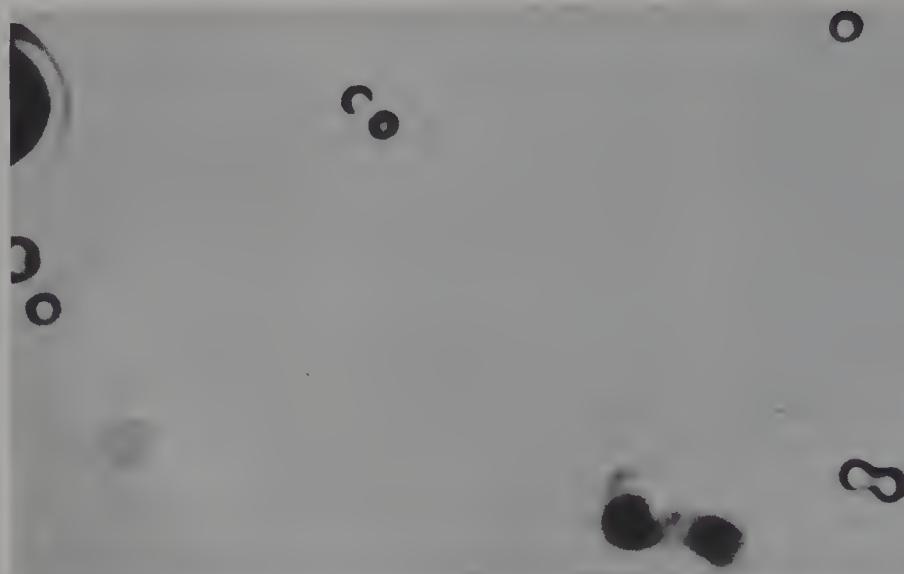


FIGURE 6-14
Buds appearing in suspension of budded microspheres after warming.

GROWTH BY ACCRETION. Liberated buds, proteinoid endomicroparticles (smaller particles within microspheres), nucleoproteinoid particles, etc. can function as centers, or as physical nuclei, for the growth of larger microparticles. Growth by accretion from crystal violet-stained buds is shown in Figure 6-15. Size and mass have clearly increased. The photomicrograph reveals the highly uniform size obtained, and that this tends to stabilize at a precisely limited size resembling that of the "parents." The temperatures that bring about release of buds and accretion thereof are less than would be provided by a large diurnal fluctuation in temperature and can even now be found in some hot zones of the Earth.

PROLIFERATION THROUGH BUDDING. Figure 6-16 shows a second-generation bud that appeared on a second-generation microsphere. We can thus visualize how processes led to polyamino acids, and thence to self-assembled microsystems that in turn proliferated with extreme simplicity.

The units studied are unable to manufacture protein; they instead obtain proteinoid from the environment. Contemporary cells, of course, synthesize protein. A protocell, however, did not need to synthesize its own protein.

Several modes of "primitive" proliferation have been identified in proteinoid microspheres (Fox, 1973).

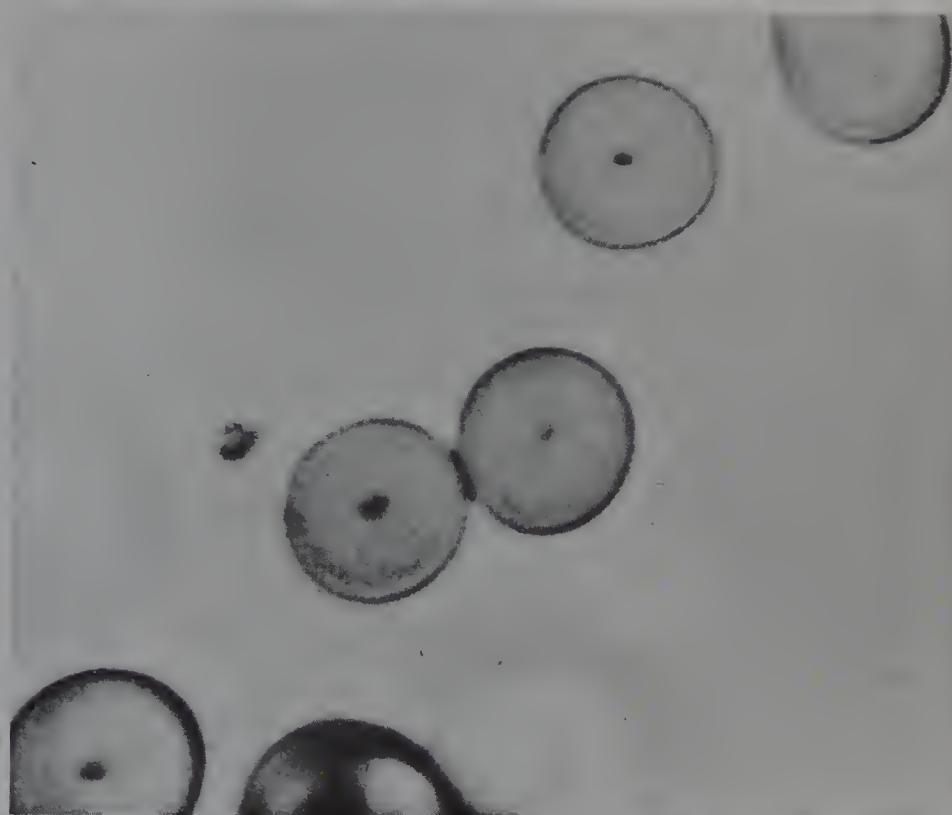


FIGURE 6-15

Microspheres that have grown by accretion around crystal-violet-stained buds.



FIGURE 6-16

A second-generation bud on a second-generation microsphere.

FORMATION OF JUNCTIONS, AND RELATED PHENOMENA. The proteinoid microspheres possess intrinsically the capacity to form junctions with each other (Hsu et al., 1971). They tend to approach and to repel each other in aqueous suspensions. Such behavior is illustrated under a coverglass on a microscope slide in Figure 6-17. A and B in this time-lapse sequence attract each other and remain in contact. C and D are joined throughout the sequence. E and F join, repel, and rejoin. This last behavior is common, having been observed innumerable times.

The second and fourth frames indicate most clearly a junction between microspheres E and F. The possibility that they are true junctions, rather than optical artifacts, requires a highly dynamic character that would seem, in turn, to require a more on-the-spot synthetic activity than the heterotrophic processes discussed in the preceding section. The fact that increasingly permanent connections are indeed formed indicates that the proteinoid in the microspheres is thus dynamic to a degree well beyond that which might have been anticipated.

Figure 6-18 presents some of the evidence for the permanence of the junctions. One of the junctions in this photomicrograph, A, is seen to be intact. Another, B, is cracked. A third, C, has been sundered. These results leave no doubt that a new structure has formed between the now joined microspheres. The ease with which this process occurs appears to be partly a function of the age of the microsystems. The junction formation, in fact, requires hardening during aging (Hsu et al., 1971).

Figure 6-19 presents the evidence that very small proteinoid microparticles can pass through the junctions, and that the connections must therefore be hollow. When water is drawn to one side of the slide as by application of a piece of filter paper, the interiors of the microspheres diminish to leave particles such as A and B. These are endoparticles, or endomicroparticles. The process occurs also through currents developing spontaneously in the liquid.

The transfer of endoparticles results from Brownian motion within the microsphere, which continues until the endoparticle happens to reach a junction. Repeated observation of this process has established that the transfer occurs within the assembly, not above nor below it.

Inasmuch as the endoparticles are composed of proteinoid, and the proteinoid has been shown to be informational (page 247), the phenomenon constitutes a model of the origin of communication, and a simulation of an early link in the evolution of biological information (Fox, 1974). This model relates to either intercellular communication or intergenerational communication; in fact, the phenomenon suggests the simultaneous origin of both types of communication (Hsu et al., 1971).

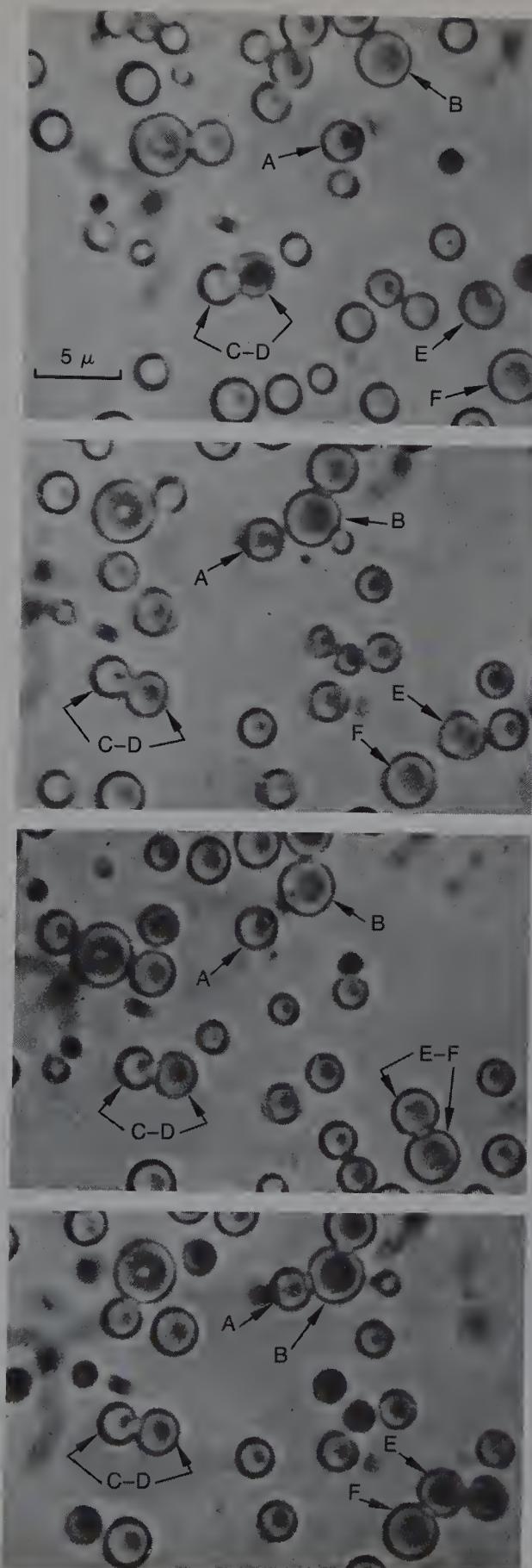


FIGURE 6-17
Proteinoid microspheres forming junctions, 10-second time lapse between successive photographs.

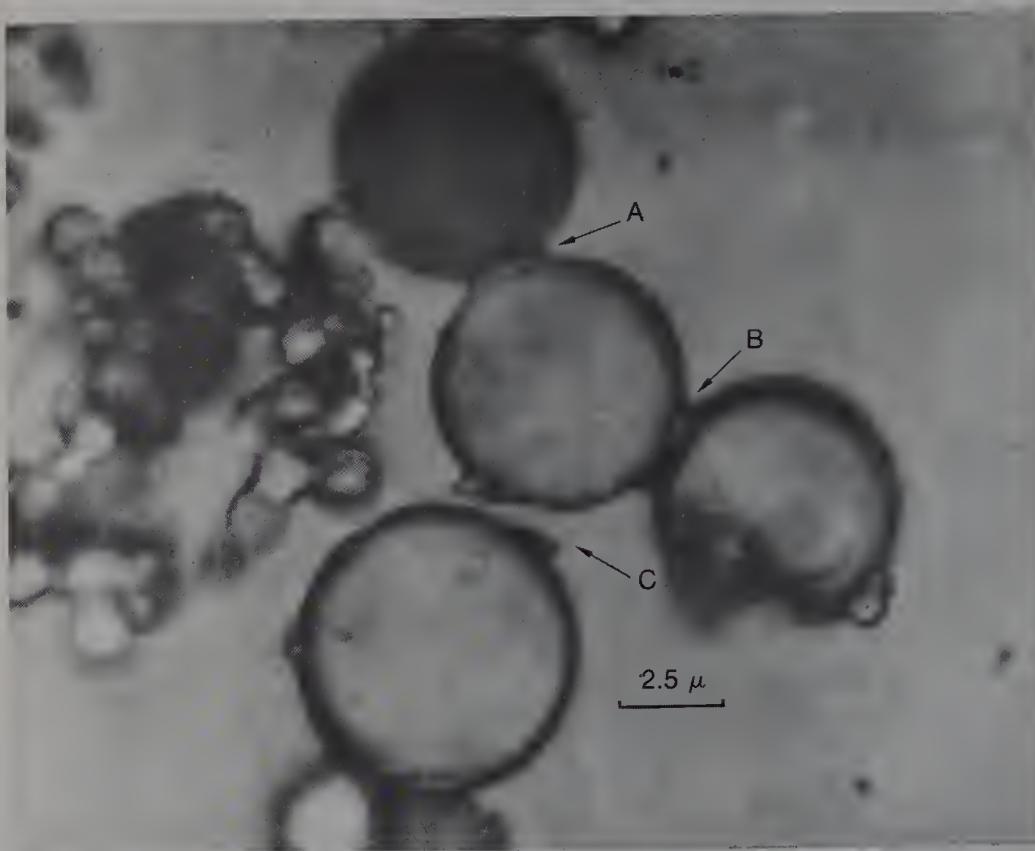


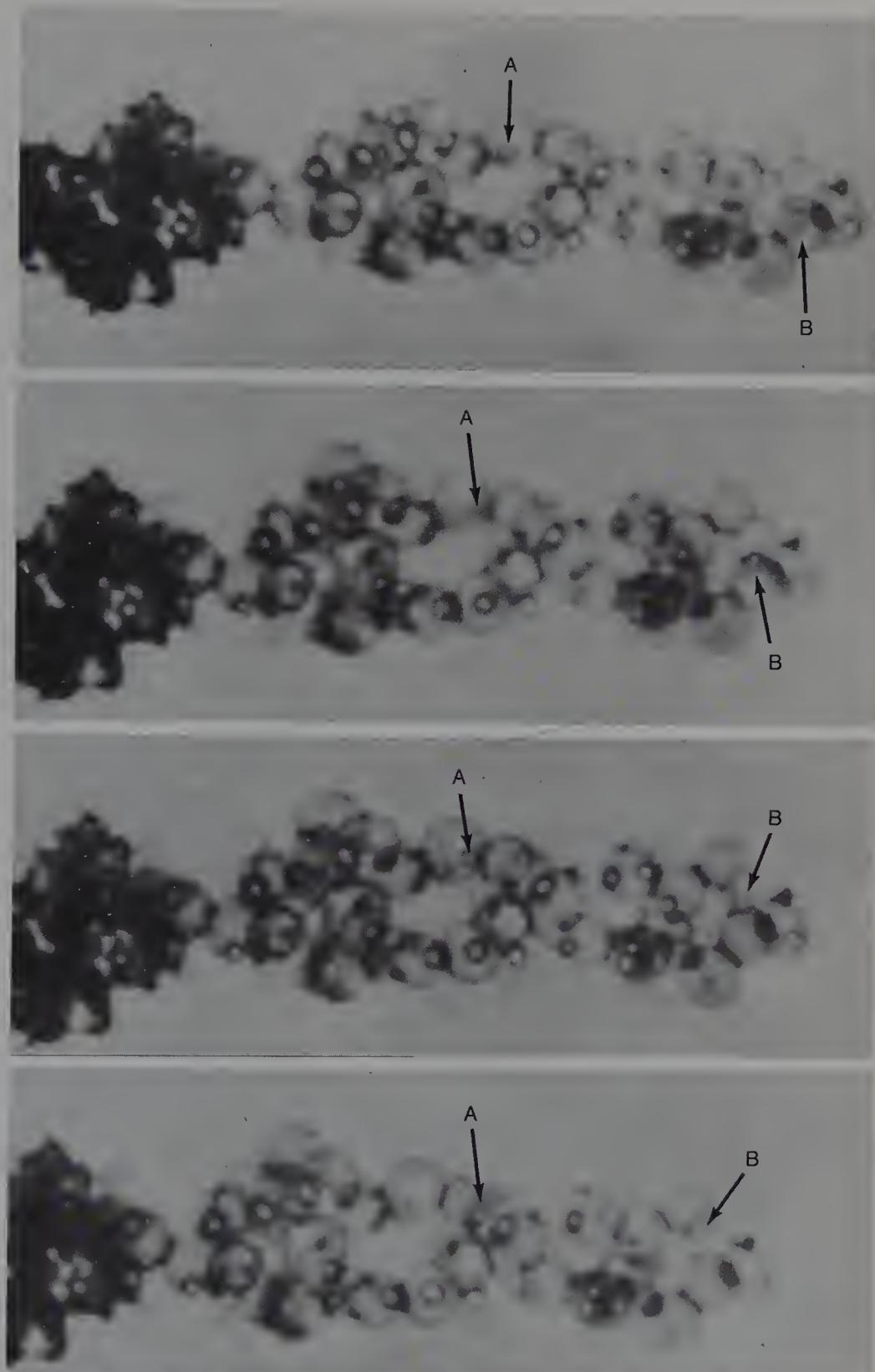
FIGURE 6-18
Junctions between proteinoid microspheres. Source: Hsu et al. (1971).

COACERVATE DROPLETS

The best known of the cell models and protocell models in the past have been Oparin's coacervate droplets. These were introduced as models for the contemporary cell by the Dutch colloid chemist de Jong and his colleagues (1930). Oparin (1965, 1966) and his students (Evreinova and Bailey, 1968; Serebrovskaya, 1968; see also Liebl and Lieblova, 1968) have extensively studied coacervate droplets as models of precellular organization.

The coacervate droplets have been made typically by combining solutions of oppositely charged colloids, e.g., gelatin and gum arabic. Each solution is uniform, but when the two are brought together they interact to form "swarms" or "clusters." When these clusters reach a certain size they separate from the solution in the form of droplets; they then constitute the organic-rich phase of the coacervate.

Table 6-5 summarizes some work done by Evreinova and Kuznetsova (1961, 1963) that shows the relative concentration of the organic-rich phase and of the aqueous phase in coacervate droplets made from

**FIGURE 6-19**

Transfer of endoparticles (A and B) between microspheres. Each frame is from a time-lapse sequence taken at 10-second intervals. Endoparticles oscillate within confining microspheres until they happen to pass through hollow junctions.

various polymers. These concentrations were determined by interference microscopy. The concentration of the polymer in a droplet is from about ten to hundreds of times greater than the concentration in the solution.

After coacervate droplets have formed, they can take up materials from the external medium. As Oparin (1966) has indicated, this ability to concentrate substances leads rather quickly to equilibrium. The coacervate droplet thus becomes a passive system. It is not very stable, being likely to break up on standing.

One of the more interesting experiments that has been performed with coacervate droplets results from allowing those droplets containing enzymes to stand in the presence of monomers, which are then converted to polymers within the droplet. For instance, Oparin and his associates have made coacervate droplets from gum arabic and

TABLE 6-5
Composition and Size of Coacervate Droplets

Diameter of Droplet 10^{-4} cm	Weight of Droplet 10^{-12} cm	Percentage Concentration of Polymer in Droplets	Ratio of Conc. Droplet to Conc. Solution
<i>Serum Albumin Plus Gum</i>			
3.1	5.2	34	50
6.2	12.9	21	31
<i>Clupein Plus Gum</i>			
2.0	1.5	36	37
3.2	5.4	32	33
<i>Histone Plus Gelatin</i>			
3.3	2.6	14	13
7.9	21	8	7
<i>Histone Plus RNA</i>			
1.8	1.5	58	116
15.8	100	4	8
<i>Gelatin Plus Gum Plus DNA</i>			
34.5	4800	22	33
62.1	12,800	10	15

Source: Evreinova and Kuznetsova (1961, 1963).

histone at pH 6.2 and have included phosphorylase during the formation of droplets. When glucose-1-phosphate is dissolved in the equilibrium liquid, the droplets are found to have begun to store starch. This product of synthesis has been verified by the starch-iodide test. After 30 minutes, such a droplet may increase in weight by 50 percent while its volume increases by more than 150 percent. Droplets of the same sort have been prepared with both phosphorylase and β -amylase. The starch that formed from the entering glucose-1-phosphate then decomposed into maltose, which diffused into the external medium where it was detected (Figure 6-20).

Similarly, polynucleotides were synthesized enzymically within coacervate droplets. The polynucleotides were decomposed by ribonuclease in a manner comparable to the way in which starch was broken down. Thus, experiments with the coacervate droplet have provided some sense of the special benefits of encapsulation of enzymes. Proximity of enzymes to each other and the presence of surfaces provides effects that have not always been considered by enzymologists studying their catalysts in dilute aqueous solution in flasks instead of in cells.

In emphasizing the meaning of organization in living units, through such studies as we have been discussing, Oparin has added another significant contribution to the major one of giving initial stimulus to investigating the subject of the origin of life (page 5). Also, he has emphasized (Oparin, 1957, 1965) the special significance of "open systems," a point made early by Prigogine (1955).

How proteinoid microspheres compare with coacervate droplets has frequently been asked. Comparisons and contrasts have been

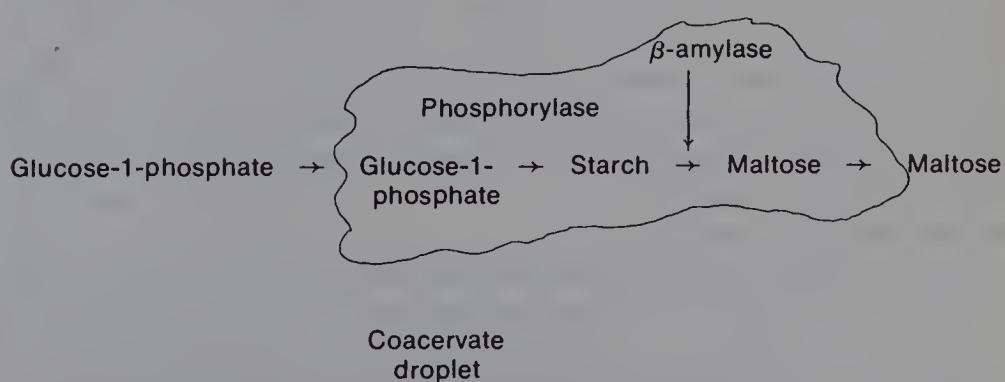


FIGURE 6-20
Diagram of synthesis and hydrolysis of starch by phosphorylase and β -amylase.
Source: Oparin (1966).

treated extensively, as well as the question of whether the proteinoid microspheres are a kind of coacervate droplet (Fox, 1970).

Each of the two models has been shown to have kinds of dynamic behavior, as described in this chapter. The coacervate droplet lacks stability; it falls apart easily on standing. The crucial difference is that the coacervate droplet is made from one or more polymers obtained from living systems while the proteinoid microsphere is made from polymers that had been made, in turn, from monomers—under conditions germane to the Earth throughout its long history. The origin of the latter kind of system is the only one that can be expected to answer exactly how cells emerged in evolution in the absence of parental cells (Lehninger, 1975). Since proteinoid microsystems are dynamic and capable of accumulating information (page 247), both their origin and their further development interdigitate conceptually with the evolutionary stream.

SULPHOBES

Many fields of science have accorded only belated credit to the contributions of pioneers. In the area of the origin of life, this is true especially of the work of the Mexican scientist, A. L. Herrera (1868–1942). Herrera investigated models of protocells over a period of 43 years; the laboratory used for this research was known as the Laboratory of Plasmogeny.

A large proportion of the 218 papers in the complete bibliography of Herrera's work (Beltran, 1968) concern models of the protocell. Some of his early studies used "silica gardens," and movements of mixtures of olive oil, gasoline, and aqueous alkali—obviously no more than laboratory curiosities. Later, however, Herrera produced "sulphobes" by evaporation of thin films of ammonium thiocyanate in aqueous formaldehyde. The sulphobes had cell-like morphology (of 6000 varieties), and Herrera (1942) claimed that they contained amino acids and starch-like material. Oparin (1957) criticized this work on the basis that plasmogeny (synthesis of protoplasm) was an unsupported title, and that the sulphobes had no organized metabolism and could not reproduce themselves.

Herrera did not assert that plasmogeny had been attained, and his writings indicate that, for his time, he was thorough and adept in physiology, chemistry, and other sciences. His understanding of chemistry is documented by the fact that in an early book (Herrera, 1924) he mentioned the synthesis of adenine by the pentamerization of HCN (page 109). The criticism of Herrera's use of the term plasmogeny can be applied also to "origin of life" in several book titles in which it appears.

The outstanding virtue of Herrera's morphological units is their origin from small molecules. Herrera did not, as did so many before (e.g., Crile, 1936) and since, employ polymers from once-living cells. Additionally, he was far ahead of his time in his choice of ammonia, formaldehyde, and water. These are constituents of interstellar matter (page 327). A more suitable factual beginning for experiments simulating molecular evolution is difficult or impossible to identify.

OTHER MODELS FOR PROTOCELLS

Attempts to coconceptualize proteinoid microspheres and coacervate droplets have been made by Smith and Bellware (1966). Related experiments and interpretations have been offered in considerable depth by Young (1965). Young, especially, has observed that proteinoid microspheres result from chilling solutions of proteinoid to below room temperature (Figure 6-3). This fact could have special evolutionary significance. Other modified proteinoid microspheres have also been reported.*

Other morphological units that have received attention include Goldacre's vesicles (1958) made from proteins and lipids, and spherules formed from gases in sparking experiments by Grossenbacher and Knight (1965). The authors believe these spherules to be glassy, due to low carbon content, but they found some associated organic matter that was hydrolyzable to ninhydrin-positive material.

An exhaustive listing of models of protocells would take us back into the era of "spontaneous generation" in the nineteenth century sense, and would include numerous studies of reconstituted cell extracts of various kinds.

In summary, the coacervate droplets are systems mostly reconstituted from extracts of cells. They have been discussed here at some length because they have provided useful information and ideas, and because they have received much attention historically. Herrera's sulphobes have been described because they are true models of protocells, having been produced from small molecules of a kind imputable to the primitive Earth. The proteinoid microsystems have been described at length because they are relevant to the basic questions and because the extensive information accumulated indicates their appropriateness to evolutionary pathways.

*K. Bahadur (Synthesis of Jeewanu, Ram Nurain Lai Prasad, Allahabad, 1966) has claimed to have produced some microscopic aggregates showing life-like morphology and function. He first began to prepare these objects from thermal proteinoid-molybdate complexes while he was a research associate at the Institute for Space Biosciences in Tallahassee, Florida. He has named his microparticles Jeewanu (Sanskrit for particles of life).

MICROSYSTEMS FROM BASIC AND ACIDIC PROTEINOIDS, PLUS OTHER COMPONENTS

The morphology and other characteristics of proteinoid microsystems are often markedly altered by the inclusion of other materials such as contemporary lipids, polynucleotides, and calcium. Special effects have been obtained also by mixing acidic and basic proteinoids, and the resultant microsystems in turn have been influenced by the addition of other substances. Some of the various altered systems that have been studied are described in this section. Because of the special significance of polynucleotides and polynucleotide mechanisms to the evolution of a contemporary cell from a protocell, relevant polynucleotide experiments are described, in the next section. Some description of microsystems composed of acidic proteinoid and basic proteinoid was presented earlier in this chapter under the heading "Gram stain" (page 211).

When microspheres are prepared from crude undialyzed proteinoid, they contain some proportion of an adventitious lipidlike material, as revealed by the fact that the crude proteinoid gives up some of its material of this kind to extraction with alcohol or with acetone. The properties of microspheres containing such material appear to include a greater plasticity than others, and a tendency to age more rapidly, which is consistent with the rapid aging of unpurified preparations of proteinoid itself.

Electron micrographs have been obtained of proteinoid microspheres into which have been absorbed adventitious lipids such as contemporary α -lecithin. One of these is reproduced here as Figure 6-21. What can be seen in the interior is a structure resembling that of myelin figures (Dowben, 1969).

A popular kind of complex coacervate droplet has been that in which there have been combined histone and gelatin or histone and other macromolecules, including polynucleotides. With the availability of synthetic acidic proteinoids and of basic proteinoids each of the components of the gelatin-histone type of droplet has been successively substituted. Figure 6-22 shows on the left a histone acidic proteinoid microsphere (Miquel et al., 1971); on the right is one in which acidic proteinoid is combined with basic proteinoid. Each microsphere pictured is composed of two polymers, each of which was produced under appropriate geological conditions without the assistance of contemporary organisms.

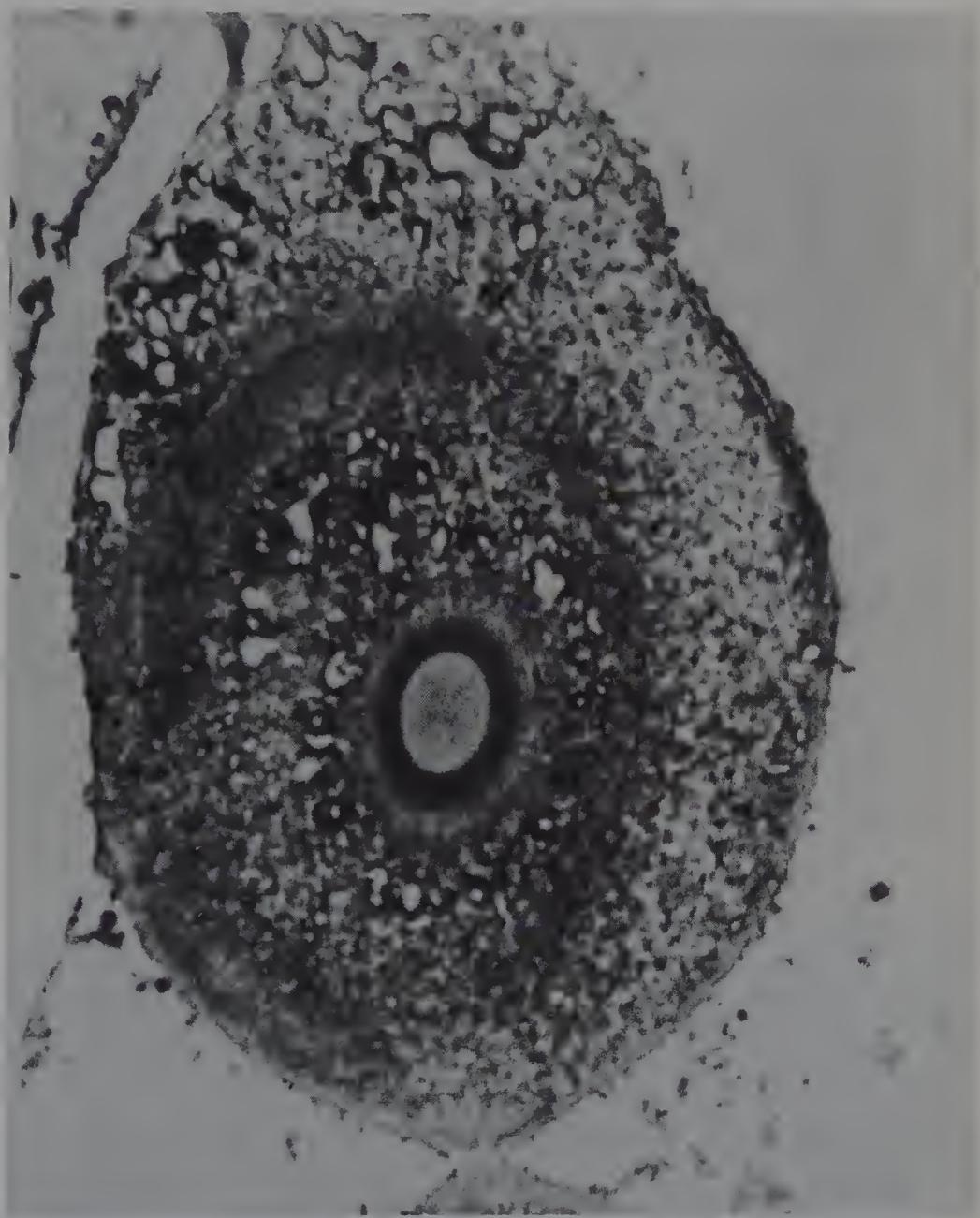


FIGURE 6-21

Electron micrograph of proteinoid microsphere containing α -lecithin.

The microsphere produced from a mixture of acidic and basic proteinoid was described earlier in the discussion of the Gram stain. The properties of such microspheres have also been described (Table 6-2). Most notable is the greater stability to elevated pH of such complex proteinoid microsystems as compared to that of the simpler type from an acidic proteinoid alone. However, greater stability to elevated pH has been found also in microspheres produced from neutral proteinoid alone. Another notable feature of the combination of acidic and basic polyamino acids is that when the proportions are suitable (Fox and Yuyama, 1963b), some of the microspheres are found to be Gram-positive, some to be Gram-negative (page 211), and many to have an intermediate reaction to the test. This kind of result suggests that the formation of such particles is not governed solely by the statistically predicted interactions of molecules, and that a Darwinian type of variation is possible within a single preparation at the level of complex microspheres.

Added cations affect the morphology and other properties of microspheres produced either from acidic or mixed proteinoids. Figure 6-23 shows the influence of various concentrations of calcium ion (Miquel et al., 1971). The effects of calcium or magnesium upon the stability, morphology, and plasticity of these systems is in some cases profound.

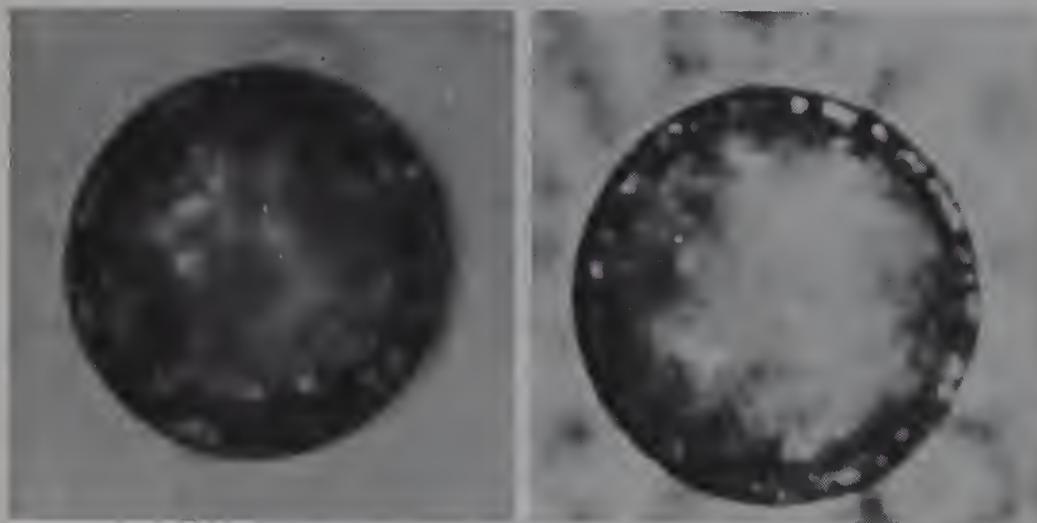


FIGURE 6-22

Photomicrographs of microspheres. *Left:* Acidic proteinoid and histone. *Right:* Acidic proteinoid and basic proteinoid. From Miquel et al. (1971).

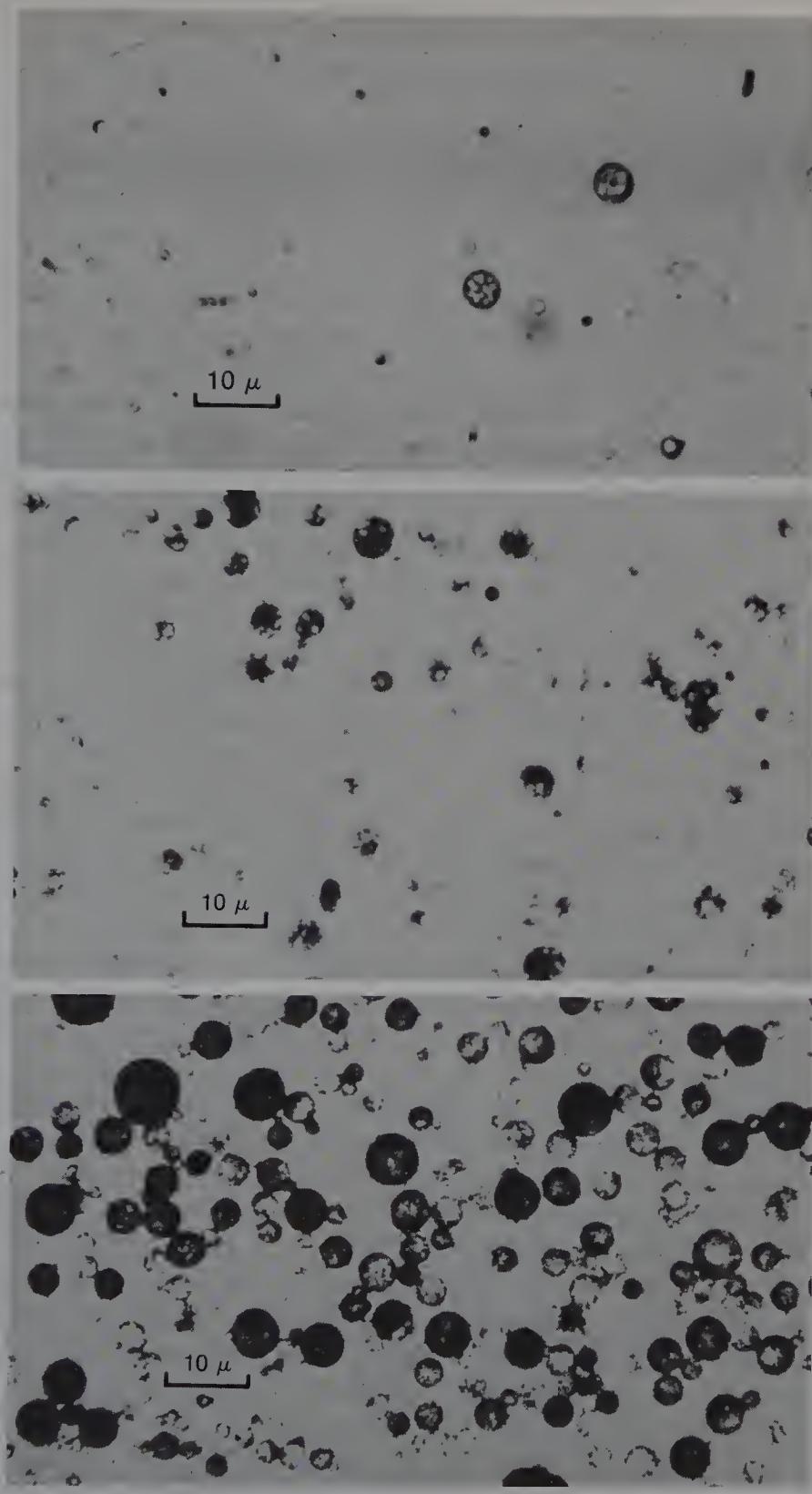


FIGURE 6-23

Photomicrographs of microspheres produced from mixtures of acidic and basic proteinoid under the influence of various calcium ion concentrations (magnification, $\times 440$). Top: 0.01 M CaCl_2 . Center: 0.1 M CaCl_2 . Bottom: 1.0 M CaCl_2 .

MICROSYSTEMS FROM BASIC PROTEINOIDS AND POLYNUCLEOTIDES

The significance of nucleic acid-containing cells and of nucleoprotein organelles for contemporary life has stimulated the conduct of experiments designed to model such units (page 225; Oparin, 1966; Liebl and Lieblova, 1968). The inclusion of proteinoids in models for organelles or cells has permitted focussing attention upon systems that can be, at least in part, held to be protobiogenic. The understanding of the origin of the components of the two necessary kinds of polymer is, however, incomplete; experiments with the already formed polynucleotide have almost entirely employed enzymatically synthesized homopolynucleotides. A consistent set of interpretations has nevertheless emerged to permit the charting in outline of an evolution from a primitive proteinaceous protocell to a contemporary nucleic acid-containing cell. Experiments have revealed that polyionic complexes composed of polyamino acids and polynucleotides have stability under elevated temperature in aqueous solution and under elevated pH such as is not possessed by particles composed of polyamino acids alone. The emergence of such polyionic complexes, therefore, could have provided a selective advantage in evolution simply on the basis of the new degree of stability. Although a primordial organism could have replicated and bequeathed information to offspring without containing nucleic acid (page 214), evolution to a more contemporary cell that used nucleoprotein organelles in the storage and readout of information would have required that new structures and functions would have entered the evolutionary stream.

Understanding of the origin of nucleoproteins, however, tends to emphasize basic proteins more than it does neutral or acidic proteins (Allfrey, 1971). In this respect, our understanding of the potential origin of the necessary basic amino acids is less satisfactory than that of the acidic and neutral amino acids. The possibilities for the origin of nucleic acids have been neither exhaustively explored, nor ignored, in experimental studies. Partial explanations for an origin of nucleic acids have been given (page 253). While at least one set of preparations is sufficiently natural in its linkages that it is attacked by ribonuclease and venom phosphodiesterase, possibilities for the development of prebiotic nucleic acids are incompletely described. Nucleic acids appear rather to have emerged late in molecular evolution, or as Ehrensvärd (1962) has suggested, as a byproduct of earlier evolutionary developments. Experiments extending this concept have been reported with the aminoacyl adenylates (page 179), which represent a form of the universal intermediate of protein biosynthesis (Meister, 1965; Krampitz and Fox, 1969; Fox, 1969; Nakashima et al., 1970).

The aminoacyl adenylate is of interest not only as an essential precursor to an intermediate for protein synthesis; it contains in conjugated form one each of the monomers of each of the two important classes of polymer. These are among the facts which permit visualizing that information could have evolved through a proteinaceous system to a nucleoproteinaceous system. The concepts of proteins-first (Moody, 1970) and of cells-first (Lehninger, 1970) will be discussed later (page 243). What is significant is the possibility that information could have flowed originally from proteins to nucleic acids as well as in the reverse direction. In the design of experiments, this possibility had to be kept in mind, as well as the premise that reductionistic thinking tends to be the inverse of the actual evolutionary progression.

The proportion of lysine was systematically varied in heteropoly-amino acids. When the various lysine-containing proteinoids were tested for their ability to form particles with RNA, this ability was found to appear at a proportion of basic to dicarboxylic amino acid slightly greater than 1.0 (Waehneldt and Fox, 1968). When a sufficiently lysine-rich basic proteinoid was allowed to interact with calf thymus, DNA fibers resulted; the same basic proteinoid with yeast RNA gave globular particles under the same conditions (Figure 6-24).

These complexes from basic proteinoid and RNA were found to be subject to dissociation under the influence of change in pH and of salt concentration in a manner comparable to that for contemporary nucleoprotein complexes (Waehneldt and Fox, 1968). In the course of studies of such dissociation the thermal polycytidylic acid (page 189) was found also to participate in such complex formation and to yield particles having quantitatively unique characteristics. Such a particle was composed entirely of two polymers, each of which could be explained as having a geochemical origin. The next question is—do these microparticles exhibit specificity in their formation or in their reaction with other materials?

The different morphologies displayed by particles formed from mixtures of basic proteinoids and polynucleotides represent one kind of specificity (Figure 6-24). Selectivities dependent upon varying amino acid composition and upon varying polynucleotide composition have been studied with lysine-rich proteinoids containing arginine but lacking lysine, and with arginine-rich proteinoids lacking lysine (Yuki and Fox, 1969). A typical result is shown in Table 6-6. This table reveals that lysine-rich (arginine-free) proteinoids interact to form particles with polypyrimidines, poly C and poly U, and that arginine-rich (lysine-free) proteinoids interact much more readily with polypyrimidines to form such particles under the same conditions. These results show selectivities dependent upon a variation

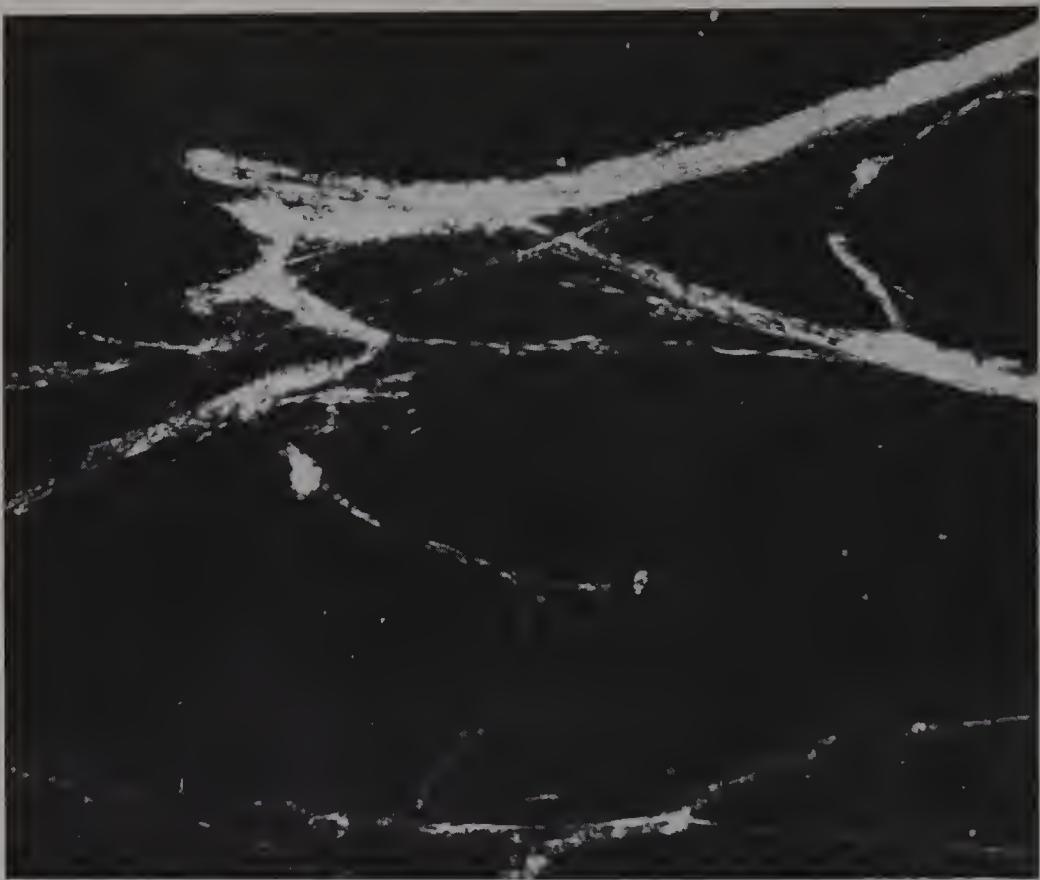


FIGURE 6-24

Photomicrographs of products of proteinoid complexes. *Top:* Product of calf thymus DNA and lysine-rich proteinoid. *Bottom:* Globular structures from yeast RNA and some basic proteinoid.

either in nucleotide composition or in amino acid composition. Recognition in protocells thus could have, in principle, existed between polyamino acids and polynucleotides in each direction.

In order to test whether such particles might be selective in the interaction with other polynucleotides, complexes from a lysine-rich proteinoid and polynucleotides of each of three kinds were prepared. Each was then allowed to bind under standard conditions ^{14}C -polycytidylic acid and ^{14}C -polyadenylic acid. Table 6-7 demonstrates that the relative proportion of polycytidylic acid and polyadenylic acid absorbed by the complex is a function of the kind of polynucleotide within the complex.

The nucleoproteinoid microsystems have been viewed as models of precursors of ribosomes (Waehneldt and Fox, 1968). Ribosomes in an early evolutionary stage could hardly be expected to have functioned in the highly sophisticated manner of contemporary ribosomes. The possibility exists, however, that a kind of selectivity or specificity might be apparent from models. An additional premise in the consideration of these microsystems as models of early ribosomes was the concept that the first particles functioning in this way might have had both messenger and transfer activity. In a search for such selectivities, Nakashima (Fox et al., 1971b) found that various nucleoproteinoid microsystems prepared as has been described did indeed show selectivities. These were expressed in preferences for the polymerization of individual aminoacyl adenylates. Many kinds of result were obtained. Empirical studies showed that some interactions could be described on the basis of a codonic relationship, others on the basis

TABLE 6-6
Comparison of Lysine-rich Proteinoid with Arginine-rich Proteinoid in Forming Microparticles with Polynucleotides

Polyribonucleotide	Turbidity	
	Lysine-rich (Arginine-free) Proteinoid	Arginine-rich (Lysine-free) Proteinoid
Poly C	0.253	0.002
Poly U	0.050	0.058
Poly A	0.001	0.060
Poly G	0.003	0.218
Poly I	0.003	0.248

Source: Yuki and Fox (1969).

Note: Proteinoid concentration 1.0 mg/ml, polynucleotide concentration 0.1 M/ml, 0.05 M tris buffer, pH 7.0, 25.0°C.

TABLE 6-7

Binding of ^{14}C -Polycytidylic Acid and ^{14}C -Polyadenylic Acid by Complexes of Lysine-rich Proteinoid with Poly A, Poly U, or Poly I

Complex	^{14}C -Poly C (counts/5 min/filter)	^{14}C -Poly A (counts/5 min/filter)	C/A
A	8,043 (7.1%)	23,232 (12.6%)	0.346
U	4,403 (3.9%)	30,709 (16.7%)	0.143
I	7,974 (7.0%)	34,860 (18.9%)	0.299
Total used	(100.0%)	(100.0%)	0.614

Source: Fox et al. (1971c).

Note: Complexes from 700 μg of proteinoid and 50 μg of polynucleotide in 2.0 ml of 0.033 M sodium chloride. Separated on a Millipore filter through which was passed ^{14}C -poly A and, in parallel experiments, ^{14}C -poly C.

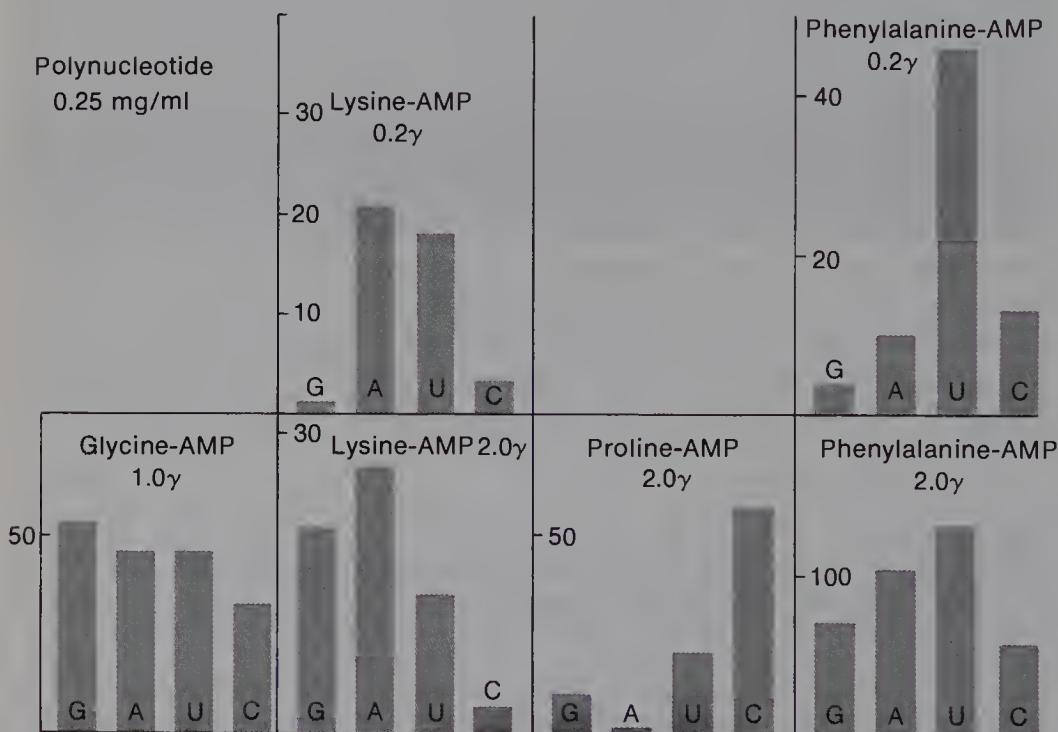


FIGURE 6-25

Relative effects of microparticles of lysine-rich proteinoid and individual homopoly-nucleotides on polymerization of each of four aminoacyl adenylates. The correspondence under these specific empirically determined conditions is qualitatively codonic.

of an anticodonic relationship, while many others yielded a more irregular type of relationship. Nevertheless, throughout these early experiments differences in selectivities were observed. Eventually, conditions were found (Figure 6-25) which yielded for each of the four homocodonic amino acids glycine (GGG), lysine (AAA), phenylala-

lanine (UUU), and proline (CCC) interactions suggestive of a stereochemical basis for the genetic code (Nakashima and Fox, 1972). In each case, under these empirically identified conditions, the amino acid corresponding to the codon is the one most favored in the compared condensations. This kind of experimentation is a kind of selection of conditions. Evolution itself, however, is a selection process. The constructionist has the advantage that he is, in advance of his experiment, aware of the relationship of phenomena that evolution has already selected. In this case the experiments were repeated with four different lysine-rich proteinoids, each yielding the same kind of result in each set of experiments.

SUMMARY

The properties of models of the protocell from various laboratories have been reviewed. The method of preparation, implications for origin, and properties of simple proteinoid microparticles have been described in some detail. The special properties of other more complex proteinoid-containing systems have been described in a context of understanding the origin of ribosomes, protein biosynthesis, and the genetic code. Table 6-8 lists the properties of simple proteinoid microparticles and Table 6-9 lists the properties of mixed proteinoid microsystems.

TABLE 6-8
Properties of Proteinoid Microparticles

Stability (on standing, during centrifugation and sectioning)
Microscopic size
Variability of shape
Uniformity of size
Numerousness
Stainability
Producibility as Gram-positive or Gram-negative
Tendency to shrink or swell in atonic solutions
Boundaried structure
Ultrastructure (visible under the electron microscope)
Selectivity of passage of molecules through boundary
Assembly from catalytically active polymer
Patterns of association
Budding and fission
Growth by accretion
Ability to propagate through budding and growth by accretion
Ability to form junctions
Ability to transfer informational molecules

TABLE 6-9
Properties of Microsystems Formed from Proteinoids Plus Other Substances

Acidic proteinoid plus basic proteinoid	
Producibility as Gram-positive or Gram-negative	
Solubility similar to that of Gram-positive bacteria	
Morphology comparable to that of histone-rich acidic proteinoid particles	
Polynucleotide plus basic proteinoid	
Nucleoproteinoid composition	
Morphology resembling that of nucleoprotein organelles	
Stability greater than that of simple proteinoid microsystems	
Microscopic size (0.5–1.5 microns)	
Uniformity of size	
Numerousness	
Selectivity in formation dependent upon identity of polynucleotide and polyamino acid	
Ability to form junctions	
Selectivity in the promotion of condensation of aminoacyl adenylates (related to the contemporary code)	

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CHAPTER 7

*Interpretations from
Experiments with
Proteinoid Microsystems*

In this chapter, the experiments leading to and from proteinoids are interpreted to explain the origin of the first reproducing protorganism. This tissue of interpretations is the only comprehensive, detailed theory of the origin of the first cell (cf. Florkin, 1975; Lehninger, 1975), and it is a theory derived from close interpretation of experiments performed under geologically relevant conditions. Concepts and partial theories that are said to oppose, or compete with, this one have been advanced. Some of the competing concepts are entirely or largely without experimental demonstration or observational support (Florkin, 1975; Fox, 1974a).

Although the interest in the origin-of-life problem has spread to command the attention of molecular biologists and others (e.g., Crick, 1968; Monod, 1971; Eigen, 1971; Lipmann, 1972; Szent-Györgyi, 1974), the matrix of information obtainable from the contemporary living cell has not provided an appropriate basis for most of the inferences that have been made. The relationships explaining this statement are depicted in Figure 7-1. The contemporary molecular biolo-

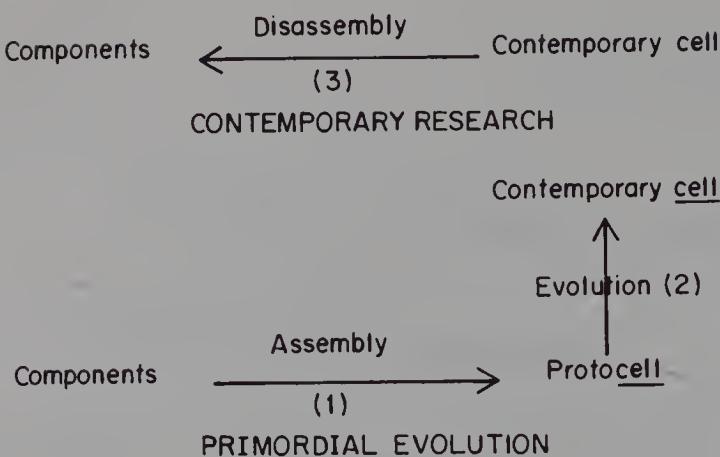


FIGURE 7-1

The flow of events in contemporary research and in early evolution.

gist, biochemist, etc., begins research by first disassembling the complex contemporary cell into its simpler components (Step 3). The catalog of information obtained by such study is, indeed, almost all of our life sciences, a truly extensive knowledge. However, the *direction of evolution* to the first cell and to the contemporary cell, on the other hand, was from simple to complex.

Accordingly, the use of an information base derived solely from analysis of the contemporary cell* not only appears not to be the way to answer the key questions; it is not the way to ask them. What was necessary was an experimental assembly of components (Step 1). What was needed also was an inferential looking forward to the present from the earlier matrices (Steps 1 and 2); one could not infer nor reproduce the primordial assembly (Step 1) by extrapolating backwards from the present (reverse of Step 2).

We have no known primordial organism at hand for comparison. Although a simulated protocell was assembled approximately twenty years ago (Fox et al., 1959), a contemporary cell has not yet been reassembled from its components (reverse of Step 3). Nor would such a successful reassembly tell us how life originated on Earth (Step 1).

We were therefore left with the need to design constructionistic experiments on the basis of inductive reasoning. Although most scientists are more comfortable with deductive, or analytic, reasoning,

*The contemporary cell is, however, essential to provide (a) clues for the molecular precursors of a primordial cell, and (b) a standard against which to judge experimental progress.

there simply were no apparent alternatives to employing constructionistic research in solving this problem.

SIGNIFICANCE OF AN EARLY PROTOCELL

Of central significance to the unified theory presented here is the appearance of a first, minimal, cell. This emphasis is possible because of (a) the great ease with which protocells (minimal cells) came into existence as judged from experiments, and (b) the numerous advantages conferred upon the evolving processes by the existence of such a cell. Many of these advantages are listed in Table 7-1.

Benefits to the evolutionary processes in addition to those of Table 7-1 can be visualized. A number of authors (ref. 2 in Fox, 1975) have independently reviewed the "remarkable" properties of proteinoid microspheres. These properties underlie the evolutionary advantages. Any two or more of the benefits listed in Table 7-1 would be sufficient to confer upon a cellular type of evolution a huge total evolutionary advantage (Zuckerndl, 1975) over any noncellular evolution. This advantage would, accordingly, have been of crucial significance if some kind of evolvable cell could have arisen easily. The answer to this question was provided by experimental demonstration of how very simply proteinoid microspheres come into existence (page 203), by contact of polymer with water.

The young student may be unaware of the historical difficulty that existed in the acceptance of the concept of an early cell. As recently as 1964, however, a symposium on the evolution of genes and enzymes omitted cells from the title (Bryson and Vogel, 1965) in accord with biological thinking popular at the time. This omission did elicit comment (Fox, 1965a). One may speculate that the complexity of contemporary cells, biomacromolecules, and even their modeled primordial precursors would have been responsible for such a view. Complexity of structure or mechanism, however, does not signify complexity of process. It is simple processes that make plausible the spontaneous transition from a purely chemical world to the first organic world.

Even before these demonstrations in the laboratory (Chapters 5 and 6), the idea of "cells-first" was explicit or implicit in the writings of Wald (1954), van Niel (1955), Oparin (1957), Ehrensvärd (1960), and others. What was needed was a clear statement that a beginning cell did not need to be a contemporary cell, and some experimental back-up of this idea. Following a first laboratory demonstration of possibilities (Fox, 1965a), Lehninger (1970, 1975) has stated the potentialities with clarity.

TABLE 7-1

Benefits to Evolution of a Cellular Structure (Membrane-enclosed)

Physical protection of contained organic material
Juxtaposition of enzymes, organelles, etc.
Organization of chemical reactions by enzyme complexes
Thermodynamically favorable hydrophobic zones
Compartmentalization of functions
Maintenance of kinetically favorable concentrations
Reproduction at the level of the microsystem
Adaptive selection of individual variants
Screening of macromolecules from diffusible molecules in the environment
Controlled dynamic interactions between environment and cellular contents

For the reductionist who begins study with the organized cell, which is then disassembled for analysis, it might seem at first that a cell would be the last product of a primordial assembly. Figure 7-1 explains that innumerable jigsaw puzzle-type testings of all relevant constructionistically derived concepts uniquely provide another answer. Cellular evolution, which was in a "forward" direction, had to intervene between protocell and contemporary cell.

The so-called "chicken-egg" question of biochemistry has been that of whether nucleic acids or proteins arose first. Some authors have tended to think of proteinoid as a simulation of prebiotic protein and to treat it as the protein in the context of "proteins-first." However, proteinoid is not protein. The one, or unique, assembly of concepts presented here defines proteinoid as a copolyamino acid that could aggregate to a protocell in which nucleic acids and proteins, much as we know them, arose approximately simultaneously. The experimentally indicated early event, which fundamentally modifies the chicken-egg question, is that of cells-first.

Part of the reason that the chicken-egg question existed is the need for understanding the priority in the appearance of two functions: catalysis and ordering. In the chicken-egg alignment of concepts, one asks how nucleic acids were formed without protein (enzymes) to make them, or how proteins were formed without nucleic acids to govern the order of amino acids. Such a question is partly a problem for more contemporary evolution, but it is nevertheless a question needing an answer at some stage in molecular evolution.

The experimentally established answer is that the necessary instructions for the first copolyamino acids (proteinoids) existed in the interactions of mixtures of stereochemically individual amino acids; nothing as complex as nucleic acids was required (nucleic acids, in fact, are significant as repositories and readout devices for such instructions in the overall view, but not for the origin of such informa-

tion). This concept has been touched on earlier (page 152) and will be explained in greater depth later (page 247).

Crick (1968) focused his attention on the "origin of the genetic code." He considered two explanations for that origin: (a) stereochemistry and (b) a "frozen accident." Since it has been possible to demonstrate selective recognitions between polynucleotides and thermal polyamino acids experimentally (page 232), it has been further possible to infer that the basis for the genetic code is rooted in the stereochemical forces, or reactivities and shapes, of molecules. The determination of dominance of nucleotide by (a) content of lysine in copolyamino acid and (b) the reflexive determination of content of lysine by identity of nucleotide in polynucleotides (Fox, 1974b) suggests that these two complementary recognitions would have been locked into an evolutionary feedback process. Such a process would, then, have the quality of a "frozen" event. The processes that were frozen were fixed on the core of interactions between amino acids and nucleotides, each of which was part of a more complex set of substances and mechanisms.

These experiments thus lead us to the view that an important primitive development was frozen stereochemistry, to paraphrase Crick. But the freezing was no accident; the nonrandom interaction of molecules of different shapes (page 248) was the predisposing cause rather than an accidental occurrence (Fox, 1975). The argument for chance (Monod, 1971) in evolution or for "accident," has essentially been refuted by Pauling and Zuckerkandl (1972).

This book treats the origin of the genetic code in terms of the origin of a genetic mechanism for coding (page 254). The reasoning underlying this view is that the stereochemical nature of molecules was and is "forever;" only the mechanism translating them could have originated in evolutionary time.

SEQUENTIAL COMPATIBILITY AB INITIO

The validity of answers to a problem concerned with terrestrial events that occurred three billion years ago requires special attention (page 258). At this point in our construction of theory we can focus, albeit incompletely, on the necessity for sequential compatibility, in conditions and processing, of all of the steps in an evolutionary sequence. Prolonged and repeated tests of theory have led to the view that the sequential compatibility must be honored at the very outset of the sequence, that is, *ab initio*, much as it is in the conceptualizations of quantum chemistry (Fox, 1975), from which the term is borrowed. This view is similar to that of *biochemical predestination* (Kenyon and Steinman, 1969).

This point may be explained most clearly by reference to the erstwhile popular idea that life began with DNA (Muller, 1966) or, in the words of Miller and Horowitz (1962), "a self-duplicating molecule of DNA would be the first living organism." Such an assumption typically leaves open the question, whence DNA? It is in the attempt to answer this question that we face the *ab initio* requirement of informational origins. The need for alternatives then appears (Lederberg, 1959; Ehrensvärd, 1960). The question is then broadened to include consideration of the origin of the first informational macromolecule, which our comprehensive review of experiments, results, and varied premises indicates was not a nucleic acid.

We are also thus brought to focus our attention on the micromolecules (Chapter 4) that preceded the macromolecules (Chapter 5) and the supramolecular systems (Chapter 6).

Reappraisal of experiments concerned with the origin of micromolecules (Florkin, 1975) has raised a question about the value of producing representatives of each of the major types of biomolecule. Although much success has attended such efforts (Stephen-Sherwood and Oro, 1973), the new question raised is the relevance of such broad demonstrations (Hartman, 1975; Fox, 1974b). The earliest cells may have been able to make many of the compounds they needed. Open to more unqualified criticism has been a tendency to discuss the origin of, for example, a few amino acids as if that were an answer to the question of the origin of life. A few authors have regarded such demonstrations as overinterpretations in various ways (Abelson, 1957; Mora, 1965) and one has referred to the demonstrations as "trivial" (Green, 1976).*

Despite the gap from micromolecules to the complex contemporary living systems, the amino acids are widely accepted as initial entities in a series of reactions that led to living systems. The fact that many experiments demonstrate easily the production of sets of proteinous amino acids, but not of sets of nucleotides, may be significant in suggesting what the *ab initio* molecular bricks were. A point deserving of reemphasis is that many fewer than eighteen proteinous amino acids could have served at the outset. Inclusion of eighteen types in many thermal condensations in the laboratory primarily demonstrates the versatility of the synthesis.

*Green (1976) has, however, also claimed that the questions of the first cell are "unanswerable." An antidefeatist point of view was earlier expressed by Tiselius (1964) who said, in respect to investigation of vital processes, "But there, more than in most fields, a tendency has showed itself to consider the unexplained as inexplicable." Indeed, at the time Green made his defeatist statement, several other biochemists had discussed the answers which already existed to some of Green's "unanswerable" questions.

It now appears from the experiments that some units and materials arose in what were probably rapidly successive stages. For example, proteinoids arose, yielded protocells, and the latter, with the aid of protoribosomes, produced the first true proteins. Protocells themselves were capable of modulation to more advanced entities.

The First Informational Macromolecule

In this volume, we use as a definition of information the capacity of a molecule or system to interact selectively with other molecules or systems.

In the past, some biologists have tended to think of information as found only in living systems. Other biologists have not; the definition used here reflects the broader perspective. Some biologists have tended to think of nucleic acids as the only informational macromolecules. The correctness of regarding proteins as informational is a premise expressed in the "molecular logic" presented in such textbooks as that of Lehninger (1970), and must be borne in mind in identifying the first informational macromolecules.

The special value of recognizing the informational quality of proteinoids, like that of the structurally related proteins, is that a continuity of information from geochemical realm to organism can be traced via:

Mixed amino acids (in prebiotic realm) →
Proteinoid → Protocell

The fact that proteinoids are informational is manifest in their selective interactions with enzyme substrates (Fox, 1974b) and with each other (Hsu and Fox, 1976).

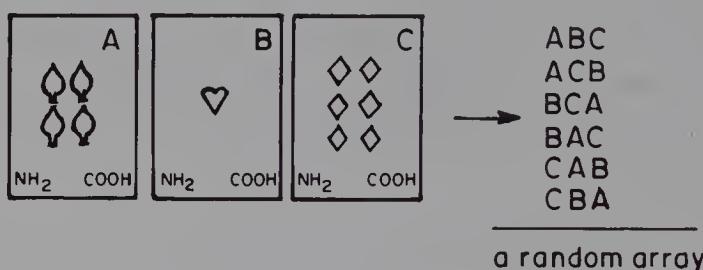
Because of the crucially significant position of the first informational macromolecules in the evolutionary sequence, it is appropriate that much evidence has been accumulated for nonrandomness in the proteinoids (page 158), as supplemented by more recent work. As Melius (1977) has stated in a review of proteinoids, "A consistent finding of all investigators is that the polymerization of the amino acids is non-random."

This awareness of nonrandomness has conflicted with a popular tendency to assume that polymerizations are random (Fox, 1973a). Senior polymer chemists do not begin with such an assumption (Flory, 1953; Billmeyer, 1962); they report a few instances of nonrandom, or what they tend to call nonideal, polymerization, especially in thermal condensations. Very probably, the large body of data now

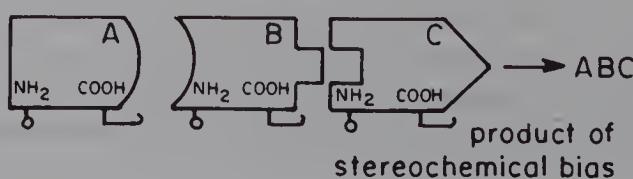
accumulated for the nonrandom thermal polycondensation of amino acids is the largest body of evidence on this issue. Study of heterogeneity in thermal copolyamino acids, furthermore, has been aided by the extensive technology that has been developed for fractionating and characterizing proteins and peptides.

The finding that nonrandom polycondensation is normal also conflicts with a popular view that types of amino acid can be compared to different denominations of playing cards, or to colored beads. This is the a priori theoretical point of view, early popularized by Gamow (1955). If we think of each of twenty amino acids as playing cards, and reduce the twenty to three for the sake of simplicity, we can examine this premise in Figure 7-2. Arranging the cards corresponding to amino acids A, B, and C from left to right, we do indeed see an equal likelihood for each of the six possibilities: ABC, ACB, BCA, BAC, CAB, and CBA. Nothing distinguishes A, B, and C, except the superficial label; identical shapes permit a random array of arrangements.

The fallacy in this conceptualization is that the various amino acids do not have identical "shapes," that is, structures and reactivities. In



AMINO ACIDS WITH THE SHAPES OF
PLAYING CARDS



UNITS OF DIFFERENT SHAPES,
AS IN AMINO ACIDS

FIGURE 7-2

Random association of playing cards (all of same shape) vs. nonrandom association of amino acids (each of unique shape). In actuality, nonrandomness is not total. The uniqueness of shape of the amino acids is exaggerated.

an exaggerated form, the varied shapes of amino acids A, B, and C are represented in the lower half of Figure 7-2. While the individual shapes are fanciful, the fact that they are different is brought out, plus the fact that they must interact in nonrandom, or selective, fashion.

The evidence that the interactions are selective has been given (page 158) in contradiction to the *a priori* assumption of randomness, exemplified by playing cards (Fox, 1973a) or a "Cosmic Casino" (Fox, 1975). The assumption of randomness bears a relationship to the phrase, "order out of chaos" (Fox, 1973a). Our evolutionary problem is, however, how one kind of order became another kind of order, and how entropic developments were influenced by the transfer of energy into organisms (page 252).

The Origin of Reproduction

Examination of contemporary organisms has revealed that they reproduce at two levels—the cellular and the molecular (DNA replication). It has been easy to assume that the molecular replication occurred first and evolved to replication of the more complex and inclusive entity, the cell which contains DNA. As we have seen (page 243), however, the experiments indicate that a reproducing protocell arose before DNA, certainly before cellular DNA. In this unified view, the first DNA-like precursor of DNA had to appear in the protocell. A number of early reviews (Lederberg, 1959; Calvin, 1962; Thimann, 1963; Buchanan, 1965; Granick, 1965; Lipmann, 1965; Tatum, 1965) already left open the possibility that DNA arose in evolution subsequent to copolyamino acids; the proteinoid experiments show how that order of evolution could have occurred.

The proteinoid microspheres are capable of four kinds of protoreproduction, in all of which growth is by accretion: budding, binary fission, and two others (Fox, 1973b). Each of these has the appearance of an evolutionary precursor of a contemporary process, in which the mechanism is much more complex.

The experiments make another point; that is, the potentially great rapidity with which the *ab initio* processes could have gone from generating amino acid precursors to yielding a protoreproductive organism. It has been fashionable to hypothesize that plenty of time was available for the complex processes of primordial evolution. The experiments have shown that, while the products and mechanisms are complex, the processes are simple, and they are fast (Fox, 1973c). The appearance of reproducing protocells on the primitive Earth (cf. Rohlfing, 1976) was, in summary, simple, frequent, and rapid, and appropriate to many terrestrial locales (cf. also Turcotte et al., 1974).

Experiments and interpretations of the origin of natural selection in proteinoid microspheres have been presented (Kenyon, 1974; Hsu and Fox, 1976).

Emergent Properties in Proteinoid Microspheres

The individual properties found in proteinoid microspheres have been judged by a number of authors (ref. 2 in Fox, 1975). The adjective used most often is "remarkable." What is far more remarkable is the association of a number of emergent properties within the single microsystem (Chapter 6; Table 7-1).

The proteinoid microsphere, and by inference the protocell, share with the contemporary cell the properties of organization and of quantum unity. The quantum leap from amorphous copolyamino acid to organized protocell has some of the quality that evokes "feelings" of vitalism. One may sense this large difference upon dismantling a contemporary organism; the difference observed in seeing the functions emerge from assembly is more dramatic to the observer.

Which properties would emerge in a nucleic acid-free cell could be determined only by experiment. The results (cf. Szent-Györgyi, 1972; Kenyon, 1974; Lehninger, 1975; Florkin, 1975) suggest that the primordia of all or most of the properties now known for the contemporary cell are found in proteinoid microspheres. For example, the proteinoid microsphere contains no nucleic acid, but the polymer (proteinoid) is known to have the ability to produce (a) simulated protocells and (b) internucleotide bonds (Jungck and Fox, 1973).

These observations are not at all an argument for vitalism. They are only an explanation for why some observers might long ago have sensed a huge functional difference between the cell and its components.

PLAUSIBLE EVOLUTIONARY PRECURSOR OR PRIMITIVE SYNTHETIC ORGANISM?

Numerous comments, many unpublished, on whether proteinoid microspheres represent a laboratory synthesis of a protoorganism (cf. Sylvester-Bradley, 1973; Sherman and Sherman, 1975) require that this question be examined in light of the experiments. Since the terms "life" or "living" are not yet objectively definable, they are here used sparingly.

The preceding discussion has emphasized that there is a difference between a primordial organism and a contemporary organism; failure to make this distinction is often a source of difficulty in discussions.

First, we can say, categorically, that proteinoid microspheres are *not* contemporary organisms. Are they examples of models for primordial life? Our answer to this question is that they are simulations for primordial life or for, more strictly speaking, the unit of primordial life—the protocell. However, the definition of primordial life is in one way even more difficult to come by than the definition of contemporary life. We do not, to our knowledge, have at hand a sample of primordial life for comparative purposes. Therefore, what has been produced in the laboratory can be, on the basis of conceptual analysis, no more than a simulation for a type of primordial life on the Earth.

For a genuine model of primordial life, a protocell, it is either preferable or necessary that that model be evolvable to a contemporary cell. We do not have a total body of evidence to establish that the proteinoid microsphere could so evolve, but we do have a conceptual analysis of what would be required (page 252). Experiments demonstrate that some of the evolutionary gap between the primordial and contemporary cell has been bridged. Accordingly, we believe that the proteinoid microsphere is capable of evolution to a contemporary cell, even though that capacity has not been fully demonstrated.

Independent assessments of this question are available. Two comments by A. L. Lehninger (1970) are relevant: "microspheres . . . are self-organizing systems . . . of much value as models of the first primitive cell-like structures. . . ." and, "Although such models of primitive cells are very plausible, they could not evolve very far without a genetic system." Also pertinent are comments by C. A. Knight (1974), "Proteinoids are readily converted in hot water to spheroidal structures . . . which possess a surprising number of features of cells . . . Such protocells, rather than a primitive virus, may in fact have developed into the first 'living' cell." The essential defensible statement is that the proteinoid microsphere is a plausible, or the most plausible, model for the evolutionary precursor of the contemporary cell.

A perhaps fuller assertion of the same kind is one that a proliferative (page 214) protoorganism has been synthesized in the laboratory. Again, a full demonstration of total evolvability to a contemporary cell is yet to be made.

Pirie (1954) published a paper titled, "On Making and Recognizing Life." He first explained why astronomers and other physical scientists tend to oversimplify biology. In speaking of eobionts, his term for the first synthetic biosystems, Pirie said, "In these systems

phenomena will in time be observed that will be taken as evidence for an eobiont . . . Recognition and acceptance of this is likely to be slow."

Pirie then stated as requirements: "The system should be or should contain liquid and should work at temperatures below say 200°C." He would further accept most readily systems containing water and protein. The system must be able to do something beyond simple growth, be able to catalyze at least five or six reactions, and have the ability to reproduce. He then said, ". . . if it does this much it will be doing enough to start an argument."

Other experts would also require reproduction and metabolism, but would add ability to mutate (Stanley, 1964). Irrespective of arguments about how closely the functions fit the a priori requirements, the various properties of the proteinoid microsphere have been identified, and reported in separate years. The significant fact is that, although individually identified by investigation in separate years, several functions were usually present in association in a single unit at the same time, or could combine as proteinoids combined (Hsu and Fox, 1976).

THE GAP BETWEEN PROTOCELL AND CONTEMPORARY CELL

In any interpretation of the roster of criteria met by the proteinoid microsphere, further research demands a determination of how the microsphere was converted to a contemporary cell. The functions of the proteinoid microsphere are sufficiently well catalogued that the salient ones can be subtracted from the salient functions of the contemporary cell. When the subtractions are performed, the needs for bridging the gap are defined, as shown in Table 7-2.

Conversion of Solar Energy to Cellular Energy

Our present biota is dependent for energy on that multitude of cells and organisms that give this planet its green cover on land, and its photosynthetic organisms in the sea. Some bacteria derive energy from chemical reactions, but Broda (1975) has reasoned that nonphotosynthetic bacteria are descendants of photosynthetic bacteria. The conversion of solar energy now takes place almost entirely through chlorophyll. Prior to chlorophyll mechanisms, however, other pigments could and, according to analysis of the possibilities, did serve as solar-energy transducers (Krasnovsky, 1974; Hall et al., 1974; Evstigneev, 1975).

TABLE 7-2
Functions Needed to Bridge the Gap from Proteinoid Microsphere (Protocell) to Contemporary Cell

Conversion of solar energy to cellular energy
Cellular synthesis of nucleic acid
Cellular (ribosomal) synthesis of protein
Genetic mechanism for coding

The photoactivating effect of a pigment found in all thermal proteinoids (Wood and Hardebeck, 1972) provides explanation for the ability of the earliest proteinoid cells to transfer solar energy to reactions carried on within them. This energy input should have been available to synthesize ATP or inorganic polyphosphate (Baltscheffsky, 1974; Keosian, 1974; Kulaev, 1974), whichever was the first product of photophosphorylation.

Cellular Synthesis of Nucleic Acid

Not much progress has been made toward the *ab initio* synthesis of nucleic acid, but the production of internucleotide bonds, some of them 3' — 5', has been reported in a number of simulation experiments (Schwartz and Fox, 1964; Stephen-Sherwood and Oro, 1973 for review; Jungck and Fox, 1973). In the last cited of these studies, the synthesis of internucleotide bonds in adenine dinucleotide and adenine trinucleotide has been reported in proteinoid microspheres. This finding of synthesis of oligonucleotides in a simulated protocell is in accord with the conceptualization of Ehrensvärd (1960).

Although a trinucleotide is already large enough to have coding ability, the prognosis for visualizing an evolutionary surge in nucleic acid-governed biochemical events suggests the need for much larger polynucleotides.

Cellular (Ribosomal) Synthesis of Protein

The stage of understanding of development of cellular synthesis of protein is also not far advanced. Simulated protoribosomes (page 232) have been produced. Those units made from poly A and lysine-rich proteinoid are able to bring about the synthesis of phenylalanine peptides from ATP and phenylalanine (Fox et al., 1974). In principle, then, we can now understand how the protoribosomes would have arisen (page

260), how adenosine would have been generated in steps (Oro, 1960, Gabel and Ponnamperuma, 1967; Fuller et al., 1972) and how the adenosine would have been photophosphorylated (page 253). A stronger overall demonstration, however, is wanted.

To this point, we can summarize by stating that we understand the *ab initio* origin of a reproductive protoorganism, that we have the first experimental indications of a bridging of the gap to a contemporary organism, and that much more is needed to bridge that gap fully.

The Genetic Mechanism for Coding

When we turn our attention to coding, we need to discuss the experiments and theory in a somewhat different mode than the synthesis of ATP, nucleic acid, or protein. Aspects of the basic problems have already been treated (page 247).

Part of what we know is that selective interactions have been demonstrated (Fox, 1974b) between nucleotides and amino acids when each of them is present in polymeric, or derivatized (Ralph, 1968), form.

Many kinds of genetic code might have been tested originally (Nakashima and Fox, 1972); only one survived. We interpret this selection as due to favored steric interactions and evolutionary feedback (page 245).

In the first edition of this volume, it was pointed out that selective recognition by amino acids of nucleotides and of nucleotides by amino acids could be observed. This differs from the directionality of the contemporary function of the genetic code, in which information flows via DNA → RNA → protein. The experiments demonstrate, however, an informational recognition of

polyamino acids ↔ polynucleotides,

in either direction. From this latter observation a concept of reverse translation was inferred. Repeated testing of overviews suggests, however, that while bidirectional recognition was possible, unidirectional translation was a consequence of the appearance of the (proto) ribosome. This view is discussed further under what was learned by constructionistic experiments (page 255).

The Flowsheet for Primordial Events

Figure 7–3 presents the flowsheet for primordial events.

This panel of evolution now interdigitates with general evolution at each end. The earliest reactants are increasingly understood as aris-

ing from the Earth, the Moon, meteorites, and interstellar matter, since common pathways are found among carbon compounds (Chapter 11). The simulated protocell more closely resembles contemporary prokaryotes than eukaryotes (Margulis, 1971). Accordingly, one can visualize prokaryotes at the upper end of the flowsheet. (The protocell may be referred to as a prokaryote).

The experimental results and the conditions that yield them suggest that, at least to the stage of the horizontal line, primordial cells arose simply, often, and in innumerable locales on this planet. According to this view, cells did not arise once (Fox, 1973a); rather, each time they arose, they resembled closely cells that arose on other occasions. In bioevolution, this similarity in bioevolutionary pathways is known as parallel evolution. The evidence is strong for parallel evolution also at the prebiotic molecular stage. The inference provides a logical basis for the principle of biochemical unity (Florkin and Mason, 1960).

A Review of the Unique Contributions of Constructionistic Research

As stated on page 244, questions of the origin of the cell can neither be answered nor comprehensively asked by reductionistic methods only. At this stage in the research, we can list a number of phenomena or principles that have been identified by constructionistic studies, and which had not been appreciated from reductionistic approaches. These new findings establish that the construction of a simulated primordial cell need not have, or could not have, waited until an analysis of the cell (of whatever evolutionary vintage) was complete. The new views are listed in Table 7-3.

Perhaps most fundamental of the new principles is the ordering of amino acids in polymers by the steric forces in the monomers. An early objective in the prefatory research was the understanding of order in proteins; this was not achieved by amino acid sequence analysis (Fox, 1976a). Nor was the potentiality for identifying of principles first suggested by results of thermal polycondensation of amino acids. Rather, this possibility emerged from enzyme-activated synthesis of peptide derivatives (Fox, 1974b); those studies, however, were themselves constructionistic.

Somewhat less surprising than the finding of internal order in proteinoids were the arrays of enzymelike activity identified in several laboratories (page 171). These were partly expected at about the time the means for condensing mixed amino acids was established. The fact that a proteinoid could display a three-dimensional active site

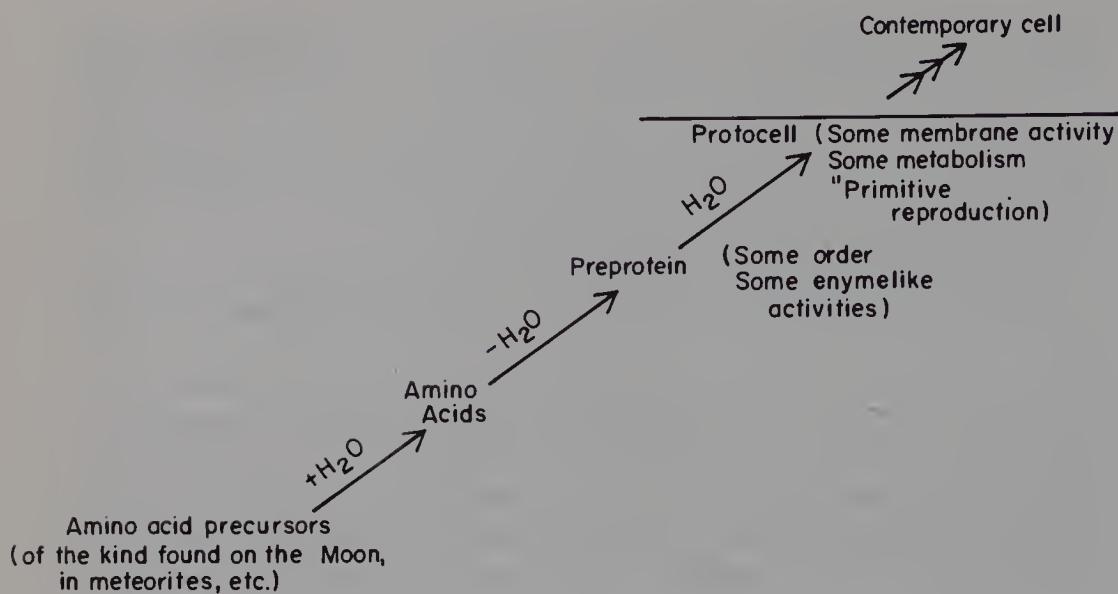


FIGURE 7-3

Flowsheet of processes from primary reactants to first informational macromolecule (preprotein = proteinoid in laboratory) and thence to reproducing protocell (proteinoid microsphere in laboratory). The model beyond that stage to the contemporary cell is incomplete, but indications of how the bridging occurred have been obtained (page 252).

TABLE 7-3

Awarenesses Resulting from Constructionistic Research

-
- Ordering of amino acids during thermal condensation thereof
 - Enzymelike, hormonelike, and photoactivating activities of thermal copoly-amino acids
 - Aggregating ability of thermal copolyamino acids
 - Reproductive nature of proteinoid microspheres
 - Presence of associated properties in proteinoid microspheres
 - Pigment-promoted metabolic reactions in proteinoids and microspheres
 - Existence of stages in the primordial development of proteinoid microspheres
-

(Rohlfing and Fox, 1967) was more unexpected, as was the finding of hormonelike activity (Fox and Wang, 1968). Photoactivation of some reactions (page 253) was also more unexpected, and took more years to establish. This last could hardly have been forecast from analytic studies, especially since the nature and capabilities of the pigment were unknown.

The finding that thermal copolyamino acids would readily *aggregate* to cell-like structures was not foreseen. The possibility of a similar process, akin to denaturation, was indeed predicted (Fox, 1957) but

that mechanism is thought to be unlikely. What brings about the aggregation is now viewed as strong binding between macromolecules. Nothing as strong as this binding is inferred for contemporary cellular structures; part of the reason for this view is that dismantled contemporary cells have not been reassembled. The powerful binding exhibited by proteinoids, incidentally, creates technical difficulties in fractionation in the laboratory. It seems likely that both the difficulties and the benefits have their roots in this same strong tendency toward binding. Such powerful binding may well have characterized the first prebiotic polyamino acids, but the degree of affinity is, quantitatively, rare or absent in today's proteins. The strong binding, as manifest in aggregation phenomena could, thus, have served only early in evolution; it is clearly not a property that would have been identified by reductionism.

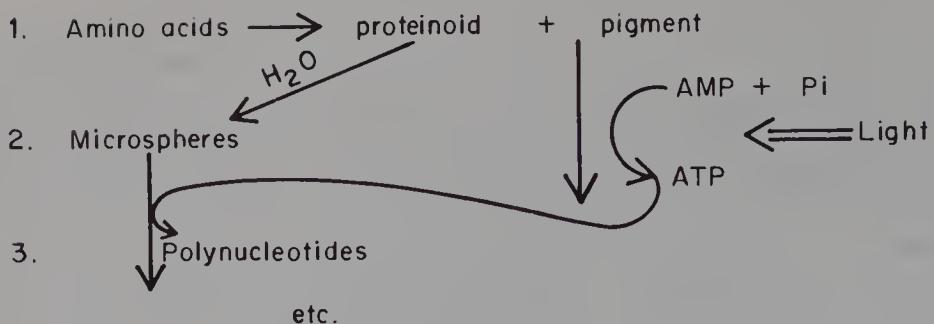
The significance of these interactions cannot be overestimated. They provide an answer to L. Pasteur's famous and basic question (page 5): "Can matter organize itself?"

The reproductive nature of proteinoid microspheres also could not be deduced from analytic studies. Indeed, the apparently logical hierarchical level at which to seek the simplest replication has been the molecular (page 249). Reservations about the meaning of the finding might lie in the well-known binary fission of soap bubbles, mercury droplets, etc. The appearance of buds on microspheres, however, is what suggested looking for a bud-mediated reproduction. In both types of proliferation, binary fission or budding, growth by accretion was already suggested. The tendency of all microparticles to grow by accretion to a precisely balanced size was learned of only by experiment.

It could not have been forecast that so many properties, plus unit boundary structure, etc., would be found in association in simulated protocells, an association approaching the extent in contemporary cells. This degree of association imputes a quantum indivisibility to the protocell (page 250). No individual property found was as astonishing (ref. 2 in Fox, 1975) as the total simultaneous complex. The large number of units formed, also unexpected, and the slightness of their variations as seen upon examination, suggest an ideal evolutionary matrix.

Knowledge of the ability of pigmented proteinoid and of microspheres to activate ultraviolet-mediated syntheses derives from the finding of pigment formation in polycondensation of amino acids, and could not have been predicted at the outset of the research. An especial significance of this finding is that it illuminates the sequence of events in the origin of autotrophism and heterotrophism.

As a final example, we can visualize a sequence of primordial events, such as:



We do not see how such a precise step-by-step sequence could have been visualized from the results of reductionistic research.

In making this case for constructionistic research, we recognize that all exploratory research yields unpredicted results that in turn lead to other experiments. Where construction is involved, the exploration takes on a quality of its own.

Criteria of Validity and Plausibility

A number of areas of science have had to develop their own criteria of validity and plausibility. The new science of experimental protobiogenesis is not an exception.

The fact that the problem is a multidisciplinary one provides rigor not available to most other scientific problems in the past. Valid answers must simultaneously meet the constraints of physics, astronomy, geology, chemistry, and biology. Fortunately, the geological conditions necessary for the experiments are widespread on the surface of the Earth today. This fact does not guarantee that those conditions existed on the early Earth. When the conditions are examined (Chapters 5 and 6), the likelihood of the presence of those conditions throughout the last three billion years, however, seems assured (cf. also Rohlffing, 1976). Indeed, the question properly arises, as to whether life could arise now. To this question, answers have been provided by Darwin, Keosian, Oparin, Wood, etc. (Fox and McCauley, 1967).

Another way of applying multidisciplinary constraints is to require that any flowsheet be sequentially compatible *ab initio* (page 245).

The conditions need be thermodynamically as well as geologically relevant. It is on this score that closed flasks tend to be inadequate (Florkin, 1975).

Criteria of plausibility include affirmative answers to the questions of whether the proposed processes are efficient. Lehninger (1970) has explained how efficient processes agree with a principle of evolutionary continuity. Chemical reactions that proceed easily to yield, say, 3% conversion to product convey more plausibility than do those that yield 0.03%. One may not defensibly rule out any low-yield reaction, but those of higher yield are more plausible, provided they are also compatible with a total sequence. In figure 7-3, the models for the second and third steps typically provide yields of 10–40%. Moreover, those reactions are carried out in systems that are geologically relevant and thermodynamically feasible, in that the glassware is as open as the geological realm. Most syntheses of amino acids (first step) do not meet such criteria of plausibility. The yields are typically below 0.1%.

This fact is interesting to compare with the situation in evolved organisms like man, in which the blood level amino acid nitrogen is only 0.0035 to 0.0050 percent. Even so, the principal organic matter of man is in the form of polyamino acids, i.e., protein. This fact suggests that evolution has developed sequences through a biochemical funneling of amino acids.

The greater the simplicity of a process, the more plausible it is as a spontaneous occurrence on the primitive Earth. If only water or heat are needed to activate a reaction, the simplicity is maximal. Most prebiotic simulation experiments meet the requirement of simplicity, even though many do not meet the other requirements of validity and plausibility.

Some Opposing Concepts

The theory presented in this volume is the only one that is experimentally based, comprehensive, and in which the steps are sequentially compatible ab initio. Opposing concepts and clusters of concepts have been advanced. They are numerous (Fox, 1973a, 1974a); mostly the frequently stated ones will be treated here.

The discordance between the concepts of proteinoids-first and nucleic acids-first is more apparent than real. Through the protoribosome, especially, we can see how the phenomena of molecular evolution presented here could have interdigitated with the Central Dogma sequence of molecular biology.

This rapprochement (Fox, 1975) is explained in Figure 7-4. Much could have happened without nucleic acids. Ordering of preprotein was by instructions of the reacting amino acids. The order that result-

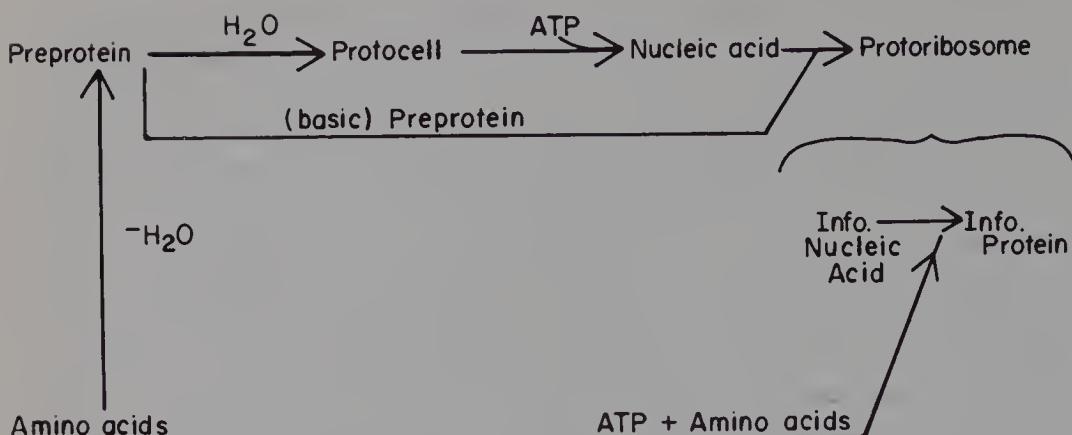


FIGURE 7-4

The processes identified in research on molecular evolution evolved to processes identified in research on molecular biology, through the (proto) ribosomes.

ed permitted (a) the formation of reproductive protocells, (b) the formation of polynucleotides, and (c) the interaction of those two to yield protoribosomes which would then function in accord with the Central Dogma, in a series of reactions at surfaces. This flow was irreversible, in contrast to mere polynucleotide-polyamino acid recognitions, which are bidirectional.

However, the steric forces operating for amino acids at different stages of macromolecular evolution would be similar, resulting in similar compositions. The compositions of thermal proteinoids and of adenylate proteinoids (page 179) are seen on inspection to resemble those of proteins (Table 7-4). Mikelsaar (1975) has evaluated this similarity quantitatively, and has suggested that the genetic code fixed the chemically determined prebiotic ratios.

The above is an answer to the "DNA-first" concept, the latter having no experimental support (Florkin, 1975). Many other opposing concepts have been discussed in the literature (Fox, 1973a, 1974a; Fox, 1976b); they largely cluster around a few main premises.

One of these is the geological relevance of conditions employed in the laboratory. The defensible views have been discussed in this volume (page 179). A recent paper summarizing most of the facts on thermal polycondensation (Rohlfing, 1976) reiterates many of these and provides new data.

Another focus of discussion has been the evolutionary flow of information and the identity of the first informational biomacromolecule. This has been treated in several places. (pages 158 and 247) on the basis of a large accumulation of data. The reliance on unproven concepts of random polymerization is in accord with an a priori sto-

TABLE 7-4
Compositions of Average Protein, Equimolar Adenylate Proteinoid, and Thermal Proteinoid from Equimolar Mixtures (mole % ammonia not included)

Amino acid	Average protein (Vegotsky and Fox, 1962)	Adenylate proteinoid (Krampitz and Fox, 1969)	Thermal proteinoid (soluble fraction) (Fox and Waehneldt, 1968)
Lysine	5.9	6.5	8.9
Histidine	1.8	2.4	3.5
Arginine	4.9	4.2	4.1
Aspartic acid	9.7	10.3	5.0
Threonine	4.8	4.9	0.4 ^a
Serine	6.0	4.2	0.2 ^a
Glutamic acid	12.7	9.7	11.0
Proline	6.2	5.1	2.9
Glycine	12.6	11.1	9.0
Alanine	9.6	14.3	13.0
Valine	5.9	7.3	10.2
Methionine	1.8	0.7	7.2
Isoleucine	6.0	4.5	6.4
Leucine	6.0	9.6	6.4
Tyrosine	2.3	0.1 ^b	5.4
Phenylalanine	3.7	4.5	4.9

^aLargely destroyed.

^bReaction of tyrosine to form adenylate is abnormal.

Note: Irrespective of mode of synthesis, such amino acids as glutamic acid, alanine, glycine, and aspartic acid (one exception) tend to exceed 6% and dominate composition, while histidine, phenylalanine, arginine, and methionine are each <6% by all methods.

chastic premise (see Hoffmann, 1975), but not with the evidence from evolution at any stage.

Other opposing assumptions are that complex products and mechanisms, which are protected in organisms by internal biosynthesis, arose in an increasingly complex order over extended geological time. One can cite as opposite examples, consistent with experiments, the cell, informational copolyamino acid, and photochemical reactions. The experiments show that a primitive form of any of these arose early in evolution.

The Principle of (Internal) Molecular Selection

One principle that is vividly apparent from results of experiments in molecular evolution is that of molecular selection, i.e., the capacity of the molecules themselves to confine the possible ranges of molecular

type. This has probably been underappreciated in biological mechanisms of selection for at least two reasons. One of these is the historically first emphasis on adaptive selection, in which the environment, as an outside agent, functions as a passive selector. Darwin himself did not intend that adaptive selection be an entire explanation for organismic evolution. The other reason is undoubtedly that today's organisms are sufficiently complicated by many substances and many functions that the central principle of molecular selection cannot be easily discerned. Even so, indications of internally constrained mutation are known, for example, in the work of Weissman et al. (1973).

The basic idea of internal selection has been described in various ways, as self-organization (Lehninger, 1975, Eigen, 1971), self-instruction (Eigen, 1971), biochemical predestination (Kenyon and Steinman, 1969), internal selection (Whyte, 1965; Fox, 1965b), etc. The basic cause seems to be the "shapes," and charges, of molecules; i.e., internal selection is stereoselection (Fox, 1975). Wherever we look in our experiments or in biochemistry, the "shapes" of molecules are at work: in the selective condensation of amino acids, in interactions between polynucleotides and polyamino acids (Fox, 1975) and in the interactions of contemporary enzymes and their substrates (Koshland, 1973).

SUMMARY

At this point, we can summarize the understanding that has been gained relative to a laboratory explanation of the spontaneous origin of primordial life on Earth.

The unit of contemporary life is the cell. The unit of primitive life, by back-extrapolation, was the protocell. The assumption that the protocell was a late development in evolution is superfluous in view of the indication that the development of precursor macromolecules and their assembly into protocells was a sequence of two maximally simple and direct processes. With preprotein present on the primitive Earth, only contact with water was necessary to produce a protocell. The advantages provided by a (proto) cell are such as to confer a huge selective advantage to a protocellular line of evolution over any other. The proteins-first concept is thus replaced by a preprotein → protocells-first concept.

When we review the proteinoid microsphere as a system, we see that it is composed of heteropolyamino acids in which the precursor amino acids had ordered themselves to yield polymers of a limited degree of heterogeneity. The material possesses arrays of enzymelike

activities, limited or familial heterogeneity of the molecules, and lipid quality. The microsystems formed, in turn, from proteinoid also possess arrays of enzymelike activities plus the possibility for compartmentalization of these reactivities, membranous ultrastructure, lipid quality, selective retention of macromolecules including those that are enzymelike, and the ability to proliferate in a primitive manner.

The complexity that has for so long been imputed to the problem of the origin of life (that is, primordial life) is seen still to apply to the material and the units, but not to the processes by which these came into existence. These processes are complex, when an attempt is made to analyze them mechanistically. Their operation, however, is simple. The necessary conditions are familiar. Such simplicity and familiarity are geological in their nature, the conditions existing contemporaneously as well as being highly inferrable for earlier eons. If we assume amino acids were present, the steps of polymerization and spherulization (page 201) would have been inexorable in many locales of the primitive terrestrial crust. The processes were rugged and they were very fast, easily occurring in less than half of a diurnal cycle. The products were so numerous as to be expressible only by exponential numbers. Some of the products would have formed during the night and thus would have been protected from destructive solar radiation; overlying water would have then protected the organic supramolecular units after they had assembled by contact with water.

Research that demonstrates how the first living systems could have arisen from polymers which, in turn, arose from monomers, is in opposition to earlier research in which physical models, e.g., coacervate droplets, were produced from polymers obtained from contemporary organisms. The older kind of research is a reductionistic study amplified by reassembly. The newer research, with proteinoid microspheres, is constructionistic and emphasizes a first assembly. In their directionality, these latter processes resemble spontaneous molecular evolution, which is essentially what must have occurred in a similar mode on the primitive Earth. The constructionistic approach has served to emphasize that living systems are characterized by their being assemblies of materials and functions, many of which can be found individually outside of living systems. Other functions are emergent ones; they required heuristic research for identification (Table 7-1).

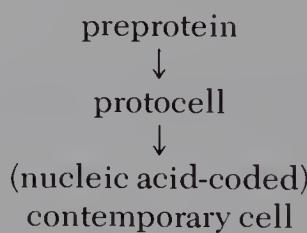
The first cells are regarded as the first "individuals" to have appeared in the geological realm. Some of the information intrinsic to those first individuals was derived from the precursor polymers, which, in turn, obtained their instructions from the diverse amino acids. In this way, the information arose from the prebiotic geological

realm. This interpretation leads to the inference that the order did not totally arise out of disorder; it was transduced from partial order of another kind. Information, like matter then, evolved from other kinds of nonrandom relationships. Such relationships can be traced back to the nonrandom compositions of mixtures of amino acids such as are produced in experiments, and thence to the interstellar matter present in the Galaxy.

One central problem, then, has been that of discovering the mechanism of emergence of informational, thermodynamically open microsystems, when no parental microsystems existed to produce them. The production of proteinoid microspheres illustrates how easily primordial, but complex, largely open microsystems would have arisen in the (thermodynamically open) geochemical realm. The research also emphasizes how rapidly complexity could have evolved from simplicity. It further suggests that cells, contemporary or primordial, have the appearance of great complexity, but that this appearance results from association within the same unit of relatively few materials and functions, each of which is itself the result of interactions.

For the evolution from a protocell to a contemporary cell, experiments have begun to suggest how energy-trapping mechanisms, protein biosynthesis, nucleic acid biosynthesis, the ribosomes, and the genetic code entered the evolutionary stream.

The sequence



is evolutionary in that it proceeds from simplicity to complexity. The essence of the contribution from constructionistic experiments has its roots in our ability to learn which functions (ultrastructure, metabolic activity, replicative ability, etc.) appeared at which stage. In the reductionistic studies, cells have been disassembled. This taking apart results in the simultaneous disappearance of a number of emergent functions; one is not able therefore to determine in what order they appeared during evolutionary assembly. One can hope to learn the order of appearance from experiments carried out in an evolutionary, i.e., constructionistic, direction. The experiments emphasize nonrandom selection as an evolutionary prelude to, and an underlying process of, contemporary adaptive selection of organisms.

It is now possible to review the unique consequences of constructionistic research without which, it is believed, some of the principles of assembly would have failed to enter our knowledge, for at least a very long time.

The experiments in simulated molecular evolution reveal, more vividly than any other kind of study, the manner in which the evolutionary processes project themselves. Although words like self-ordering, self-instructing, self-organizing, and self-reproducing are not strictly correct (since some environmental component is present), a sequence of self-ordering → self-organizing → self-reproducing phenomena is a more accurate representation of the evolutionary development than any other that has received attention.

The self-organizing forces evident at each stage evidently restricted the scope of earliest evolution to a narrow pathway. Although the experiments and geological considerations suggest that a protoorganism arose on numerous occasions in numerous and differing locales on the Earth, the types would have been limited by internal constraints. The results could then be interpreted, eons later, as a single source whereas what was operative was an almost single pathway at each *de novo* generation.

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CHAPTER 8

*Origin and Evolution
of Optical Activity*

Optical activity in molecules isolated from living systems has long been regarded as a distinguishing characteristic of such systems, and a sure sign of life or of life-related substances. The first spontaneous syntheses of molecules containing one or more asymmetric carbon atoms theoretically yielded essentially racemic compounds. Since contemporary organisms contain substances such as amino acids and carbohydrates that are optically pure, the problem of evolution from racemic compounds to single enantiomorphs has vexed chemists. The plausible answers that are now available in the pertinent literature are fairly numerous. Many of the processes that have been proposed to account for the origin and evolutionary development of configurational one-sidedness can be viewed as having reinforced other processes.

Special possibilities for the explanation of the origin of optical activity are rooted in the long known observations that some phenomena in nature are basically asymmetric. One assumption is based upon the finding that natural light is circularly polarized, though to a

very small degree, by reflection from the sea, or by reflection from atmospheric particles, and/or under the influence of the magnetic field of the Earth (Byk, 1904, 1909, 1925). In an appropriate set of reactants, circularly polarized light can cause either a slightly stereospecific synthesis, or the slightly stereospecific destruction of one member of a pair of racemates.

A number of experiments (Kuhn and Braun, 1929; Kuhn and Knopf, 1930; Tsuchida et al., 1935; Karagunis and Drikos, 1933; Tenney et al., 1935; Tenney et al., 1945) to produce or destroy optically active compounds with circularly polarized light were initiated in this connection by Van't Hoff (1908) early in this century. However, the stereochemical effects thus produced were very small. Furthermore, although the reactions may be interpreted to demonstrate a principle, most of these reactions cannot be directly related to molecular evolution because the reactants were unnatural. An exception, perhaps, is the conversion of diethyl fumarate into (+)-tartaric acid by oxidation with hydrogen peroxide under irradiation with right-handed circularly polarized light (Tenney et al., 1945). An important deficiency of the role ascribed to circularly polarized light in the evolution of optical activity is that natural light has such an extremely low degree of polarization that its contribution was probably not significant (Wald, 1957).

The nonparity of fundamental physical particles (Lee and Yang, 1956) has led to another suggestive inference concerning the origin of optically active compounds (Fox, 1957; Ulbricht, 1959; Haldane, 1960). The electrons from radioactive β -decay are predominantly left-handed. Consequently, the Bremsstrahlung that they produce is to a low degree circularly polarized (Goldhaber et al., 1957). If these polarized radiations interact directly with a racemic mixture of organic compounds, a stereospecific destruction of isomers could theoretically result. But a direct chemical interaction of the original radiation quanta occurs only at an extremely tiny rate inasmuch as almost all of their energy is converted into that of compton electrons and photoelectrons. These secondary electrons have no significant chance to be polarized. Moreover, in a dilute aqueous solution the major part of chemical effects is produced by diffusion-controlled processes of indirect action (Bacq and Alexander, 1959). This emphasis explains why several experiments to produce optically active materials by use of radiations from asymmetric decay of elements have been unsuccessful.

Garay (1968), however, claimed to have found that D-tyrosine was decomposed more than L-tyrosine by the polarized β -rays from ^{90}Sr when the dilute alkaline solutions of the optically active isomers were

incubated for as long as 18 months. The results are questionable and need reconfirmation, since most of the effects are produced by indirect action processes. The possibility of microbial contaminations cannot be fully excluded, although some precautions were taken. Moreover, the crucial experiment with racemic DL-tyrosine was not carried out.

This chapter will consider the theory relating to stereoselectivity in small molecules that existed before organisms, stereoselectivity in macromolecules that existed before organisms, and stereoselectivity in cells in the biological era.

ORIGIN OF OPTICAL ACTIVITY IN MICROMOLECULES

The possibility of spontaneous resolution during crystallization of racemates is an explanation that has often been brought forward for the origin of optical activity. A number of experiments have been offered as pertinent. The favoring of a single stereoisomer of a particular molecule during the crystallization from a supersaturated solution of a mixture is a well known result in the laboratory (Harada, 1970). Supersaturated solutions of DL amino acids especially have been studied in such experiments. For example, a crystal of L-glutamic acid may be added to a supersaturated solution of DL-glutamic acid, thereby shocking the L-glutamic acid out of solution while leaving the preponderance of the D-glutamic acid in solution.* DL-Glutamic acid is unusually susceptible to this operation since the D and L forms compose a mixture rather than a compound. Many, if not most, pairs of enantiomorphs interact to form compounds. Such resolution procedures do not work effectively in the cases of DL compounds. Glutamic acid, however, has been resolved on a commercial scale by the seeding process. The method is highly reliable and reproducible. Harada (1965) has studied the resolution by inoculation of DL-aspartic acid, DL-asparagine, DL-glutamic acid, and DL-glutamine. As Table 8-1 shows, he has been able to obtain some pure enantiomorphs by a single treatment. This method is, therefore, a very powerful one.

Questions do arise, however, about the relevance of such chemist-directed resolutions to the reconstruction or discovery of spontaneous processes that produced optical activity in the geological realm. One criticism has stated that the pools of amino acid solution would have contained only DL amino acids; the probability of seeding by one form

*Such resolutions have provided examples of oscillating processes; compounds of the D and L forms precipitate alternately (Radke et al., 1954).

TABLE 8-1
Optical Activities of Resolved Amino Acids

Amino Acid	Configuration Obtained	$[\alpha]_D^{25}$ (hydrochloric acid)	Percent Optical Purity
DL-Aspartic acid	L	+24.6° (6 N)	99
	D	-23.6° (6 N)	95
DL-Glutamic acid	L	+28.5° (6 N)	91
	D	-27.5° (6 N)	88
DL-Asparagine	L	+28.6° (3.6 N)	93
	D	-29.7° (3.6 N)	97
DL-Glutamine	L	+32.8° (1 N)	100
	D	-31.4° (1 N)	96

Source: Harada (1965).

Note: All elemental analyses of resolved amino acids agree with the theoretical value after one recrystallization.

would merely balance the probability of seeding by another in other pools. However, as Pasteur (1852) long ago showed for ammonium hydrogen malate, some racemates tend to crystallize side-by-side in essentially equal numbers as D crystals and L crystals. Once this had occurred in a prebiotic locale, geological influences could have separated the two, leaving one behind to function as the seed for a supersaturated solution. As two papers have proposed (Fox et al., 1956; Northrop, 1957), such forces as the wind could easily have been responsible for the separation. The wind would thus replace the famous pair of tweezers used by Pasteur. A second criticism, voiced by Wald (1957) and Ulbricht (1959), points out the rarity of supersaturated solutions. Such supersaturation seems, however, not too difficult to imagine. We can surmise that evaporation would have been rapid in pools at warm surfaces and that supersaturation would have been an inevitable consequence. Recession of the seas and recession of lakes and ponds are common on the surface of the Earth, as is drought. That water evaporated from pools on the primitive Earth is very likely. Harada (1965) has shown that supersaturation is greatly aided by the presence of ammonium formate, which is a compound that would have been present on the primitive Earth. As the laboratory organic chemist knows, contaminating compounds present as impurities often promote supersaturation. Opportunities for supersaturation must have been very numerous in the pools that contained unused organic compounds before life appeared.

STEREOSPECIFIC SYNTHESIS AND MACROMOLECULES

Relevant experiments in stereospecific synthesis of polyamino acids have been performed by Blout et al. (1957) and by Blout and Idelson (1956). A comparison was made between the rates of polymerization of the DL forms and of the D or L forms of benzyl-N-carboxyglutamic acid anhydride. The rate of polymerization of the mixture was approximately $\frac{1}{20}$ of the rate of the D form or of the L form. In this polymerization, the DL form appears to have been inhibited from assuming a helical configuration. Moreover, the DL polymer was found to be considerably shorter than the polymers of the single stereoisomer. Finally, the polymer from the racemic amino acid derivatives is less stable than those from the L or D forms. As has long been known (Astbury, 1940), the L configurations of amino acid residues in protein allow a staggered relationship of side chains. This is a configuration that is least sterically hindered. Such steric influences might well have operated in the polymerization of amino acids under primordial conditions. Although examination of steric effects in model systems has not been extensive, thermal polymerization of L amino acids yields polymers (page 167) in which a small fraction of total activity has been conserved (Fox et al., 1962; Rohlfing, 1967). Evolution to polymers containing single configurations of the amino acid monomers is more easily visualized than from those with no optical activity.

EVOLUTION OF OPTICAL ACTIVITY IN CELLS

The evolutionary type of explanation is superposable on other explanations. That evolutionary processes operated in the biological era to assist in the establishment of optical activity has been regarded as certain. A minute disproportionation of D and L, which would be consistent with statistical expectations, would have been sufficient as a starting point for an evolutionary drift to a single configuration (Langenbeck, 1935). The organism that happened to have an excess, however slight, of one form would react selectively with substrates that happened to have a configurational bias (Fox et al., 1956). The tendency for such a development to enrich a very slight excess of configurational drift is readily explainable on the basis of the uncounted generations of evolution (Fox et al., 1956). Nor is there reason to suppose that the extreme disproportionation, the occurrence of a single molecule of one asymmetric type in cells, has been a rare

event. A cell that had evolved to the stage in which it contained ribosomes met the requirement of the single molecule in a sensitive biosynthetically functional unit. The individual units of ribosomes each contain single molecules of protein and single molecules of RNA (Osawa, 1968). If optical activity had not established itself before the contemporary ribosomes evolved, it would necessarily have done so at that time.

For macromolecules in the first organisms, the "single molecule" explanation applies most forcibly. Let us assume for one or more types of macromolecule that only one individual macromolecule is present. Each monomer containing an asymmetric carbon atom can be either D or L, but it cannot be both. This is, in fact, the extreme case of disproportionation of enantiomorphs mentioned above. Extension of the particular configurational bias of a single monomer to interactions with enzymic substrates or polynucleotides would trigger formation of other configurational biases.

SUMMARY AND OVERVIEW

Not all questions are answered by this treatment of the origin of optical activity. Not all possible or plausible mechanisms are included in this treatment. The answers given, however, are examples sufficient to indicate how optical activity of the kinds found in nature might have come into existence. One way to define the problem at present might be to determine how many and which of the processes mentioned, as well as others, were operative in the origin and evolution of optical activity in biochemically significant molecules. Another problem that arises at the integrative level, provided we assume that no basic bias for optical activity exists in nature (page 269), is posed by the possibility that individual pools of, for example, supersaturated solutions might yield some D pools and some L pools upon seeding, in separate events. This problem is greatly attenuated by the considerable likelihood that for the planet as a whole throughout two-thirds of its history, a unitary biosphere must have existed. Organisms have constituted this biosphere in the atmosphere and the hydrosphere. At present, the ocean covers approximately two-thirds of the surface of the Earth. When we consider the circulation of water through the atmosphere and the way in which this water, when condensed, runs to the ocean, the visualization of a unitary biosphere becomes easier. If there were numbers of individual small pools of configurationally biased molecules of each type, the same preference should have been manifested throughout the unit biosphere.

This brief excursion, then, into experiments and interpretations

on the origin of optical activity has dealt with the possibilities that can be visualized for micromolecules, for macromolecules, and for the supramolecular organization of the cell. The latter could have been very effective in enriching a minute configurational bias through the evolutionary process. Also effective could have been the powerful influence of single molecules, trigger effects, and a unitary biosphere.

The belief that optical activity is a sure sign of life seems not to be durable. Moderately highly evolved life without optical activity is hardly thinkable. Optical activity without life, however, is highly plausible, as has been explained. This view, expressed clearly as early as 1957 (Fox, 1957) has been reappraised in 1973 (Pincock and Wilson, 1973). Actually, highly efficient "spontaneous" resolutions have been described (Harada, 1970). For what occurred on the primitive Earth, we have plausible answers, though we do not have sure ones (Elias, 1972).

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CHAPTER 9

*Perspectives on Molecular
Evolution in Organisms*

When we consider the whole of molecular evolution in organisms, we find that we must take into account a large variety of organisms and of individual (macro) molecules, but a relatively small number of classes of macromolecule. The reader acquainted with biochemistry is aware of this molecular tapestry. For a more extensive awareness, he can refer to the treatise by Florkin and Mason, *Comparative Biochemistry* (1964).

While the micromolecules are the currency of metabolism, the essential storage and readout of information of the organism are manifestations of the biopolymers. We thus focus our attention, in this chapter, on proteins, and on their relationship to nucleic acids.

TEMPLATE-FREE PROTEIN SYNTHESIS

We are not without some clues on the transition from primitive adenylate condensation to contemporary protein biosynthesis. In particular, Lipmann and his associates (Gevers et al., 1969) have shown that

a strain of *Bacillus brevis* can condense amino acids by ATP to form Gramicidin S without direct involvement of a (nucleic acid) template. This mechanism may be an evolutionary vestige of a bridge from primitive to contemporary systems. An alternative explanation is that this type of peptide synthesis has evolved from ribosomal protein synthesis at a later stage. The only informational macromolecules directly involved in the biosynthesis of Gramicidin S are the enzymes, the polypeptide intermediates, and the complexes composed of these. The synthesis of these enzymes, however, is coded by *m*-RNA. Such determination of order calls into purview enzyme-peptide experiments that yielded the concept of ordering due only to enzymes and peptide intermediates (Fox et al., 1953), i.e., zymosequential specificity;* cf. Haurowitz (1963). The experimental basis provided by such enzymic experiments, in fact, was utilized in the initiation of the program of production of self-ordered proteinoids (Fox, 1956; Krampitz and Fox, 1969) that has represented much of the material of this volume and the one totally sequential theory of the origin of life.

EVOLUTION OF PROTEINS IN ORGANISMS

The topic of this section will be treated by way of representative examples. A large bank of relevant information has accumulated in recent years. In the 1969 edition of *The Atlas of Protein Sequence and Structure* (Dayhoff, 1969), for instance, 192 pages are required to present the collected condensed data on sequences in proteins, and there are many more pages of other kinds of data including sequences for 18 transfer RNA structures, and numerous chapters of interpretation. The large number of protein molecules represented is nevertheless a very minute fraction of those conceptually possible. If we think of a protein molecule of 200 units, (i.e. having a molecular weight of approximately 20,000), we realize that it could have 20^{200} different isomers constructed from the 20 common amino acids. Since one mole contains fewer than 10^{24} molecules, simple calculations reveal that the volume of the Earth is too small to hold even a minute fraction of all the isomers of such a protein molecule (page 143).

The choice of the isomeric possibilities is a function of evolution, and evolution of protein molecules is an exceedingly slow process (Zuckerkandl and Pauling, 1965; Margoliash et al., 1969; Vegotsky

*Amino acid sequence at any point in the growing polyamino acid chain is determined by interaction of enzymes and sequence to that point.

and Fox, 1962). Accordingly, the appearance of protein molecules has been itself a nonrandom process, when compared to the *a priori* possibilities. An important factor in the evolutionary development of many types of protein seems to have been that certain combinations were favored in the reactions of adenylate condensation (page 179). The control effected by nucleic acid templates (page 254) introduced additional restriction on the possibilities.

We are able to form some judgments of rates, limits, and possibilities by examining the information in the light of codon-amino acid relationships. One source of such collected information is the book by Jukes (1966).

CODON-CONTROLLED EVOLUTION OF PROTEINS

As will be supported later (page 283), comparison of many sequences in proteins reveals many stepwise substitutions of amino acid residues (Table 9-1). The recognition of this pattern (reviewed by Vegotsky and Fox, 1962) suggests the existence of a genealogical tree for protein molecules. One example of genealogical relationships, the human hemoglobins (Table 9-1), is given.

The understanding of the stepwise evolution of protein molecules is to be sought in the relationship to the corresponding set of changes in purine-pyrimidine base. Table 9-1, which illustrates the human hemoglobin family of molecules, permits comparing the substitutions with the number of necessary changes in the corresponding aromatic bases. Thirty-three changes in amino acid residue were found (Jukes, 1966). The kinds of change agree closely with the hypothesis of stepwise mutation through single-base changes in coding triplets. The kind of evidence that bears on this is that of the comparison of the amino acid residue changes with the necessary changes in coding triplets (Jukes, 1965). In the hemoglobins, for example, these tend to be single-base changes, and the number of two-base changes is such as would be expected by random occurrence of a second change following a one-base change.

Much of the summary of Jukes' analyses of the hemoglobins is in Table 9-2. This and other analyses of cytochromes (Table 9-3), TMV protein, and other proteins indicate that the stepwise pattern of amino acid interchanges is explained by the introduction of mutations one step at a time through single-base changes in those proteins. Such results are based on the quite rigorous information obtained from sequence assignments in proteins and from the development of

TABLE 9-1
Amino Acid Sequences of Human Myoglobin and Alpha, Beta, and Gamma Hemoglobin. (In each chain, every tenth residue, and the last residue, are numbered.)

<i>Protein</i>	Myo	NH ₂ -gly	leu	ser	glu	gly	glu	try	glN	leu	10	leu	his	val	try	ala	lys	val	glu
α -Hb	NH ₂ -val	leu	ser	ala	ala	asp	lys	thr	asN	10	10	lys	ala	ala	try	gly	lys	val	gly
β -Hb	NH ₂ -val	his	leu	thr	glu	glu	lys	ser	ala	val	10	thr	ala	leu	try	gly	lys	val	
γ -Hb	NH ₂ -gly	his	phe	thr	glu	glu	asp	lys	ala	thr	ilu	thr	ser	leu	try	gly	lys	val	
<i>Protein</i>																			
Myo	pro	asp	val	ala	gly	his	gly	asp	asp	ilu	leu	arg	leu	phe	lys	gly	his	pro	
α -Hb	ala	ala	gly	glu	tyr	gly	ala	glu	ala	leu	glu	arg	met	phe	leu	ser	phe	pro	
β -Hb	asN	val	asp	glu	val	gly	gly	glu	ala	leu	gly	arg	leu	leu	val	val	tyr	pro	
γ -Hb	asN	val	glu	asp	ala	gly	gly	glu	thr	leu	gly	arg	leu	leu	val	val	tyr	pro	
<i>Protein</i>																			
Myo	glu	thr	leu	glu	lys	phe	asp	lys	phe	lys	his	leu	lys	ser	glu	asp	glu	met	lys
α -Hb	thr	thr	lys	thr	tyr	phe	pro	his	phe	...	asp	leu	ser	50	50	50	50	50	met
β -Hb	tyr	thr	gln	arg	phe	phe	glu	ser	phe	gly	asp	leu	ser	thr	pro	asp	ala	val	
γ -Hb	tyr	thr	gln	arg	phe	phe	asp	ser	phe	gly	asN	leu	ser	ala	ser	ala	ala	ilu	met

(Continued)

TABLE 9-1 (Cont'd)

Myo	ala	ser	glu	60	asp	leu	lys	his	gly	val	thr	val	leu	70	ala	leu	gly	ala	ili	
α -Hb	gly	ser	ala	gln	val	lys	gly	his	gly	lys	lys	val	ala	asp	ala	leu	thr	asN	ala	
β -Hb	gly	asN	pro	lys	val	lys	ala	his	gly	lys	lys	val	leu	gly	70	ala	phe	ser	asp	gly
γ -Hb	gly	asN	pro	lys	val	lys	ala	his	gly	lys	lys	val	leu	thr	70	ser	leu	gly	asp	ala
<i>Protein</i>																				
Myo	leu	lys	lys	lys	80	gly	his	his	glu	ili	glu	leu	lys	pro	leu	ala	gln	ser	his	ala
α -Hb	val	ala	his	val	asp	asp	met	pro	asN	ala	leu	ser	ala	leu	ser	asp	leu	his	ala	
β -Hb	leu	ala	his	leu	asp	asp	asN	leu	lys	gly	thr	phe	ala	thr	leu	ser	glu	leu	his	cys
γ -Hb	ili	lys	his	leu	asp	asp	leu	lys	gly	thr	phe	ala	gln	leu	ser	gly	leu	his	cys	
<i>Protein</i>																				
Myo	thr	lys	his	lys	ili	pro	ili	lys	tyr	leu	glu	phe	gln	ser	glu	ala	ili	ili	ser	
α -Hb	his	lys	leu	arg	val	asp	pro	val	asN	phe	lys	100	leu	leu	ser	his	cys	leu	leu	val
β -Hb	asp	lys	leu	his	val	asp	pro	glu	asN	phe	arg	leu	leu	gly	asN	val	leu	val	cys	
γ -Hb	asp	lys	leu	his	val	asp	pro	glu	asN	phe	lys	leu	leu	gly	asN	val	leu	val	thr	

TABLE 9-2

Variation in Amino Acid Residues and in Base Changes in the Hemoglobins

Number of Interchanges at Given Locus	Number of Loci Having Number of Interchanges Shown	Percent of Total Interchanges Observed	Calculated Percent of Total Interchanges
0	6	4.0	8
1	29	20.0	20
2	49	33.0	25
3	32	22.0	21
4	19	13.0	14
5	8	5.4	7
6	3	2.0	3
7	2	1.4	1

Source: Jukes (1966).

TABLE 9-3

Structural Variations in Amino Acid Residue Sequences in Interior Peptides from Cytochromes c

Species	Sequence
Cow	NH ₂ -Val-Glu-Lys-Cys-Ala-Glu-Cys-His-Thr-Val-Glu-Lys-NH ₂
Horse	-Lys-Cys-Ala-Glu-Cys-His-Thr-Val-Glu-Lys-NH ₂
Pig	-Lys-Cys-Ala-Glu-Cys-His-Thr-Val-Glu-Lys-NH ₂
Salmon	NH ₂ -Val-Glu-Lys-Cys-Ala-Glu-Cys-His-Thr-Val-Glu-
Chicken	NH ₂ -Val-Glu-Lys-Cys-Ser-Glu-Cys-His-Thr-Val-Glu-
Silk-worm	NH ₂ -Val-Glu-Arg-Cys-Ala-Glu-Cys-His-Thr-Val-Glu-
Yeast	-Arg-Cys-Glu-Leu-Cys-His-Thr-Val-Glu-
<i>Rhodospirillum rubrum</i>	-Cys-Leu-Ala-Cys-His-Thr-Phe-Asp-Glu-

firm understanding of correspondences between amino acid residues and coding triplets (Nirenberg and Jones, 1963; Ochoa, 1963).

Rigorous evidence for the view that changes in amino acid sequence resulted from changes in the corresponding nitrogen bases was first supplied by Fraenkel-Conrat (Funatsu and Fraenkel-Conrat, 1964). Wittmann and Wittmann-Liebold (1963) have studied the effect of nitrous acid on frequency of mutation, in the manner introduced by Fraenkel-Conrat. The data of the Wittmanns are summarized in Table 9-4. For those studies, the tobacco mosaic virus molecule was treated with nitrous acid. Changes in nucleic acid bases were then understood as occurring largely through deamination of amino groups such as exist in cytosine and adenine. The changes in amino acid composition could then be correlated directly with the changes in the purine or pyrimidine bases. The results are in agreement both with the code and with the known changes in molecular structure induced by the chemical mutagen, nitrous acid. Table 9-3 shows that the majority of changes induced by nitrous acid correspond to deaminative single-base changes in coding triplets.

The direct governance of amino acid sequence in proteins by sequence in nucleic acids is thus supported by the accumulated evidence.

The results of the reported studies of amino acid sequence in proteins are consistent with the inference that all or most of the evolution of protein molecules occurred during the codonic era, the era in

TABLE 9-4
Amino Acid Replacements in Tobacco Mosaic Virus Resulting from Treatment with Nitrous Acid

Amino Acid Replacement	Change in Coding Triplet	Times Observed
Threonine → isoleucine	Cytosine → uracil	9
Serine → phenylalanine	Cytosine → uracil	4
Proline → leucine	Cytosine → uracil	3
Proline → serine	Cytosine → uracil	3
Serine → leucine	Cytosine → uracil	2
Aspartic acid → glycine	Adenine → guanine	2
Threonine → methionine	Cytosine → uracil	3
Asparagine → serine	Adenine → guanine	2
Aspartic acid → alanine	Adenine → guanine	4
Isoleucine → valine	Adenine → guanine	3
Isoleucine → methionine	Adenine → guanine	1
Leucine → phenylalanine	Cytosine → uracil	1
Glutamic acid → valine	Adenine → uracil	2
Glutamic acid → glycine	Adenine → guanine	1

Source: Wittmann and Wittmann-Liebold (1963).

which cells employed the "bicameral" macromolecular system. This consistency is not proof. Forces resulting in single-base changes might also have operated in zymosequential specificity (Haurowitz, 1963) or in selective interactions of amino acids, as in the formation of proteinoids (page 163). In fact, there are reasons for inferring that nucleic acids serve to govern or select amino acid sequences that would arise in a nonrandom manner anyhow.

GENEALOGY OF PROTEINS

We may now ask the questions of how many stages existed in protein synthesis. The idea of prebiotic protein synthesis has been treated in a number of ways.

The evolution of organismic protein molecules can now be described in systematic detail (Dayhoff, 1969). We may also discern some principles. Since the early attempts were made to trace the genealogy of protein (Vegotsky and Fox, 1962), the collected data have proved to be immense (Dayhoff, 1969). Our attention here will focus on descriptive principles.

Already emphasized (page 278) is the stepwise pattern of protein evolution. Another important point is that protein evolution was extremely slow (page 277). Qualitatively and subjectively, this slowness seems disparate with the vast number of species of organisms. Nature may have seen a few dozen kinds of hemoglobin, a few dozen kinds of cytochrome, a smaller number of trypsins, etc. When, however, total cellular aggregates of the various potential combinations of these and other macromolecules were tested in natural experiments, the potentiality was very much greater due to interactions. At either the macromolecular level or the organismic level the number of types was, however, a minute fraction of the theoretical possibilities that can be calculated on an *a priori* basis when the evolutionary effects are omitted from the theory (page 277).

In Table 9-5 are presented the relationships between the glycyl chains of insulins of various species. The amino acids in three positions, 8, 9, and 10, are variant; the others are invariant. The interchanges represent single-base changes (page 278). The shift from glycine to serine, or from alanine to threonine, represents minimal structural changes in the amino acids. The same is true for the threonine-valine-isoleucine group of position 10 as each of these is branched at the β -carbon.

A comparative study of active sites of enzymes (Dayhoff, 1969) also leads to a new emphasis. The active sites of a number of enzymes are

TABLE 9-5
Structures of Glycyl Chains of Insulins from Various Species

Species	Sequence
Cow	NH ₂ Gly-Ileu-Val-Glu-Glu-Cys-Cys-Ser-Ala-Ser-Val-Cys-Ser-Leu-Tyr-Glu-Leu-Glu-Asp-Tyr-Cys-Asp
Pig	NH ₂ Gly-Ileu-Val-Glu-Glu-Cys-Cys-Thr-Ser-Ileu-Cys-Ser-Leu-Tyr-Glu-Leu-Glu-Asp-Tyr-Cys-Asp
Sheep	NH ₂ Gly-Ileu-Val-Glu-Glu-Cys-Cys-Ser-Ala-Gly-Val-Cys-Ser-Leu-Tyr-Glu-Leu-Glu-Asp-Tyr-Cys-Asp
Horse	NH ₂ Gly-Ileu-Val-Glu-Glu-Cys-Cys-Thr-Gly-Ileu-Cys-Ser-Leu-Tyr-Glu-Leu-Glu-Asp-Tyr-Cys-Asp
Whale, sperm	NH ₂ Gly-Ileu-Val-Glu-Glu-Cys-Cys-Thr-Ser-Ileu-Cys-Ser-Leu-Tyr-Glu-Leu-Glu-Asp-Tyr-Cys-Asp
Whale, sei	NH ₂ Gly-Ileu-Val-Glu-Glu-Cys-Cys-Ser-Ala-Ser-Thr-Cys-Ser-Leu-Tyr-Glu-Leu-Glu-Asp-Tyr-Cys-Asp

Source: Vegotsky and Fox (1962).

TABLE 9-6
Active Sites of Enzymes

Enzyme	Serine Site	Histidine Site
Bovine trypsin	—Glu-Gly-Asp-Ser-Gly-Gly-Pro-Val—	—Ala-Ala-His-Cys-Tyr-Lys—
Ovine trypsin	—Glu-Gly-Asp-Ser-Gly-Gly-Pro-Val—	—Ala-Ala-His-Cys-Tyr-Lys—
Porcine trypsin	—Glu-Gly-Asp-Ser-Gly-Gly-Pro-Val—	—Ala-Ala-His-Cys-Gly-Val—
Bovine chymotrypsin A	—Met-Gly-Asp-Ser-Gly-Gly-Pro-Leu—	—Ala-Ala-His-Cys-Val-Asp—
Porcine elastase	—Glu-Gly-Asp-Ser-Gly-Gly-Pro-Leu—	—Ala-Gly-His-Cys-Gly-Thr—
α -Lytic protease (<i>Sorangium</i>)	—Asp-Ser-Gly-Gly—	
Protease (<i>S. griseus</i>)	—Asp-Ser-Gly—	
Protease (<i>Arthrobacter</i>)	—Ser-Ser-Gly—	
Subtilisin	—Gly-Thr-Ser-Met-Ala-Ser-Pro—	
Phosphoglucomutase	—Thr-Asp-Ser-His-(Asp?)—	
Alkaline phosphatase	—Asp-Ser-Ala—	
Human carbonic anhydrase		—Leu-Thr-His-Pro-Pro-Leu—

Source: Dayhoff (1969).

presented in Table 9-6. These data and others (Fox and Wang, 1968) lead to the inference that biological activity in proteins is to be correlated more directly with the spatial arrangement of amino acid residues than with the sequence. The complete active site is assumed to be a juxtaposition of appropriate reactive groups made possible by folding of the three-dimensional structure of the protein molecule. On this basis, much of the sequence serves a function of providing a foldable conformation so that aspartylseryl sequences can, for instance, be in close proximity to histidine residues (Hess, 1969). The portion of the peptide chain not in the active site also functions to constitute a molecule large enough that it will be retained by the membranes of the cell (Rohlfing and Fox, 1969). The potentialities in the possible modes of folding represent the scope of series of natural evolutionary experiments, dealing with macromolecule-macromolecule interactions in the cell, with permeabilities, and with enzymic activities. The latter are, however, fine-tuned expressions of limited numbers of amino acid residue sidechains. For such fine tunings, spatial arrangement is accordingly more significant than sequence alone.

The fact that the rate of evolution of proteins is exceedingly slow (Zuckerkandl and Pauling, 1965)* suggests that the possibility for mutation has been greatly decreased as evolution has proceeded. This may be due to the construction of an increasing number of constraints within the cell. The picture is consistent with the inference that the coding mechanism represents superposed constraints. In fact, this overall view is conceptually an extrapolation of that idea.

Another perspective that comes into purview is the extent of variation necessary for significant variation in phenotypic expression by the organism. A striking and historical example of the potential represented is found in the example of hemoglobins (Ingram, 1963), in a case of "molecular disease." A single mutation replacing glutamic acid with valine in position 6 of β -hemoglobin (Table 9-1) results in sickle-cell anemia (Pauling, 1955). This is a serious and widespread form of anemia. It is a serious departure from the norm physiologically; but it is only a single mutation in the form of a single-base change, in the middle letter of the triplet. The variation corresponds however, to only one residue in a total structure of 660 residues. This example gives us some sense of dimensional correspondence between mutational site and physiological variation.

The two perspectives of composition and sequence provide us

* Kimura (1969) estimates the average rate of amino acid substitution as 1.6×10^{-9} per amino acid site y^{-1} , for seven proteins studied.

TABLE 9-7
*Relationship of Twenty-Four Heterologous Proteins
 by Correlation Index*

Item	Index of Correlation
Complete identity	1.00
Six seed globulins	0.97
24 varied heterologous proteins	0.63
Complete independence	0.00

Source: Fox and Homeyer, (1955).

Note: For an explanation of correlation index, the paper cited should be consulted. The index has been devised so that complete identity = 1.00 and total randomness = 0.00. One example of complete independence is that in which the proteins being compared have mutually exclusive compositions.

with an opportunity to obtain a further "fix" on the evolutionary relationships. Compositions of protein in evolution have shown a tendency toward conservatism (Table 9-7). We may infer that compositions of heterologous (those of different function) proteins are more alike than they are different (Fox and Homeyer, 1955; Smith, 1966). On the other hand, a number of groups that have studied the relationships have come to the conclusion that sequences within heterologous proteins are random or almost random (Gamow et al., 1956; Williams et al., 1961; Vegotsky and Fox, 1962; Bull, 1965; Jungck, 1968). This seeming discrepancy may have been resolved by Jungck (1970). His statistically controlled comparisons have shown that the variation in sequence is random only in the sense that the frequencies of combination of any two amino acids fit closely with those calculated from compositional frequencies of component amino acids. Thus, the sequences are essentially as nonrandom as the compositions when viewed independently of the compositional frequencies. The greatest uncertainty that attaches to this analysis pertains to the question of whether any proteins are truly heterologous (Vegotsky and Fox, 1962). The net view is that proteins are partly nonrandom and have not yet evolved to the stage at which they would be random or completely disordered (nor need they do so as long as they are produced in open energy-utilizing systems).

SUMMARY

The thesis presented in Chapter 7 and in this chapter may not necessarily exclude other primordial sequences (page 259). In the conceptualization presented, however, information flowed from amino

acids in the geochemical matrix to polyamino acids to organized cell-like units. Irrespective of the stage, or stages, at which nucleic acids arose, the protein (proteinoid) molecules of most effectiveness were selected into the evolutionary development. The experiments show that specific assemblies of proteinoid with polynucleotides were then possible in a simple way, and that the information of these proteinoid molecules could be fed into storage in nucleic acid molecules. This information could subsequently be read out. The grand coded mechanism of protein biosynthesis may have developed gradually or in sudden steps from discernibly simple beginnings.

An outline of evolution of protein and other molecules in organisms can be limned. An introduction to this now large field of knowledge has been presented.

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CHAPTER 10

*Organismic and
Molecular Fossils
in Ancient Sediments*

The investigation of molecular evolution comprises both laboratory experiments, such as have already been discussed in detail, in which a primitive geological and chemical environment is simulated and field research in combination with laboratory studies carried out by paleontologists and geochemists. To relate the finding of microstructures in early Precambrian sediments, in particular, to the existence of primitive life or even protolife is, however, difficult for at least two reasons. First, most geological materials have been metamorphosed many times since the Earth was formed. Therefore, the chances of finding material that has not been reworked and contaminated during the past 3 billion and more years are extremely low. Second, we do not know how to define and classify the systems these microstructures resulted from; that is, we have to draw lines relatively arbitrarily between three categories. These are (1) nonliving systems (microstructures that are artifacts), (2) protoliving systems (microstructures for which the proteinoid microsphere has been viewed as a model; page 250), and (3) fossilized living systems (microstructures that are the remains of cellular organisms in the conventional sense).

FOSSILS OF THE PRECAMBRIAN

The gap in knowledge about the Earth from its beginning (approximately 4.5 to 5 billion years ago) through the Precambrian (which ended 0.6 billion years ago) is tremendous (Tilton and Steiger, 1965). The fossil-poor Precambrian era spans about 90 percent of the Earth's history. With respect to molecular and early biological evolution, only tiny bits of information are available for the first third of the Earth's history, that is, the first 1.5–2 billion years. No firm evidence is at hand to date the time when biological evolution emerged from prebiotic evolution; we can only say that it was at least 3 billion years ago. We cannot even be entirely sure that this event first took place on Earth, because we cannot exclude the possibility, suggested by Robinson (1966, 1967), that the "germs of life" were already present in the cloud of planetesimals, dust, and gases that aggregated to form the Earth and the other bodies of our Solar System. According to Robinson's speculations, the first microorganisms came with the primeval matter from outer space and grew on abiogenic hydrocarbons and related compounds. This concept is not a revival of the nineteenth-century idea of "panspermia" as described by Oparin (1957). Panspermia holds that the "germs of life" are universal and eternal, implying that the question of the origin of life cannot be subjected to experimental research. The modern concept of a possible extraterrestrial origin, however, does not preclude experimental attack. Unquestionably, evidence needs to be supplied for the determination of whether the origin of life was extraterrestrial or terrestrial. This problem, however, is at present of no significance to the manner in which laboratory experiments bearing on the origins of life have to be planned and carried out on the Earth.

The following data on Precambrian fossils are reviewed in order to present some paleontological information that is needed to set up a time scale for the earliest and most crucial phases of molecular evolution on the Earth, and also to elucidate the difficulties just mentioned.

Organismic and Molecular Fossils of the Early and Middle Precambrian

The task of estimating the age of fossil material is extremely complex. The difficulties further increase when the fossils represent microorganisms rather than more advanced multicellular organisms,

as seemingly is the case for most Precambrian fossils. Whether material under investigation is truly of biogenic origin and not an artifact is often doubtful. Within the past few years, new and improved techniques, in combination with traditional micropaleontological and geological observations, have reversed the old inference that the Precambrian is "unfossiliferous." Fossils found in the Precambrian suggest that during this era living things were not highly organized. These observations suggest, in turn, that the surface of the Earth was populated during the first half of the Precambrian Era by predecessors of contemporary microorganisms in various stages between protolife and life.

The most ancient rocks that appear to contain fossils of microorganisms belong to the Onverwacht Series (Swaziland System) of South Africa (Engel et al., 1968). The rocks of the Onverwacht Series are older than 3.2 billion years. Perhaps they are the oldest unmetamorphosed sedimentary rocks on Earth. They lie at a depth of approximately 10,000 meters stratigraphically beneath the Fig Tree rocks. The microfossils found in the rocks of the Onverwacht Series are interpreted as being alga-like; the fossil spheroids and filaments are found to be best preserved in black carbon-rich cherts and siliceous argillites interlayered with lavas. Fractions of the carbonaceous materials of these rocks were extracted and analyzed by gas chromatography (MacLeod, 1968). The resulting fractionation diagram shows a relatively large number of irregularly distributed hydrocarbons in the range from $n\text{-C}_{16}$ to $n\text{-C}_{25}$ with a maximum at about $n\text{-C}_{20}$ and a tail towards $n\text{-C}_{31}$. Superimposed on this continuous curve are rather specific peaks at the location of C_{19} , C_{18} isoprenoid, and the normal alkanes. This kind of distribution might be expected for a mixture of abiogenic (the continuous Gauss-curve) and biogenic (the superimposed peaks) hydrocarbons. However, more detailed studies of these materials seem necessary before conclusive inferences may be drawn.

While studying some black carbon-rich cherts from the sequence of sedimentary rocks known as the Fig Tree Series, near Baberton, South Africa, Barghoorn and Schopf (1966) discovered that these ancient sediments contain the entrapped and petrified remains of minute bacterium-like structures. The occurrence of these in largely unmetamorphosed early Precambrian sediments dates them as being more than 3.1 billion years old. These rod-shaped microfossils have been named "*Eobacterium isolatum*" (Figure 10-1). They are about $0.5 \mu\text{m}$ long and $0.25 \mu\text{m}$ in cross-sectional diameter. The application of the electron microscope in combination with a special double-replica technique revealed that "*E. isolatum*" has a two-layered cell

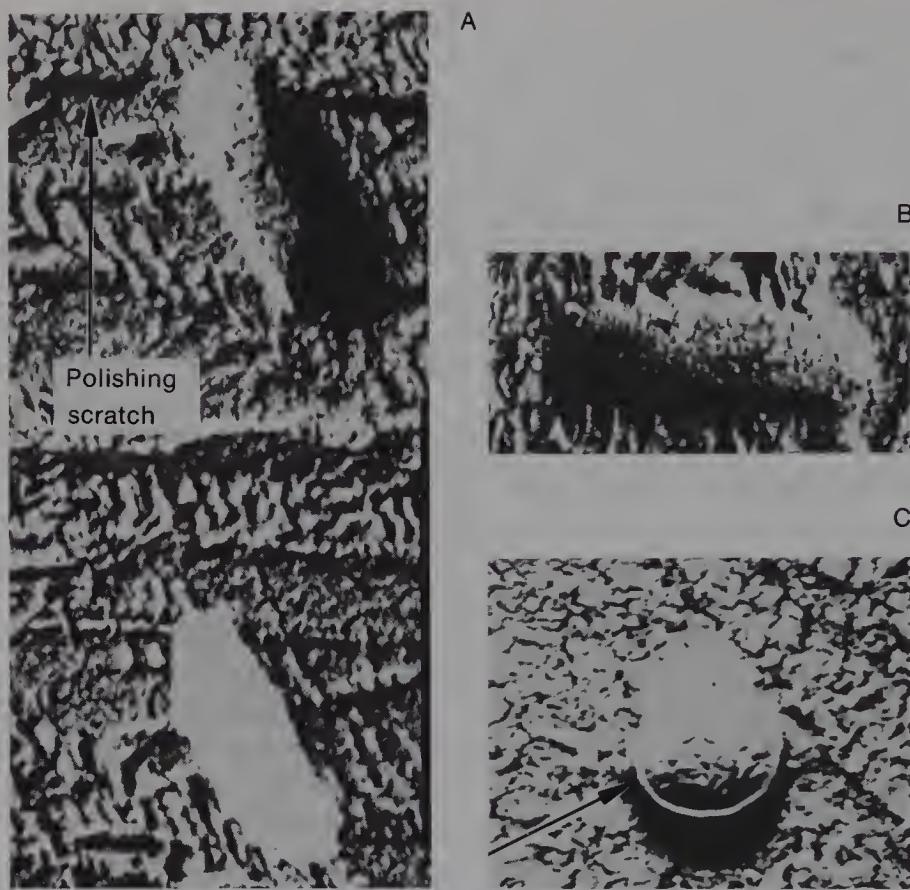
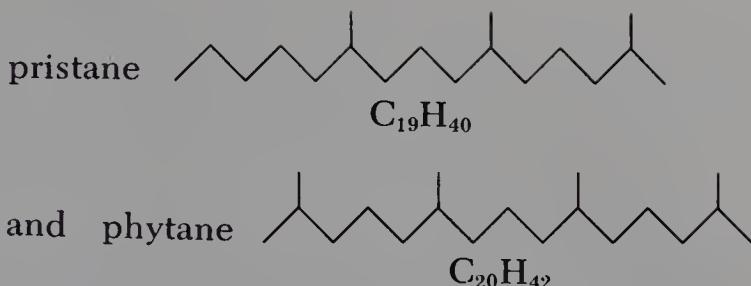


FIGURE 10-1
Electron micrographs of *"Eobacterium isolatum"* from the Fig Tree chert (3 billion years old): A, structurally preserved cell (white) and its imprint, transgressed by a polishing scratch in the rock surface; B, well-preserved rod-shaped cell; C, transverse section of the fossil organism showing the preserved cell wall. Source: Barghoorn and Schopf (1966).

wall, like many types of contemporary bacteria. Proteinoid microspheres can also have a two-layered cell wall (Figures 6-7 and 6-9). The Fig Tree chert is black and relatively rich in carbonaceous materials, which are mostly insoluble, though small amounts can be extracted. Using gas-liquid chromatography, Hoering (1966) and others (Calvin, 1969) analyzed the extracts of the sedimentary rocks that contain the microfossils and detected small amounts of long-chained alkanes, which show a fairly uniform distribution from about 14 to 25 carbon atoms in length, with pronounced peaks for the *n*-alkanes. They further discovered two small fractions, which are probably identical with the isoprenoid hydrocarbons,



The two hydrocarbons may result from the geochemical alteration of the phytyl alcohol portion of chlorophyll, the porphyrin-magnesium complex (Figure 10-2) that functions in photosynthesis. However, vanadium-porphyrin complexes that would result from geochemical alteration of chlorophyll, as frequently observed (Dunning and Moore, 1957; Hodgson et al., 1967; Hodgson et al., 1968), have so far not been detected in the Fig Tree chert. The role of chlorophyll as a source of the two isoprenoid hydrocarbons is questionable because some nonphotosynthesizing organisms are capable of synthesizing pristane and, apparently, phytane. Therefore, the evidence for the presence of photosynthesis at this remote date is not yet conclusive, although the increased $^{12}C/^{13}C$ ratio in the carbonaceous material, which is abundantly present in these Precambrian sediments, may have been caused by photosynthetic processes. Contemporary plants tend to utilize $^{12}CO_2$ rather than $^{13}CO_2$. Thus a partial enrichment of the lighter carbon isotope in plant tissues is opposed to its relative deficiency in the atmosphere, hydrosphere, and in carbonate rocks of inorganic origin.

Besides the hydrocarbons, significant amounts of the amino acids glycine, alanine, and valine (total amino acid content about 0.09 μg amino acids per gram of rock) were also extracted from the Fig Tree rocks by Pflug et al. (1969).

The existence of photosynthetic organisms about 400 million years after Fig Tree sediments were deposited may be indicated by the occurrence of stromatolites (MacGregor, 1951)—provided stromatolites are truly fossils of photosynthesizing algae, as has been proposed (Hoering, 1962). Stromatolites have been found in limestone 2.7 billion years old near Bulawayo, Southern Rhodesia.

More significant data are available for the iron formation of the Soudan rocks of upper Michigan and Minnesota. These rocks, about 2.7 billion years old, are dense materials with graphitic or carbonaceous inclusions (Cloud et al., 1965). Belsky et al. (1965), Johns et al. (1966), and Calvin (1969), all in Berkeley, extracted hydrocarbons from the carbonaceous materials and fractionated them, mainly by gas chromatography. Among the fractions that they identified is the C_{21}

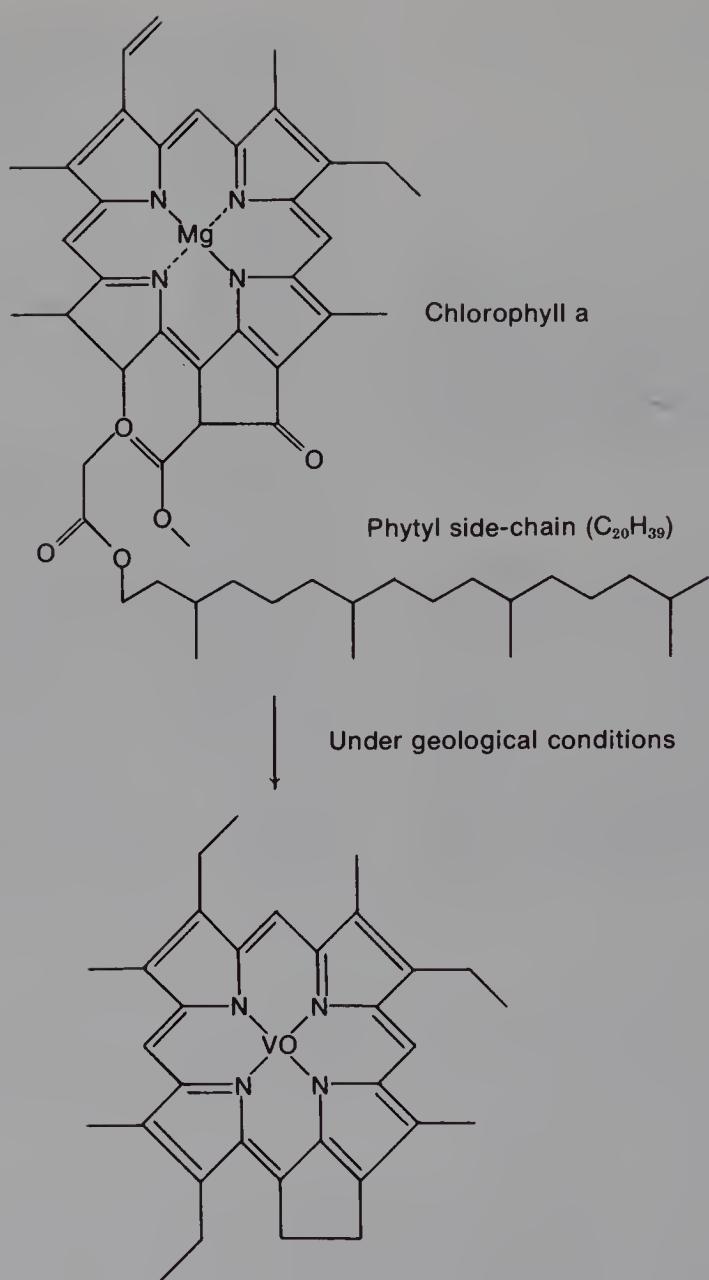


FIGURE 10-2
Relationship between chlorophyll and the petro-porphyrins. Source: Calvin (1969).

isoprenoid 2,6,10,14-tetramethylheptadecane, which may have been formed by the cracking of solanosane, or solanosol, or both (C_{45} isoprenoids), or of the C_{40} isoprenoid lycopene (lycopane), the precursor of vitamin A. However, a cracking of the lycopene skeleton at the C_{17} junction to give rise to a C_{21} isoprenoid would be unusual (Calvin,

1969), and solanosane is a relatively rare material. The geochemical precursor of the C₂₁ isoprenoid is, therefore, still regarded as being unknown, although it is agreed that it cannot be derived from phytane or squalene, but may be derived from isoprenoid compounds related to solanosane.

Another problem concerns the identification and the origin of a branched C₁₈ hydrocarbon found by gas chromatography in the same extracts. A similar (or identical) fraction was isolated from the blue-green alga *Nostoc*. A microfossil that appears to be related to *Nostoc* is present in some Precambrian rocks (Han, 1969), but has not been found in the Soudan shale. Besides these two peculiar hydrocarbons, the C₁₉ and C₂₀ isoprenoids (pristane and phytane) and the straight-chain alkane C₁₇ were found by gas chromatography. The carbonaceous materials show a deficiency of the heavy isotope ¹³C, which may be interpreted as indicating the presence of photosynthetic activity during the time these materials were deposited (cf. Calvin, 1969).

The microstructures found in the Soudan shale do not have a more organized appearance than the algae of the Fig Tree Series (Cloud et al., 1965). However, in the sedimentary rocks of the Witwatersrand System of South Africa, an iron formation like the Soudan shale and of similar age (2.7 billion years), various microstructures resembling monocellular organisms with complicated inner structures were discovered (Saager, 1968; Saager and Mihalik, 1967; Schidlowski, 1965). In the same material Pflug et al. (1969) found significant amounts of leucine, isoleucine, threonine, and serine, besides alanine, glycine, and valine, totaling about 1 µg of amino acids per gram of rock. According to Hare (1969), serine and threonine are not sufficiently stable to be indigenous to rocks of this age. Furthermore, L-isoleucine is expected to have isomerized partially to D-alloisoleucine since Precambrian times. The finding of D-alloisoleucine, however, was not reported by Pflug et al. (1969). At least some of the amino acids that these authors found in Precambrian rocks are probably the result of diffusion of amino acids of more recent origin into the ancient sediments (Hare, 1969).

More conclusive data are available about some middle Precambrian microorganisms whose remains are preserved in black cherts of the Gunflint Formation at the northern shore of Lake Superior. Radiometric determinations of age by ⁴⁰K/⁴⁰Ar and ⁸⁷Rb/⁸⁷Sr ratios indicate that this formation is approximately 1.9 billion years old. Its microfossil assemblage has been studied by Barghoorn and Tyler (1965). The assemblage appears to consist largely of photosynthetic algae, which grew in laminar sheets or mats in shallow disturbed waters. The organisms were preserved relatively unaltered by being

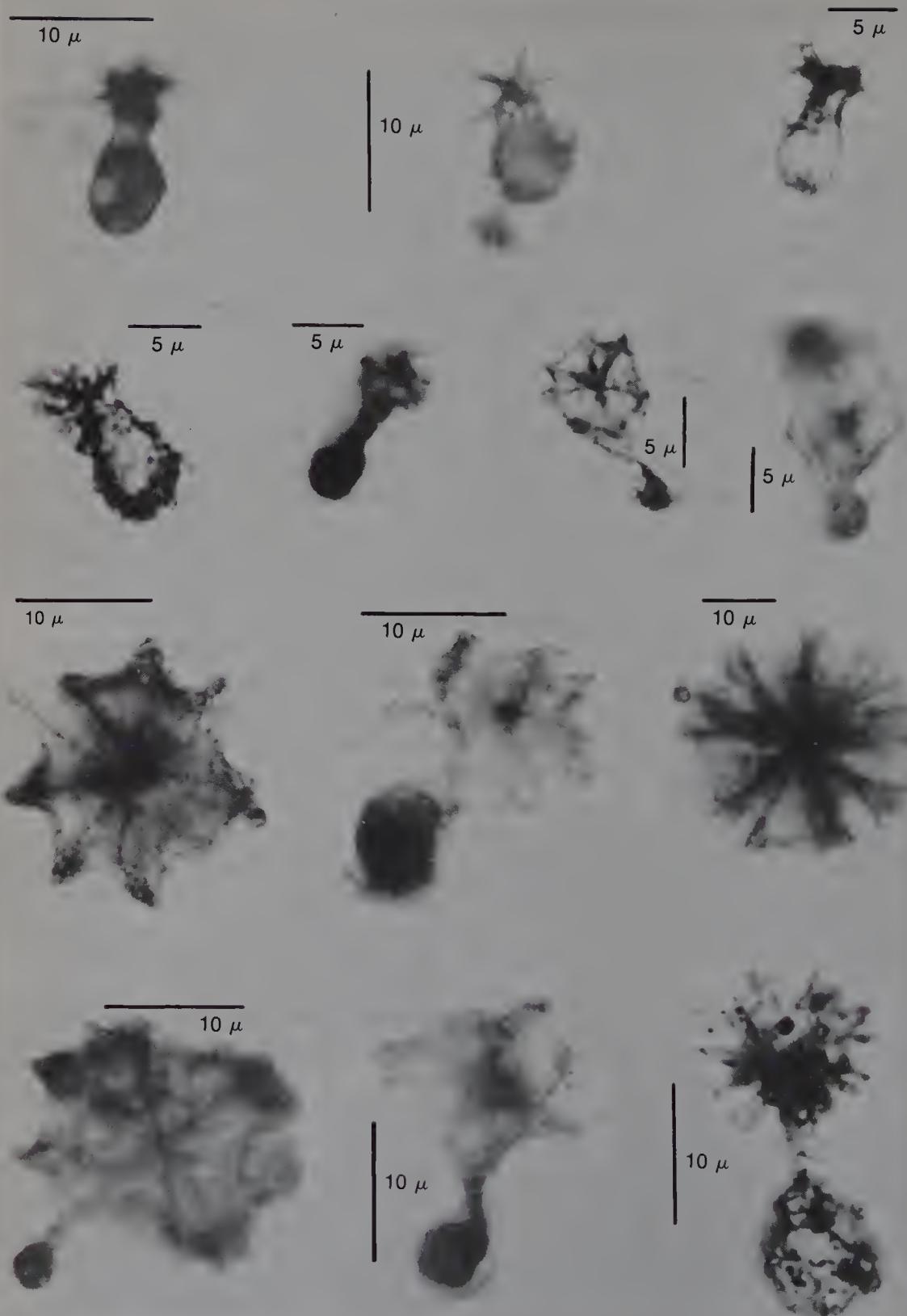


FIGURE 10-3

Various fossils of *Kakabekia umbellata* Barghoorn. The affinities of this organism are uncertain. Source: Barghoorn and Tyler (1965).

encased in colloidal silica, which later petrified and became chert. Eight genera and twelve species of morphologically distinct micro-organisms have been recognized in the assemblage. Some of the fossil filaments of the Gunflint rocks seem morphologically comparable to contemporary blue-green algae such as *Oscillatoria*. Their diameter ranges from 0.5 to 6.0 μm and they are several hundred μm long. Other bodies, spherical in shape and from about 1 to 16 μm in diameter, are found in the assemblage irregularly distributed throughout the matrix. Morphologically, they are comparable to some contemporary species of blue-green algae. They also show some similarity to the poorly preserved alga-like spherical units of the Fig Tree chert. In addition, the presence of well-preserved fossils of what appear to have been rod-shaped and coccoid bacteria was revealed by electron microscopy. Both bacterial forms seem to be similar to contemporary iron-depositing microbes. The biological affinities of some other genera of fossils from microorganisms of Gunflint chert are somewhat less certain, although one of them, called *Eoastrion*, seems to resemble a contemporary manganese- and iron-oxidizing bacterium found at several Karelian lakes (Barghoorn and Tyler, 1965; Cloud, 1965). A second fossil is called *Kakabekia umbellata* (Figure 10-3). That there was photosynthetic activity during the time when the Gunflint rocks were being deposited is suggested by the substantially higher proportion of ^{12}C in the organic matter than in organic carbonate from the same rock sample. Gas chromatography of heptane-soluble and total alkane fractions from Gunflint argillite shows the dominance of normal hydrocarbons, although there is evidence indicating the presence of some branched types, or some other fairly large hydrocarbons (Belsky, 1966). Two peaks, just ahead of the $n\text{-C}_{18}$ and $n\text{-C}_{17}$ positions, have been tentatively identified as pristane and phytane (Oro et al., 1965). This result may indicate the presence of photosynthetic algae at the time of deposition of the Gunflint rocks. This suggestion seems well backed by the finding of the remains of microorganisms resembling photosynthetic blue-green algae, although it is not fully backed by molecular evidence and renders more and detailed investigations necessary.

Organismic Fossils of the Late Precambrian

Microfossils are more abundant in the late Precambrian rocks (about one billion years old) than in those deposited earlier. Pflug (1964, 1965, 1966) described the well preserved microfossils,

probably the remains of blue-green algae and aquatic fungi, present in organically rich rocks of the 1.1 billion year old Belt Series of south-central Montana. In addition, spore-like cysts, possibly related to primitive flagellated protozoans or unicellular algae, and microscopic multichambered objects, probably the fossils of foraminiferans, have also been discovered. These seem to be the oldest remains of protozoan cells found in acknowledged fossil records, although some of the Gunflint organisms also appear morphologically similar to certain modern protozoa.

Microfossils derived from a microflora of filamentous blue-green algae and spheroidal, primitive green algae associated with algal stromatolites (and the remains of aquatic fungi and rod-shaped and coccoid bacteria) have been discovered in black cherts from the 0.8 billion year old Bitter Springs Formation of central Australia (Barghoorn and Schopf, 1965). Several types of filamentous blue-green algae appear to be morphologically indistinguishable from certain living species of *Oscillatoria*. The similarity in morphology, however, does not necessarily mean that these organisms have remained evolutionarily unaltered through the past billion years of Darwinian evolution, i.e., they may have undergone an evolutionary change of their biochemical performance that is not directly reflected by their morphology.

To the biochemist, the detection of what are certainly chlorophyll derivatives in the organic material of the billion year old Nonesuch shale of northern peninsular Michigan is of particular interest (White and Wright, 1964). The sediments contain oil seeps, oil encased in the limestone, and oil enclosed in the calcite crystals that can be removed only by grinding the rock. The three types of fractions of oil have been extracted separately and analyzed by gas chromatography (Eglinton et al., 1964a, 1964b). The chromatogram shows the major peaks at and around the *n*-C₁₇ position. However, no odd-even dominance or odd-even alteration is found. This property differs from that of the 50 million year old Green River shale (Calvin, 1969). The oils of the Nonesuch shale seem to contain some farnesane (C₁₅ isoprenoid). So far, however, most hydrocarbons are unidentified. The compounds have to be identified before a discussion of the origin of this oil can be meaningful. It is likely, however, that at least some of the oil is of biogenic origin, because optically active compounds have been found in it. In addition, vanadium-porphyrin complexes associated with pristane and phytane were found in the same samples. This is a fairly sure indication of the presence of photosynthetic organisms at least one billion years ago.

The Emergence of Late Precambrian Invertebrates

Paleontological evidence so far available permits the conclusion that the first metazoan invertebrate animals evolved from flagellated protozoan ancestors towards the close of the Precambrian, about 0.6 billion years ago. This evolution of animal life has been correlated to a simultaneous increase of the atmospheric oxygen to a level above the "Pasteur point" (Berkner and Marshall, 1964).

The "Pasteur point" is the minimal oxygen partial pressure above which oxidative metabolism can take place in aerobic organisms. The "Pasteur point" is not a general constant; it varies from organism to organism, depending on environmental factors and adaptation. For most contemporary aerobians it is of the order of a few percent of the present oxygen concentration. The "Pasteur point" of late Precambrian and Cambrian invertebrates is unknown. Berkner and Marshall assume a value of one percent of the present O₂ level. The order of magnitude may be correct, but the assumption of an average value to characterize an unknown variety of unknown species can be taken only as a first approximation. The geological evidence so far presented to demonstrate that anaerobic conditions were widespread on the Earth during the period 0.6–3 billion years ago is not fully conclusive. Photosynthetic—that is, oxygen-producing—organisms have probably existed on the Earth for more than 3.0 billion years. The first photosynthetic activity may have yielded only fugitive local accumulations of oxygen. The conjunction of evolution of animal life and increase of atmospheric oxygen level as proposed by Berkner and Marshall (1964), and by others (page 45), is an attractive speculation, but the existence of significant levels of oxygen prior to the evolution of animal life cannot be completely excluded.

Conclusions and Summary

The pertinent information on Precambrian evolution is summarized in Figure 10–4. The abundant fossil record of the Cambrian and later periods is not discussed here because it seems less relevant to intrinsic questions of biogenesis. Some data on "chemical fossils" are included for comparison.

With respect to the problem of verifying the origin of both the microstructures and the organic compounds in ancient sediments, two points deserve special consideration.

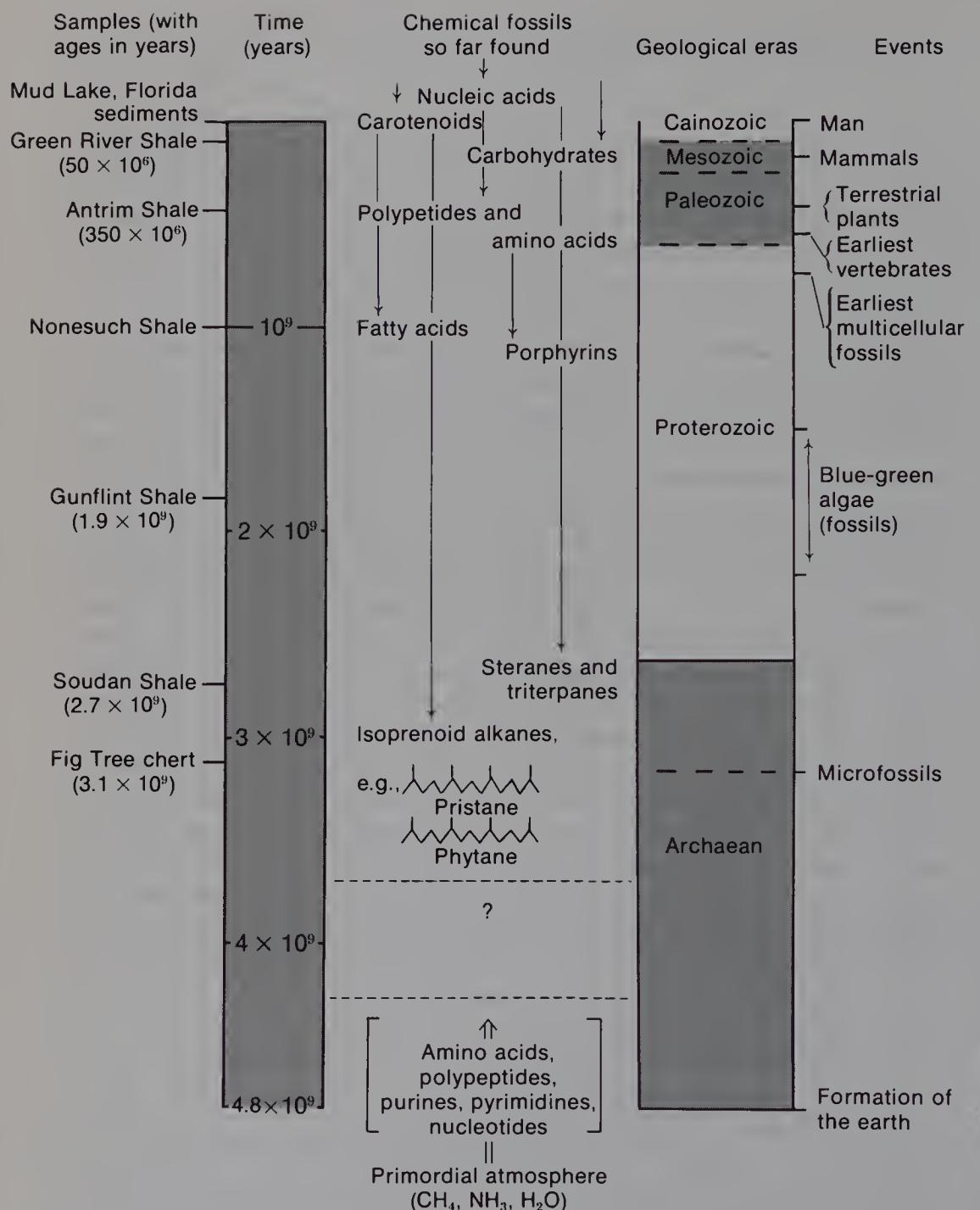


FIGURE 10-4

Geological time-scale for chemical and biological evolution. Source: Calvin (1969).

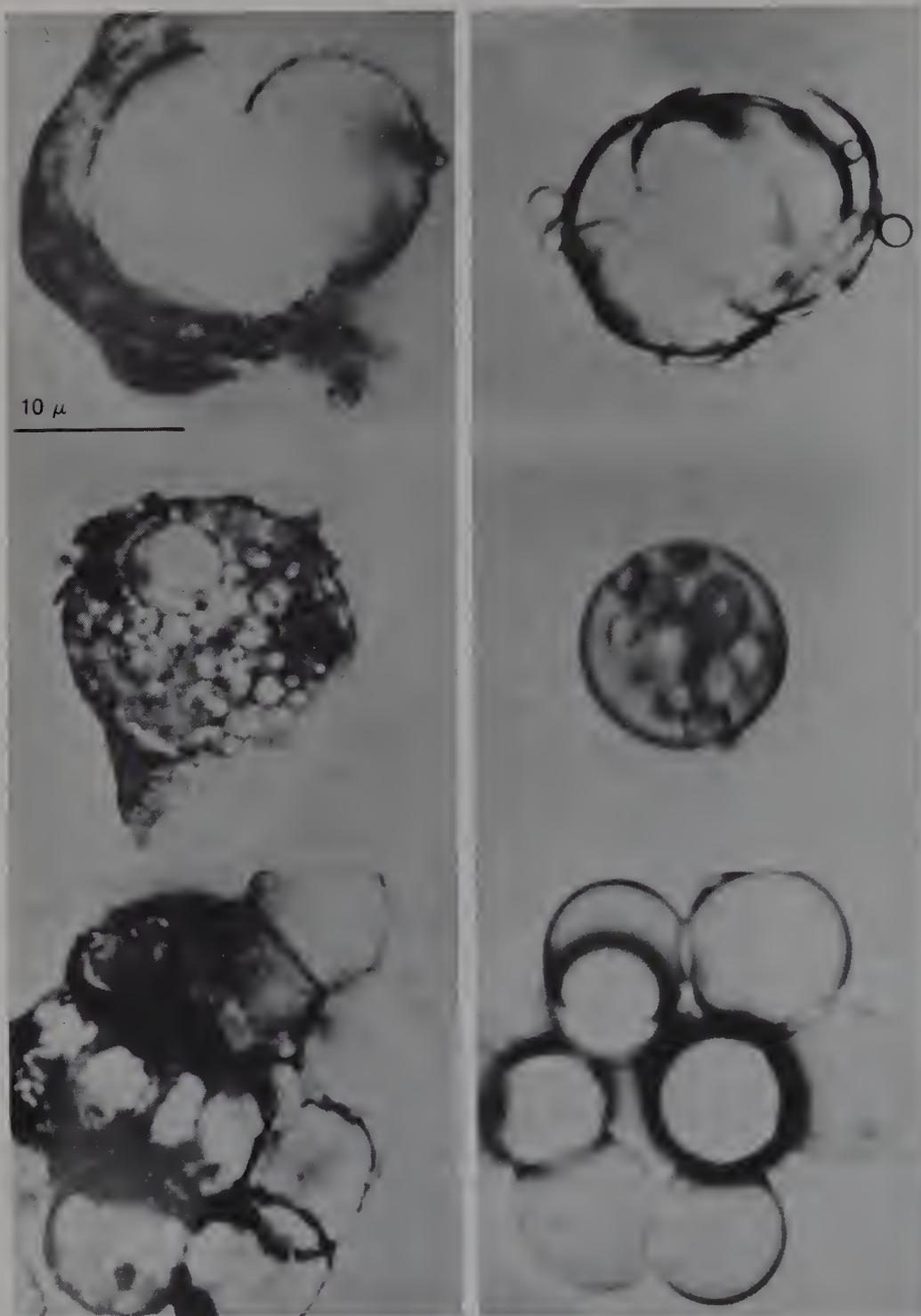
First is the question of whether the organic compounds associated with fossils are truly indigenous. The possibility of contamination by migration of organic compounds after sedimentary deposit must be taken into account (McCarthy and Calvin, 1967). The isolation and identification of any organic compound from sedimentary rocks is of debatable significance, unless it is evident that the material was laid down at the time of sedimentation. Articles have been written describing what the authors propose are examples of diffusion of recently produced amino acids into older strata (e.g., Hare, 1969).

Second, the biogenicity of "organized elements" in terrestrial materials needs to be established rigorously. Paleoecological and geochemical tests have to be met in addition to evident morphological requirements. Generally, morphological evidence alone appears insufficient to establish the true origin of microstructures contained in sedimentary rocks. Structures that are morphologically related may originate from artifacts, from primitive cells, or from protocells. Many microstructures of the Precambrian rocks show a striking similarity to proteinoid microspheres. These resemblances may be superficial, in that even the various types of proteinoid microspheres are found to be indistinguishable by microscopic examination alone from certain extant microorganisms (Figure 10-5; see also Fox, 1969). After more paleontological information is accumulated, particularly from materials older than 3 billion years, comparisons of microstructures with models of primitive cells, such as proteinoid microspheres, should become increasingly profitable. The microstructures may be rightfully referred to as the remains of organisms, if "organisms" is taken to mean self-organized structures that grow and proliferate. Their greatest significance may reside in their being the remains of precursors of the first organisms of a contemporary type (page 250).

In a related consideration, the biogenicity of the "organized elements" indigenous to the carbonaceous chondrites has already been questioned because of their resemblance to proteinoid microspheres.

Hydrocarbons, so far, appear to be the only definable organic compounds indigenous to all Precambrian sediments. A summary of the occurrence of the various hydrocarbons in Precambrian sediments is best given by comparison of typical gas chromatograms. The distribution of hydrocarbons in the extracts of various sedimentary materials seems to be closely related to age and geochemical history.

Figure 10-6 shows a typical chromatogram of a mixture of abiotic hydrocarbons produced by the action of hydrochloric acid on iron carbide. The diagram represents approximately a Gaussian distribution of isomers of different chain length with a maximum at

**FIGURE 10-5**

Photomicrographs of microfossils of Barghoorn et al. on the left; proteinoid micro-particles from the laboratory on the right.

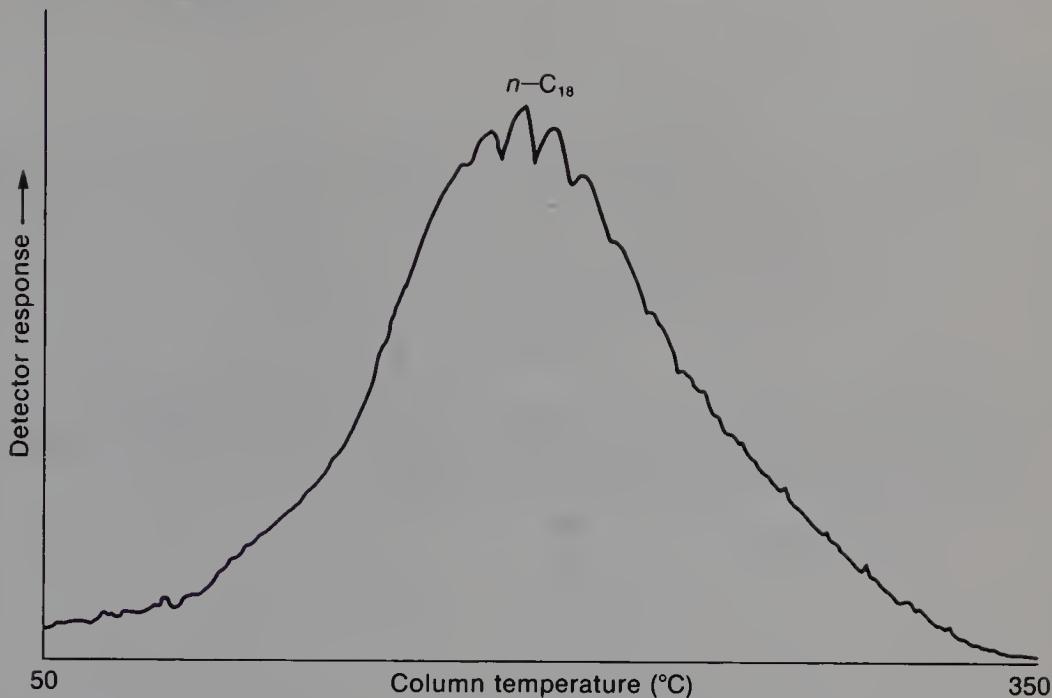


FIGURE 10-6

Gas chromatogram for a hydrocarbon fraction produced by the action of hydrochloric acid on iron carbide. Source: Hoering (1966).

the $n\text{-C}_{18}$ position. Some small peaks are superposed. They indicate that some of the hydrocarbons are formed nonrandomly. The hydrocarbons extracted from the oldest known sedimentary rocks (Onverwacht Series, more than 3.2 billion years old) show a similar overall pattern (Figure 10-7). A number of discrete peaks superpose non-randomly the basic Gaussian curve. This similarity, however, does not necessarily indicate an abiogenic origin, inasmuch as geochemical diagenesis appears to convert mixtures of a few discrete hydrocarbons into a related mixture of branched isomers. The fine structure of the chromatograms increases—as will be shown— inversely to the age of the material. The more the discrete peaks dominate in the chromatograms, the less the biogenic hydrocarbons are altered geochemically.

Figure 10-8 shows a gas chromatogram of alkanes from the Fig Tree shale. The fine structure is still superposed on a relatively high Gaussian curve with a maximum in the range of $n\text{-C}_{23}$. Figure 10-9 shows gas chromatograms of heptane-soluble and total alkane fractions from Gunflint argillite, about 1.9 billion years old. The Gaussian curve is significantly lowered and the discrete peaks clearly dominate.

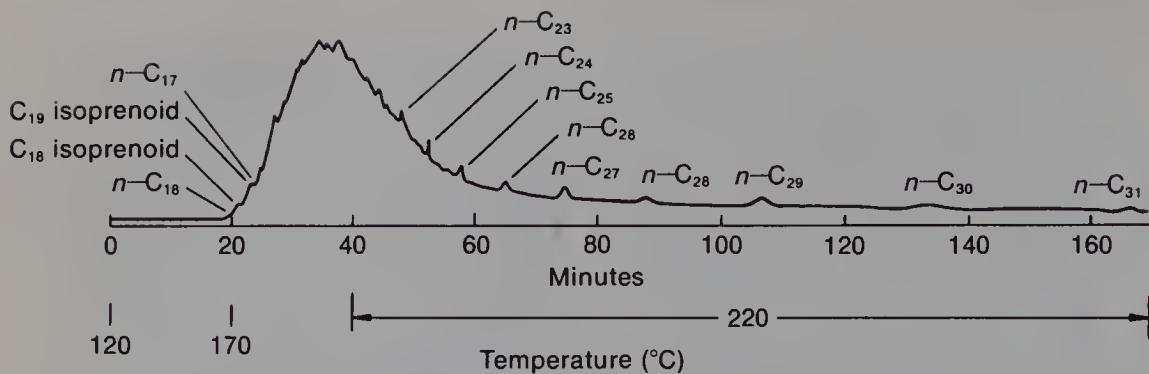


FIGURE 10-7

Saturated hydrocarbon extract from sedimentary rock of the Onverwacht Series, temperature programmed on a 15 foot \times 0.020 inch i.d. SCOT column coated with Apiezon L. Source: MacLeod, cited in Calvin (1969).

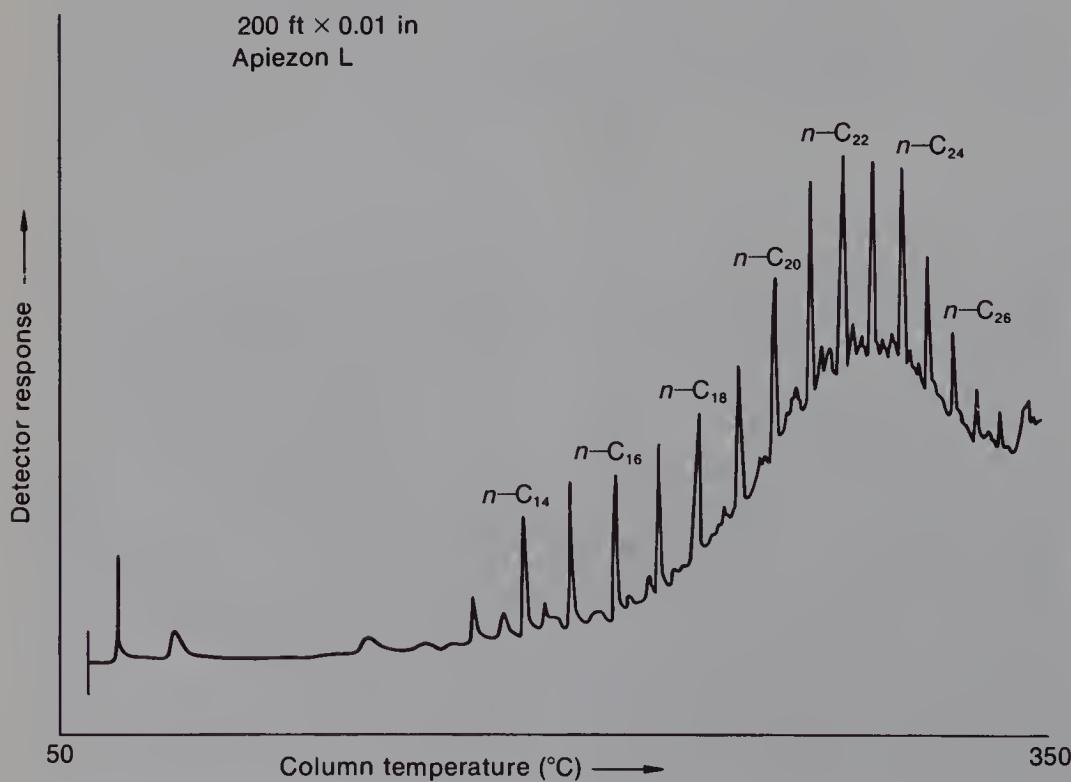


FIGURE 10-8

Gas chromatogram of alkanes from Fig Tree shale. Source: Hoering (1966).

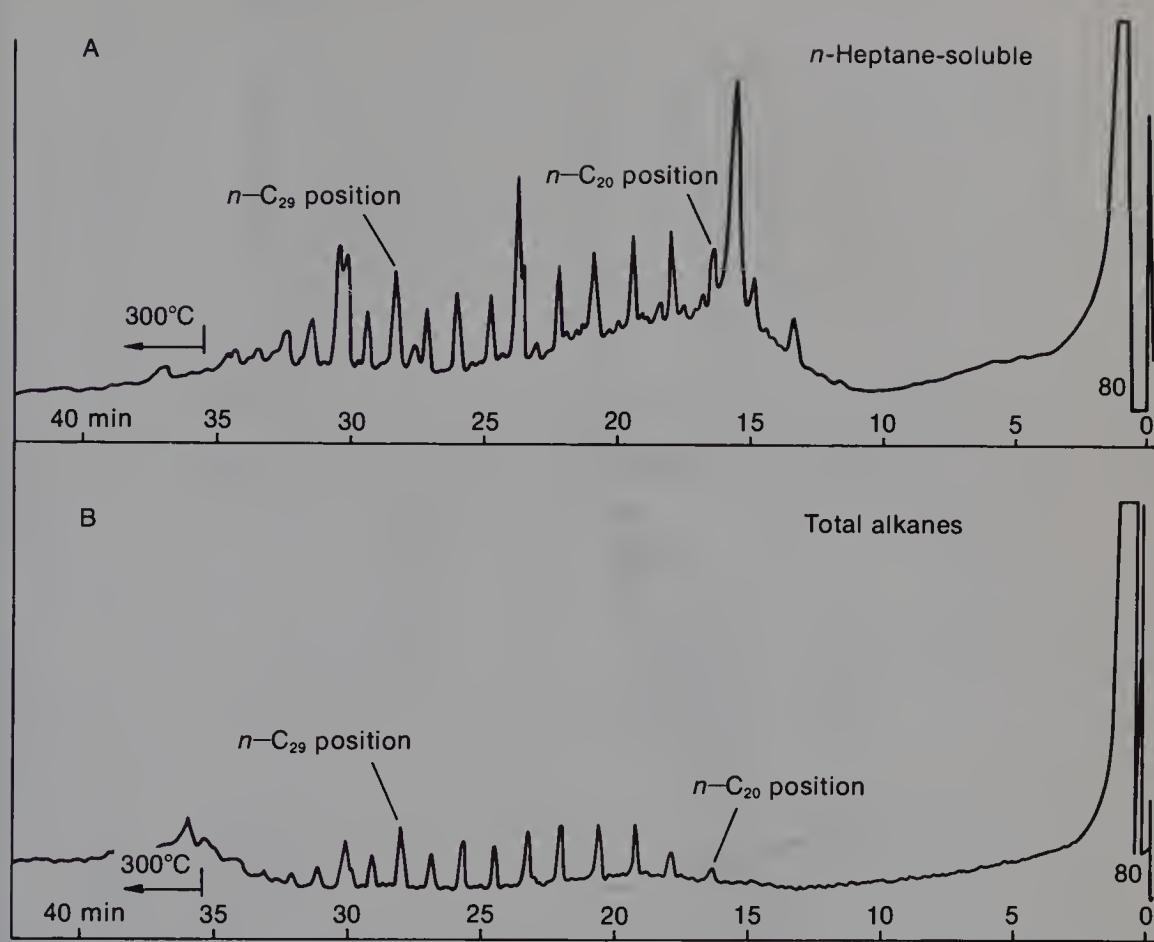


FIGURE 10-9
 Gas chromatograms of alkane fractions from Gunflint argillite. A: Heptanesoluble fraction. B: Total alkane fraction. Source: Calvin (1969).

This predominance of discrete peaks increases for the alkanes of the Nonesuch shale. Figure 10-10 shows high-resolution capillary chromatograms of this material. The distribution of alkane fractions from Precambrian materials may now be compared with the distribution of hydrocarbons from the Green River shale, Colorado (Eocene, approximately 60 million years old), and alkanes in contemporary sediments of the Florida Mud Lake (Figures 10-11 and 10-12). The last two patterns may be taken as typical examples for the distribution of biogenic hydrocarbons that underwent only a small degree of diagenesis.

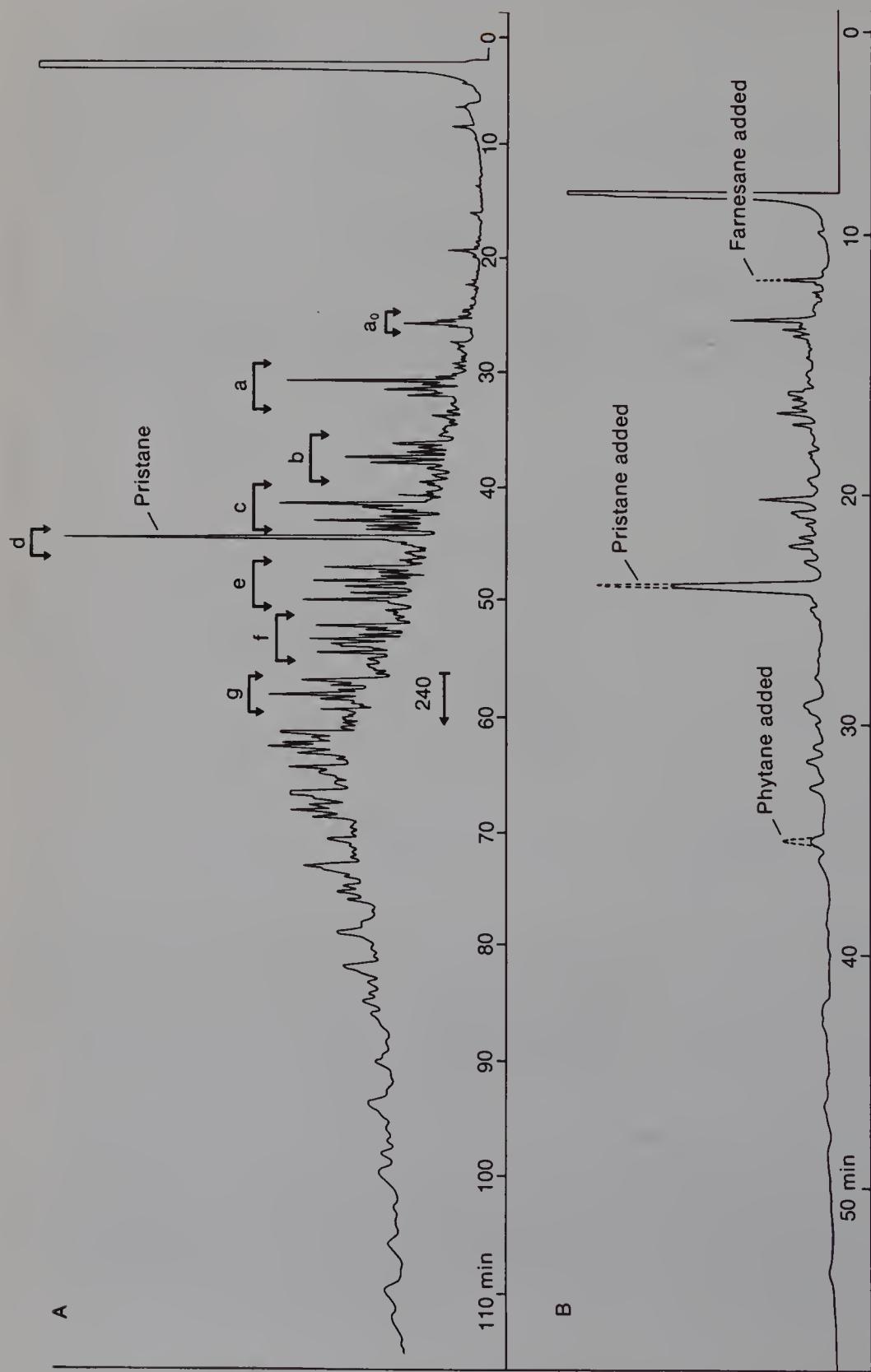


FIGURE 10-10
High-resolution capillary chromatograms of alkanes from oil in Nonesuch shale:
A, total fraction; B, combined group of fractions from a₀ to g. Source: Calvin (1969).

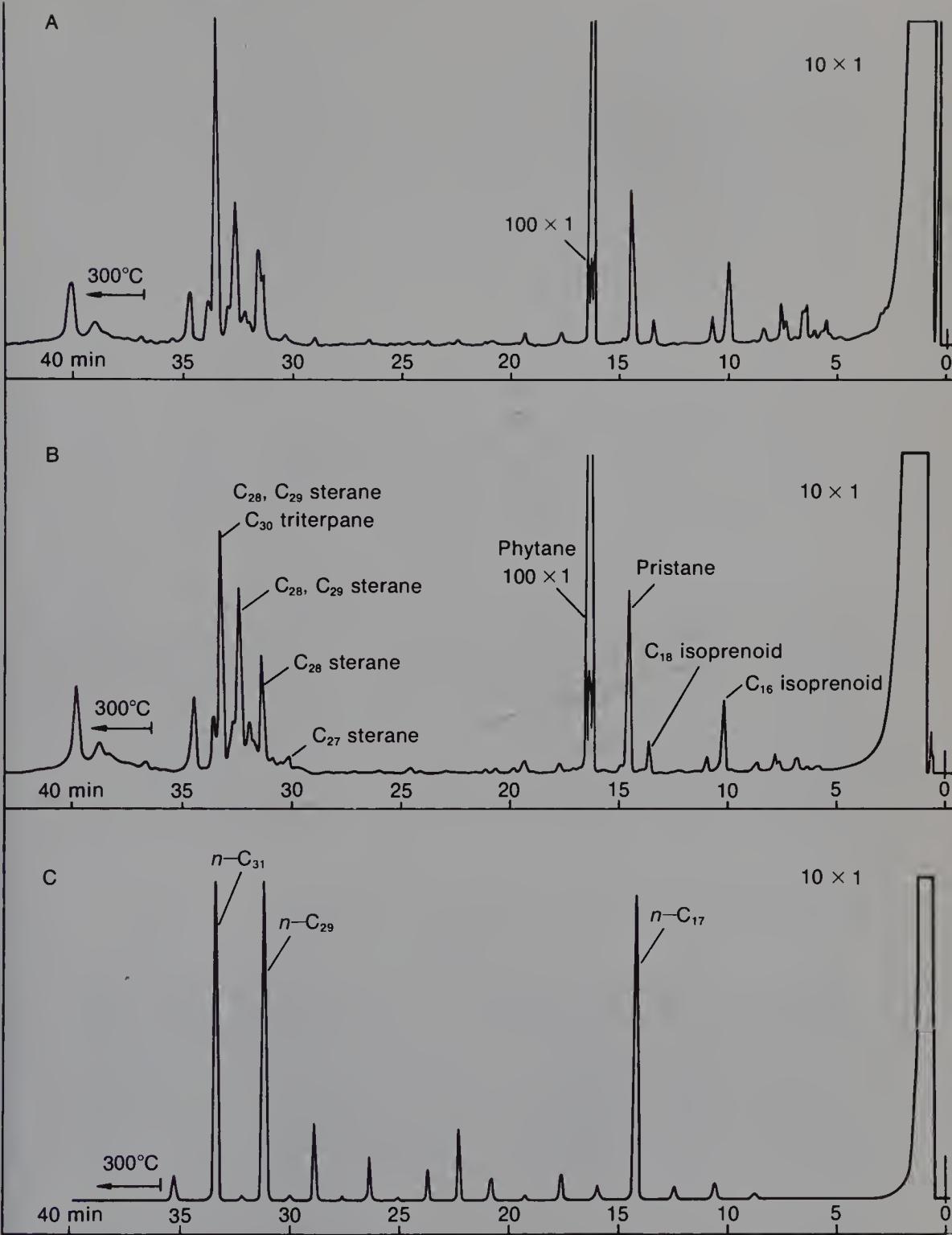


FIGURE 10-11

Gas chromatograms for total hydrocarbons from Green River shale, Colorado (Eocene, approximately 60 million years old). A: Total hydrocarbons. B: Branched-cyclic fraction. C: Normal fraction. Source: Calvin (1969).

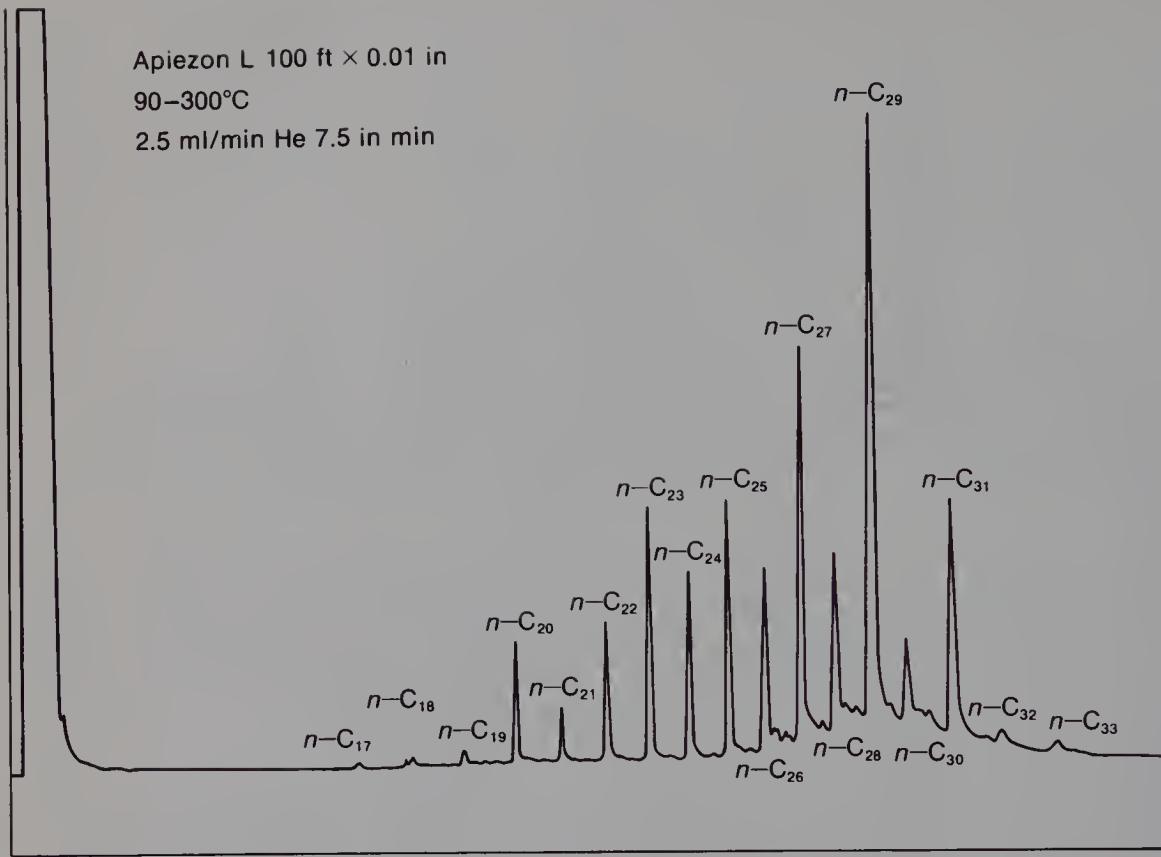


FIGURE 10-12
Gas chromatogram of total alkanes in Mud Lake (Florida) sediments. From Calvin (1969).

MOLECULAR FOSSILS IN SEDIMENTS OF CAMBRIAN AND YOUNGER PERIODS

The finding of various hydrocarbons, and other organic compounds (biogenic or abiogenic, indigenous or introduced) in ancient sediments has revived interest in the diagenesis of such compounds. Many reports on this subject have been delivered, although as yet only a few conclusive results have been obtained. It is not within the scope of this volume to present a detailed review on the geochemistry of organic compounds. This discussion of the geochemistry and fossil occurrence is restricted to organic compounds that can be related directly to their origin. (For a more detailed report, see Breger, 1963, and Eglinton and Murphy, 1969.) Except for a number of hydrocarbons and porphyrins, none of the organic compounds indigenous to Precambrian sediments has been traced back to the biogenic

mother compounds. Most of the biogenic compounds were altered geochemically during significantly younger geological periods. They were finally converted into ill-defined oils, kerogens, bitumens, asphaltites, mineral waxes, and coals. These materials often disappear from the locales of their original deposit either by transport processes or by further degradation. The content in carbon of Precambrian rocks can be as low as 10 parts per million. Such low concentrations, however, could indicate that Precambrian sediments were never as rich in organic carbon as sediments of relatively recent times.

Amino Acids, Peptides, and Proteins

Table 10-1 shows the principal amino acids found in sediments deposited during the Recent and Oligocene Epochs. The amino acids are arranged in order of decreasing abundance. The Recent sediment is a typical shallow-water marine deposit, probably a few thousand years old. It was collected on the inner continental shelf of the Gulf of Mexico from a narrow layer at a depth of 120 centimeters below the surface of the sediment, which is below the zone of major bacterial activity. The Oligocene sediment is of similar origin and probably about 30 million years old. It was obtained from a section of a marine-shale core cut from the Anahuac Formation in Fort Bend County, Texas, at a depth of 5000 feet.

The Recent sediment contained about 3.0 μ moles of amino acids per gram; the Oligocene sediment had only 0.51 μ moles per gram. Table 10-1 shows that the unsubstituted aliphatic amino acid and the

TABLE 10-1
Principal Amino Acids in Recent and
Oligocene Sediments

Recent	Oligocene
Valine	Alanine
Leucines	Glutamic acid
Alanine	Glycine
Glutamic acid	Proline
Aspartic acid	Leucines
Glycine	Aspartic acid
Proline	
Tyrosine	
Phenylalanine	

Source: Erdman *et al.* (1956).

two common acidic amino acids are the most stable amino acids. Cystine (and cysteine), methionine, and tryptophan, on the other hand, seem to be the most unstable amino acids under most geological conditions. Only traces, if any at all, of these amino acids have been found in soils during the course of investigation over the past fifty years (Abelson, 1963). This result agrees with the well-known chemical instability of these three amino acids. Fossilized materials such as shells or bones have been analyzed more quantitatively than sediments for their amino acid contents. Some relevant data are summarized in Table 10-2. The data in Table 10-2 also demonstrate that environmental factors often are of more crucial importance than the mere lapse of time.

The stability of amino acids in aqueous solution under controlled laboratory conditions has been studied in detail by Abelson (1957), Conway and Libby (1958), and Vallentyne (1964). The principal degradation reaction is the decarboxylation. Alanine yields ethylamine and CO_2 . This reaction is first order and follows the Arrhenius equation:

$$k = Ae^{-E/RT}.$$

In this equation k represents $dC/C \times dt$; C is the concentration of

TABLE 10-2
Amino Acid Content of Various Fossils

Fossil	Period	Age (yr $\times 10^{-6}$)	Amino Acids ($\mu\text{moles}/\text{gram}$)	Principal Amino Acids
<i>Plesippus</i> (prehistoric horse bones)	Late Pliocene	5	0.6	Ala, gly
<i>Plesippus</i> (prehistoric horse tooth)	Late Pliocene	5	1.5	Gly, ala, leu, val, glu
<i>Mesohippus</i> (prehistoric horse tooth)	Oligocene	40	0.31	Ala, gly
<i>Mosasaurus</i> (dinosaur)	Cretaceous	100	1.8	Ala, gly, glu, leu, val, asp
<i>Anatosaurus</i> (dinosaur)	Cretaceous	100	2.8	Ala, gly, glu
<i>Stegosaurus</i> (dinosaur)	Jurassic	150	0.26	Ala, gly, glu
<i>Dinichthys</i> (fish)	Devonian	360	3.0	Gly, ala, glu, leu, val, asp

Source: Abelson (1963).

alanine, t is the time, A is the frequency factor (about 10^{13} seconds), E is the activation energy, T is the absolute temperature, and R is the gas constant. In Figure 10-13 the degradation time, the time required for initial concentrations to diminish to $1/e$ or 37 percent (log scale), is plotted against the temperature, in °C. The results presented are in agreement with the Arrhenius equation. An activation energy of $E = 44,000$ calories per mole and a frequency factor of $A = 3 \times 10^{13}$ has been found for the overall degradation of alanine according to Vallentyne (1956).

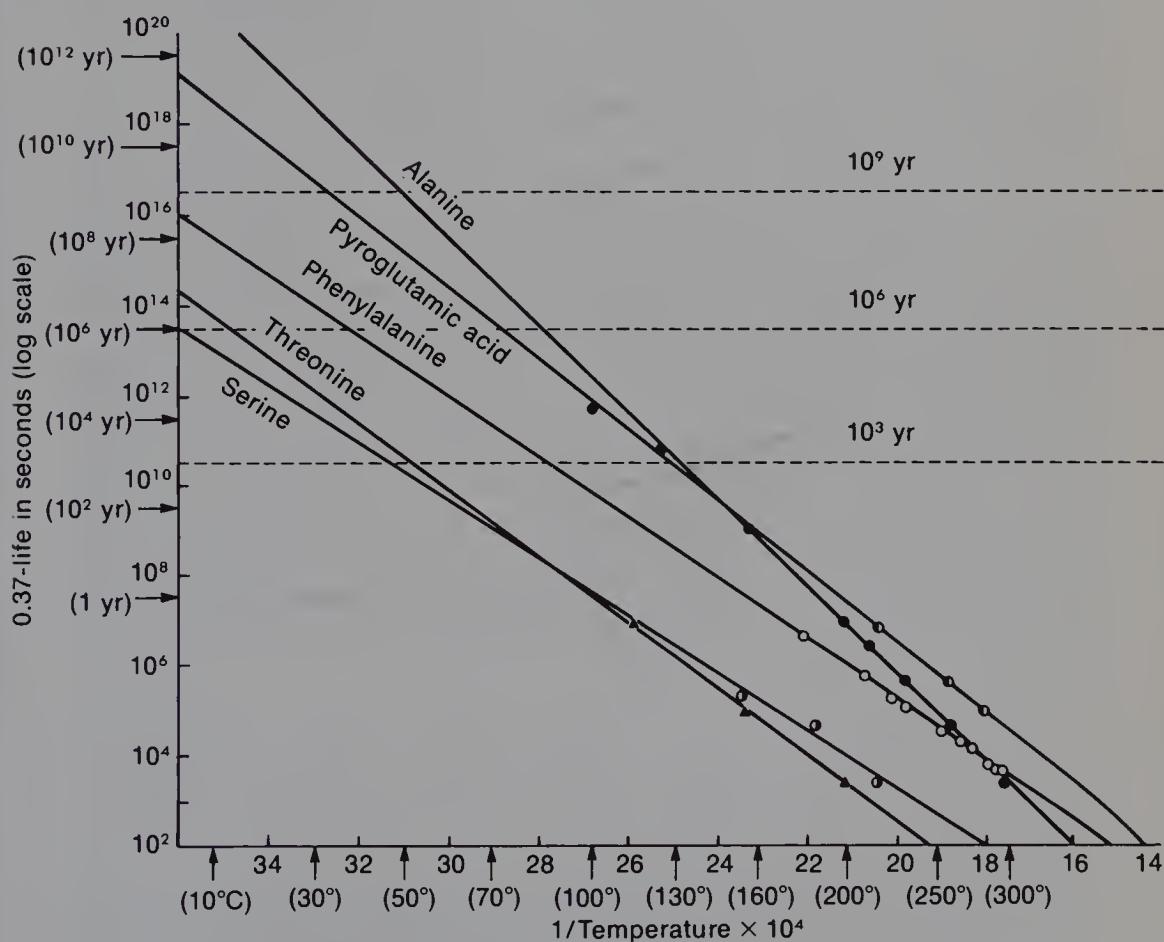


FIGURE 10-13

Linear regression lines based on Arrhenius equations for the thermal decomposition of five amino acids in 0.01 M aqueous solution. Vertical axis: 0.37-life in seconds (years in parentheses) plotted on a logarithmic scale. Horizontal axis: the reciprocal of the absolute temperature multiplied by 10^4 (values in parentheses give the corresponding temperatures in °C). Open squares: threonine; half-solid circles, dark on right: serine; open circles: phenylalanine; solid circles: alanine; half-solid circles, dark on left: pyroglutamic acid. Source: Vallentyne (1956).

Provided that the linear plots may be extrapolated to ambient temperature, the data suggest that solutions of alanine would persist more than one billion years; serine would survive through the course of one million years under the same conditions. In context with geological reasoning, however, it would be desirable to study this degradation in a medium other than distilled water. The incubation of amino acids with minerals, carbohydrates, or other compounds would lead to different results. Alanine in aerobic aqueous solution and at ambient temperatures would last only about 100 thousand years (Abelson, 1957). Pflug et al. (1969) have isolated substantial proportions of glycine and alanine from various Precambrian sediments more than 2 billion years old. These amino acids might be in all instances indigenous inasmuch as the thermolabile serine, threonine, methionine, and isoleucine, but no alloisoleucine (Hare, 1969), were found.

The degradation times of proteins and polypeptides are even shorter than those of most of the amino acids with reactive side chains. At the death of an organism, degradative processes are set into action, mainly by a variety of hydrolytic enzymes. Later these enzymes are destroyed by proteinases, which, in turn, destroy themselves in a final phase of enzymic decay. Under natural conditions, however, the selfdestruction of a dead organism is seldom completed, mostly because the body usually provides food for other organisms, in particular microorganisms. Even if protein molecules or polypeptides escaped biological degradation, they would be hydrolyzed in the presence of traces of water. Hydrolysis is catalyzed by many minerals. Hydrolysis of peptide bonds is an exothermic reaction, although the activation energy in water is as high as 25,000 calories per mole (Borsook and Huffman, 1944; Abelson, 1963).

The complete hydrolysis of peptide bonds in different geological environments may require as long as many million years. Partial hydrolysis of peptide bonds will, however, change the identity of any protein in relatively short time even under favorable conditions — e.g., if it is protected from water, oxygen, and microorganisms by being in the interior of a clamshell. Abelson (1955) reported the isolation of altered proteins from clams about one thousand years old. Extraction of contemporary shells of the clam *Mya myarenaria* with dilute hydrochloric acid yields a filamentous light-colored, denatured, protein after lyophilization. The thousand-year-old specimens yield an amber-colored and considerably less filamentous peptidic material of apparently identical amino acid composition. Some quantitative studies were made with the shells of the clam *Mercenaria mercenaria*, which lives today and is also represented by fossil specimens dating

TABLE 10-3
Amino Acid Content of Shells of *Mercenaria mercenaria*

Age		Amino Acid Content (μ moles/gram)		
Yr $\times 10^6$	Period	Bound in polymeric matrix	Soluble peptidic polymer	Free amino acid
0	Recent	33.0	1.5	<0.35
0.1-1.0	Pleistocene	2.1	2.25	1.0
~25	Miocene	0	0	0.75

Source: Abelson (1963).

back at least 25 million years. Some characteristic results are summarized in Table 10-3. As can be seen, the amino acid content and the amount of polymer yielding amino acids after hydrolysis decrease with age. Only low proportions of amino acids are found in the material 25 million years old.

Insoluble keratinous materials, proteins immobilized in shell or bone, are protected from hydrolytic degradation and contact with microorganisms. Whether the materials are encased or not, diffusion of water to the inside and diffusion of hydrolyzed amino acids to the outside of the remains of the organism will finally yield a complete degradation (Figure 10-13). Determining whether proteins or polypeptides were preserved in sediments older than 100 million years seems, therefore, quite hopeless. Materials for analysis of the amino acid sequence in peptidic compounds of that age will probably not be available.

Nucleic Acids and Degradation Products

No conclusive results are available concerning the geochemistry of nucleic acids. Nucleic acids are found in small amounts in Recent soils either as such or partly degraded; however, no report is available regarding the survival of nucleic acids and their constituents in any older geological material (Breger, 1963; Eglinton and Murphy, 1969). Two types of easily hydrolyzable links are found in nucleic acids: (1) the glycosidic link between the nitrogen of the aromatic base and the carbon atom C-1 of ribose (in RNA) or deoxyribose (in DNA); (2) the ester links between phosphoric acid and carbon C-3 or carbon C-5 of the sugar moieties. All of these links are thermodynamically

unstable in the presence of water and are even more quickly hydrolyzed than peptide bonds or ester bonds in lipids. From the data for the geochemical stability of peptides and esters of the fatty acids, we must infer that finding even fragments of nucleic acids, that is, oligonucleotides, nucleotides, nucleosides, or ribosephosphates, in pre-Quaternary material, unlikely.

Carbohydrates

Although the geochemistry of carbohydrates has been studied in much detail since Gothan (1922) isolated a carbohydrate (cellulose) from fossil material in Miocene deposits, our information on this subject is far from complete. Recently the geochemistry of fossil carbohydrates has been reviewed by Swain (1969). Lignitic cellulose of the Pliocene and Miocene (up to 30 million years old) has been found in such abundance that the feasibility of manufacturing paper from it has been seriously considered (Beyschlag, 1938). The theories and observations related to the conversion of cellulose into coal (Cooper and Murchison, 1969) have drawn widespread attention. Besides the conversion into coals, the hydrolytic decay of carbohydrates is of interest. As early as 1939, Staudinger and Jurisch found that the degree of polymerization of cellulose falls from an assumed value of 2000–3000 glucose units in contemporary samples to 200–300 units in most preparations of Miocene samples. The extent of degradation, however, varies with local factors as well as the age of the material.

Other than the cellulose-containing remains of woody tissues, carbohydrates in fossils have received only little investigation. That chitin is present in fossil marine invertebrates is often suggested by geologists, although supporting evidence is usually not presented. Glucosamine, which is released from chitin after hydrolysis, has been discovered by Abderhalden and Heyns (1933) in middle Eocene deposits (about 50 million years old). Drozdova and Kochenov (1960) found glucosamine in fossil fish bone. Using color tests, Vinogradov and Boichenko (1943) also presented some evidence for the presence of pectins and other polysaccharides in fossil algae. Primitive algae may have been rich in pectins. This suggestion, however, needs confirmation.

A variety of carbohydrates were isolated from carbonaceous chondrites. These and other findings of organic compounds in meteorites will be discussed in more detail in Chapter 11.

Lipids

The lipids are the third major group of biochemical compounds quite widely spread in geological materials (Breger, 1963; Eglinton and Murphy, 1969) the first two being amino acids and carbohydrates. Hydrocarbons occur abundantly in mineral oils but may also be found, though in smaller amounts, in any sedimentary material of any period including the Precambrian (page 292). Attention may be called to the fact that the saturated fatty acids found in some kinds of petroleum are also the principal acids in the lipids of sharks and whales. The lipids of these vertebrates, which were abundant in the Mesozoic and Tertiary periods (up to 225 million years ago), possibly were converted into petroleum by biochemical and geochemical processes. The remains of both animals and plants are believed to be principal starting materials for petroleum, although we cannot exclude the possibility that some hydrocarbons are of abiogenic origin (page 318). Discussing in detail the origin of hydrocarbons in mineral oil or geological settings of periods younger than the Cambrian is beyond the scope of this volume, because the problem of molecular evolution is to be correlated to the earliest—that is, the Precambrian—occurrence of these materials (page 292; for hydrocarbons in meteorites see page 337; for origin of petroleum see Breger, 1963; Eglinton and Murphy, 1969; Fox and Maier, 1959).

The fossil occurrence of terpenoids, like that of other hydrocarbons, largely depends on their molecular weight and vapor pressure. So far, low molecular weight terpenoids, such as mono- and sesqui-terpenes, have not been found in Precambrian sediments, although terpenoids containing four or more isoprene units, such as pristane and phytane (page 292) have been found to be associated even with the oldest known fossils. More widely distributed in many younger remains of plants and animals are the triterpenoids. A special group of these, the steroids, is of particular interest. Ross (1911) was probably the first to provide experimental evidence for the occurrence of steroids in petroleum. The optical activity of petroleums first observed as early as 1835 by Biot depends largely on their contents of steroids and other terpenoids.

Being esters of glycerol and fatty acids, typical lipids such as triglycerides are not stable under geological conditions. Mostly they are saponified within geologically insignificant periods of time. However, the resulting fatty acids and related lipid compounds are the only organic materials found to remain long after autolysis, bacterial activity, and geochemical and geophysical processes have

metamorphosed and removed most of the other organic components of an organism. (The mixture of material that is mostly lipid left after decay of a body often is referred to as adipocires.) No conclusive results are available concerning the further fate of adipocires over geologically significant periods of time. Closely related to the geochemistry of fats is the practice of the Irish and Scandinavians of burying in bogs large quantities of butter encased in wooden containers. In this moist environment the butter gradually saponifies, the water-soluble constituents, including glycerol and acids of lower molecular weight, e.g., butyric acid, seep out through the wooden container, and eventually, bog butter, a reportedly well-tasting, cheese-like material of the consistency of tallow, is left. According to its composition, bog butter is closely related to adipocires. Conclusive results on its further fate over times of geological significance are not at hand.

Lignite and peat waxes, another group of fossil lipids, are mostly of plant origin. In living plants the leaves, needles, trunks, and fruit are protected by waxes that are extremely unreactive and water-insoluble. These properties explain the persistence of plant waxes through geological times. Consistent with their origin, plant waxes are often associated with resins. In certain minerals such as pyropisite the wax content may be as high as 75 percent. Therefore, special names such as "wax-coal" or "lipolithes" have been given to these organic minerals. Their major constituents are wax acids, wax alcohols, and oxidized resin acids accompanied by terpenoids, resin alcohols and acids, solid paraffins, and other aliphatic hydrocarbons. Higher plants, the main source of the kinds of waxes described here, first appeared in abundance during the Carboniferous Period, 280 to 345 million years ago. Little is known about the fossil occurrence of plant waxes of more ancient origin.

ORGANIC COMPOUNDS OF ABIOTIC ORIGIN IN GEOLOGICAL MATERIALS

Most organic substances of firm biogenic origin are found in relatively young sediments including anthracite coals, lignite-peats, asphaltites, asphalts, fossil resins, petroleum, and fossil waxes. Notably, they all can be characterized by O/C ratios below 0.5. Among hydrothermal and igneous deposits, however, an important group of organic phases, the tucholites, is found. These cannot be easily related to a biogenic origin. Tucholites are characterized by O/C ratios above 0.5 and H/C ratios below 0.3, according to Mueller (1963). Three requirements for

their formation are identified: high pressures, high temperatures, and the presence of uranium. The high pressure and high temperature necessary would have accelerated the deterioration of the evidence from which their possibly biogenic origin might have been traced. If tucholites are not of abiogenic origin, they represent a final stage of an extreme conversion of biochemical substances.

Two other groups of heavy hydrocarbons that show some evidence of abiogenic origin are the carbonaceous compounds in hydrothermal veins, also called hydrothermal "minerals," and the igneous hydrocarbons occurring in association with alkaline or basic intrusions. The hydrothermal hydrocarbons are bitumens. They do not have such a high O/H ratio as the tucholites. That these hydrocarbons, often associated with Permian igneous activity, have been juvenile in the parent magma has been suggested (Dons, 1956). However, the possibility exists that they have been derived from Cambro-Silurian or other life-bearing sediments. Among the igneous hydrocarbons (Kropotkin, 1959), bitumens similar to crude oils and volatile fractions consisting of light hydrocarbons, CO and H, have been found. The suggestion has been made that they are syngenetic and were formed by the hydrogenation of dispersed carbon (or of carbon dioxide) during crystallization of the magma. Whether hydrocarbons including bitumens and asphalt could subsequently have formed from presumably juvenile CH₄, H₂, H₂O, and CO₂ under various pressures within magmatic materials is a question of chemical equilibria, provided the conditions are those of a thermodynamically closed system. Calculations of these equilibria have shown that the formation of many organic compounds from CH₄, H₂, H₂O, and CO₂ is quite favored at low pressures and at temperatures between 300°K and 1000°K, preferentially close to 300°K (Eck et al., 1966).

GENERAL CONCLUSIONS

Reviewing our knowledge of the occurrence of fossil biochemicals, we note that molecular fossils that have been converted under geological conditions may be found in relative abundance in sediments deposited during the Cambrian Period (about 500 to 570 million years ago) and since then; however, only a few biochemical traces can be found from Precambrian organisms. The biogenic origin of at least some of the hydrocarbons found to be associated with the microfossils of the Fig Tree Series and Onverwacht Series (more than 3.2 billion years old) is debatable (page 292).

Even more questionable would be the origin of carbonaceous

compounds in sedimentary rocks older than those of the Onverwacht Series if unmetamorphosed rocks of this age could ever be found. No direct evidence is yet available with regard to the occurrence and origin of carbonaceous materials during the era of prebiotic and early biotic evolution on Earth or on any other celestial body.

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CHAPTER 11

*Extraterrestrial
Molecular Evolution*

*Man is formed not only of the biblical dust
of the ground; he can now trace his origins
to the dust of stars.*

Our understanding of the molecular basis for protobiogenesis has three main sources. One is the cosmic abundance of radicals containing the bioelements hydrogen, carbon, oxygen, and nitrogen. Another is the presence in interstellar space of substantial amounts of formaldehyde, ammonia, hydrocyanic acid, and other compounds. The third is the production in the laboratory of many kinds of biologically significant molecules from simple intermediates. Terrestrial and extraterrestrial observations have led to the conclusion that the probability is extremely low that molecular evolution followed any imaginable alternative mode, whether based on silicon instead of carbon, whether requiring liquid ammonia instead of water, or whether proceeding from antimatter.

The question of whether life evolved independently on Earth or was brought to the Earth from outer space early in the Earth's history

seems of secondary importance in a broad evolutionary context. Conditions favoring the emergence of primitive life now appear, in any event, to have existed on the Earth.

Many ideas concerning place, time, and circumstances of the first emergence of life in the Universe have been put forward throughout human history. Particularly long-lived were the doctrines of the ancient Greek philosophers, among whom Aristotle is the most cited author. According to Aristotle, living systems are produced by the combination of the passive principle "matter" with the active principle "form." The active principle comprises the self-ordering forces (*entelechies*) that vivify matter. These forces are already present in the primary elements (earth, water, air, and fire) of which all things are made. The vitalistic doctrine that was widely held during the nineteenth century is closely related to Aristotle's thesis. The vitalists believe that a vital principle or a vital force, being distinct from all physical and chemical forces, is a crucial and supraphysical attribute of all living things. The coming into existence of a "life force" as a supernatural process is represented by literal interpretations of Genesis. This latter concept is not susceptible to experimental examination and has, therefore, not been discussed in this volume. Similarly, the original doctrine of panspermia cannot be tested by experimental investigation. This doctrine postulates that life on Earth once arose from "germs of life" brought to Earth from outer space. According to the nineteenth century doctrine of panspermia these germs of life, like the life force, are distinguishable from common matter, but in contrast to the life force they are something substantial. Until the beginning of the twentieth century their existence was believed to be eternal and universal.

The panspermia hypothesis was originally developed by Richter in 1865. Meteorites could indeed carry in their interior germs of life. While travelling in outer space, the interior of a meteorite would be relatively safe from the deadly cosmic rays and from extreme changes of temperatures. Even when their speed is rapidly slowed by collision with the Earth's atmosphere, the heat of friction will cause only some superficial heating of the meteorites (page 331). However, all attempts to isolate viable germs of life indigenous to a meteorite have failed so far.

Another hypothesis of panspermia has been put forward by the Swedish physical chemist Arrhenius. He suggested that spores of life, together with particles of cosmic dust, would travel through the universe propelled by the pressure of stellar rays. His reasoning faced serious objections by later investigators. One important argument against Arrhenius' hypothesis is the fact that any known terrestrial

organism would not survive the longterm effect of ultraviolet and other rays, provided it would survive extreme differences in temperature and the condition of an ultrahigh vacuum.

With two modified concepts, the classic hypothesis of panspermia has found a revival in our time. The doctrine that germs of life are universal and eternal is not held any longer. Thus, the question of the origin of life can be subjected to research. On the basis of data available we cannot entirely exclude the possibility, suggested by Robinson in 1966, that the germs of life were already present in the cloud of planetesimals, dust, and gases that aggregated to form the Earth and other bodies of our solar system almost five billion years ago (page 26). Also, we cannot safely deny that life was brought to us by meteorites, cosmic particles, or other natural means.

In the age of extraterrestrial flight, another modification of the panspermia hypothesis has been proposed—the concept of “directed panspermia.” According to the latter hypothesis, life has been deliberately brought to Earth by intelligent beings from an astronomically remote civilization.

One interesting argument in favor of (directed) panspermia has been developed by Crick and Orgel (1973) on the basis of molecular biological data. The genetic code and the machinery of protein biosynthesis are universal. The same genetic code, the same machinery of protein biosynthesis, and the same principal biochemical reactions operate in all living things on Earth. Theoretically there would be many alternatives to the contemporary genetic code. But since no alternatives are known on Earth one might conclude with Crick and Orgel that all contemporary life has evolved from one source. (An alternative view is represented on page 265). This germ of life could have been brought to Earth from outer space by (directed) panspermia. Crick and Orgel have argued that the germs of life probably evolved in some other part of the universe because there are apparent discrepancies between the concentrations of metals in certain biological systems and their abundance in the Earth. This argument, however, has been explicitly rejected by Banin and Navrot (1975).

Another criticism that has been voiced is that moving the origin of life to an extraterrestrial site also moves the problem to that locale. Only by the broadest interpretation invoking organic chemical precursors can the site be stretched to such a distance.

In yet another view, mechanism is directly opposed to vitalism. The mechanists teach that all natural phenomena are exclusively controlled by the laws of physics and chemistry. The principal mechanistic concept pertinent to the subject of this monograph is that the first living thing would have come into existence by the coincidental accumulation and aggregation of randomly distributed small molecules.

The classical mechanistic hypothesis has not yet been elaborated to take into account the molecular and submolecular forces that selectively and specifically would have brought together the various types of molecule during the processes of self-assembly of a protocell. The statistical chance of coincidental formation of a living system out of randomly distributed and nonspecifically interacting matter, according to this concept, was only extremely small. Accordingly, a long time and a large amount of starting material would have been needed to give the formation of a protocell a significant chance. According to this line of reasoning, the occurrence of such an event cannot be tested by a laboratory experiment.

Related to this classical mechanistic concept, and pertinent to the subject of the monograph, is the evolutionary explanation of the origin of life. The evolutionary theory is also entirely based on faith in the laws of chemistry and physics. It takes particularly into account the existence of specific submolecular and molecular forces that determine the self-ordering, self-organization, and self-assembly of matter (page 265). These concepts are evolutionary because they postulate that organization of matter into a living system evolved by a sequence of events beginning with the cosmic formation of the first elements (pages 19, 349). At least one rendition of astrophysical theory proposes that molecular evolution was already nonrandom at its cosmic outset (Sakharov, 1965). The first living thing thus emerged from non-random processes. The sequence of events was largely determined at first by the surroundings in which these processes took place. In later stages, the evolution of organisms became increasingly controlled internally (page 265). The evolutionary theory has also been referred to as the materialistic theory, because this theory arose out of materialistic philosophy. In the materialistic view, life resulted from a continuous evolution of matter. Emergence of living systems from inanimate matter led directly into Darwinian evolution (Oparin, 1953, 1957; Engels, 1940; Urey, 1962).

Life may have evolved on Earth only, or forms of life akin to terrestrial life may exist on other planets of the Universe. The latter inference is based on the rationalization that life would be found with some degree of certainty on any planet not too dissimilar, chemically and physically, from the Earth. The number of stars in the observable Universe is about 10^{20} . At least 10^{18} stars are presumably surrounded by planets. According to Shapley (1963), about 10^{10} planets would be like our Earth in almost all respects and, therefore, likely capable of supporting life. We do not know, however, with what mathematical probability life might emerge on any of these planets. For these reasons, no realistic estimates can be given of the number of life-carrying celestial bodies, not to speak of the number of celestial

bodies carrying "intelligent" life. One goal of space research is to identify conditions that have led to the emergence of life, as well as conditions that have not led to its emergence; these two kinds of study contribute to each other.

More information may be available in the foreseeable future, after our closest neighbors in the Solar System, the Moon and the planets Venus and Mars, have been explored more closely (page 48). The Moon, however, is thought not to have been capable of bearing life since it was formed. The Moon has neither an atmosphere nor a hydrosphere (page 342). The conditions on Mars have appeared to be, at best, capable of supporting only the most lowly living organisms. The conditions on Venus are generally believed to be too hot (page 481) to permit any but very special life, although in regions near the poles the conditions may be more favorable (Libby, 1968a, 1968b; Brink and Stein, 1968). Even if none of these three celestial bodies at present bears living systems, a search for fossilized organisms and biochemical compounds on them might yield valuable information. Comparisons of such extinct forms of extraterrestrial life with terrestrial life should supply evidence for or against various details of the concepts of the origin of life (as would, of course, comparison with any extant forms). The lunar samples that have been examined so far leave no hope that fossil organisms will be found on the Moon (Barghoorn et al., 1970; Cloud et al., 1970; Fox et al., 1970; Schopf, 1970).

The exploration of our Solar System, which has begun in earnest, presents us with a number of formidable technological problems. These problems will expand enormously should we ever be able to send a spaceship to the planetary systems of the nearest single and Sun-like stars, Epsilon Eridani and Tau Ceti. Although close by astronomical standards, these stars are approximately 11 light years away. A round trip would require more than 22 years—probably exceeding the life span of some of the human members of the expedition, provided such a trip were to become technically feasible. A radio exchange with hypothetical beings at such distance would also require 22 years.

The probability of finding intelligent life in outer space naturally increases with the distance from the Earth, but even if such life were found, the difficulties of communicating with it over cosmic distances would be almost insurmountable. Even if we could radio an intelligible message to another civilization less than 100 light-years from the Earth, we would have to wait at least 100 years for an answer. Various attempts have been made to receive radio messages that might have been sent into space by other civilizations in order to contact us or someone else. So far, however, none of the radio signals

received from other stars can be interpreted as evidence for the existence of civilizations. Most likely for all of these extraterrestrial signals there are explanations within the framework of our present knowledge of chemistry and physics.

As yet we have no evidence proving the existence of extraterrestrial life. Three lines of evidence, however, guide us to the concept that chemical evolution has taken place and still takes place in outer space. This evidence is supplied by the data on interstellar matter, meteorites, and lunar samples and will be presented in the following sections.

INTERSTELLAR ORGANIC MATTER

During 1968 and 1969, astonishing discoveries of interstellar organic matter resulted from microwave spectroscopy. The first reports on polyatomic organic molecules came from two groups: Snyder et al. (1969) and Cheung et al. (1968, 1969). Microwave spectroscopy has revealed water, formaldehyde, and ammonia in separate regions in our galaxy. A most striking finding is the existence of formaldehyde in substantial amounts.

Formaldehyde is the first organic molecule to be identified in the interstellar medium. It was found in approximately 60 percent of the 22 sources searched at first. Essentially what was observed were large regions filled with clouds of formaldehyde, at concentrations of approximately 10^3 molecules per cubic centimeter. The kinetic temperature* in these clouds was found to be less than 10°K . Because of this finding, plus the finding of water and ammonia, the authors have pointed out that chemical evolution in interstellar space must have proceeded much further than previously had been thought (Snyder et al., 1969).

Ammonia has been detected in clouds having kinetic temperatures below 100°K . Some of the measurements have indicated that there are 10^3 molecules per cubic centimeter, approximately the same value found for hydrogen atoms in the same clouds. Perhaps one percent of the total nitrogen in such interstellar clouds is present as ammonia. Some measurements were made for the ammonia cloud

*The density of the interstellar clouds is about 14 orders of magnitude less than the density of the terrestrial atmosphere at sea level. The interstellar clouds are actually under the conditions of an ultra high vacuum. So far, such low pressures have not been reached in the laboratory, and a temperature cannot be ascribed to gases under such pressures. But the few atoms or molecules that are always present in a sufficiently large evacuated system will travel with a given speed, which depends on the energy content of the system. The kinetic energy of these atoms or molecules theoretically corresponds to a "kinetic temperature."

against a microwave background from Sagittarius A. These measurements indicated that the kinetic temperatures were in the range 17–30°K. The presence of water was also confirmed by a method that rules out the reading from water in the terrestrial atmosphere. The authors conclude that ammonia, water, and formaldehyde are each widely distributed throughout the galaxy. Both ammonia and formaldehyde have been identified as occurring in a number of sources of microwave signals (Townes, 1970). Since these compounds are present at extremely low densities and very low kinetic temperatures, we may infer that collisions of their molecules causing further reactions are relatively rare. According to Donn (1969), therefore, these compounds are in a state far from equilibrium.

The existence of formaldehyde, ammonia, and water in the galaxy is to be viewed in association with the expectation that clouds containing these compounds would at some point undergo physical condensation, possibly bringing into being mixtures of types of molecule. Such condensation, causing chemical reactions, might well have occurred before formation of planets, the Solar System, and the other bodies of the Universe. Moreover, the results indicate that raw materials for the synthesis of amino acids, polyamino acids, nitrogen bases, and nucleic acids probably were and are widespread throughout the Universe. The likelihood that molecular evolution would begin, even extraterrestrially, with a source of carbon that is already partly oxidized is considerably increased over what had previously been inferred. Evaluation of theoretical possibilities about the kinds of chemical reaction that would prevail during gradual condensation of interstellar clouds consisting in part of HCHO, NH₃, HCN, H₂O, H₂, and N₂ is difficult. A study of possible reactions under physical conditions simulating those prevailing in the clouds is not feasible at present because the necessary high vacuum techniques are insufficiently advanced to permit such experiments. What was probably necessary in order for molecular evolution to proceed was the physical condensation of the clouds. The possibility of this taking place raises a fundamental question: could the organic materials present in a condensing interstellar cloud survive under the prevailing conditions of the protoplanets (page 28)? We may recall that the proto-Earth is believed to have lost its primary atmosphere due to the heat produced during its aggregation (page 38). Also conceivable is the possibility that some “back-evolution” from the partially oxidized carbon to more reduced carbon would have occurred during chemical condensation in the presence of excess hydrogen. On the other hand, as warming proceeded escape of hydrogen would have been favored.

Experiments on production of amino acids by ultraviolet radiation of mixtures of ammonia, formaldehyde, and water at normal pressures

had earlier been reported (Table 4-12; Pavlovskaya and Pasynski, 1959; Reid, 1959). Several amino acids were found. More recently, heating of these reactants has been found to produce six to ten amino acids more readily than amino acids are produced by the other forms of energy (Fox and Windsor, 1970). The relevance of these experiments to chemical reactions in the interstellar medium is difficult to evaluate, as already indicated. Nevertheless, the data from studies of the interstellar medium is difficult to evaluate, as already indicated. However, these data provide us with a more factual basis for experiments than assumptions made by reasoning backwards from geological observations, or by theoretical inferences based on equilibrium constants for a Universe that is not in equilibrium.

The finding of cyanoacetylene is especially striking because of the fact that it had earlier been shown in the laboratory to function as an intermediate in the synthesis of cytosine, aspartic acid (Sanchez et al., 1966), and nicotinamide (Ferris et al., 1969).

Table 11-1 summarizes the molecules identified in interstellar matter up to late 1971. These were recognized almost entirely by microwave spectroscopy (Snyder and Buhl, 1970), with only a little confirmation provided by use of other methods.

The presence of SiO has been used to explain grains of silica in the interstellar clouds. This has been correlated with the postulate that such grains have served as sites for synthesis of organic compounds. The silica might conceivably also shield organic matter within clouds from destructive radiation.

METEORITES

Origins, Composition, and Classification of Meteorites

The first extraterrestrial materials available on the Earth for direct investigation were meteorites, meteoritic dust, and cosmic dust. Meteoritic dust comprises abrasion products of meteorites; cosmic dust is a mixture of primary finely dispersed matter. Both are found all over the Earth. Within even the extraterrestrial matter collected from Antarctic ice, however, terrestrial contaminations are evident. Cosmic dust collected by rockets would more likely be free of such contamination; it would thus be more appropriate material for investigations in an evolutionary context. No investigations of such materials have, however, so far been reported. Astronomers agree that meteorites originate within the Solar System. Most meteorites derive either from the 10^{15} asteroids of mass greater than 50 kilograms, or from

TABLE 11-1
Molecules Found in Interstellar Space

Molecule	Year	Authors
H ₂	1970	Carruthers
¹⁶ OH	1963	Weinreb et al.
¹⁸ OH	1966	Rogers and Barrett
SiO	1971	Wilson, Penzias, et al.
H ₂ O, water	1969	Cheung et al.
NH ₃ , ammonia	1968	Cheung et al.
¹² CH ⁺	1937	Dunham
¹³ CH ⁺	1969	Bortolot and Thaddeus
CH	1937	Dunham
CN	1938	Adams
C ¹⁶ O	1970	Wilson, Jefferts, et al.
¹³ C ¹⁶ O	1971	Smith and Stecher
¹² C ¹⁸ O	1971	Penzias, Jefferts et al.
CS	1971	Penzias, Solomon et al.
H ¹² C ¹⁴ N, hydrocyanic acid	1971a	Snyder and Buhl
H ¹³ C ¹⁴ N, hydrocyanic acid	1971a	Snyder and Buhl
OCS	1971	Jefferts et al.
H ₂ ¹² C ¹⁶ O, formaldehyde	1969	Snyder et al.
H ₂ ¹³ C ¹⁶ O, formaldehyde	1969	Zuckerman, Palmer et al.
H ₂ ¹² C ¹⁸ O, formaldehyde	1971	Gardner et al.
HNCO, isocyanic acid	1971b	Snyder and Buhl
H ₂ CS, thioformaldehyde	1971	Sinclair et al.
HCOOH, formic acid	1971	Zuckerman, Ball et al.
HC≡C—C≡N, cyanoacetylene	1971	Turner
CH ₃ OH, methanol	1970	Ball et al.
CH ₃ C≡N, methyl cyanide	1971	Solomon et al.
HCONH ₂ , formamide	1971	Rubin et al.
CH ₃ C≡CH, methylacetylene	1971b	Snyder and Buhl
CH ₃ CHO, acetaldehyde	1971	Ball et al.
X-ogen ^a	1970	Buhl and Snyder
HNC, hydrogen isocyanide	1971b	Snyder and Buhl

Source: Snyder (1972).

^aUnidentified relative to laboratory compounds.

their fragments. These asteroids orbit between Mars and Jupiter. A lunar origin has been postulated for certain meteorites (Urey, 1959), but this hypothesis is not consistent with the results of lunar analysis so far reported. Each year an estimated 5000 meteorites of one kilogram mass or more, each, are distracted into Earth-collision orbits after their movements have been perturbed by interaction with the gravitational fields of Mars and Earth. Of these meteorites, less than one percent are recovered. Meteorites enter the atmosphere at speeds of 12 kilometers per second or even less, or as fast as 70 kilometers

per second, depending on the direction of their movement relative to the Earth's orbit. In any event, their speed is rapidly slowed by friction; they hit the ground at comparatively low velocities—about 0.1–0.2 kilometers per second (Mason, 1962a). The friction produces intense heat causing some superficial melting and brilliant light phenomena. The exposure to frictional heat is so transitory, however, that material a few centimeters below the surface of the meteorite may show no evidence of thermal alteration. The speed of meteorites of mass 10 tons and more is not significantly slowed by friction. These heavy meteorites explode on impact with the Earth's crust. About 35 structures on the Earth have been identified, some only tentatively, as meteoritic craters. The Arizona Meteor Crater, which is 1.2 kilometers in diameter and 140 meters deep, is a well-known example.

As meteorites are exposed to cosmic rays, nuclides such as ^{36}A , ^{21}Ne , ^{36}Cl , and ^3He are produced in amounts which depend upon the time of exposure. Measuring the amounts of these nuclides in meteorites found on the Earth gives an indication of their age: stony meteorites were exposed to cosmic rays in space for 10–50 million years; most iron meteorites were exposed for $0.1\text{--}1 \times 10^9$ years. Since these time periods are shorter than the age of the Solar System ($4.5\text{--}5.0 \times 10^9$ years), the matter of meteorites must have been part of larger celestial bodies where it stayed protected from exposure to cosmic radiation almost from the beginning of the Solar System until an interplanetary disaster led to the destruction of these celestial bodies. Isotopic dating by the U-Pb or the ^{40}K - ^{40}A method (pages 39, 56, 57) has frequently yielded ages for the meteorites that are close to the age of the Solar System. The ^{40}K - ^{40}A method allows determination of the time interval during which the meteorites were not hot enough to outgas argon. The U-Pb technique yields ages that include the periods during which the meteorites were molten. Age measurements with these and similar methods have shown that the time between formation of the elements and final cooling of the meteorites probably was less than 10^8 years.

Meteorites are divided into four groups according to their iron content and their structure. These groups are (1) chondrites (stones); (2) achondrites (stones); (3) irons; (3) stony-irons. Of these groups, only the chondrites are of interest in the evolutionary context. This interest centers on the carbonaceous chondrites, a class within the group. Chondrites, in general, are composed of or contain chondrules, which are spheroids about $10 \mu\text{m}$ in diameter, of ultrabasic minerals. Chondrites are quite uniform in chemical composition. Mason's (1962b) analysis of a typical chondrite, Kyushu, revealed the following percentage composition: SiO_2 , 39.93; MgO , 24.71; FeO , 15.44; Al_2O_3 , 1.86; CuO , 1.70; Na_2O , 0.74; K_2O , 0.13; Cr_2O_3 , 0.54; MnO , 0.33;

TiO_2 , 0.14; P_2O_5 , 0.31; H_2O , 0.27; Fe metal, 6.27; Ni metal, 1.34; Co metal, 0.05; FeS, 5.89; C, 0.03. The composition of carbonaceous chondrites typically differs from these data mainly in that their carbon content varies from 0.2 to 5.0 percent; their sulfur content varies from 1.8 to 6.7 percent; and their water content (they contain hydrated minerals) varies from 0.1 to 22 percent. According to their composition, carbonaceous chondrites are divided, in turn, into three subclasses (Table 11-2).

Carbonaceous chondrites have elicited wide interest for at least two reasons: the debatable origin of the constituent organic compounds—that is, whether it was biotic or abiotic; and the debatable origin of the organized structures—that is, whether they are fossilized organisms or artifacts. Unfortunately, only a limited number of carbonaceous chondrites have been discovered in the past 150 years. On or after impact, the materials are contaminated to varying extents by compounds deriving from terrestrial organisms. Storage and handling in museums over the decades certainly further increased contamination. These facts make evaluations of the origin of the carbonaceous materials (Hochstim, 1963) and of the organized structures in any meteoritic materials tenuous. More than 20 carbonaceous chondrites have been collected and investigated during the past 150 years. Data about most of these are listed in Table 11-3.

Carbonaceous Chondrites and Organized Structures

The controversy over whether the carbonaceous materials in meteorites are of biotic or abiotic origin began early in the nineteenth century. Berzelius (1834b) suggested that the organic material is not of biotic origin. Wöhler (1860), however, reported evidence that he interpreted as indicating that the material did originate from living organisms. Towards the end of the last century, Hahn (1880, 1882)

TABLE 11-2
Partial Composition of Carbonaceous Chondrites

Carbonaceous Chondrite	Percent Carbon	Percent Sulfur	Percent Water
Type I	2.7-5.0	5.2-6.7	18-22
Type II	1.1-2.8	2.3-3.7	8-17
Type III	0.2-0.6	1.8-2.4	0.1-1.5

Source: Wiik (1956).

TABLE 11-3
Carbonaceous Chondrites Collected on the Earth

Name and Place of Fall	Date and Time of Fall	Coordinates of Place of Fall	Approximate Mass of Collected and Preserved Portion
1. <i>Alais</i> , France	1806, Mar. 15, 5 P.M.	44°7'N: 4°5'E	6 kg
2. <i>Cold Bokkeveld</i> , S. Africa	1838, Oct. 13, 9 A.M.	32°5'S: 19°20'E	several kg
3. <i>Crescent</i> , Oklahoma, U.S.A.	1836, Aug. 17, 7 P.M.	35°7'N: 97°35'W	80 g
4. <i>Felix</i> , Alabama U.S.A.	1900, May 15, 11:30 A.M.	32°32'N: 87°10'W	7 lb
5. <i>Haripura</i> , India	1921, Jan. 17, 9 P.M.	28°23'N: 75°47'E	500 g
6. <i>Indarch</i> , USSR	1891, Apr. 7, 8 P.M.	39°55'N: 46°40'E	27 kg
7. <i>Ivuna</i> , Tanganyika	1938, Dec. 16, 5:30 P.M.	8°25'S: 32°26'E	several kg
8. <i>Kaba</i> , Hungary	1857, Apr. 15, 10 P.M.	47°21'N: 21°18'E	3 kg
9. <i>Lance</i> , France	1872, July 23, 5 P.M.	47°42'N: 1°4'E	
10. <i>Mighei</i> , Ukraine	1889, June 13, 8:30 A.M.	48°4'N: 30°58'E	8 kg
11. <i>Mokoia</i> , New Zealand	1908, Nov. 26, 12:30 P.M.	39°38'S: 174°24'E	10 lb
12. <i>Nawapali</i> , India	1890, June 6, 6 P.M.	21°15'N: 83°40'E	60 g
13. <i>Nogoya</i> , Argentina	1879, June 30, afternoon	32°22'S: 59°50'W	about 4 kg
14. <i>Orgueil</i> , France	1864, May 14, 8 P.M.	43°53'N: 1°23'E	several kg
15. <i>Santa Cruz</i> , Mexico	1939, Sept, 3, noon	24°10'N: 99°20'W	?
16. <i>Simonod</i> , France	1835, Nov. 13, 9 P.M.	46°5'N: 5°20'E	
17. <i>Tonk</i> , India	1911, Jan. 22, 4 P.M.	24°30'N: 76°52'E	8 g

Source: Briggs and Mamikunian (1963).

Note: The names of the other carbonaceous chondrites, which were not included in this list because data on them are not available, are Boriskino, Warrenton, Ornana, Bali, St. Caprais, Al Raïs, Erakot, and Vigarano.

claimed that certain stony meteorites contained indigenous fossils. His critics, however, insisted that the structures under discussion were inorganic artifacts and that some of the fossil-like structures were merely chondrules.

Pasteur apparently was one of the first to investigate whether life in the form of bacterial spores was brought to the Earth with meteorites (Becquerel, 1924). His results were negative as were those from later studies (Gallipe and Souffland, 1924). In the nineteen thirties, Lipman (1932, 1936) claimed the detection of a number of bacterial species in the centers of surface-sterilized meteorites. These reports naturally elicited some controversy. Roy (1935) isolated *Bacillus subtilis* and *Staphylococcus albus* from three of twelve meteoritic samples. Most likely, however, these microorganisms entered the meteoritic materials after the fall. Imshenetsky (1966) reported that a heat-sterilized meteorite was infected by lying on the soil of Moscow. Within four days it had been infected not only on the surface, but also throughout the interior.

More recently, Briggs and Kitto (1962) reported that the interior of the meteorite Mokoia is sterile. Sisler (1962) detected some unidentified aerobic species in the interior of the meteorite Murray. Rubchikova (1962) reported the isolation of viable, unidentified microorganisms from the interior of another meteorite (Mighei), which had been sterilized by heating the surface to 150°C. However, most of these results need further confirmation, because they are not based on sufficiently rigorous evidence. Terrestrial contamination is one obvious explanation for the findings of microorganisms in meteoritic material.

Also, the observation of organized structures, particularly in carbonaceous chondrites, has continued to stir many controversies up to the present time. Soon after Nagy, Meinschein, and Hennessey (1961) concluded that the hydrocarbons of the Orgueil meteorite are of biotic origin (as will be discussed in the next section), Claus and Nagy (1961) and Nagy et al. (1963) claimed that the organized elements that they found in Type I carbonaceous meteorites are also of indigenous biotic origin.

Fitch and co-workers (1962) and Fitch and Anders (1963), on the other hand, maintained that the microstructures in the Orgueil meteorite are mineral artifacts. Briggs and Kitto (1962) concluded that the microstructures are open to interpretations of both biotic and abiotic origin. Urey (1962) postulated that meteorites may have ultimately originated from the Moon, which, in turn, might once have been contaminated by waters from oceans of the Earth or other planetary bodies. The evidence indicating that the microstructures in meteorites may be of biotic origin is no more than suggestive as yet. In

more recent studies, Nagy and his associates (Nagy et al., 1963; Nagy, 1966a,b) try further to support the claim that the organized elements are indigenous to the meteorite and do not result from contamination. This interpretation agrees with the studies of Tasch (1964). However, rigorous evidence which allows exclusion of the possibility that biotic terrestrial materials contaminated the meteorite during the decades of time of storage and handling prior to the recent analysis is not presented by the authors.

Another approach to the study of the organized microstructures in meteorites has been used by Fox and Yuyama (1963). The kind of morphology observed in the microstructures in meteorites can be compared with the morphology of proteinoid microspheres. After pictures of the formed elements were published (Claus et al., 1963; Claus and Nagy, 1961), Fox and Yuyama (1964) searched photomicrographs of proteinoid microparticles and found several bearing resemblances to those of meteoritic microstructures. Some examples for comparison are given in Figures 11-1 to 11-3.

The similarity of the pairs of structures in Figures 11-1 to 11-3 is evident. The diameters are in the range of many microns in all cases. Figures 11-1 to 11-3 permit judgment of such comparisons. The unit shown on the left at the bottom. In Figure 11-3,A is what Nagy described as "two daughter bodies" or "organized elements" that appeared to undergo "cell division." Figure 11-3,B shows an artificial structure produced in the laboratory. Several inferences can be drawn from these comparisons. One is that the organized structures in the meteorite are indigenous but never were alive. They could

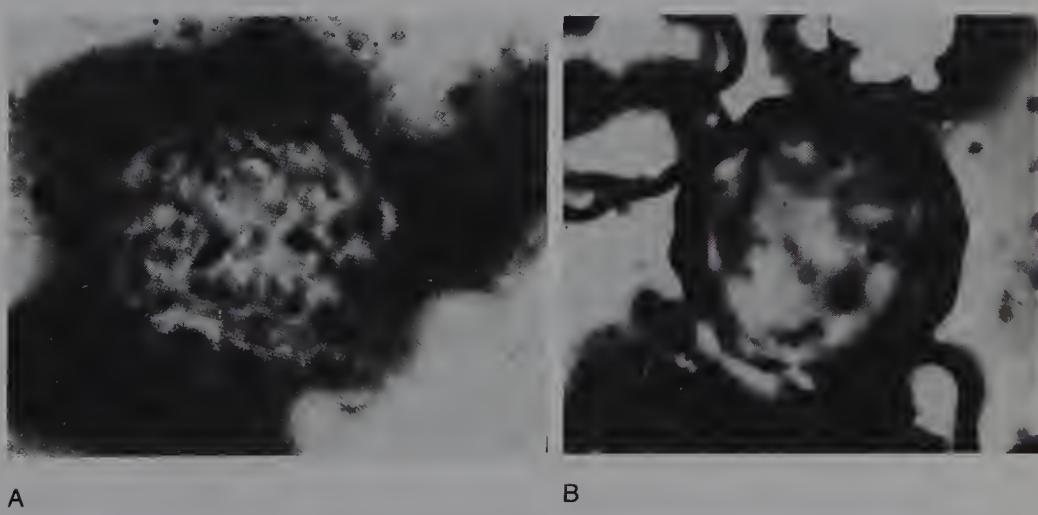


FIGURE 11-1
Organized microstructure: A, from a carbonaceous chondrite (courtesy of Bartholomew Nagy); B, from the laboratory.

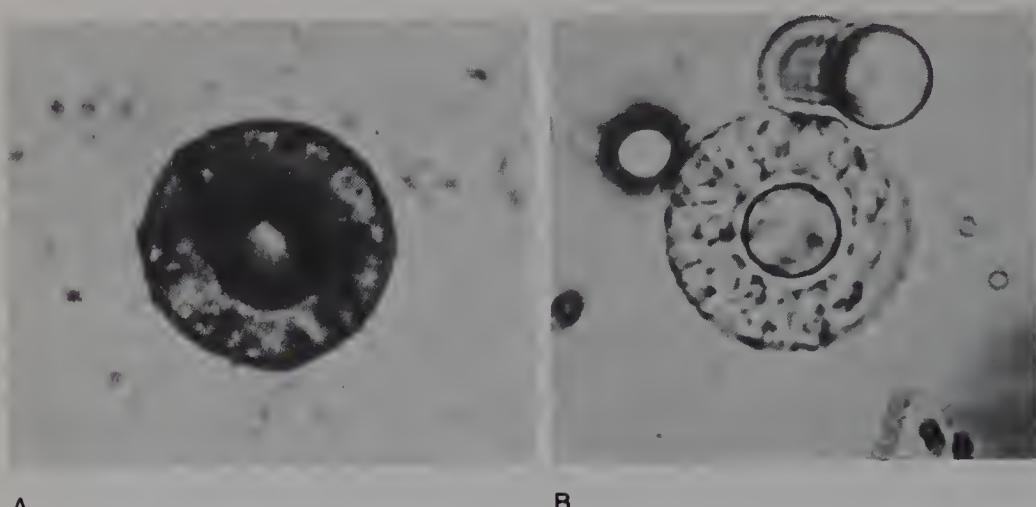


FIGURE 11-2

Organized microstructure: A, from a carbonaceous chondrite (courtesy of Bartholomew Nagy); B, from the laboratory.

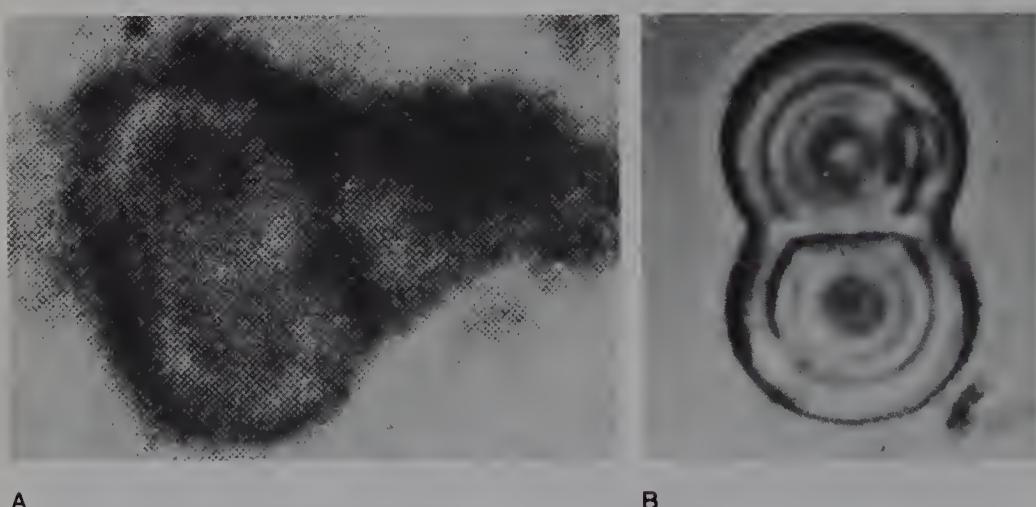


FIGURE 11-3

Organized microstructure exhibiting division: A, from a carbonaceous chondrite (courtesy of Bartholomew Nagy); B, from the laboratory.

have been organic polymers in supramolecular complexes or mineral substitution products thereof. A second inference is that the similarities strengthen the belief that the laboratory experiments are akin to natural processes. The necessary conditions to produce the structures such as presence of water, although now chemically bound, and thermal gradients, are found in the carbonaceous chondrites and possibly existed on their parent bodies (Mason, 1962b). Complex microstructures with morphological resemblances to contemporary cells are easily produced in the laboratory from polyamino acids (page 196).

Relationships have been observed between microstructures found in old sediments and ones obtained in the laboratory (page 304; Fox, 1969). Kremp (1968), on the basis of his laboratory experiments on mineralization of blue-green algae, suggests that the fossil-like structures in the Orgueil meteorite were formed from prebiological systems, perhaps like coacervates or proteinoid microspheres. Some of the microfossils found in Precambrian sediments could have a similar origin (page 303; Fox, 1969). We have to bear in mind, however, that meteorites are not sedimentary materials. This fact diminishes the likelihood of finding the same types of fossilized cells or protocells in meteorites that paleontologists find in Precambrian sediments.

Organic Compounds in Meteorites

Since Berzelius (1834a) reported his first results, a large number of organic compounds have been isolated from meteorites by treatment with substances that dissolve carbon compounds or by exposure to high temperatures (Berthelot, 1868; Calvin, 1961; Calvin and Vaughn, 1961; Hayes, 1967; Mueller, 1953; Smith, 1876). Among the organic materials that have been isolated are aromatic compounds, straight-chain and cyclic paraffins, urea and acetamide, chlorinated hydrocarbons, nitrogen bases, and low-molecular-weight aliphatic fatty acids and their derivatives, including esters and amino acids. Even porphyrins have reportedly been isolated from the Orgueil meteorite (Hodgson and Baker, 1964). Many of the organic compounds isolated from meteorites are of biological significance. In connection with the finding of organized structures in those same meteorites, a number of investigators have claimed that the organic materials are of biotic origin. Nagy et al. (1961) have reported that the distribution of the various molecular species of hydrocarbons from both the Orgueil and Murray meteorites resemble the hydrocarbon distribution found in some materials of terrestrial biotic origin, including recent sediments. From these results, Nagy and his associates concluded that the hydrocarbons found in meteorites are of biotic origin. This inference has been criticized in at least two ways. Anders (1961) has pointed out that the agreement between the gas chromatograms of the meteoritic materials and those of various samples of terrestrial biotic origin was not very close.

Another criticism is that the unbranched paraffins, which are abundantly released from each kind of material, do not necessarily indicate a biotic origin. Although unbranched fatty acids are typically produced by contemporary organisms, A. T. Wilson (1960) has shown that they are also produced under special conditions in model prebi-

otic experiments. Because secondary C-radicals are more long-lived than primary C-radicals, branching of hydrocarbons is favored when, e.g., electric discharges act on a mixture of methane, ammonia, and hydrogen. However, if the reaction takes place above a conducting salt solution, so that the discharge continually strikes the liquid-gas interface, the formation of unbranched hydrocarbons is favored. The mechanism seems to entail the initial collection of low-molecular-weight hydrocarbons on the surface of the aqueous phase where they tend to become stacked parallel to each other and perpendicular to the plane of the aqueous interface. In this way only the end of the hydrocarbon chain points towards the reaction zone. The small alkyl radicals produced in the atmosphere therefore probably combine with the terminal carbon atoms of the liquid hydrocarbons. Thus, unbranched hydrocarbon chains grow out of the liquid layer.

The presence of hydrocarbons can also be explained as due to contamination. Some of the samples, such as Orgueil, may have been dusted with oily rags in the museum; others may have been contaminated with crumbs of wax (Fox, 1966).

Another controversy has arisen over the question of whether the organic materials in some of the first meteorites to be examined have optical activity. Nagy et al. (1964) reported observation of an optical rotation of $-0.023 \pm 0.005^\circ$ in an extract from the Orgueil carbonaceous chondrite, which is chiefly composed of organic acids and hydrocarbons. They concluded: "It seems reasonable to connect optical rotation in Orgueil with biological activity, either of indigenous origin or perhaps still related to terrestrial contaminations." However, Hayatsu (1964, 1965) was unable to verify the findings of Nagy et al., and concluded that the fatty acids and hydrocarbons in Orgueil do not have a detectable optical rotation. The highest rotation that he found was -0.002° , less than that of optically inactive controls. Hayatsu suggested that "the 'rotations' observed by Nagy may have been caused by scattered depolarized light from colloidal particles, reduced instrument sensitivity due to low transmittance of the solutions, or a combination of both." These two sources of error have been pointed out by others as well (Rouy et al., 1963; Rouy and Carroll, 1966). In addition, Hayatsu suggested that these negative results are of no significance with respect to the question regarding the origin of the organic materials. Most optically active carboxylic acids have large specific rotations. In a sample containing a mixture of acids, however, mutual cancellation of optical rotation will reduce the net values considerably. Perhaps for this reason, Nagy et al. (1964) were unable to find any optical rotation in a fatty acid preparation from brown algae or in the naphthenic acids (of presumed biotic origin) from petroleum.

More recently Nagy (1966b) reported reexamining the organic material from the Orgueil meteorite. After eliminating most of the indicated sources of error, he came to the same conclusions, although a satisfactory explanation cannot be offered. Even if there actually is optical activity, its interpretation is still open to question. The optical activity may originate from terrestrial contamination; it may stem from extraterrestrial and indigenous matter, or both explanations may pertain simultaneously to the same material. Moreover, reasons for not accepting the inference that optical activity is associated with biotic origin were presented in Chapter 8.

Another result has been obtained for the amino acids from the Orgueil meteorite (Anders et al., 1964; Kaplan et al., 1963). If these amino acids consisted of only one enantiomeric form, rotation of as little as 0.0046° would result for a given extract. The actual value was found to be even below 0.001° .

Hodgson and Baker (1964) reported that they found evidence for the occurrence of porphyrins in the Orgueil meteorite and suggested a biotic origin. As already demonstrated, however, this suggestion is not conclusive, since porphyrins are also formed abiotically under natural conditions (page 123).

Similarly, Studier et al. (1965, 1966), after reinvestigating the composition of various meteoritic samples, suggested that the organic materials of carbonaceous chondrites are of abiotic origin. According to these authors, two alternatives for the origin of the organic compounds must be considered, provided their abiotic origin from the primordial gas (page 327) is accepted: equilibrium processes and nonequilibrium processes. The analytical results on the amino acids and hydrocarbons from the Murchison meteorite have been interpreted to suggest an abiotic origin of these organic compounds (Kvenvolden et al., 1970, 1971). The Murchison meteorite fell September 28, 1969, near Murchison, Victoria, Australia. Some stones that had the least apparent contamination and the fewest cracks were selected to be analyzed were organic compounds. Among the amino acids identified were glutamic acid, proline, glycine, sarcosine, alanine, valine, and 2-methylalanine. The total concentration of glycine, alanine, valine, proline, and glutamic acid was $15 \mu\text{g/g}$. The presence of almost equal amounts of D and L enantiomers of alanine, valine, proline, and glutamic acid suggested that these amino acids are not recent biological contaminants. The discovery of 2-methylalanine, sarcosine, and other nonproteinous amino acids supports the conclusion that the amino acids were produced abiotically. Some of these compounds have been produced in evolutionary model experiments and simulated atmospheres (page 69; Miller and Urey, 1959).

An abiotic origin is also indicated by the gas chromatograms of alkanes isolated from benzene-methanol extracts of the Murchison meteorite. These chromatograms resemble the gas chromatograms of aliphatic hydrocarbons synthesized by the action of electrical discharges on methane (page 100; Ponnamperuma et al., 1969).

Contamination by either terrestrial organisms or organic compounds in the Earth's atmosphere or on the soil has not been ruled out. The Murchison meteorite lay on the ground before being collected and analyzed; Imshenetsky (1966; page 334) had shown the ease and rapidity with which the (porous) carbonaceous chondrites become contaminated. No sterility tests were reported for the Murchison chondrite.

Contamination by organic substances in the atmosphere (break-down products of bacteria, for example) and chemical conversion of such materials is a real possibility in view of the relatively porosity of many chondrites and the heat of their anterior surfaces during travel in the air. The speed of travel is such that compounds in the atmosphere, or their conversion products, might be driven into the interior. As is well known, the anterior surface is at red heat, although the posterior surface is cool in large meteorites. Such a thermal gradient favors organic chemical conversions, especially since chondrites contain chemically bound water (Mason, 1962a), which may become involved in some of the conversion processes. Racemization of amino acid residues is also favored by heating; the rate of racemization under conditions of atmospheric friction is not known.

The $\delta^{13}\text{C}$ content (Calvin, 1969) of carbonaceous materials from meteorites has been used to characterize these materials and to differentiate them from terrestrial material, as in Table 11-4. The relative proportion of ^{13}C in meteoritic amino acids was not reported, only the total carbon, the carbonate, and organic soluble carbon. Libby (1971) has calculated that terrestrial carbon has the same isotopic composition as that in meteorites, i.e. $\delta^{13}\text{C} = -6.9\text{ ‰}$. This is in contrast to the + values for lunar fines which, Oro states emphatically, is indicative of nonterrestrial carbon.

The evidence is stated to "suggest the indigenous nature of amino-acids and hydrocarbons in the Murchison meteorite." This evidence consists of the particular amino acid profile in which glycine is prominent, the positive $\delta^{13}\text{C}$ values in the extractable carbonaceous matter, the finding of amino acids that are close to being racemic, and the presence of a number of nonproteinous amino acids (Kvenvolden et al., 1971).

The bill of health of the meteorite is not improved by the carbon isotope studies. All of the other kinds of evidence can be explained by reference to chemical reactions in a hot, moist meteorite. Perhaps

TABLE 11-4
Carbon Content of Meteoritic Samples and Others

Meteorite or Other Source	Permil $\delta^{13}\text{C}^a$
Ivuna	- 7.5
Orgueil	-11.6
Mighei	- 5.6
Cold Bokkeveld	- 7.2
Erakat	- 7.6
Murray	-10.3
Mokoia	-18.3
Murchison	- 7.2
Average indigenous terrestrial sample containing biotic carbon	-24.0
Apollo 11 lunar fines	+13, +18.5

Source: Libby (1971) except the lunar fine values, which are from Oro et al. (1970).

^aPermil $\delta^{13}\text{C}$ expresses the relative divergence between the content of the isotope, ^{13}C , in a given carbonaceous sample and a carbonate standard that is stored in the National Bureau of Standards in Washington, D.C.

the amino acid profile is of most interest. Studies of the course of decomposition of contaminating peptides deserve to be carried out.

Despite the reservations expressed, the likelihood that amino acids, or precursors hydrolyzable to amino acids, are indigenous to the meteorites seems relatively high. Systematic testing of the uncertainties will, however, require much time, and the results may have to be verified on meteorites yet to be captured.

Equilibrium concentrations of more than 100 organic compounds for various proportions of carbon, hydrogen, and nitrogen in the atmosphere and at temperatures and pressures ranging between the limits 300–1000°K and 10^{-6} –300 atm have been calculated by Dayhoff et al. (1964; see also Table 4-15). When comparing the calculated equilibrium distributions (for a C:H:O ratio of 1:1:1.33) with the distribution of volatile organic compounds from carbonaceous chondrites, Studier et al. (1965, 1966) found the latter in good general agreement with the calculated values.

The equilibrium calculations of Dayhoff et al. (1964), however, do not account for all organic compounds found in meteorites. Among those compounds not accounted for are butene, toluene, diethylbenzene, trimethylanthracene, and long-chain fatty acids (Nagy and Bitz, 1963). Also, chlorinated hydrocarbons that have been found in

carbonaceous chondrites were not included in those calculations. Organic compounds containing amino nitrogen, such as amino acids or nitrogen bases, have not been considered because they are produced in insignificant amounts under the equilibrium conditions considered by Dayhoff et al. (1964), although they have been found in significant proportions in carbonaceous chondrites (Anders et al., 1964; Calvin and Vaughn, 1960; Hayatsu, 1964; Kaplan et al., 1963; Vallentyne, 1965). To account for these nitrogen compounds, non-equilibrium reactions have to be considered. By exposing a mixture of NH_3 , CH_4 , and H_2O briefly to a temperature of about 1000°C , several investigators have obtained amino acids from intermediates (page 82). Similar nonequilibrium reactions probably account for aliphatic hydrocarbons—the amounts of which cannot be satisfactorily explained by equilibrium reactions alone (Studier et al., 1965).

Urey and Lewis (1966) have criticized the reports of Studier et al. (1965, 1966) and Dayhoff et al. (1964) by stressing that only graphite would be stable under the conditions considered by the investigators. In a strict sense, this objection cannot be rejected when the term equilibrium—rather than nonequilibrium—is used. However, as Studier et al. (1966) pointed out in their reply to the criticism of Urey and Lewis, the formation of graphite proceeds extremely slowly but the equilibrium state of the organic reactants is reached much faster, in many cases within seconds, under the conditions postulated.

According to Urey and Lewis (1966), the organic compounds in meteorites “could only have been produced by high-energy radiations or in some instances by living organisms of either terrestrial or extraterrestrial origin.” So far, however, no radiation chemical synthesis in significant yield of complex, aromatic compounds, asphalt, or unbranched hydrocarbons from a primordial gas mixture can be visualized (although the production of such materials by heat is plausible). In such processes, the formation of light alkanes is likely to dominate the production of heavy alkanes or of aromatic compounds. Furthermore, the formation of branched hydrocarbons occurs more frequently than that of the unbranched, due to cross-linking between hydrocarbons (Swallow, 1960).

SURFACE OF THE MOON

The Apollo program provided an opportunity for a series of repeated analyses of similar individual extraterrestrial samples. Because the samples were collected and processed with great care, they were not subject to the doubts that apply to the meteorites, which neces-

sarily arrive through the Earth's bacteria-laden atmosphere and lie on unclean soil for periods sufficient for contamination.

The results of analyses of samples from twelve collections from Apollo 11, 12, 14, 15, 16 and 17 are summarized in Table 11-5. These analyses are of samples that yielded less than 50 parts per billion of amino acids; new details of nanotechnology based on ion-exchange separation, and using the discriminatory ninhydrin reagent, were developed for this study.

The early results of the Apollo investigation for amino acids were confused by controversy. Moreover, the earliest findings of minute proportions of amino acids in hydrolyzates of aqueous extracts of lunar samples contrasted to the anticipation of a number of authors (e.g., Sagan, 1960; Ponnamperuma, 1972). The high expectations were largely based on the experiments of Miller (1955), in which substantial amounts of several organic molecules were found. The fallacy lay in extrapolating such results to the surface of the Moon (or of a planet). Furthermore, had a substantial layer of organic matter ever formed on the Moon, the high hopes also underevaluated the rate at which solar radiation can activate the decomposition of organic compounds.

The experiments in the laboratory were carried out in a closed flask in order to retain the reactant gases; indeed, at the end of the experiment the gases contained up to 75% H₂ (Florkin, 1975). No such closed system exists or existed on the surface of the Moon; it is for this reason that the laboratory results were not transferable.

Some investigators discounted as due to contamination the first positive, but minute, results obtained by two analytic teams (Fox et al., 1970; Nagy et al., 1970). One team, however, obtained repeated and consistent results on twelve collections from all of the six missions (Fox et al., 1973, 1976). The controversy at first revolved around methodology.

The sample preparation was standardized by extracting with hot water and hydrolyzing the extract. (Direct hydrolysis of geologic samples had been known to result in loss of amino acids as judged by the final assay.) The main material in the lunar fines is properly regarded as amino acid precursor(s), rather than as amino acids per se, because hydrolysis is needed to liberate or form the amino acids.

Some of the early confusion was resolved by a confrontation between two groups in the laboratory. Analyses were performed in two ways by ion-exchange chromatography (IEC), and by gas-liquid chromatography (GLC) of volatile esters of the amino acids on a special sample carefully collected during Apollo 14. This special sample was opened under rigorous conditions in a clean-room in

TABLE 11-5
*Systematic Analyses by Ion Exchange Chromatography of Amino Acid Contents in Hydrolyzates of Returned
 Lunar Samples (molar ratios)*

Apollo Mission	Sample	Glycine	Alanine	Glutamic Acid	Aspartic Acid	Serine	Threonine	Others	Total Amounts of Amino Acid (ng per gram of sample)	
									50	25
11	10086	49	16	27	1	1	1	0	19	19
12	12033	62	20	12	4	1	0	0	19	19
14	14003	47	26	20	6	1	0	0	30	30
14	14163	63	15	6	11	4	0	0	5-7 ^a	5-7 ^a
14	14240	57	7	13	7	10	2	4	37	37
14	14298	61	6	16	6	6	2	3	7	7
15	15012	73	8	3	2	7	2	4	12	12
15	15013 ^b	56	6	19	5	8	1	5	12	12
16	66041	83	12	0	3	1	0	1	30	30
17	70011 ^b	70	11	7	7	3	1	0	10	10
17	72501									

^aDetermined under atypical conditions.

^bFrom beneath descent engine.

Berkeley. The values obtained by IEC were confirmed by GLC (Figure 11-4). These latter results showed, however, that the GLC method (Gehrke et al., 1975) could be used only for confirmation, not as a basis for primary identification. The IEC method revealed few or no peaks larger than those due to amino acids, whereas the GLC method showed many peaks larger than those from amino acids. Accordingly, primary identification was possible only with the more discriminatory ninhydrin-based ultramicro IEC method.

Potential contamination could be classified into two kinds, biological and chemical (rocket exhaust). The biological was hypothesized as due to astronauts, sample handlers in Houston, or to analysts. This possibility was effectively ruled out by several kinds of evidence: (a) the profile of 5–6 amino acids obtained was unlike that of 17–19 types found for contamination (fingerprints; Figure 11-4), (b) human contamination before hydrolysis (amino acid precursors) closely resembles contamination after hydrolysis (free amino acids), while the lunar extracts show manyfold to infinite increases on hydrolysis, (c) the regularity of the results from analyses of twelve successive collections, and (d) the finding of 30–200 times as much amino acids from analytical samples as from blanks carried through the entire sample preparation and analyses. Independent, firm summary statements that the results were not due to biological contamination were made by 1975 (Hamilton and Nagy, 1975; Fox, et al., 1976).

Critical attention could be paid to the chemical source (rocket exhaust) after lengthy arguments were settled regarding the basic fact that there were positive results, and the irrelevance of human contamination. Also, at about that time, suitable samples from Apollo 15 and 17 (near to the descent engine and maximally distant) were collected. What was learned from these samples is that the amounts found in analyses of samples collected at 4 km (Apollo 15) or 6 1/2 km (Apollo 17) from the descent engine were at least 50,000–100,000 times too high to be accounted for by conversion of the rocket fuel. This assessment (Fox et al., 1976) required some new auxiliary data on HCN, the one exhaust product which appeared to be hydrolyzable to amino acids.

The amount of conversion of cyanide to amino acids under conditions of hydrolysis of the samples was determined. The principal assumption then made was that all of the exhaust products were implanted into the surface of the Moon during the 12-minute descent of Apollo 17. Since it is obvious that most of this product must have been lost to the high vacuum of outer space, the assumption is an extremely conservative one. The proportion of calculated maximal contribution of exhaust to the analysis (less than about .001% of the amino acids found) would, accordingly, be yet smaller. These thor-

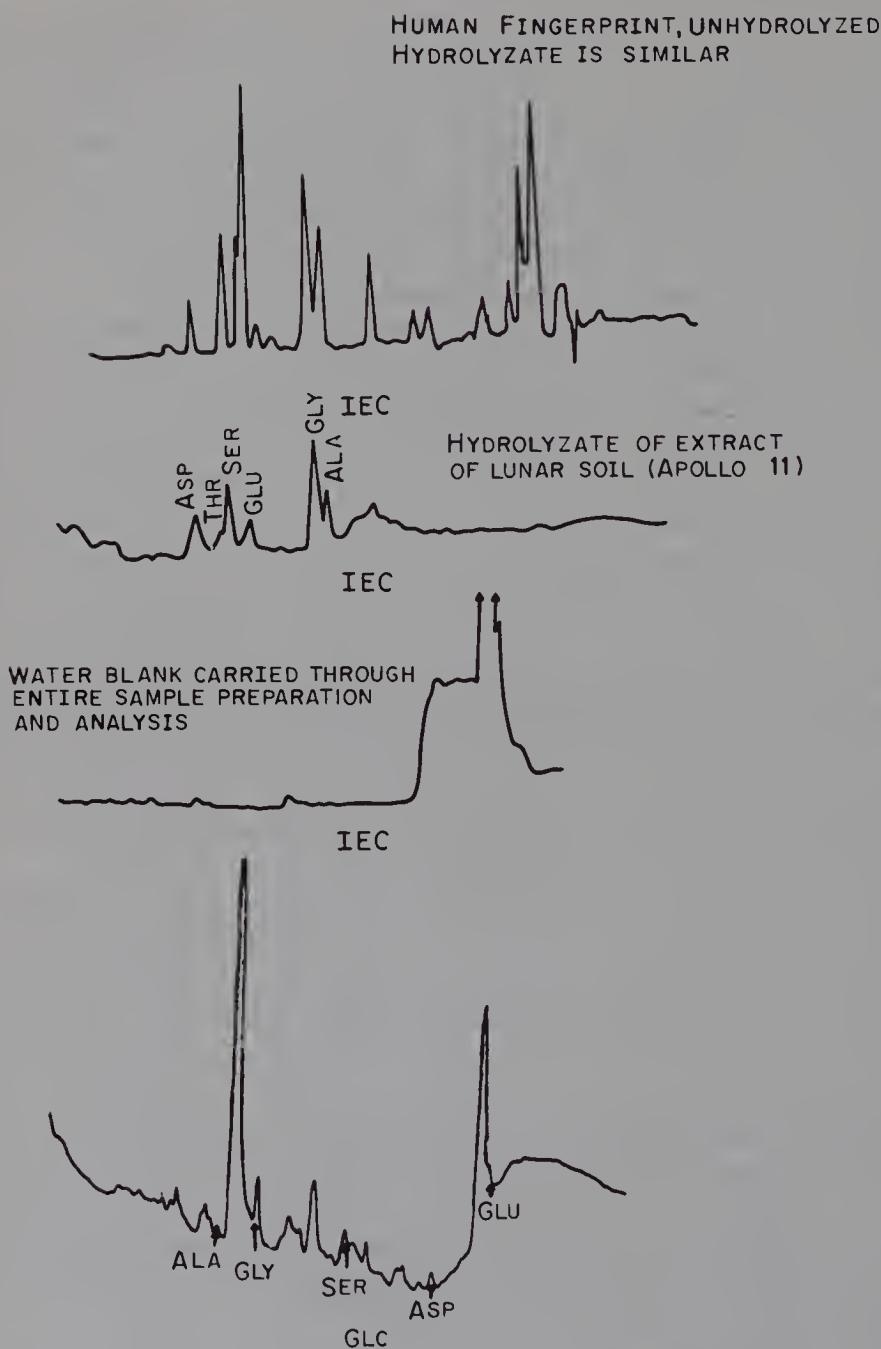


FIGURE 11-4.
IEC of hydrolyzate of extract of soil from Apollo 14, and GLC of volatile derivatives of hydrolyzate of extract.

ough and tested results yield the conclusion that the amino acid precursors obtained are predominantly indigenous to the lunar surface.

The results from lunar fines can be compared with those from the Murchison, Murray, and Allende meteorites; results from the anal-

ysis of earlier meteorites have been set aside mainly because the need for clean handling was not adequately recognized at the time they were analyzed. Some questions of cleanliness, however, apply also to the more recently collected material. All meteorites, carbonaceous chondrites included, arrive at land through the Earth's atmosphere, which is somewhat laden with bacteria. All meteorites have lain on the soil for some hours, days, or more. Imshenetsky (1966) has shown that bacteria penetrated into the interior of a meteorite sample placed on the soil of Moscow for a few days. The degree of infection in other specific instances has not been determined. Chemical changes may also have occurred during the meteoric travel, in view of the fact that the anterior portions of meteorites are heated to incandescence by friction in the Earth's atmosphere. Indeed, some nonproteinous amino acids look like decarboxylation (β -alanine) or rearrangement products. The fact that amino acids from meteorites are predominantly DL suggests they are nonbiological. The conditions of heating, however, leave open the question of thermal racemization.

Despite these reservations, a major indication of a set of a few proteinous amino acid precursors in meteorites is observed. The correctness of this observation is further enhanced by its analytic similarity to the discovery of amino acid precursors in lunar samples. It is also possible to compare amounts and types from meteorites with those from the Moon, especially since some of the meteoritic samples have been prepared for analysis by the procedure first developed for Apollo 11.

In comparing the total amounts, it is necessary to relate them to the amounts of carbon. When this was done, the total amounts of proteinous amino acids, as well as the species, were found to be similar for the two extraterrestrial sources. Both extraterrestrial sources, Moon and meteorite, thus provide evidence for extraterrestrial amino acid precursors. The findings from the Moon have been easily repeatable when the samples were properly prepared and analyzed by IEC (Table 11-5); much confidence can be placed in such repeated findings.

Although early publications spoke of *amino acids* in lunar dust and meteorites, the proper designation is that of *amino acid precursors* when hydrolysis is used to liberate (or produce) them. The compounds are precursors in an operational sense; they are produced by hydrolysis which is catalyzed, for convenience, by HCl. Hydrolysis, however, is a frequent geological process due to the long term presence of water. Precursors in the sense of laboratory operations are thus, with likelihood, evolutionary precursors.

The low range of amino acid precursors present in extraterrestrial samples suggests that the precursors may have been captured at the

surface of the Moon from interstellar clouds of organic matter (Snyder and Buhl, 1970) or from solar wind (Chang et al., 1971) which is very likely similar material. The presence of small amounts of organic material can thus be understood as due to a somewhat steady-state replenishment of material whose survival on the lunar surface was necessarily of limited duration.

Although the geophysical history of the Moon is undoubtedly different from that of the meteorites, the stage of cosmochemical evolution of carbon compounds is found to be similar. This is the first hard evidence, from two extraterrestrial sources, of a common evolution within the Solar System at the molecular level. Such findings have a number of significances (Fox, 1973). The indication for a common cosmochemical "metabolism" preceding the well-known unity of organismic biochemistry is especially enlightening. It betokens a unity in the Universe, rather than chemical chaos.

PROSPECTS FOR ASSESSING STAGE OF MOLECULAR EVOLUTION ON MARS

The data on Mars from the Viking vehicles had not been evaluated at the time this book was assembled in revised form. Because of earlier indications of a very thin atmosphere surrounding Mars, a greater likelihood for organic material (and cells) on Mars than on the Moon, has often been inferred in the past.

The probability of finding definable organic compounds was and is less for Mars than for the Moon. In the Apollo program, which was concerned with the Moon, samples were returned by astronauts. Reiterated analyses and scientist-directed selections of later samples were accordingly possible. Samples collected on Mars, on the other hand, have had to be analyzed *in situ*; the data were telemetered to Earth. Repeated analyses will require future activity in space.

The chemical tests run by remote control on Mars consisted of pyrolysis of surface samples and recognition of pyrolytic products by gas chromatography and mass spectrometry. Tests for cells that metabolize were also included in Viking. Because of the known catalytic activities of minerals and polyamino acids (page 173), any indications for such cells will require further examination (Fox, 1964). Telemetered Viking pictures on the surface of Mars have revealed it as comparable in barrenness to the surface of the Moon, although the Martian surface provided more indications of features interpretable as former rivers.

COSMOCHEMICAL SEQUENCES

A first tentative construction of a cosmochemical flowsheet now becomes possible (Figure 11-5; Fox, 1972). Ammonia and formaldehyde, and hydrocyanic acid and water, are abundantly available in

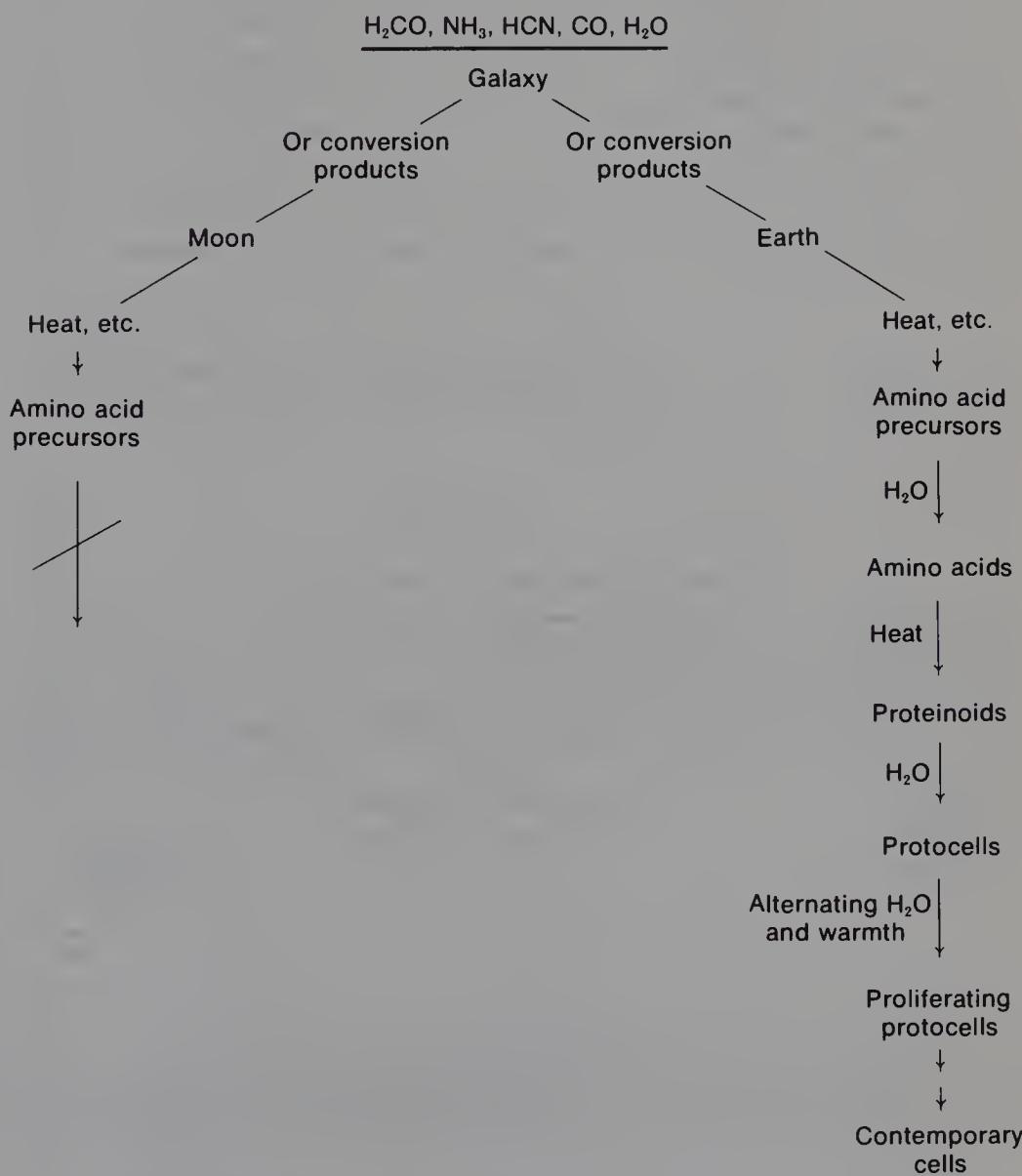


FIGURE 11-5

Biochemical origins within cosmochemical unity. On the Earth the flow may have proceeded as indicated on the right; on the Moon it may have terminated as indicated on the left.

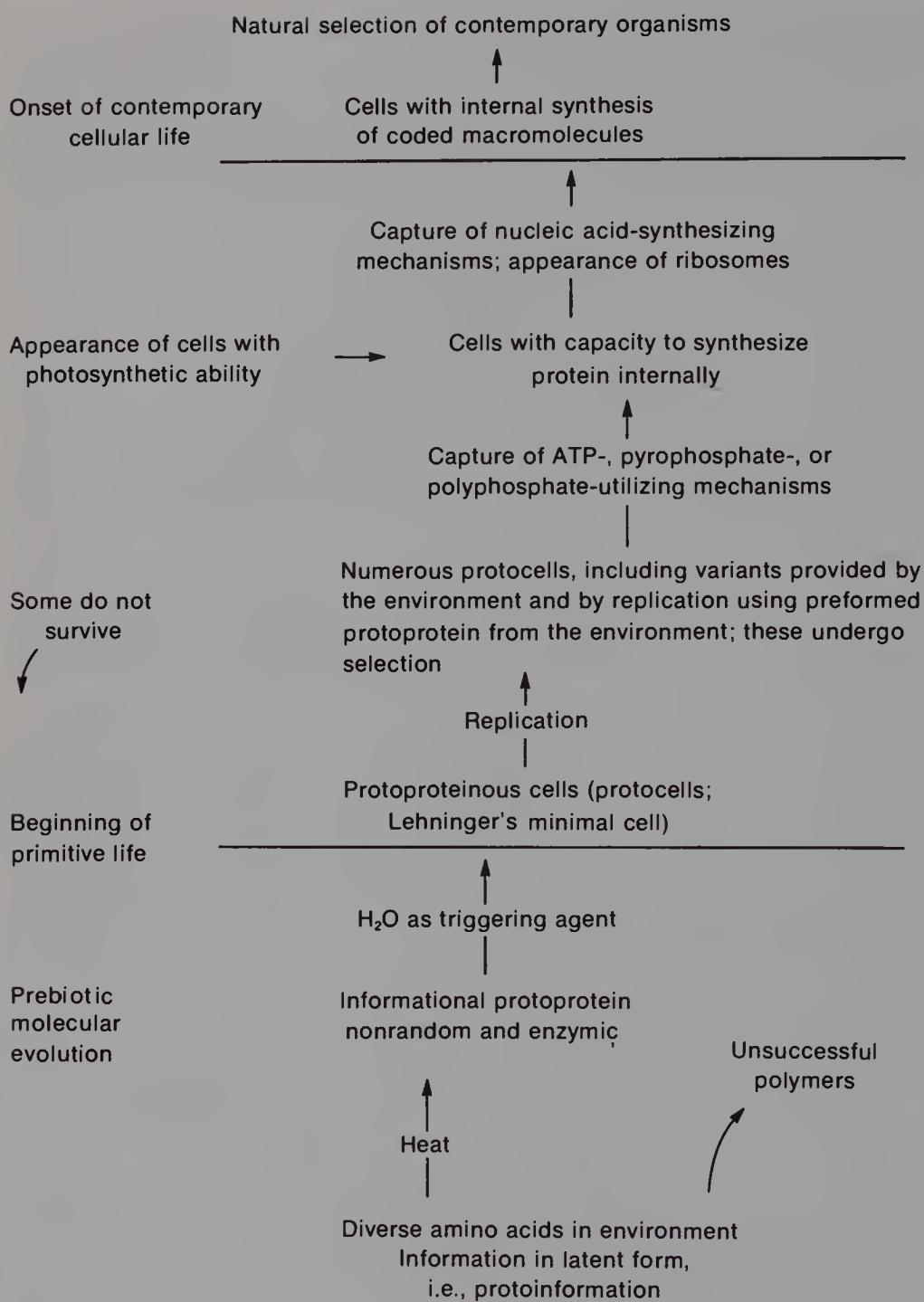


FIGURE 11-6
Flow chart of principal stages of molecular evolution and the origin of life.

the Galaxy to serve as precursors of amino acids. Amino acids could have arisen from each of these two pairs of intermediates, or from others. Since several pathways lead to amino acids, the direction is one of chemical convergence to that point. For a planet such as the Earth we can visualize, on the basis of laboratory experiments, that a pathway would have been followed to the emergence of primordial, and subsequently contemporary, living systems. For the traversing of this pathway simple organic compounds were needed, followed by appropriate alternations of heat and water. In fact, the special role of water in this series of reactions has been crucial, as much of this monograph has explained in detail. If instead of the Earth, the celestial body happened to be the Moon, the same cosmic pathway might have been followed, but it would have been terminated at the point indicated on the left of Figure 11-5 because the necessary material for the next step, water, was not present or not sufficiently abundant in the free state.

In this manner, we can hope to construct a theory in which both successful and unsuccessful traverses to living systems will provide us with comprehension. When we compare the carbon chemistry of the Earth, the Moon, the meteorites, the planets, and interstellar matter we examine cosmochemistry comparatively. In the most advanced overview, comparative biochemistry forms an evolutionary continuum with comparative cosmochemistry.

When we review the contents of this chapter and the contents of earlier chapters, we are reviewing what is reaching conceptually toward the two magnificent frontiers of science—outer space and the human mind. The latter has begun to understand how it emerged from the former (page 357).

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APPENDIX

Condensed Directions for Preparations in the Laboratory and Some Suggestions for Investigation

Preparation of proteinoid or other polyanhydro- α -amino acids

Heat a mixture of amino acids, 3.0 g, for 2-5 hr at 180°C. This mixture can consist of 1.0 g each of aspartic acid and glutamic acid plus 1.0 g of any other amino acid or of a mixture of amino acids.

Preparation of microspheres

To hot polymer, add 10 ml boiling 1.0% NaCl solution slowly with stirring (care!), boil 30 sec, stir, decant hot clear solution. Allow to cool without agitation and examine the cooled solution under a high-powered microscope by oil immersion.

The proteinoid may be purified and characterized. The properties of the microparticles may be examined.

Purification of proteinoid

Proteinoid can be purified by simple dialysis, or by fractionation on DEAE-cellulose, etc. The proteinoid can then be converted to

microspheres by using the preceding instructions, and a ratio of 25–100 mg of proteinoid per ml of aqueous solution.

Characterization of proteinoid, etc.

Various properties may be examined. Many standard color tests for protein (e.g., the biuret) can be performed. Controls should be run with some protein such as serum albumin. More elaborate experiments entail amino acid analysis, catalytic activity, optical activity, etc.

Properties of microspheres

Various properties may be examined. For example, monosaccharides may be enclosed in one preparation of microspheres and polysaccharides in another preparation to permit observation of selective retention after washing with proteinoid-saturated water in a standard procedure.

Other attributes that may be examined include staining with Crystal Violet followed by splitting due to increased pH or to $MgCl_2$.

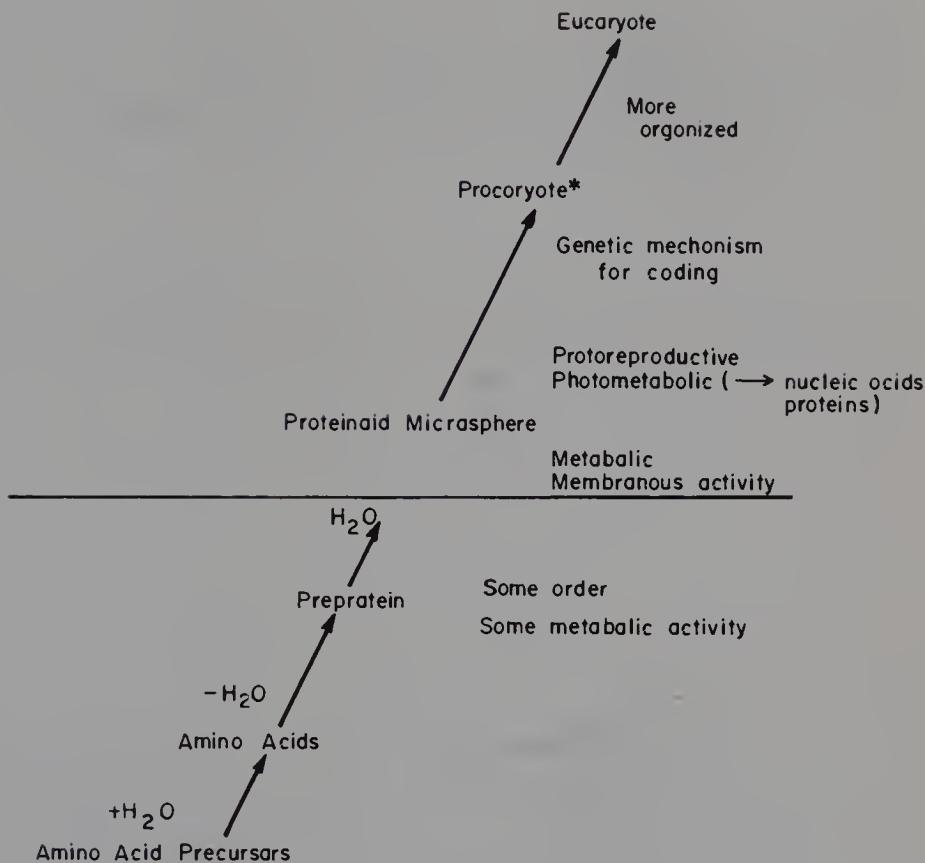
More complicated procedures are preparation of Gram-positive and Gram-negative microspheres, preparation of Zn-containing microspheres and motility in presence of ATP in aqueous suspension, "budding" and "growth," binding of basic proteinoids with polynucleotides, electron microscopy, etc.

Synthesis of a set of amino acids

There are many kinds of amino acid synthesis. Directions for one that uses compounds found in interstellar matter follow:

In a fume hood, add 5.0 ml of 28% ammonium hydroxide slowly to 15 ml of 37% formaldehyde (formalin) in a glass test tube (30 × 4 cm). After allowing the solution to cool, insert a two-holed rubber stopper with glass tubes through it for the entrance and exit of nitrogen into the top of the reaction tube. Then immerse this at least halfway into an oil bath. Nitrogen gas should flow slowly through the reaction tube both while heating and while cooling. Heat the oil bath to 185°C and let the reaction proceed for 8 hours at this temperature. During the initial heating of the bath, the water in the reaction mixture will be driven off. After heating, remove the reaction tube from the oil bath, allow to cool, add 100 ml of 6 N HCl, and reflux the mixture for 24 hr. Concentrate the liquid

GENERALIZED FLOWSHEET



to dryness, dissolve the residue in water or citrate buffer and analyze by chromatography.

The utility of sets of amino acids, including the one obtained from formaldehyde and ammonia, for thermal polymerization has been studied especially by Saunders and Rohlfing (1972).

*Similarities between proteinoid microspheres and prokaryotes (Margulis, 1971) suggest that this was the nature of the interdigititation at the onset of cellular evolution.

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about the textbook . . .

Of the various experimental studies which have been related to the origins of life, there is only one comprehensive, experimentally based theory and sequence of supporting data. Written by two of the pioneers of this sequence, *Molecular Evolution and the Origin of Life, Revised Edition* details the steps leading up to the first organisms on Earth. Embracing in its scope relevant astronomy, geology, physics, radiation science, organic chemistry, polymer chemistry, biochemistry, and cell biology, the volume outlines the results of studies which have stimulated profound revisions in general thinking about protobiology.

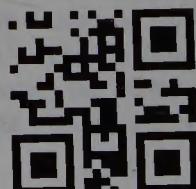
The experimental sequence described in the book begins with the origin of organic matter believed to have existed on the primitive Earth. That matter is then carried through the first informational protobiomacromolecules to the first cell-like structures which already had many characteristics suitable for protocells serving as simulated precursors of prokaryotes. The authors thoroughly explain the experiments and their interpretations. In addition, lines of thinking from other centers—including some that lack experimental support—and other relevant data, as from the Moon and meteorites, are examined.

The only thorough, scientifically supported treatment of this material in book form, *Molecular Evolution and the Origin of Life, Revised Edition* is essential reading for researchers in biochemistry, molecular genetics, cell biology, organic chemistry, and other related disciplines. Of great interest to undergraduate and graduate students in these fields, this volume is particularly useful as a textbook in origin-of-life courses, and as a supplementary text in courses on evolution, biochemistry, and biology.

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