Molecular Evolution
and the Origin of Life

Molecular Evolution and the Origin of Life

Sidney W. Fox

University of Miami

Klaus Dose

Johannes Gutenberg University

With a Foreword by

A. Oparin

W. H. Freeman and Company

San Francisco

Printed in the United States of America

International Standard Book Number: 0–7167–0163–4

Library of Congress Catalog Card Number: 76–172407

1 2 3 4 5 6 7 8 9

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

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. Rohlfing, 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 Mrs. Dorothy Butterbrodt, who typed the manuscript, and to Miss Christel Brand, Miss Judith Bruce, Mrs. Fides Dietz, and Mrs. Donna Murphy for other assistance.

SIDNEY W. FOX
KLAUS DOSE

January 1972

Contents

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

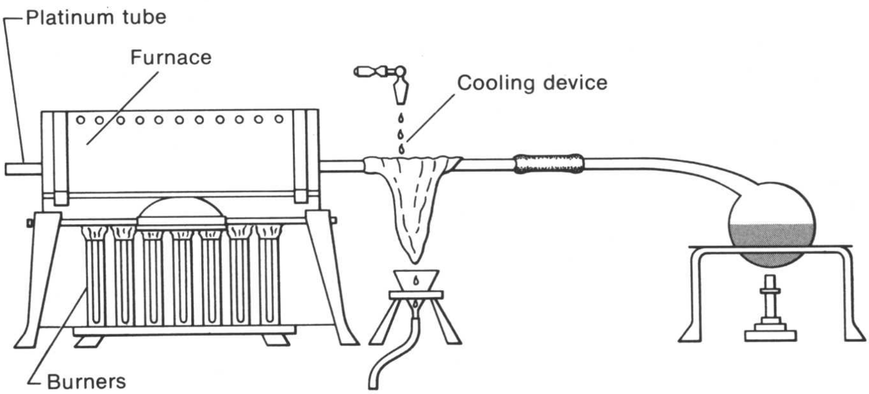

FIGURE 1–1
Pasteur's apparatus for sterilizing air. The device cools the hot air before its entry into the flask. From Keosian (1968).

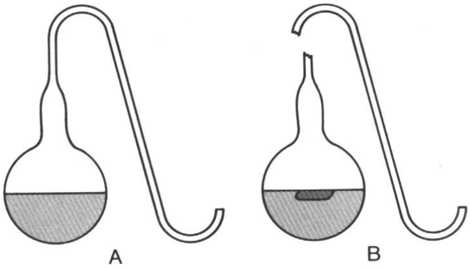

FIGURE 1–2
Flasks with S-shaped necks: *A*, unbroken neck, contents of flask remained uncontaminated in Pasteur's experiments; *B*, flask with broken neck, contents of flask became contaminated. From Keosian (1968).

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, p. 62; 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

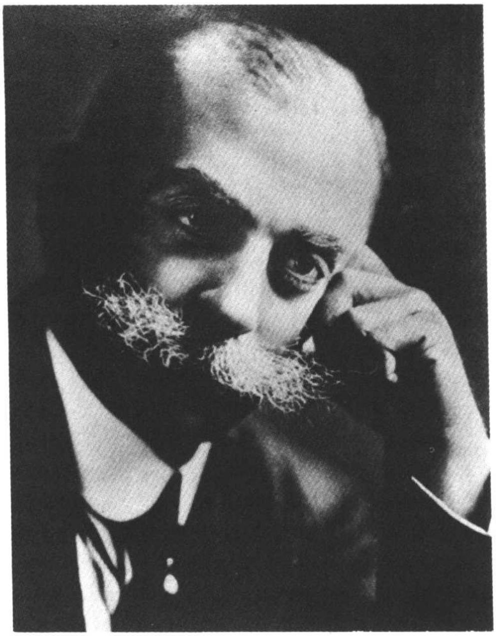

FIGURE 1–5
Alfonso L. Herrera (1868–1942).

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 composed of 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 208).

The concept of self-assembly was experimentally applied to a