Prebiotic and Biochemical Evolution

edited by

A. P. KIMBALL and J. ORÓ

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Department of Biophysical Sciences, University of Houston, Houston, Texas, U.S.A.

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PREFACE

More than one hundred years ago Ernst Haeckel proposed the nineteenth century version of the hypothesis of the "spontaneous" generation of life. At that time it was a logical extension of Wallace's and Darwin's concepts on the origin of species but had no support from either the physical, chemical or biological sciences. It is well known that the first comprehensive formulation of a theory of the origin of life in physical and chemical terms was presented by Oparin in 1924 and that the first experimental demonstration of the synthesis of amino acids under possible primitive earth conditions in support of this theory was published by Miller in 1953. Since then a substantial number of investigations have been carried out in this field of research, most of which have been collected in two volumes published in 1959 and 1965.*

The present volume is a collection of papers of research work performed during the last few years and assembled for the purpose of maintaining a continuity in this field of research. It begins by presenting important new developments in the area of abiotic synthesis of biochemical compounds and model systems of protocellular organization which attempt to trace different stages of chemical evolution before life appeared on our planet. The book then brings together some of the most relevant advances in the area of biochemical evolution which have been made by studying the nucleic acids, proteins, metabolic pathways, and other processes of living organisms. It ends, among other interesting papers on nucleic acids and enzymes, with the first successful attempt at the total synthesis of a naturally occurring polypeptide of 55 amino acids.

This chemical and biochemical research has progressed to a point where a meaningful and self-consistent picture of prebiotic and biochemical evolution starts to emerge from the experimental work done at different laboratories. We stand now on the brink of another phase of molecular evolution. We are rapidly accumulating knowledge and methods from which ways can be developed to predetermine our own evolutionary history.

This book developed from a Symposium on Proteins and Nucleic Acids (Synthesis, Structure, and Evolution) held at the University of Houston, Houston, Texas, in April, 1968, and from papers on Biochemical Evolution

* Oparin, A. I., Ed., The origin of life on the earth (Pergamon Press, Oxford, 1959). Fox, S. W., Ed., The origins of prebiological systems and their molecular matrices (Academic Press, New York, 1965).

presented at the 6th Meeting of the Federation of European Biochemical Societies, held in Madrid, in April, 1969. We are indebted to the participants who have contributed to this volume and to several journals who have permitted us to reprint pertinent papers. Some of the authors of the papers delivered at the Houston meeting were kind enough to submit updated versions. The different contributions are not only relevant today but it is hoped they will be relevant many years from now. We wish to thank Drs. R. B. Hulbert, D. N. Ward, M. D. Anderson Hospital and Tumor Institute, Houston, Texas; Dr. S. Kit, Baylor University College of Medicine, Houston, Texas; and Dr. J. R. Cox, University of Houston, Houston, Texas; for their help with the Houston meeting; and Drs. A. Sols, C. Asensio, and Prof. A. I. Oparin, for their help with the Madrid meeting. We appreciate the assistance and encouragement received in the organization of the meetings and the preparation of the book from Drs. A. H. Bartel, A. Zlatkis, R. Segura, Mrs. Marianna O'Rourke, Thomas Spoor and Henry Simon, of the University of Houston. We also acknowledge the support and encouragement received from Dr. R. S. Young and the National Aeronautics and Space Administration.

A. P. KIMBALL
J. ORÓ

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M = Meeting of the Federation of European Biochemical Societies, Madrid, 1969.
H = Symposium on Proteins and Nucleic Acids, Houston, 1968.

COACERVATE DROPS AS MODELS OF PREBIOLOGICAL SYSTEMS

A. I. OPARIN

Bakh Institute of Biochemistry, Academy of Sciences of the USSR, Moscow, USSR

Model experiments on the abiogenic synthesis of various organic substances (monomers and polymers) under the conditions similar to those which existed on the primitive Earth have led to a conjecture that long before the emergence of life on the Earth numerous biologically important compounds had been formed on our planet. This hypothesis was reliably confirmed by the discovery of similar abiogenically formed organic substances in meteorites and deep layers of the Earth's crust.

The solution of those substances in waters of the Earth's primitive hydrosphere (the so-called primordial broth) was the initial material for the development of life. However, the formation of primary organisms (eobionts) in the broth could proceed only via self-formation of relatively simple individual systems (protobionts) which in the course of long-term evolution came to be the precursors of all the living organisms on the Earth.

A good number of the systems capable of self-formation in the solution of organic substances similar to the primordial broth (bubbles of Goldacre, microspheres of Fox, coacervates of Bungenberg-de-Jong, etc.) can be not only conceptually developed but also practically reproduced in model experiments. Sometimes the self-formation is accompanied by the appearance of structures resembling those of living objects. But the important fact is the dynamic character of stability of these systems rather than this external resemblance.

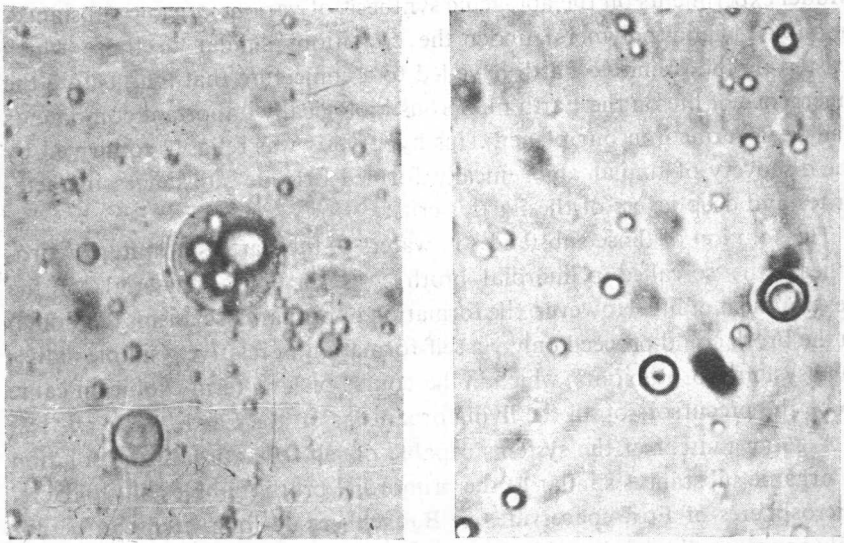
The typical feature of living objects is that they are not static but stationary open systems in which continuous decay is constantly compensated for by substances and energy of the environment.

Initial protobionts should have had this stationarity. Their structure was significant for their further development only if it determined their dynamic stability and in some cases if it also dictated the growth of a given open system at the expense of its interaction with the environmental medium.

From this viewpoint, coacervate drops seem to be the most promising (though not the only possible) models for studying evolution of protobionts. In the medium similar to the primordial broth coacervates are readily self-formed via aggregation of varied polymers (e.g., polypeptides and poly-

nucleotides) to drops of microscopic size. Of key importance for the self-formation is the level of polymerization but not the intramolecular structure of pclymers. Fig. 1 presents, for instance, drops formed from monotonously built polymers (polyadenine and polylysine).

The drops are separated from the surrounding solution by a well outlined surface but are capable of interacting with the surrounding medium, selectively adsorbing such substances as amino acids, sugars, mononucleotides and releasing products of reactions developing within them.

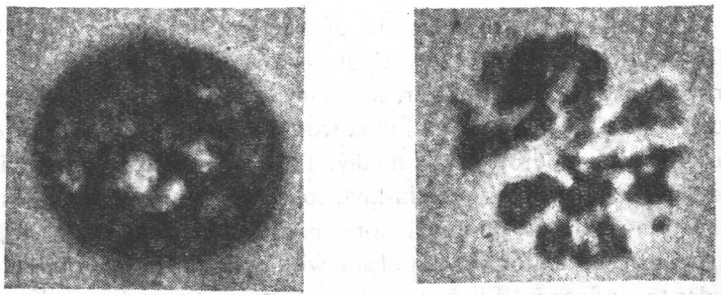


(a) (b)

Fig. 1. Coacervate drops containing polypeptides and polynucleotides. (a) RNA + polylysine; (b) Poly-A + polylysine.

Without this interaction coacervate systems being static systems, are unstable. For instance, polymers are readily decomposed both spontaneously and under the influence of catalysts included in the drops. As a result, the drops disintegrate partially, as can be seen in the electron-micrograph, or disappear completely (fig. 2).

Despite disintegration processes, coacervate drops can long persist, acquiring dynamic stability due to the entry of matter and energy from the environment. In the simplest event, the disintegration occurring in the drop can be compensated for by ready-made polymers entering the system from the surrounding medium [in our experiments at the expense of a polymer (RNA) added to the equilibrium fluid].



(a) (b)

Fig. 2. The coacervate drops containing serum albumin, gum arabic, RNA and RNA-ase. (a) no incubation; (b) 20 min incubation.

In other cases, drops adsorb energy-rich monomers, and their dynamic stability depends on the polymer synthesis occurring within the system. If the synthesis rate is balanced up by the disintegration rate, drops develop dynamic stability and can persist for a long time; if synthesis prevails over disintegration, drops increase their volume and weight, growing before our eyes.

Here is a scheme of one of the experiments (fig. 3).

A coacervate drop, formed from histone and gum arabic, is placed into the glucose-1-phosphate solution. Under the influence of glucosyl-transferase incorporated in the drop, glucose-1-phosphate transforms into starch, at the expense of which the drop grows, if the synthesis rate (a) exceeds the disintegration rate (b). This scheme illustrates another procedure.

In fig. 4 a drop consisting of histone and RNA adsorbs ADP from the surrounding medium. Due to the polymerization reaction (poly-A formation) the drop growth occurs which can be easily recorded.

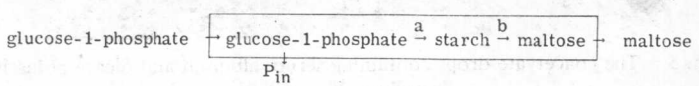


Fig. 3. Synthesis and hydrolysis of starch in the coacervate drop.

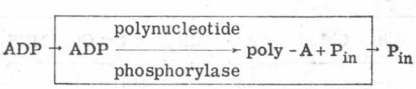


Fig. 4. Scheme of polyadenine synthesis by polynucleotide phosphorylase in the coacervate drop.

In the examples described the rate of reactions developing in the drop is mainly dictated by the activity of incorporated catalysts (in laboratory experiments: enzymes). However, the indicated rate can depend on other events, e.g., on the light energy influx from the environment, occurrence of photosensitizers in drops and, finally, their intermolecular organization. This can be exemplified by protein-lipid coacervates incorporating sensitizers of reduction–oxidation photoreactions. Fig. 5 shows coacervate drops consisting of protein and potassium oleate with chlorophyll incorporated.

Under the influence of light the photoreaction of ascorbic acid oxidation and methyl red reduction develops in the drop according to the following scheme: fig. 6.

In this system the photoreaction rate in the drop is 60 times as high as in the equilibrium fluid. This occurs mainly due to an increase of the concentra-

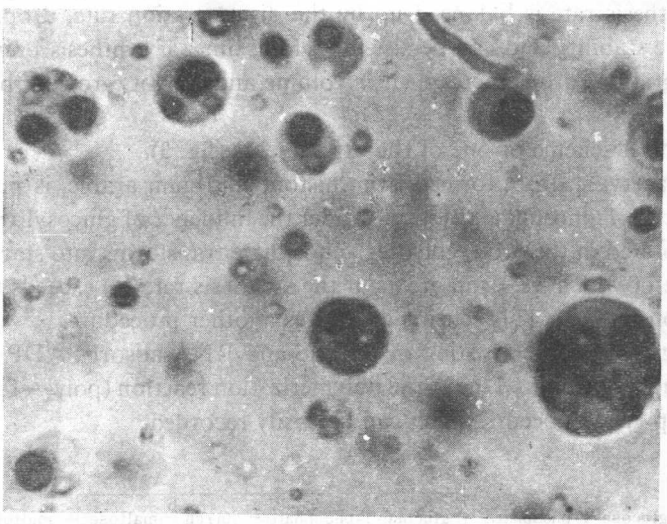


Fig. 5. The coacervate drops containing serum albumin and oleate potassium, including chlorophyll.

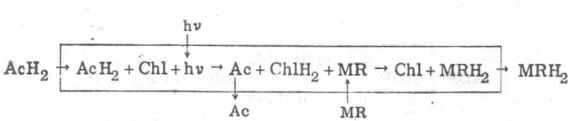


Fig. 6. Scheme of ascorbic acid oxidation in the coacervate drop, containing chlorophyll (red light).

tion of the sensitizer in coacervate drops which can be demonstrated by direct assay.

The situation is different if coacervate drops involve lecithin. In this case the pigment location on phosphatic micellae becomes orderly as a result of a fixed position of the phytol tail of chlorophyll between two hydrophobic residues of lecithin. This more perfect spatial intermolecular organization of coacervate drops increases immediately the rate of the reactions by two orders of magnitude. In this event the photoreaction proceeds approximately by 100 times faster than it is required for the pigment concentration. In the above case the role of the phytol group is indicated by the fact that the chlorophyll replacement by the phytol-free pigment – porphyrin IX – does not lead to the indicated increase of the photoreaction rate in the lecithin coacervate.

The intermolecular spatial organization seems to be an intermediate stage in the course of development of structures visible under the electronic microscope.

The effect of the spatial organization on the rate of reactions occurring in the coacervate drop can be also distinctly seen if two or more reactions proceed in the system. As an example we reproduced the beginning of the pentose cycle in coacervate drops composed of lecithin and enzyme proteins in which the hexokinase and glucose-6-phosphate dehydrogenase action was combined (fig. 7).

The diagram in fig. 8 presents curves characterizing the development of the reactions in homogeneous and heterogeneous conditions.

We can see that, if the two reactions develop together under homogeneous conditions, then the rate of the first reaction increases due to the presence of glucose-6-phosphate dehydrogenase which oxidizes glucose-6-phosphate formed in the first reaction. The acceleration is, however, comparatively low.

Simple incorporation of hexokinase into coacervate drops enhances significantly the effect of the enzyme as compared to that of homogeneous conditions. This reveals the role of the spatial organization. It becomes apparent upon a combination of the effect of the two enzymes in coacervate drops. In such a case the combined effect greatly exceeds that observed in homogeneous

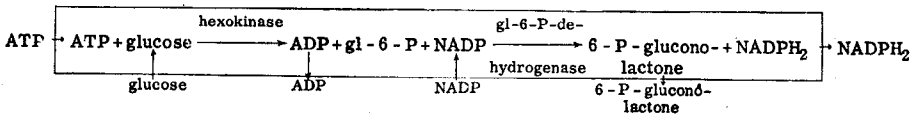


Fig. 7. First steps of pentosephosphate cycle in the coacervate drop.

conditions. A similar phenomenon was detected in the case of a combined effect of hexokinase and polynucleotide phosphorylase, as shown in the scheme (fig. 9).

Here we can clearly see the effect of the spatial organization on the rate of the polymer synthesis, and therefore on the rate of the growth of coacervate drops.

On the basis of the above scheme, we carried out an experiment with two differently organized systems which were built of histone and enzyme proteins. They differed in the fact that one of them incorporated glucose and a

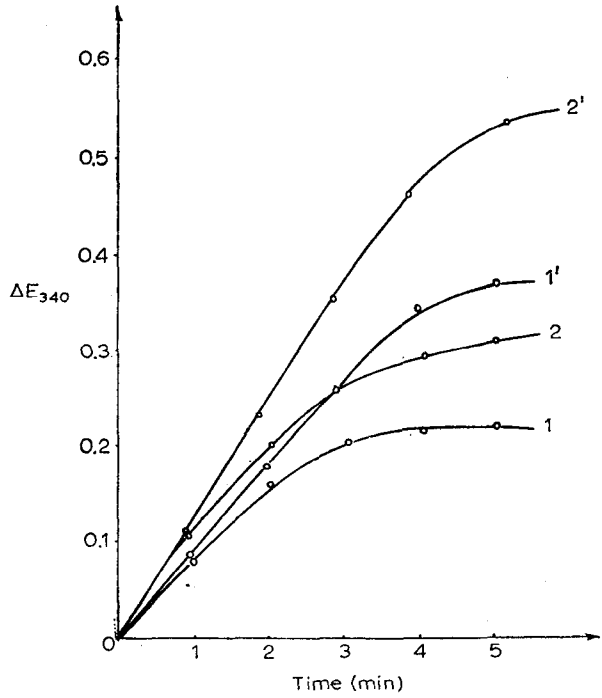


Fig. 8. The first step of pentose cycle in the coacervate drops (curve 2').

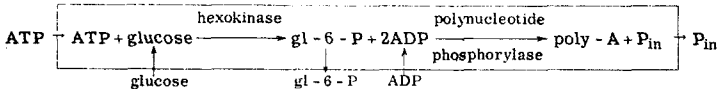


Fig. 9. Scheme of polyadenine synthesis by polynucleotide phosphorylase in the coacervate drop in presence of hexokinase.

combination of two enzymes (polynucleotide phosphorylase and hexokinase) whereas the other involved polynucleotide phosphorylase alone. Both drops were placed into a solution of commercial ATP which contained up to 20% of ADP (fig. 10).

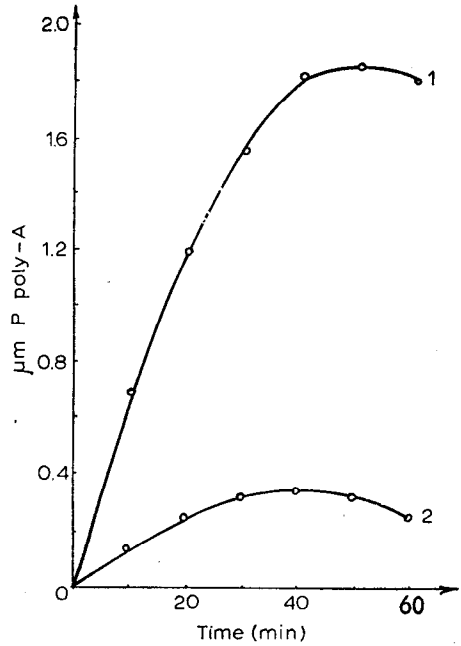


Fig. 10. The dependence of the synthesis of poly-A by polynucleotide phosphorylase in the coacervate drops on the time of incubation. (1) histone/polynucleotide phosphorylase/hexokinase; (2) histone/polynucleotide phosphorylase.

This diagram shows that drops having more perfect internal organization (curve 1) grow much faster than those having less perfect organization (curve 2) though they are in the same environment.

Here we can already distinguish the beginnings of new principles of development originating at the junction of chemical and biological evolution, the beginnings of prebiological natural selection. These principles underlie the entire further evolution of protobionts.

SELF-ASSEMBLY OF THE PROTOCELL FROM A SELF-ORDERED POLYMER*

SIDNEY W. FOX

Institute of Molecular Evolution,
University of Miami, Coral Gables, Florida, U.S.A.

The problem of the origin of life, or in truly perceptive nineteenth century terms, the problem of spontaneous generation, has often been regarded as one of overwhelming complexity. Upon analysis, with the aid of hindsight, this problem loses some of its imponderability. The aspect of evolution which first received major attention was that of the progression, in principle, from primitive cell to contemporary cell and to contemporary multicellular organisms. This stage is the one that has been illuminated mechanistically by Darwin's theory of selection. We can now regard this stage as far more intricate and involved than the emergence of primitive life from the primordial reactant gases. By such an analysis, the primordial cell is emphasized, the highly ramified later stages are removed from purview, and the limits of the meaningful problem are identified.

The preorganismic stage can also be analyzed. For intellectual convenience, it may be divided into two or three parts. The first of these parts is that of the spontaneous organic synthesis involved in the production of the small organic molecules which are necessary for contemporary and, presumably for, primitive organisms. The second step is the spontaneous synthesis of the polymers and of cells. This latter constitutes in turn, however, two stages. These two steps were collectively most forbidding in quality, and are particularly significant to life and therefore, to its origin. Our most modern knowledge requires that we recognize that a primitive cell can not be a synthesized entity in the true meaning of "synthesized". The precursor macromolecule can be conceived of as synthetic. When the appropriate macromolecule has been formed, the final and crucial stage, leading to a primitive organism, would then be one of self-assembly. The term "self-assembly" and the con-

* Since 1960, this research has been aided by the National Aeronautics and Space Administration, currently Grant no. NsG-689. Contribution no. 096 of the Institute of Molecular Evolution.

Paper presented to International Convention of Biochemists, Bangalore, India, 7 september 1967. (Reprinted by permission from the Journal of Scientific and Industrial Research (India).)

cept have recently been receiving increasing recognition e.g. [1] in the biochemistry of contemporary systems.

One way in which students of the total problem have dealt with the seemingly great complexity has been to postulate a long chemical evolution [2] extending over, say, 25 million years. I will explain here why our experiments lead to the interpretation that the essential steps from primordial gases → amino acids → primitive protein → a primitive organized structure having simultaneously many lifelike properties including the ability to participate in its reproduction, could have occurred many times in a very short period, say 25 hr. My immediate problem is to present the salient experimental material in an even shorter time. This problem exists because of the careful devotion to it by many associates during 14 years of continuous study in our laboratory. Accordingly, I shall rely heavily on summaries and upon examples from our laboratory and others.

Our approach to this problem was based on clues from contemporary cells. The results of experiments have been evaluated in part by how well they lead to an increasing appearance of the properties that are associated with contemporary cells. The experiments have however been based on very simply derived initial systems and simple processes. These employ conditions that have proved to be plausible not only for geologically ancient times; the conditions identified are widespread now through recorded history [3].

Models of the prebiotic synthesis of small organic compounds such as monosaccharides, amino acids, purine and pyrimidine bases, ATP, porphyrins, etc. have been described from many laboratories including those of Ponnamperuma [4]. Oró [5], Orgel [6] and our own [7]. Since the essence of life is generally recognized as being that of the biopolymers, protein and nucleic acid, this paper will focus on questions involving the primordial formation of protein and nucleic acid and on the attributes of the polymers formed in the laboratory. It will deal also with complexes of the two.

Turning first to the question of proteins, we find that a number of studies of the synthesis of peptide bonds, mostly in aqueous solution, have been carried out in a number of laboratories. Akabori [8] employed the progressive substitution of polyglycine as a model of the first protein, and Matthews [9] has reported a similar process. The model of our laboratory which relies on heat and hypohydrous conditions is the only one that has yielded polymers of molecular weight in the thousands, a content of all eighteen amino acids common to protein, several protoenzymic activities, and it is the only model which has been demonstrated to yield organized structures with a lengthy roster of the properties of the contemporary cell [10].

This synthesis has the simplicity appropriate to geologically spontaneous occurrences, and it yields both polymer and organized units in abundance.

One other synthesis, which has most of the attributes enumerated, uses as intermediates the reactive Leuchs anhydrides of the amino acids. This synthesis was also first performed in our laboratory, by Hayakawa [11]. Of these two syntheses, only the thermal process has the simplicity appropriate to geologically spontaneous occurrences.

The thermal syntheses, first attempted in 1953, were indicated as thermodynamically possible following studies of Borsook, Huffman, Ellis and Fox [12]. The results of calculations from the tabulated physical constants have shown that one could expect in an open aqueous solution only small yields of small peptides unless the reaction were somehow coupled to an endergonic one.

The fact that organisms are nearly always aqueous entities has led some to assume that hot, dry conditions would not have been appropriate to early life and they have somehow projected such thinking to precursor molecules. Our chemical experience however tells us that macromolecules can easily survive conditions lethal for ordinary cells, and our biological experience reminds us that bacterial spores are relatively resistant to heat and dryness.

The reaction involving formation of peptide bond with its attendant Gibbs free energy change is:

$$\begin{gathered} H_2NCHRCOOH + H_2NCHR'COOH \\ = H_2NCHRCONHCHR'COOH + H_2O \\ \Delta G^0 = 2000 \text{ to } 4000 \text{ cal.} \end{gathered}$$

As the number of peptide bonds per molecule increases, the equilibrium constant becomes geometrically more unfavorable. Dixon and Webb [13] have calculated that the volume of 1 M amino acid solution in equilibrium with one molecule of protein of molecular weight 12,000 would be 10^{50} times that of the Earth! Stated otherwise, uncoupled synthesis from amino acids in water should be expected to give small yields of small peptides only.

In order to shift this equilibrium to favor synthesis, one can postulate removal of either product. Theoretically, one contribution of a membrane in contemporary protein-synthesizing systems may be the overcoming of an energy barrier by separation of synthesized macromolecules from the aqueous solution. This process could not apply, however, until a membrane composed of macromolecules had first formed. Our attention therefore shifts to removal of the other product, water. This route to peptide bond synthesis can be visualized as a geochemical possibility. It has also been demonstrated experimentally [7].

One mode of removal of water, as thus suggested by the thermodynamic analysis, would be that of heating the amino acids above the boiling point of water. When this possibility was initially contemplated, the probability of gross decomposition had to be considered. Such a consequence of heating α-amino acids above the boiling point of water has been recorded in the literature a number of times and was also common knowledge. We were led to attempt the thermal condensation by employing an inference from comparative studies of organismic protein, the fact that the amino acids which most dominate the composition of proteins are glutamic acid and aspartic acid [7]. These contents were taken hypothetically to be an evolutionary reflection of a circumstance required for the primordial formation of prebiotic protein.

Another consideration that had to be dealt with was the somewhat vague feeling that, without nucleic acids present, the necessary systematic sequences of amino acid residues would not result. This problem was conceptually eliminated, in principle, by studies of enzymic acylpeptideanilide synthesis [14] which demonstrated that interactions of amino acid residues would alone select the sequence formed. (This principle and the inference that prior nucleic acids may have been unnecessary [7] has since been corroborated by Steinman [15] in another system of reacting amino acids.) Since the difficulties were thus conceptually surmountable in 1953, heating was employed (fig. 1). The discussion now deals with experimental observations.

A typical thermal condensation used at first a mixture of 1 part of aspartic acid, 1 of glutamic acid, and 1 of an equimolar mixture of the 16 other amino acids common to protein. This mixture was heated at 170°C for 6 hrs [16]. The resulting light amber glassy product, not depicted, is entirely soluble in water by salting-in, and can be purified by salting-out. Such products yield amino acids 100% by acid hydrolysis, they contain some proportion of each of the amino acids common to protein (or fewer as desired), and molecular weights of many thousands. They have many other properties of protein and are called proteinoids. The proteinoid described in this example is, because of the proportions reacted, a 1:1:1 type. More recently, Waehneldt has shown in our laboratory that aspartic acid and glutamic acid may be merely equimolar with the 16 other amino acids. Proteinoids are produced even so. Yields in the usual syntheses are typically in the range of 10–40%, higher yields being obtained by the addition of phosphates [17, 18]. Many other laboratories have repeated this synthesis and have confirmed it and its simplicity, which is in turn crucial to the geological validity. The spontaneous occurrence of a carbobenzoxy synthesis or the