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Experimentally Derived Explanations of Some Aspects of the Origin of Life*

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*This paper is essentially the subject matter presented during a USSR-USA interacademy lecture tour in April-May, 1969. In some of the footnotes are discussed a number of questions raised during discussion periods in Moscow, Putschino, Leningrad, Kiev, Yerevan, and Tbilisi. This lecture tour was reciprocal with that of Academician A. I. Oparin. Whatever anyone else has done in the past, or will do in the future, on the subject of the origin of life must rest on the platform built by Professor Oparin.

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A first set of experimentally derived answers to questions of how cells originated de novo[1] has been based on processes which are operationally simple (Fox, 1969).

The scientific question of the origins of life can be analyzed according to Fig. 1.

We see, in the later stages of Fig. 1, that one needs to concern himself with the evolution from primitive organisms to contemporary unicellular or multicellular organisms. This part of evolution is one which Darwin clarified by his selection mechanism. Looking back on the experimental research in the field of abiogenesis since 1950, I believe that the Darwinian part of the answer, explaining evolution from the first organisms, represents by far the most intricate and involved aspects. Darwinian evolution of organisms undoubtedly required hundreds of millions of years. When we focus our attention on the true chemical synthesis of an organism starting from nonbiological precursors, such as activatable atmospheric gases, we see that we have narrowed our questions. We have then stripped away the most forbidding part of what was not so many years ago easily dismissed as a hopelessly imponderable problem.

The first step from primitive reactive gases to amino acids, to the nitrogen bases of the nucleic acids, and to monosaccharides represents the area in which the largest proportion of several laboratories in the field have worked. Contributions have come from the laboratories of Oparin (1957), Calvin (1962), Ponnamperuma (1965), Oró (1965), Miller (1955), Orgel (Sanchez et al., 1966), Fox (1965, 1969), and others. The next step concerns the formation of the larger molecules, proteins, nucleic acids, and cellulose. Their formation is thought of as an appropriate type of polymerization of monomers. We can see also, by the further analysis of the large problem, that the following step is not one of true synthesis, but is rather one of structural organization of appropriate polymers. This kind of process has been increasingly referred to, by the biochemist, as an act of self assembly. On this basis we should, strictly, think and speak of the synthesis of precursor polymers and of their self assembly into protocells. Examples of self assembly of organelles of cells are now numerous (Seventh International Congress of Biochemistry, 1967). The two steps of Fig. 1 are the ones to which we have devoted most of our attention (Fox, 1965).

The primitive cell, which our experiments now tell us could have arisen from reactant gases in less than a few hours (Fox, 1969), had then to evolve to a contemporary cell. The elegant studies that have been carried out by Goulian and Kornberg (1967) and by Spiegelman (1968) involve the dismantling of a contemporary cell and the utilization of contemporary enzymes and primer nucleic acids for further synthesis of a contemporary type of RNA or of DNA, respectively. These processes do not, therefore,

answer the fundamental questions of how enzymes began in the absence of enzymes, of how cells arose in the absence of cells, or of how genes appeared in the absence of genes. Our work has been aimed at these latter questions.

Although the work of our laboratory has been primarily concerned with steps 2 and 3, we have applied much effort to the production of small molecules in the context of step 1. Table I is adapted from the book on the Origin of Life by the Armenian-born J. Keosian (1968).

This table shows the results of Miller (1955), obtained while he was a doctoral student with Urey at the University of Chicago. By electric discharge in methane, ammonia, water, and diatomic hydrogen, two amino acids, glycine and alanine, plus smaller quantities of aspartic acid and glutamic acid were obtained. These results were first reported in 1953, sixteen years ago. About 1960, several laboratories studied attempts to produce amino acids by heating gases to volcanic temperatures in fast vapor-phase reactions[2] and hydrolyzing the products.

Oro (1965), and Harada (1964) in our laboratory, each obtained in hot tubes the amino acids recorded by Miller. Indeed, the electric spark has a high temperature associated with it. Harada's results differed, however, when he packed the tube with silica, and omitted diatomic hydrogen. Omission of the hydrogen permitted the production for the first time of the hydrogen-poor tyrosine and phenylalanine. The use of silica resulted in the production of a long roster of amino acids, most of those that are found in contemporary protein. Moreover, with the exception of alloisoleucine, essentially none of the amino acids are those not found in protein.

Taube et al. (1967) has repeated this synthesis and has obtained similar results. Both he and we are studying this pansynthesis further for the production of synthetic foods.

The studies of synthesis of amino acids at high temperatures followed experiments of 1957-58 performed also at elevated temperatures, albeit below 200°. Dry[3,4] amino acids were heated in order to obtain protein-like polymers. This followed reasoning from thermodynamics (Fox et al., 1957), evolution (Fox, 1956), considerations of generation of amino acid sequence (Fox, 1956), and of organic and polymer chemistry (Fox, 1968). The thermodynamic calculations (Huffman, 1942) showed that one cannot hope to obtain and retain more than small yields of small peptides when the peptide bonds are formed from free amino acids in dilute aqueous solution. Our studies of evolution of protein molecules in organisms indicated the desirability of including sufficient proportions of aspartic acid and glutamic acid (Fox, 1960) in a heated mixture. Experiments with proteinases and amino acid

derivatives already had indicated that reactive amino acids alone might be self-ordering (Fox, 1956).

Heating of amino acids (to combine them) had long been known, however, to lead to gross decomposition (Fig. 2a). When equal parts of a dry mixture of, for example, one part glutamic acid, one part aspartic acid, and one part of the sixteen other amino acids in equimolar proportions was heated at 170° for six hours, a light amber-colored polymer was obtained. This can be freed of pigment in various ways to yield a white polymer (2b) which has molecular weight of many thousands (typically 5,000 to 10,000) and contains some proportion of each of the amino acids common to protein. These polymers have many other properties of proteins, recognized at the outset, and were therefore designated proteinoids.

More recently, we have learned (Fox and Waehneldt, 1968) that the ratio of aspartic acid and glutamic acid can be very low. Even when an equimolar mixture of all eighteen amino acids is employed, substantial yields are obtained.

One outstanding feature of this reaction is its stark simplicity. The conditions are not simply imputable to the primitive planet; they are moderately widespread on the contemporary Earth (Fox and McCauley, 1968; Fox, 1964). These experiments thus explain how a kind of protein would have come into existence in the absence of cells to produce it.

A first question to ask about the proteinoids is how disordered they are. Many chemists have assumed that the first protein-like molecules, and such polymers as might be obtained by heating, would be highly disordered, or random. Oparin (1957, p. 290), for example, has referred to "organic polymers in the shape of polypeptides and polynucleotides having, as yet, no orderly arrangement of amino acid and nucleotide residues adapted to the performance of particular functions". This point of view has been widely held (see also Rich, 1962). We also were concerned about this question, and performed the first experiments only after we had observed self-ordering effects in reactions of amino acid derivatives (Fox, Winitz, and Pettinga, 1953).

Figure 3 shows nonrandom, nonuniform elution patterns of polymers obtained by heating amino acids. A random assortment of macromolecules would, theoretically, require a horizontal line. This pattern is neither horizontal nor uniform. Other thermal polyamino acids give similar nonuniform patterns in gradient elution (Fox and Nakashima, 1969).

In Fig. 4 are seen the analytical patterns of total hydrolyzates of materials of peaks 3, 4, and 5 from the fractions of Fig. 3. Below those three chromatograms from the automatic amino acid analyzer are seen the profiles of the partial hydrolyzates. These latter are "peptide maps" or "fingerprints". These

peaks have been shown by increase in ninhydrin color following alkaline hydrolysis to represent mostly peptides. The top three chromatograms indicate that the polymer is highly uniform in composition throughout. The bottom three chromatograms require the conclusion that the polymer is highly uniform in sequence. These results are consistent with many other kinds of evidence (Fox and Nakashima, 1967) that the thermal polymers of amino acids are self-ordered.

We visualize such phenomena as resulting from the facts that each kind of amino acid has its own shape and its own distribution of charge and that steric orientations relative to the growing polyamino acid chain are highly specific. Consequently, the total process is highly selective.

In the larger context of origins, such results indicate how macromolecular order (= repeating sequences) could arise in the absence of a code. No prior nucleic acids were necessary (Fox, Harada, and Vegotsky, 1959)[5].

A next question concerns catalytic activity in proteinoids. Do they have activities that could have functioned as the first enzymes?

Six laboratories (in the U.S., West Germany, and Japan) have reported enzyme-like activities in thermal proteinoids. These have appeared in sixteen publications (Rohlfing and Fox, 1969). The activities are mostly weak relative to contemporary enzymes but weak activity was all that was required for a beginning (Calvin, 1962; Rohlfing and Fox, 1969).

In Fig. 5 is seen a representative result with proteinoid acting on pyruvic acid.

In Table II are listed the findings of catalytic and paracatalytic activities. Four kinds of reaction have been identified in publications: hydrolysis, decarboxylation, amination, and deamination. Dose (1969) has recently orally reported peroxidase activity. For each of the reactions catalyzed, Lineweaver-Burk plots have been obtained. Thus are indicated proteinoid-substrate interactions.

Through these experiments we can discern in principle how enzymes arose when there were no enzymes to make them.

The origin of metabolism is considered in Fig. 6. By assembly of some of the reactions for which proteinoid catalysts have been identified, the flowsheet (Fox, 1969) of Fig. 6 is a result. The decarboxylation of oxaloacetic acid was shown by Rohlfing (1967) to be catalyzed by basic proteinoids but not by acidic ones. In the next step, also a decarboxylation, Hardebeck, Krampitz, and Wulf (1968) have demonstrated that acidic proteinoids

are much more active than basic ones. This comparison provides an example of specificity observed in reactions of proteinoids and substrates. Also may be seen a small part of the Krebs cycle. We may thus understand, in principle, how metabolism arose.

In Table III are listed properties of thermal proteinoids found also in contemporary proteins. These constitute a large proportion of the properties of various contemporary proteins[6]. The property that is fundamentally relevant, however, is the tendency (in the presence of water) to form ultrastructured microsystems, the proteinoid microspheres (Fox, 1969).

Oparin (1957) and his school have stressed the importance of a first organization of the cell[7], and they have done many experiments with coacervate droplets in this context of inquiry. Many interesting results have been obtained; these are well known in the Soviet Union.

The proteinoid microsphere (Fig. 7) results, in a maximally simple process, by contact with water. When hot water is poured onto proteinoid, and the resultant clear hot solution is cooled, millions of microscopic spherules separate (Fig. 7). These are stable to centrifugation, they have properties suggestive of osmosis, they can be made gram-negative or gram-positive, they have catalytic powers, they can be produced so that they are motile, they bind polynucleotides as well as dyes, and they also show some selectivity in the passage of molecules through their boundaries (Fig. 8)[8].

The particles can vary somewhat from those shown. Microparticles resembling the microfossils of Barghoorn and Schopf (1965) are seen in Fig. 8. Evidence is at hand that some microfossils are more than three billion years old. The proteinoid particles depicted in the photomicrographs are much younger, and represent morphologically what is seen in either the microfossils or, alternatively, some of the "organized elements" of meteorites (Claus and Nagy, 1961).

Since the particles are stable, they can be mounted in methacrylate blocks after staining with osmium tetroxide, and can be sectioned to yield the electron micrograph of Fig. 9. As Fig. 9 shows, these boundaries are structured. One may in this figure compare a section of a proteinoid microsphere with one of Bacillus cereus under the electron microscope. Experts who are uninformed on these units often guess wrong as to which is which, reportedly because the artificial particle has a thicker boundary. In the same figure we see that the artificial boundary can be a double layer. This has permitted some review of our understanding of the Danielli model (1935) of the unit membrane of the contemporary cell, especially with regard to the contribution of lipid[9].

One kind of selectivity in the boundary is seen in Fig. 10. As the pH is raised by one unit or so, polymer in the interior diffuses through the boundary, which is itself composed of the same kind of polymer.

In Fig. 11 is recorded a cyclic phenomenon which is intrinsic to the units composed of proteinoid. In the first picture are shown microspheres which have, during two weeks in their liquor, developed "buds" which in appearance, texture, and tenacity resemble buds on yeast and some bacteria. In the second photomicrograph, the "buds" have been removed, a phenomenon resulting from heat, electrical, or mechanical shock. These are then stained with Crystal Violet and transferred to a solution of proteinoid saturated at 37° and allowed to cool to 25° over one hour. The "buds" then grow by a kind of heterotrophic accretion. In the last picture, one can see one of the microspheres with a second generation "bud". In this manner, we can visualize an evolution from the simplest physical processes acting on simply derived polymers to yield the minimal complexity required for an evolution to reproduction.

The geological conditions necessary for steps (1) and (2) of Fig. 1 are sufficiently high temperatures, much higher (ca. 800°) for step (1) than for step (2), which requires temperatures at about the boiling point of water. Step (1) is also more complex than indicated in the flowsheet, since it involves hydrolysis and evaporation. Assuming amino acids to be present, we can see that the crucial steps (2) and (3) require only moderately high temperatures acting on hypohydrous amino acids, followed by the intrusion of water. This temperature is no more than that found in thousands of terrestrial zones today at or near the surface. For the final step all that is required is rain. Accordingly, the necessary conditions need not be argued for the primitive Earth; they are moderately widespread on the contemporary Earth[9]. The evaluation is thus on a basis far more rigorous than was anticipated when the research began. Other possibilities for polymerization and formation of microparticles have been described.

The reactions are also rugged, fast, and simple. Consequently, primitive life could have arisen innumerable times. The inferred frequent emergence of primitive life is consistent with the abundance of contemporary life.

The current research deals with models of the origin of energy transfer mechanisms, internal synthesis of peptide bonds, and origin of the genetic code[10-15].

The results of the experiments however provide for the first time answers to the following major questions of the origin of life:

1. The origin of order in proteins when no large molecules and no code existed.

The experiments have demonstrated that the information necessary for primitive biologically functional large molecules arose from the monomers from which they formed. This demonstration resolves a fundamental dilemma. No code was necessary at the first stage of life. The macromolecules formed and their internal constraints constituted a simply derived precursor to contemporary genetic systems.

These results, when combined with a recognition of other phenomena described below, explain that information latent in the pre-environment would have been transferred by macromolecular synthesis and assembly to the first individual(s). The experimental findings also tend to negate the hypothesis that any discontinuity existed between pre-life and life or between nonlife and life.

2 The origin of enzymes when no enzymes to make them existed.

The experiments show that appropriate simulated geophysical conditions and mixtures of diverse amino acids yield molecules with weak enzyme-like activity. These molecules, proteinoids, have nearly all, or all, of the salient characteristics of some enzymes. The products have relatively specific behavior, of the kinds found in today's organisms. We thus have one answer to the question of how enzymes arose when there were no enzymes to make them.

3. The origin of metabolism in the absence of metabolizing cells.

By association of individual reactions catalyzed by proteinoids, the origin of metabolism can be understood. This demonstration makes the point, most vividly, that metabolism (and other functional properties) of the cell has its roots and its origin in one kind of material. That material was a sufficiently variegated polymer, or polymers, of amino acids.

For such metabolic potential to exist, prior cells were not needed. However, for fullest expression of this potential in specialization, localization in the cell, and development of very high levels of activity, the evolutionary process has subsequently required the incorporation of such activities into proliferating systems through Darwinian selection.

4. The origin of cells when no cells existed to father them.

George Wald stated in 1954 that the problem of how anything as complex as a cell could have come into existence had often been regarded as "insuperable". Wald also proposed in theory an answer to this question. His answer was based on experiments of F. Schmitt (1956) of the early 1950s. These experiments showed that protein molecules contain the information necessary to assemble themselves into subtly structured microsystems. Since the experiments of Schmitt, the general phenomena of self-assembly have come to be recognized as widespread and powerful. Wald invoked these in a general way for the first cell.

The experiments with proteinoid are, in a specific manner, consistent with this inference. Those experiments demonstrate that many kinds of polymers of many kinds of combination of amino acids, including those produced by heat, assemble themselves into microstructures having a number of resemblances to contemporary cells of the coccoid bacteria. Such features as lipids and nucleic acids, found always as components of contemporary cells, are found not to be necessary in these units. Both the order conferred by nucleic acids and the selective quality of lipids have been shown to be provided in part by the proteinoid in the assembled structure. Many other properties, such as the enzyme-like activities, are also part of the microsystem, being present through the same modulation from molecules to system of step c.

The properties found and the thorough experiments revealing them are documented in detail in the scientific literature. The assembled systems contain, simultaneously, the various properties necessary for primitive life. What has often been regarded as the most difficult of the problems of abiogenesis has thus proved to have in principle an answer of the utmost simplicity. This simplicity is operational, or phenomenological. The intimate structure, molecular or morphological, is however complex.

5. The origin of membranes when no microsystems containing membranes existed.

Some experts in cellular biology have regarded the origin of the membrane as a most difficult problem.

The proteinoid microstructures have been shown to display double layers which resemble the ultrastructure of contemporary cells (Fig. 3), permit retention of large molecules while allowing small ones through (Fig. 4), and selectively allow interior proteinoid molecules to diffuse out (Fig. 5). These are properties of membranes; the experiments thus illustrate how the origin of primitive membranes could have been intrinsic to the assembly of proteinoid microstructures.

6. The origin of reproduction.

Also inherent in the proteinoid microparticle is the tendency to participate in the reproduction of its own likeness (Fig. 6). The nature of this sequence of processes seems to be closer, in many respects, to simple physical phenomena than to the complex concomitance of events associated with contemporary reproduction. Such simplicity, however, is appropriate for the recent emergence of a "biological" type of phenomenon from the inanimate world. On the other hand, these processes are processes of systems and their complexity is such that they were not, and could not have been, predicted alone from knowledge of the behavior of macromolecules.

Perhaps of most interest is the fact that all of these answers have their roots in the same kind of material, a hetero-polyanhydro amino acid, such as could arise spontaneously by simple heating. Indeed, contemporary life itself is notable for, and has resisted analysis because of, the fact that it represents so many properties in association with each other.

Table I

Relative Abundance of Amino Acids Synthesized from Simulated Primitive Gases

Amino Acid	Thermal Synthesis		Spark Synthesis
	Harada and Fox (1030° C) %	Oró (1030° C) %*	Miller (arc temp.) %*
Glycine	24.4	61.05	55.76
Alanine	20.2	36.78	30.08
Serine	10.0	0.26	-
Aspartic acid	15.2	0.06	0.33
Glutamic acid	10.2	0.26	0.55
Threonine	3.0	0.66	-
Leucine	4.6	0.13	-
Isoleucine	2.5	0.06	-
Proline	2.3	-	-
Valine	2.1	-	-
Phenylalanine	2.2	0.01	-
Tyrosine	2.0	0.01	-
Alloisoleucine	1.4	0.06	-
Lysine	-	-	-
Beta-alanine	-	0.66	13.28

*Recalculated from the original tables of data.

Table II

Catalytic Activities Found in Proteinoids

Substrate or Reaction	Authors	Year of Publication
p-Nitrophenyl acetate	Fox, Harada, Rohlfing	1962
p-Nitrophenyl acetate	Noguchi, Saito	1962
p-Nitrophenyl acetate	Rohlfing and Fox	1967
p-Nitrophenyl acetate	Usdin, Mitz, Killos	1967
Glucose → glucuronic acid → CO_2	Fox and Krampitz	1964
ATP → ADP	Fox	1965
	Durant and Fox	1966
p-Nitrophenyl phosphate	Oshima	1968
Pyruvic acid → acetic acid + CO_2	Krampitz and Hardebeck	1966
	Hardebeck, Krampitz, Wulf	1968
Oxaloacetic acid → pyruvic acid + CO_2	Rohlfing	1967
Amination of ketoglutaric acid	Krampitz, Diehl, and Nakashima	1967
	Krampitz, Baars-Diehl, Haas, and Nakashima	1968
Dehydrogenation of glutamic acid	Krampitz, Haas, Baars-Diehl, and Nakashima	1968

Table III

Properties of Thermal Proteinoids in Common with Those of Contemporary Proteins

Limited heterogeneity
Qualitative composition
Quantitative composition (except serine, threonine)
Range of molecular weight (4000 - 10,000)
Color tests
Solubilities
Inclusion of nonamino acid groups
Optical activity
Salting-in and salting-out properties
Precipitability by protein reagents
Hypochromicity
Infrared absorption maxima
Recoverability of amino acids on hydrolysis
Susceptibility to proteolytic enzymes
Some catalytic activities
Inactivatability of catalysis by heating in aqueous solution
"Nonrandom" (nonuniform) sequential distribution of residues
Nutritive quality
Hormonal activity (MSH)
Binding of polynucleotides (by basic proteinoids)
Morphogenicity

Primordial gases $\xrightarrow[(1)]{\text{(Synthesis)}}$ small organic molecules

[amino acids, N bases, etc.] $\xrightarrow[(2)]{\text{(Polymerization)}}$ Macromolecules

(prebiotic protein or nucleic acid) $\xrightarrow[(3)]{\text{(Self Assembly)}}$

Protocells $\xrightarrow[(4)]{\text{(Reproduction and Darwinian Selection)}}$ Contemporary cells and multicellular organisms.

Fig. 1

Outline of Problem

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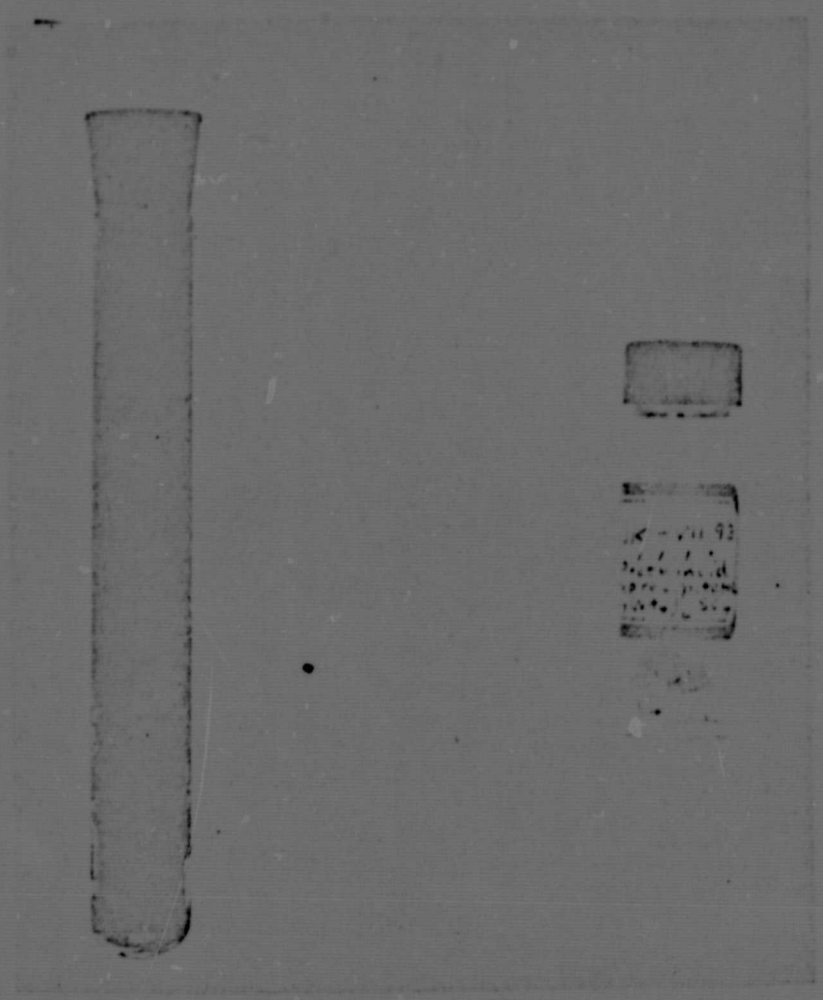

Fig. 2. Amino acids. (a) Heated above the boiling point of water. (b) With sufficient aspartic acid and glutamic acid, heated above the boiling point of water and purified by salting out.

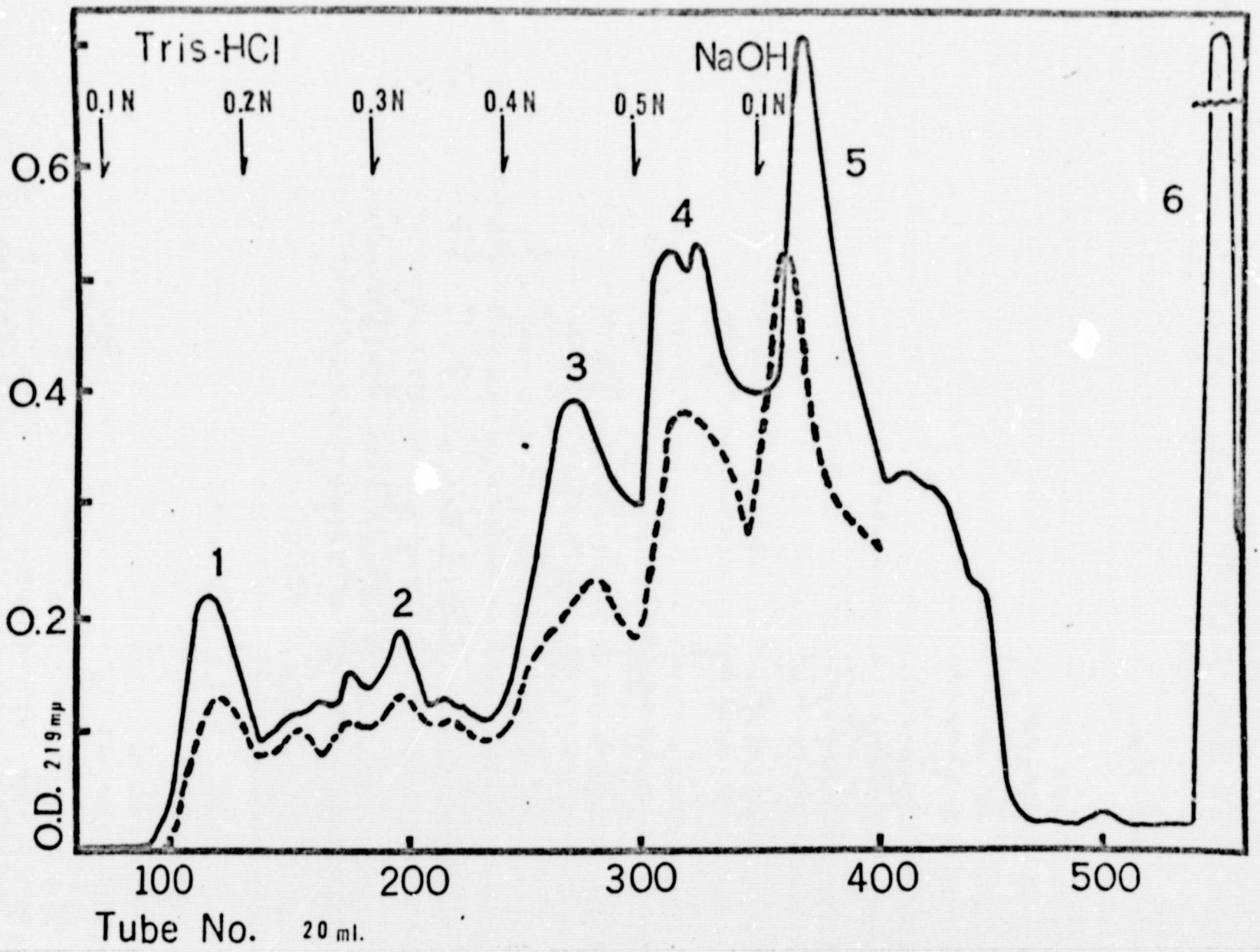

Fig. 3. Elution pattern of 1:1:1-proteinoidamide fractionated on DEAE-cellulose. The broken line is a second run.

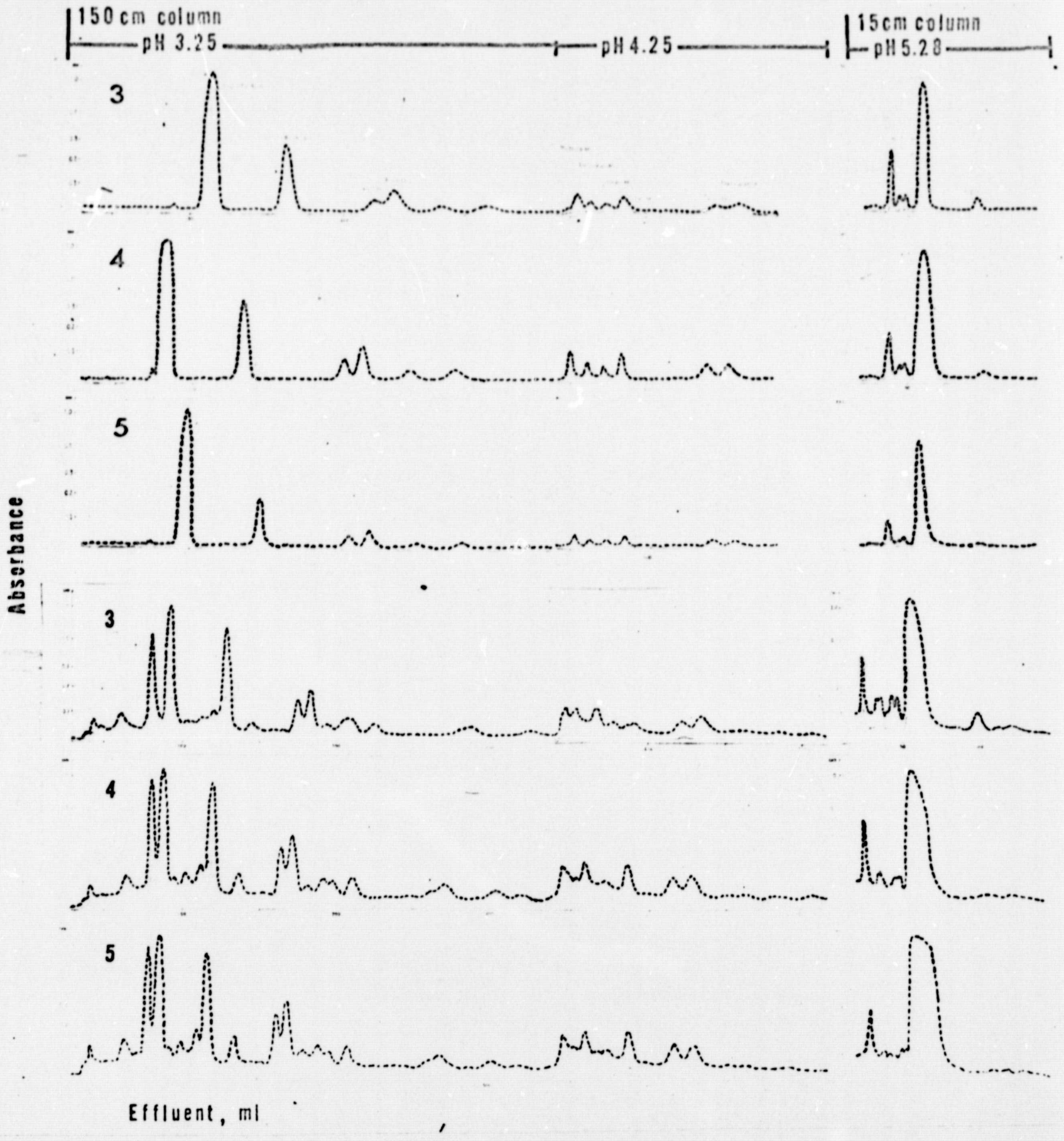

Fig. 4. Chromatograms of total hydrolyzates (top three) and of partial hydrolyzates (bottom three) of material of peaks 3, 4, and 5, from DEAE-cellulose according to Fig. 3.

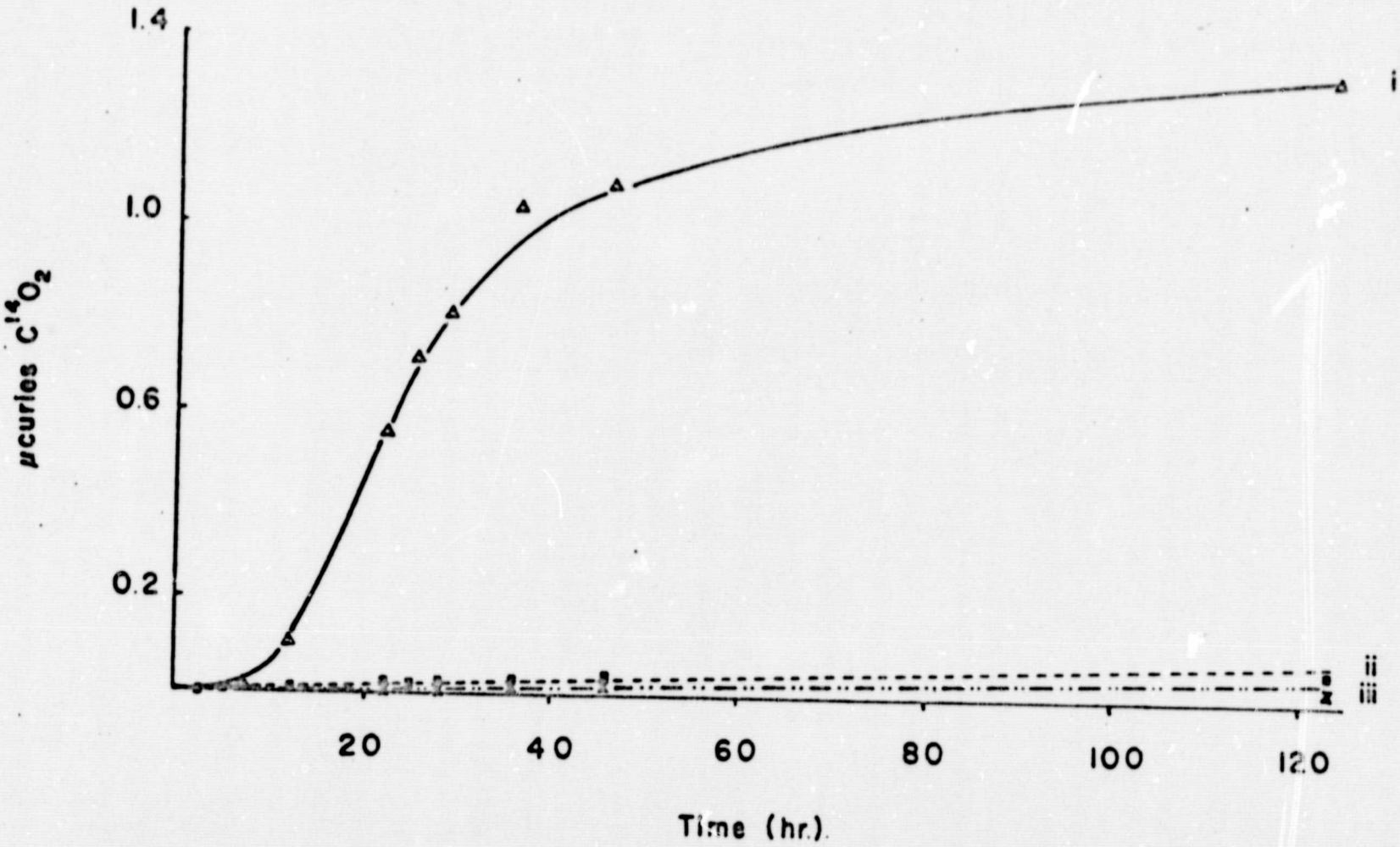

Fig. 5. Decarboxylation of pyruvic acid catalyzed by proteinoid. Controls without proteinoid and with proteinoid hydrolyzate are also shown. Such results are obtained with pyruvic acid-1-^{14}C but not with pyruvic acid-2-^{14}C or pyruvic acid-3-^{14}C.

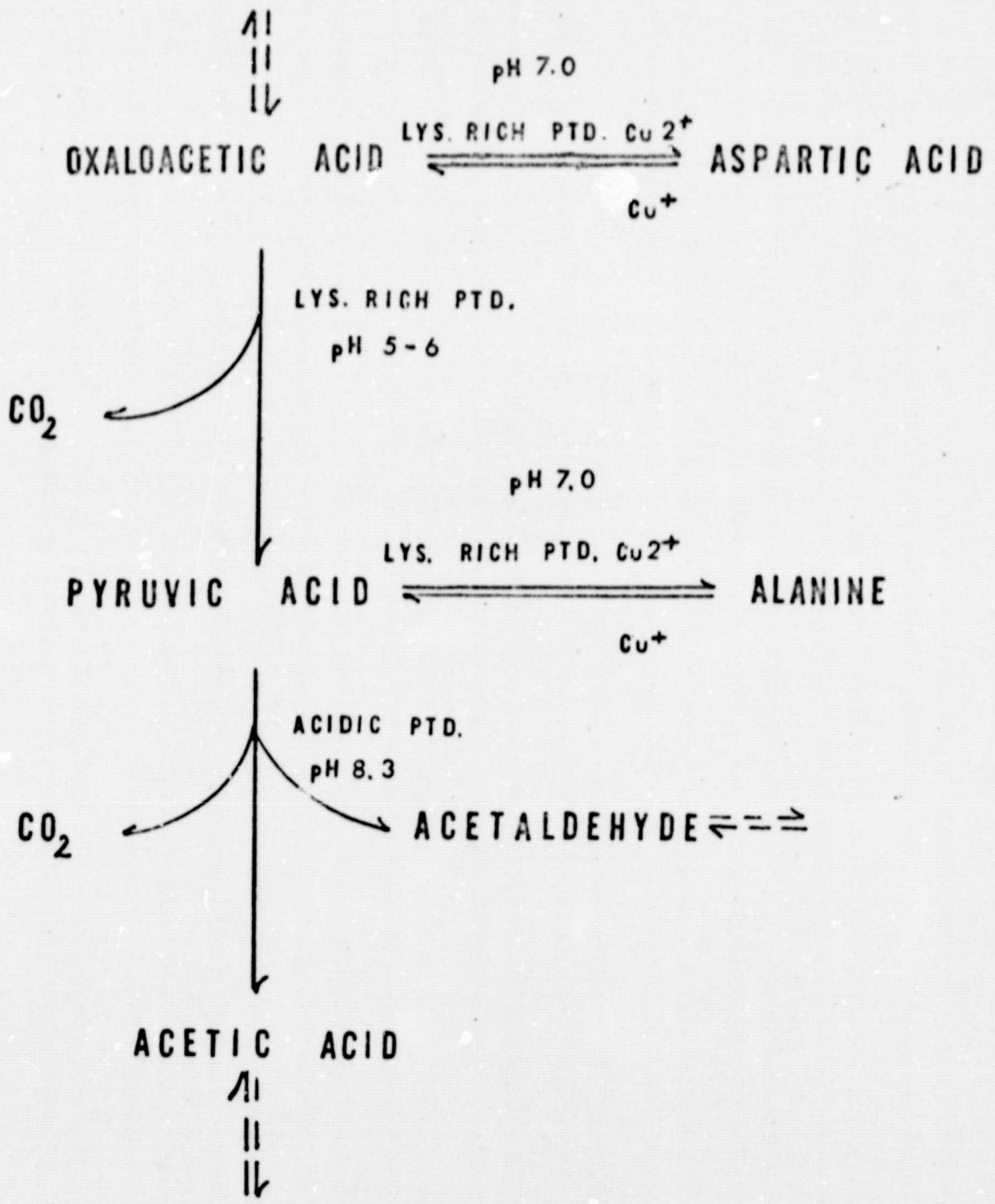

Fig. 6. Indication of an origin of some of metabolism, from data on catalytic activities in proteinoids.

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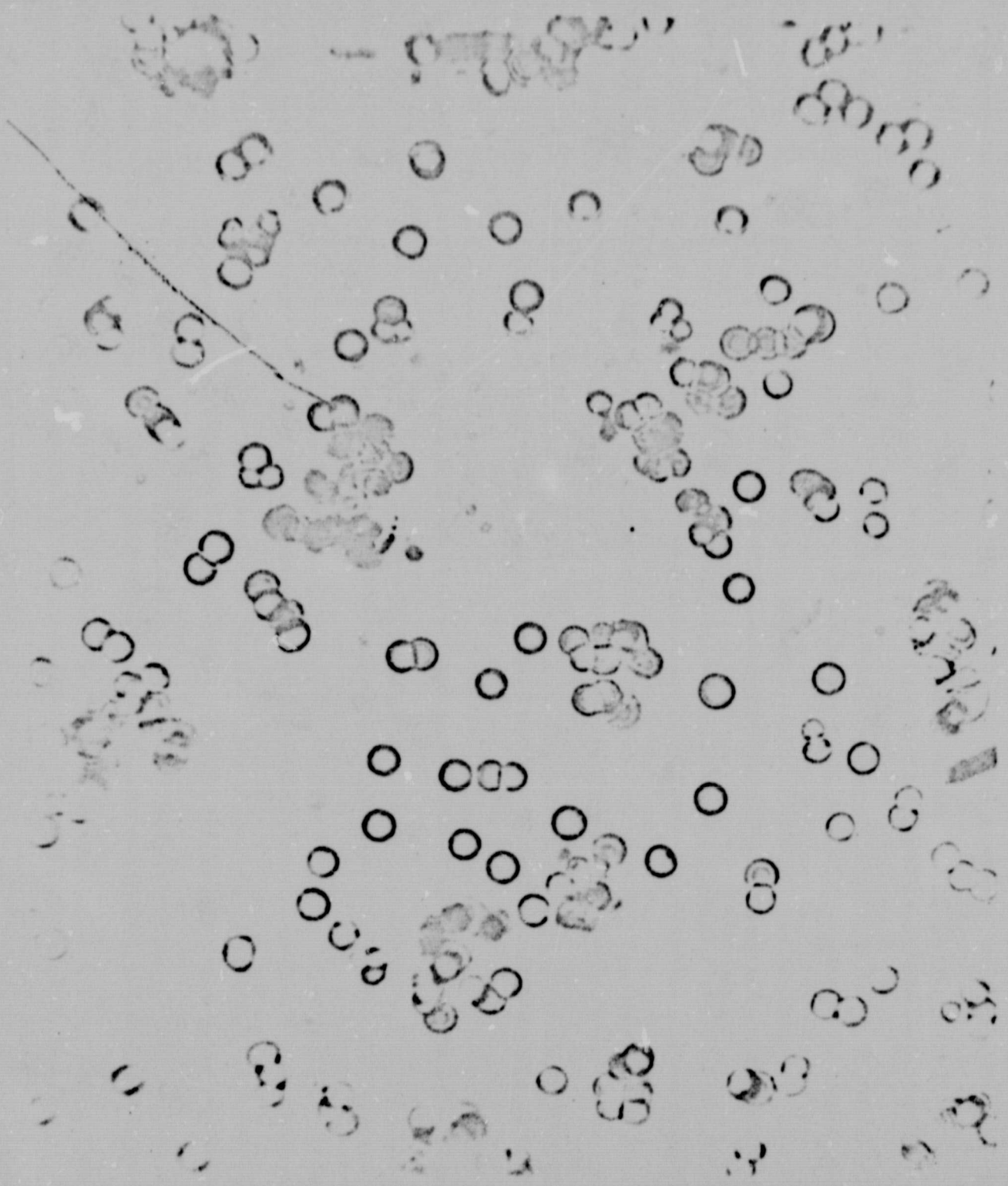

Fig. 7. Proteinoid microspheres. Approximately 2 μ in diameter.

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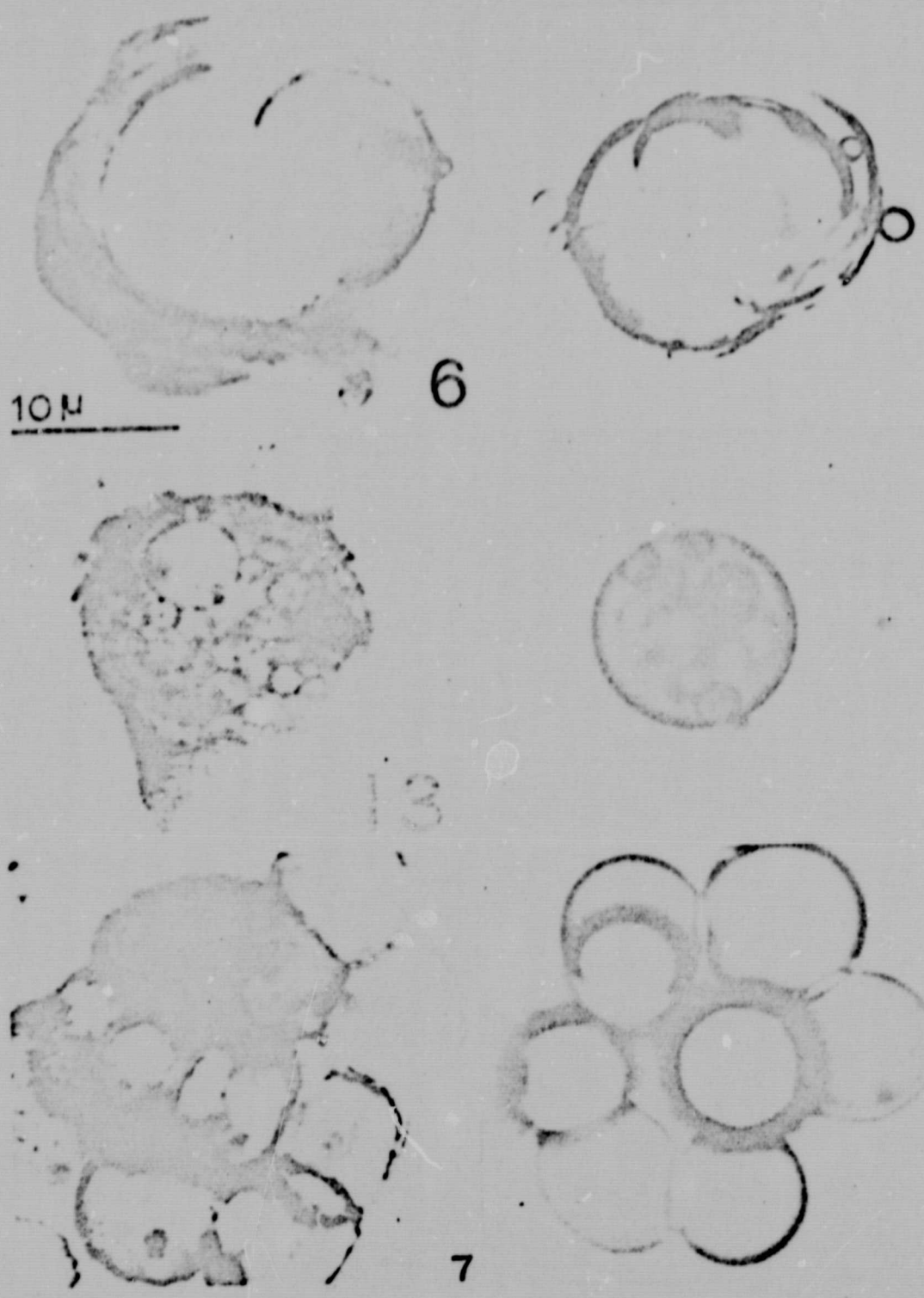

Fig. 8. Microfossils and proteinoid microspheres. Microfossils on left, microspheres on right.

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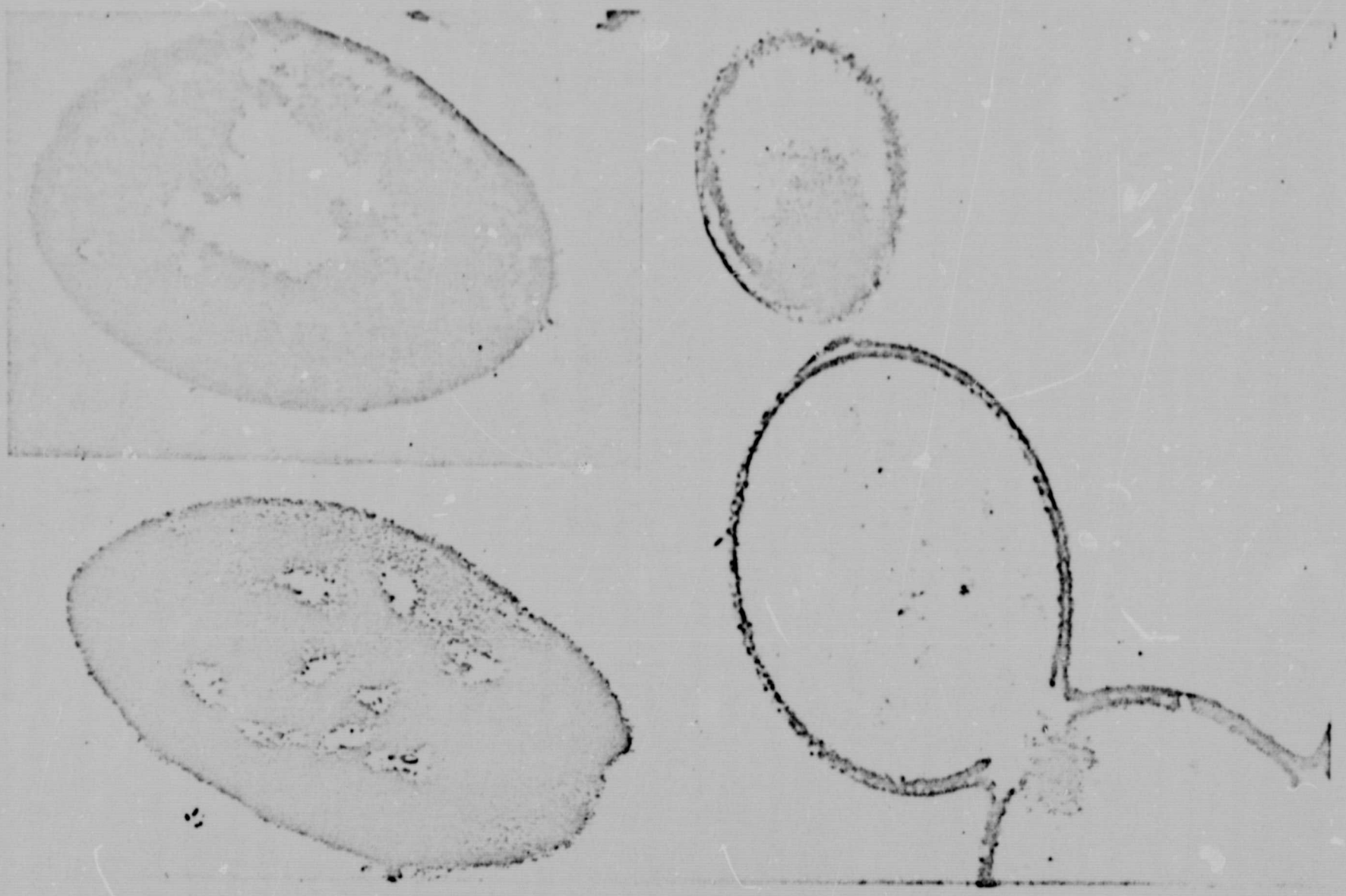

Fig. 9. Electron micrographs of Bacillus cereus and of proteinoid microsphere. Section of proteinoid microsphere in upper left hand corner, of Bacillus cereus (after Murray) in lower left hand corner. Double layered boundary in microspheres on right after pH elevation in a suspension. Fixed with osmium tetroxide and sectioned in methacrylate blocks.

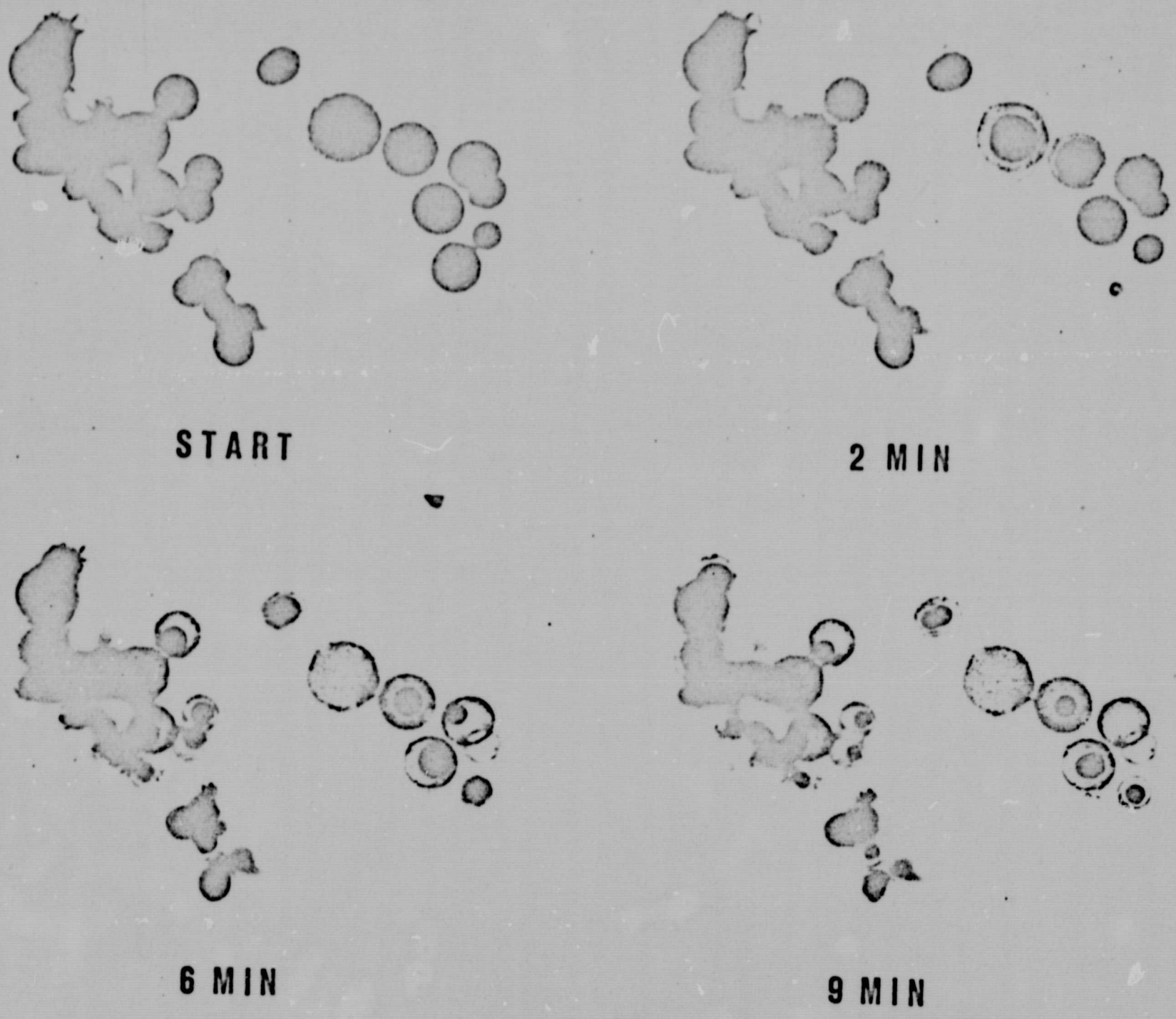

Fig. 10. Time-lapse study in ultraviolet light of diffusion of polymer outward from microsphere when pH is raised slightly. Experiment performed by Mr. R. J. McCauley with Dr. Philip O'B. Montgomery.

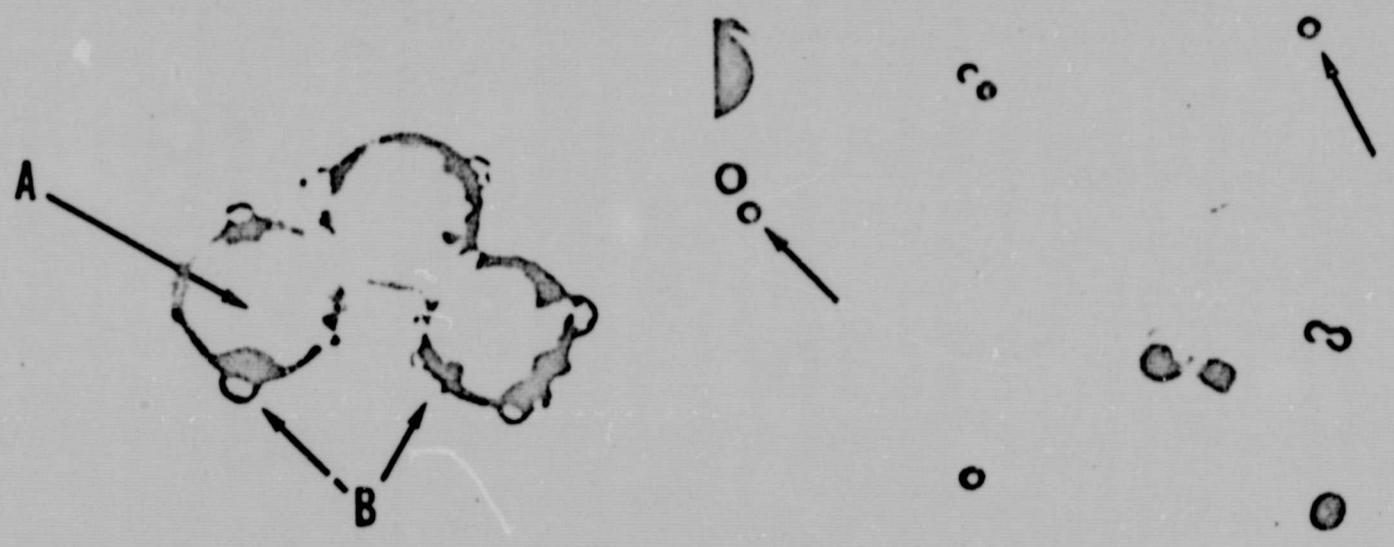

Fig. 11. Optical micrograph of proteinoid microsphere replicating by "budding" and heterotrophic growth. (a) Microsphere with buds, (b) Buds after removal, (c) Microspheres which have grown from stained buds, (d) Microsphere with second generation bud.

FOOTNOTES

1/ Discernible remaining problems include: evolution of energy mechanisms, from heat energy to predominantly phosphate bond energy transfer; development of internal synthesis of peptide bonds; and origin of a nucleic acid code. These problems are to some degree the problems of evolution of primitive life to contemporary life rather than problems of the origin of primitive life. Experiments attempting to answer these three questions are under way. This paper, however, deals primarily with a model of the origin of a protocell. The possibility of finding a second or third set of answers to questions of the first protocell is also being investigated.

2/ These experiments in open hot tubes followed by hydrolysis have their geological counterparts. The use of closed flasks which retain H_2, for example, is not an accurate simulation of what can occur in the atmosphere.

3/ One idea which persists without experimental support is the long entrenched one of a "primordial soup." Careful survey of the chemistry has led to the conclusion that many organic compounds are relatively unstable in dilute aqueous solution (Hull, 1960; Bernal, 1960; Fox, 1968a). Accordingly, in the early phase of molecular evolution, anhydrous or hypohydrous (Fox, 1960) conditions were more favorable. Moreover, many biologically significant compounds, polymers and heterocyclic rings, are formally anhydrides or anhydrodehydrogenation products (Fox, 1957).

4/ Geologically, the amino acids had not necessarily to be totally anhydrous (see Kenyon and Steinman, 1969). The thermodynamics of the situation does not require a total absence of water; it requires conditions other than dilute aqueous solution. More significantly, temperatures or other conditions which will cause condensation polymerization will also cause concentration of an aqueous solution to dryness. Although we were sure that experiments could proceed normally from amino acids polymerized after their recovery by evaporation of solution, experiments (by interested classroom students) have verified this expectation.

5/ Tatum (1963) and Strominger (1960) have each shown that polypeptides can be synthesized in vivo without templet. This fact has been used as a reason to consider a nontemplet synthesis for primitive forms (Lipmann, 1965). Such inferences lead in turn

to the concept of a primacy of protein molecules preceding nucleic acids. Also, scrapie has been proposed as a self-reproducing small protein molecule (Griffith, 1967) but adequate evidence for this proposal is lacking.

For a typical contemporary cell, I lean toward the simultaneous origin of protein and nucleic acid, as suggested by experiments based on mixed amino acid adenylates (Krampitz and Fox, 1969).

6/ The proteinoids are not highly branched, having, for example, 2-4 N-termini in molecules of 5000 weight. They are split by proteolytic enzymes but, in general, not as rapidly as are proteins. Some fractions of poly (glutamyl, glycyl, tyrosyl) have been found by Nakashima to be very susceptible to chymotrypsin and to pronase.

As yet, no antigenicity or helicity has been found in thermal proteinoids. Since those tested have had molecular weights below 10,000, this may not be surprising. Assiduous search for these properties has not been carried out since these attributes were not visualized as providing selective advantage at an early stage of evolution.

7/ In answering a number of questions on the differences between coacervate droplets and proteinoid microparticles, I would state first that some regard proteinoid microspheres as a kind of coacervate droplet. The correctness of this position of course depends upon definitions, which for coacervate droplets are not easily found in the literature. Assuming the microsphere to be a coacervate droplet, we must emphasize that the microsphere has many properties to distinguish it from most or all other coacervate droplets studied in this context:

(1) The units are uniform in size, and as such often resemble contemporary cells. Their photomicrographs have been mistaken for, e.g., algal cells, and for dividing sea-urchin eggs (upon digital pressure).

(2) They have stability. They can be centrifuged, and sectioned for electron microscopy.

(3) To exhibit catalytic activity, inclusion of enzymes is unnecessary. The component proteinoids confer catalytic activities upon the unit. Different proteinoids have different arrays of catalytic activities.

(4) The crucial difference, as a model of a primordial cell, is the source. Whereas other coacervate droplets studied in the context of origins are made from polymers from organisms already here, the proteinoid microspheres assemble from synthetic polymer. The synthetic polymer arises from monomers, not organisms. As such, proteinoid alone answers the crucial question of how cells arose when there were no cells.

As models of contemporary cells, the proteinoid unit has the advantage that the polymer can be precisely and almost infinitely varied. One consequence of this flexibility has been the development of proteinoid-polynucleotide microparticles, so that we now have experimental systems of cell models containing nucleoproteinoid organelle models (Waehneldt and Fox, 1968; Yuki and Fox, 1969).

8/ The model developed carries out its functions without nucleic acid and without added lipid. The findings of osmophilicity without lipid, and of selective retention without lipid, and of a unit structure in the boundary without lipid is not without counterpart. David Green and associates, for instance, have removed lipid from mitochondria by washing with aqueous acetone, and have found that the structure was unchanged. A number of papers from Green's laboratory have included "structural protein" in the title (Green et al., 1961; Criddle et al., 1961). One inference from the above results is that lipid was not essential for either primitive or contemporary cells, but that they contributed to the optimal state of some attributes such as selective diffusion. A related inference is that due to hydrocarbon sidechains in amino acid residues, both proteinoids and proteins have much lipid quality. We have also some evidence that separate lipophilic material is formed during the condensation of amino acids. Whether then or later, lipids would tend to associate with the lipophilic regions of proteinoids or proteins, as observed in experiments.

9/ In response to the question of whether life could begin now, we may refer to a recent article analyzing this possibility (Fox and McCauley, 1968). Such a question raises a consequent question of why do we not see de novo life now, if it is originating. Darwin explained that life might arise now if the Earth were sterile, but that de novo life (or its precursors) would be consumed by life already here. Another explanation is that life arising now would be indistinguishable from lineal descendants of primitive types already here. A further explanation is that, if a de novo form were somewhat distinguishable, systematists would merely classify it as a previously unrecognized old species.

The production of amino acids occurs best thermally in a siliceous bed. The steps of polymerization and spherulization have been carried out in a variety of earthy materials and on lava. The reactions are found to be rugged.

10/ We have elsewhere indicated that experiments suggest that contemporary proteins and contemporary nucleic acids probably arose simultaneously. In the condensation of adenylates of mixed amino acids (Krampitz and Fox, 1969), adenylic acid appears in preliminary experiments to have condensed to polymers. If this condensation were to occur simultaneously with other mononucleotides, an original nucleic acid could result. Preliminary experiments indicate also modifications by polynucleotides of the condensation to polyamino acids. Also, the occurrence of synthetase or polymerase activity in the polyamino acids would advance the molecular evolution; these activities are being sought.

The condensation products are in purview as models of evolutionary precursors of ribosomes.

11/ The experiments reported to this point explain in principle the origin on the Earth of a protocell. While this had a sufficient number of the properties of a contemporary cell to initiate some organismic evolution, some attributes of all, or the majority of, contemporary cells are missing. These include an internal synthesis of protein, a coding mechanism, and a mode of transfer of energy1/. For the model of the primitive, as defined here, the picture is relatively complete. Experiments aimed at guiding the evolution from the primitive to the contemporary are proceeding in other laboratories (Woese, 1967; Lacey and Pruitt, 1969) and in ours (Yuki and Fox, 1969; Krampitz and Fox, 1969) but this chapter of the story is incomplete. Our experiments include various polymerizations of mononucleotides (Schwartz and Fox, 1967), the polymerization of mixed amino acid adenylates as well as a search for formation of polynucleotides from the adenylates (Krampitz and Fox, 1969), selective interactions between (poly) amino acids and (poly) nucleotides (Yuki and Fox, 1969), effects of polynucleotides on peptide bond synthesis (Lacey and Fox, 1969), and studies of the origin of photobiochemistry (Weber et al., 1968).

12/ One consequence of the purely thermal experiments is that they have shown how a self-replicating, ultrastructured microsystem with a selective membrane might have been composed of, and spontaneously assembled from, polyamino acid having order (self-determined repeating sequences) plus catalytic activities.

This demonstration of how far evolution could have proceeded with polyamino acids alone does not specify at what stage nucleic acids entered the evolutionary stream. Other routes and other sequences are not necessarily excluded. However, the appearance of biopolymer, either protein or nucleic acid, without precursor monomers as seems to be suggested by some, is not visualized by this author.

13/ Leading proponents of the concept of the primacy of nucleic acid in the primordial sequence are the late H. J. Muller (1966), Rich (1962), and Crick (1968). As requested, my criticism of their expressed ideas are a failure to recognize:

(1) that any polymer, whether DNA, RNA, or protein, must first have arisen from monomers,

(2) the experimental fact now well supported, that (thermally) reacting amino acids dictate their own sequences in a manner of providing an early evolutionary form of a genetic system,

(3) the need for the origin of enzymes when no enzymes were present to make them. Hypothesizing catalytic activity in the first nucleic acids for the production of subsequent nucleic acids does not suffice for a minimal metabolism.

(4) The assumptions seem to include the idea that a cell would come later.

These concepts include unrecognized or unstated assumptions, ignore experimental facts such as self-ordering of monomers in the formation of polymers, and require placing hypotheses on other unsupported hypotheses.

14/ In attempting to answer the question of at what stage in evolution selection originated, we need to consider definitions. Many definitions of natural selection require reproduction (e.g. Ross, 1962) as the heart of the process. Selection could thus not have begun until a self-reproducing system appeared or until a process leading directly into a self-reproducing entity appeared. The selective thermal interactions are a candidate for this kind of prebiological molecular selection. Some classical biologists in my audiences have suggested that this was the beginning of selection. If one allows the concept of prebiological selection, the fact that amino acids are favored in reactions of simulated primordial atmospheres is an earlier kind of selection.

At the level of the contemporary organism, ground rules for selection have been worked out, as indicated (Ross, 1962). For the model of the primitive cell, ground rules have not been developed. If the first organism were a heterotroph as reasoned by Oparin (1957), Haldane (1929), Van Niel (1956), etc., it could not carry out varied gene-controlled biosyntheses because it was not making nucleic acids in a coded relationship with proteins, which it was also not making. During its existence as a heterotroph, however, variation in the preformed macromolecules could occur. Consequently, the assembled microspheres, or organisms, would vary. For instance, when proteinoid microspheres containing proportions of acidic and basic proteinoid on the borderline between gram-positive and gram-negative were made in experiments (Fox and Yuyama, 1963), some of the particles stained gram-negative while others stained gram-positive. The basis for selection could thus have appeared. The more gram-positive individuals might thus have been selected by attraction to high concentrations of acidic proteinoid. In these higher concentrations, budding and replication could presumably occur more readily.

This kind of selection would be a primitive type to be distinguished from contemporary selection in essentially the same features as "primitive organisms" must necessarily be distinguished from contemporary organisms.

In more direct answer to the question--since amino acids can be made in numerous ways (and so far a generally functional protein-like molecule leading directly to proliferating microsystems has been made in essentially one way) I favor the coupling of amino acids as the most significant initial stage in selection. (Earlier definitions would of course not have recognized this possibility, since the data are relatively new.)

15/ Synthetic ribonuclease need not be considered in the same context as catalytically active proteinoid. Using Webster's definition of artificial (1951), proteinoids are artificial, not synthetic, but they are more than "artificial". Webster's definition states that "artificial is applicable to anything that is not the result of natural conditions but is, in a sense, a human creation." The proteinoids are artificial in having been created by humans. They are more than artificial in being the result of natural or geological (nonorganismic) conditions. Webster's dictionary is not adequate to this concept. We are dealing with the need for a new adjective to describe what occurred by spontaneous synthesis as a beginning point for an evolution which permitted in its later stages artificial creation.

Barghoorn, E. S. and Schopf, J. W., 1965. Microorganisms from the late Precambrian of Central Australia. Science 150, 337-339.

Bernal, J. D., 1960. (Comments on Hull's paper.) Nature 186, 694-695.

Calvin, M., 1962. Communication: from molecules to Mars. Bull. Am. Inst. Biol. Sciences 12, 29-44.

Claus, G. and Nagy, B., 1961. A microbiological examination of some carbonaceous chondrites. Nature 192, 594-596.

Crick, F.H.C., 1968. The origin of the genetic code. J. Mol. Biol. 38, 367-379.

Criddle, R. S., Bock, R. M., Green, D. E., and Tisdale, H. D., 1961. Specific interaction of mitochondrial structural protein with cytochromes and lipids. Biochem. Biophys. Research Commun. 5, 75-80.

Danielli, J. F., 1936. Properties of lipoid films in relation to the structure of the plasma membrane. J. Cellular Comp. Physiol. 7, 393-408.

Dose, K., Taki, L., and Rauchfuss, H., 1969. Hemoproteinoids and their catalase activity. Fedn. Europe Biochem. Socs. Madrid, Abstract 432.

Fox, S. W., 1956. Evolution of protein molecules and thermal synthesis of biochemical substances. Amer. Scientist 44, 347-359.

Fox, S. W., 1960. How did life begin? Science 132, 200-208.

Fox, S. W., 1964. Thermal polymerization of amino-acids and production of formed microparticles on lava. Nature 201, 336-337.

Fox, S. W., 1965. A theory of macromolecular and cellular origins. Nature 205, 328-340.

Fox, S. W., 1968. Natural Polymers. Abiotic polymerization and self-organization. In Encyclopedia of Polymer Science and Technology, Vol. 9 (H. F. Mark, N. G. Gaylord, and N. M. Bikales, eds.). Interscience, New York, 284-315.

Fox, S. W., 1968a. Self-assembly of the protocell from self-ordered polymer. J. Sci. Ind. Res. 27, 267-274.

Fox, S. W., 1969. Self-ordered polymer and propagative cell-like systems. Naturwissenschaften 56, 1-9.

Fox, S. W., Harada, K., and Vegotsky, A., 1959. Thermal polymerization of amino acids and a theory of biochemical origins. Experientia 15, 81-84.

Fox, S. W. and McCauley, R. J., Aug.-Sept., 1968. Can Life Originate Now? J. Am. Museum Natural History, 26-31.

Fox, S. W. and Nakashima, T., 1967. Fractionation and characterization of an amidated thermal 1:1:1-proteinoid. Biochim. Biophys. Acta 140, 155-167.

Fox, S. W., Vegotsky, A., Harada, K., and Hoagland, P. D., 1957. Spontaneous generation of anabolic pathways, protein and nucleic acid. Ann. N.Y. Acad. Sci. 69, 328-337.

Fox, S. W. and Waehneldt, T. V., 1968. The thermal synthesis of neutral and basic proteinoids. Biochim. Biophys. Acta 160, 246-248.

Fox, S. W., Winitz, M., and Pettinga, C. W., 1953. Enzymic synthesis of peptide bonds. III. The influence of residue type on papain-catalyzed reactions of some benzoylamino acids with some amino acid anilides. J. Am. Chem. Soc. 75, 5539-5542.

Fox, S. W. and Yuyama, S., 1963. Effects of the gram stain on microspheres from thermal polyamino acids. J. Bact. 85, 279-283.

Goulian, M. and Kornberg, A., 1967. Enzymic synthesis of DNA. XXIII. Synthesis of replicative form of phage øX 174 DNA. Proc. Nat'l Acad. Sci. U.S. 58, 1723-1730.

Green, D. E., Tisdale, H. D., Criddle, R. S., and Bock, R. M., 1961. Structural protein and mitochondrial organization. Biochem. Biophys. Research Communs. 5, 81-84.

Griffith, J. S., 1967. Self-replication and scrapie. Nature 215, 1043-1044.

Haldane, J.B.S., 1929. The origin of life. Rationalist Annual.

Harada, K. and Fox, S. W., 1964. Thermal synthesis of natural amino-acids from a postulated primitive terrestrial atmosphere. Nature 210, 335.

Hardebeck, H. G., Krampitz, G., and Wulf, L., 1968. Decarboxylation of pyruvic acid in aqueous solution by thermal proteinoids. Arch. Biochem. Biophys. 123, 72-81.

Huffman, H. M., 1942. Thermal data. XV. The heats of combustion and free energies of some compounds containing the peptide bond. J. Phys. Chem. 46, 885-891.

Hull, D. E., 1960. Thermodynamics and kinetics of spontaneous generation. Nature 186, 693-694.

Ito, E. and Strominger, J. L., 1960. Enzymic synthesis of the peptide in a uridine nucleotide from Staphyloccus aureus. J. Biol. Chem. 235, PC5-PC7.

Kenyon, D. H. and Steinman, G., 1969. Biochemical Predestination. McGraw-Hill, p. 175.

Keosian, J., 1968. The Origin of Life, second ed. Reinhold, New York, p. 83.

Krampitz, G. and Fox, S. W., 1969. The condensation of the adenylates of the amino acids common to protein. Proc. Nat'l Acad. Sci. U.S. 62, 399-406.

Lacey, J., Jr. and Pruitt, K. M., 1969. Origin of the genetic code. Nature 000, 0000-0000.

Lipmann, F., 1965. Projecting backward from the present stage of evolution of biosynthesis. In: The Origins of Prebiological Systems and of their Molecular Matrices. Academic Press, New York, 259-273.

Mach, B., Reich, E., and Tatum, E. L., 1963. Separation of the biosynthesis of the antibiotic polypeptide tyrocidine from protein biosynthesis. Proc. Nat'l Acad. Sci. U.S. 50, 175-181.

Miller, S. L., 1955. Production of some organic compounds under possible primitive Earth conditions. J. Am. Chem. Soc. 77, 2351-2361.

Muller, H. J., 1966. The gene material as the initiator and the organizing basis of life. Amer. Naturalist 100, 493-517.

Oparin, A. I., 1957. The Origin of Life on the Earth. Academic Press.

Oró, J., 1965. Stages and mechanisms of prebiological organic synthesis. In: The Origins of Prebiological Systems and of their Molecular Matrices (S. W. Fox,ed.). Academic Press, New York, 137-171.

Ponnamperuma, C., 1965. The chemical origin of life. Science Journal No. 65, 39-45.

Rich, A., 1962. On the problems of evolution and biochemical information transfer. In: Horizons in Biochemistry (M. Kasha and B. Pullman, eds.). Academic Press, New York, 103-126.

Rohlfing, D. L., 1967. The catalytic decarboxylation of oxaloacetic acid by thermally prepared poly-α-amino acids. Arch. Biochem. Biophys. 118, 468-474.

Rohlfing, D. L. and Fox, S. W., 1969. Catalytic activities in thermal polyanhydro-α-amino acids. Advances in Catalysis 20, 000-000.

Ross, H. H., 1962. A Synthesis of Evolutionary Theory. Prentice-Hall, p. 99.

Sanchez, R. A., Ferris, J. P., and Orgel, L. E., 1966. Cyanoacetylene in prebiotic synthesis. Science 154, 784-785.

Schmitt, F. A., 1956. Macromolecular interaction patterns in biological systems. Proc. Am. Phil. Soc. 100, 476.

Schwartz, A. and Fox, S. W., 1967. Condensation of cytidylic acid in the presence of polyphosphoric acid. Biochim. Biophys. Acta 134, 9-16.

Seventh International Congress of Biochemistry, 1967. Symposium on The Self Assembly of Structural Subunits, plus other papers. Tokyo, Japan.

Spiegelman, S., 1967. The synthesis of living systems. In vitro synthesis of viral RNA. Chem. Eng. News 45, no. 33, 150-156.

Taube, M., Zdrajewski, St. Z., Samochocka, K., and Jezierska, K., 1967. Angew Chem. Int'l Ed. Engl. 6, 247.

Van Niel, C. B., 1956. Evolution as viewed by the microbiologist. In: The Microbe's Contribution to Biology (A. J. Kluyver and C. B. Van Niel, eds.). Academic Press, 155-176.

Wald, G., Aug. 1954. The origin of life. Sci. Amer. 191, 44-53.

Weber, A. L., Wood, A., Hardebeck, H. G., and Fox, S. W., 1968. The accelerated photodecarboxylation of pyruvate and glyoxalate by proteinoid. Federation Proc. 27, 830.

Webster's Dictionary of Synonyms, 1951. G. & C. Merriam Co. Publishers, Springfield, Mass., U.S.A., p. 75.

Woese, C., 1968. The fundamental nature of the genetic code: prebiotic interactions between polynucleotides and polyamino acids or their derivatives. Proc. Nat'l Acad. Sci. U.S. 59, 110-117.

Yuki, A. and Fox, S. W., 1969. Selective formation of particles by binding of pyrimidine polybironucleotides or purine polyribonucleotides with lysine-rich or arginine-rich proteinoids. Biochem. Biophys. Research Communs. 00, 0000-0000.