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How Did Life Begin?

Recent experiments suggest an integrated origin of anabolism, protein, and cell boundaries.

Sidney W. Fox

The scientific question of the mechanism of life's beginning is a more sophisticated version of the personal question, "Where did I come from?" This question, appropriately phrased, is one which man generally has long asked himself and which man individually asks from his early childhood. If we accept the proposition that the impetus of the scientist is truly curiosity, virtually all thinking men are to a point scientists because of their special curiosity about this problem.

One consequence of such widespread concern is the large amount of writing on the origin of life. The total number who have done little or no experimentation but have conjectured in print about this problem is remarkably large. The number who are currently active in putting ideas to experimental test is, however, remarkably small. Despite this emphasis, there are many whose thoughtful analyses should be credited with providing stimulating ideas and an increasingly favorable intellectual climate. Especially pertinent are printed speculations of Oparin (*1*), Bernal (*2*), Urey (*3*), Rubey (*4*), and Wald (*5*). Inasmuch as the experiments in our laboratory are treated here in some detail, I am pleased to acknowledge also careful and devoted collaboration, especially that of Kaoru Harada.

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The first international symposium on the origin of life was held in Moscow in 1957 under the auspices of the International Union of Biochemistry (*6*). The subject matter was at that time divided into five consecutive stages. A similar division involves (i) synthesis of organic compounds and (ii) synthesis of simple biochemical substances. The majority of the experiments which have been performed fall into these first two stages, which at times are telescoped into one stage. These experiments report production of, principally, amino acids under presumed prebiological conditions. The fact that the production of these biochemically significant organic compounds falls into one or both of the first two stages underlines the outlook that amino acids are rather far from being synonymous with life, a relationship which has not always been recognized.

Stage (iii), having to do with production of large molecules, such as proteins, has received experimental attention for almost as long as have the amino acids, with far fewer results.

It is to be expected that life will ultimately be found to have arisen in stage (iv), which has to do with organized cellular structure, or in stage (v), which concerns evolution of macromolecules and metabolism, or during both. There are in fact reasons to believe that, although it is analytically useful to think of these stages one at a time, the first life involved a simultaneous orchestration of all five.

Production of Amino Acids

Insofar as I am aware, the first bold experiments expressly constructed to provide information on stage (i) in prebiological chemistry were those of Calvin and his associates (*7*). Treatment of carbon dioxide and water in a cyclotron gave significant yields of formaldehyde and formic acid. The production of formaldehyde permitted visualization of the formation of carbon-carbon bonds and, therefrom, of a sufficient variety of organic compounds. Calvin's experiments have been criticized on the basis that the prebiological atmosphere contained only a small proportion of carbon dioxide. One answer to this objection is that no more than a small proportion of any material was needed for the germ of life. I see no adequate basis for assuming, as has often been done, that the origin of life is necessarily a general geochemical problem.

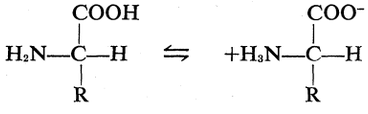
An experimental demonstration that especially focused attention upon this field of inquiry was the production of amino acids by electrical discharge in a mixture of methane, hydrogen, ammonia, and water, as reported more than six years ago (*8*). Miller obtained a few natural amino acids, some that are not found or are very rarely found in proteins, many ninhydrin spots not so far reported as identified, and other acids (*9*). Amino acids are of course more significant in our context than are formaldehyde and formic acid, and the experiments leading to production of those organic compounds are especially well known, undoubtedly for this reason.

Looking backward from 1960 we can see that, in fact, a majority of published experiments in this field have dealt with production of amino acids. These results are comprised in more than ten papers describing scores of experimental modes for production of amino acids under conditions that can be designated prebiological (*10*). One of the discernible reasons for the emphasis on amino acids is the fact that these substances are the components of pro-

Table 1. Examples of effects of thermal polymerization of amino acids.

Leucine	diketopiperazine, tar
Phenylalanine	diketopiperazine, tar
Tryptophan	tar
Aspartic acid + leucine	linear peptides
Glutamic acid + phenylalanine	linear peptides
Lysine + alanine	linear peptides
Excess aspartic acid + excess glutamic acid + 16 common amino acids	linear peptides containing all 18 amino acids

tein. It is difficult to visualize a primeval form of life which was not protein-centered in the same way that all current terrestrial life is. Another reason is the fact that amino acids, thanks to paper chromatography and ninhydrin, are easily identified. Another fundamental reason is that amino acids are relatively stable organic compounds. This stability inheres in the fact that they are organic salts. The equilibrium between

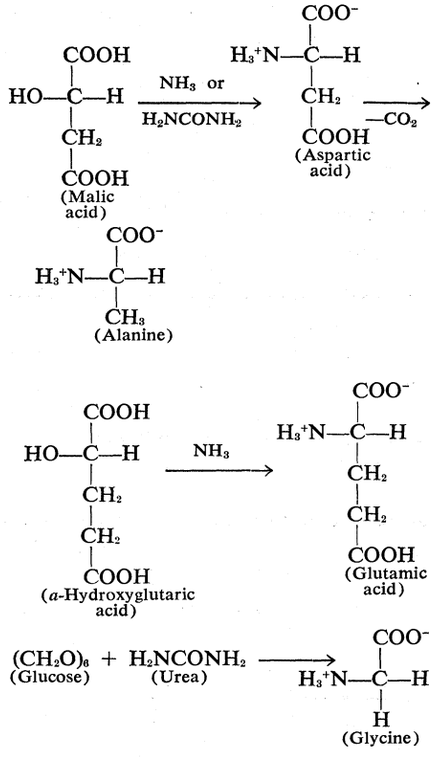


the charged and uncharged forms, has been shown to be predominantly on the side of the charged form (*11*). The salt-like structure imparts a degree of thermodynamic stability. If one provides a mixture of simple inorganic compounds of carbon, hydrogen, oxygen, and nitrogen with enough of any kind of energy to produce any organic compounds, production of some amino acids should not be unexpected, for this reason.

One of the many other modes of production of amino acids, by way of example, is that of Bahadur (*12*). Under the action of sunlight he obtained from formaldehyde and nitrate many amino acids identified by paper chromatography and three which were more fully characterized. Oro' and his co-workers have recently also used formaldehyde, with hydroxylamine, in thermal synthesis of amino acids (*13*).

Inasmuch as amino acids can be produced in so many presumably prebiological ways (see also *14*), one is unable to narrow the range of speculation about the nature of the geochemical matrix to any truly helpful degree.

Our own laboratory method of producing amino acids has been thermal (*15*). These acids arose in experiments aimed at producing protein in a prebiological context. Many other unexpected compounds were formed. With support from the literature, the results can be related in one continuum of physical conditions to all of the five stages mentioned earlier. Some of the ways in which four amino acids have been produced thermally from intermediates used also by organisms are as follows:



No special argument is offered for the thermal mode of generation of amino acids in contrast to the many other mechanisms, except for the fact that with the former method amino acids can be produced under the same physical conditions that cause their polymerization. In recovering amino acids from the reaction of ammonia or urea on a hydroxyacid, it is in fact frequently necessary to hydrolyze the polymer formed in the same treatment.

Production of Proteinoids

In view of the premise that early organisms were protein-centered, the terrestrial origin of protein is a more penetrating question than the origin of amino acids. A plausible answer to the question of the origin of protein (*16*), based on experiment, has begun to take definite shape recently. At the outset, the initial experiments unexpectedly yielded results which offered clues suggesting the comprehensive origin of biochemical systems, and more recently of organized or cellular systems.

The study leading to the syntheses of polymers with many properties of proteins was one of molecular evolution (*17*). The related interpretations included a Darwinian explanation of the known and then perplexing heterogeneity of protein molecules (*18*) and the provocative realization that the two acidic amino acids, aspartic acid and glutamic acid, are relatively dominant throughout the proteins and many internal fluids of plants and animals. The added inference that evolution of protein molecules, especially of homologous types, has been unexpectedly slow led to the carrying out of experiments to produce a presumably primordial protein. There were a number of reasons for selecting thermal energy to test polymerization of amino acids despite the fact that carbonaceous destruction of these substances is well known to be a dominant result of heating (*19*). The critical conditions that made an almost white polymer of 18 amino acids obtainable were found to be dry heat in the presence of excess dicarboxylic amino acids, aspartic acid, and glutamic acid. The temperatures used (150° to 200°C) are consistent with assumptions about the thermal history of the earth beneath or at the surface, whether one subscribes to the theory of a "hot" origin or of a "cold" origin of this globe (*20*).

The term *proteinoid* is employed to indicate a polymeric preparation containing, in peptide linkage, all or most of the amino acids common to protein.

Before we examine some of the properties of the proteinoids, mention should be made of other attempts to attain a similar objective. The most progress may seem to have been reported in experiments concerned with the action of ultraviolet light on aqueous solutions of amino acids (*21*). The simple peptides, glycylglycine and glycylalanine, were reported as products, but in trace amounts which were identified only by qualitative paper chromatography. Low conversions of amino acids to peptides are to be expected anyhow from considerations of equilibria (*22*), whereas understanding the evolution of bioenergetics requires conversions much higher than the few percent allowed by thermodynamics. Attempts by Miller to find biuret-positive material in his electrically produced products have been reported as unsuccessful (*9*). Akabori and his colleagues have ex-

perimental evidence for the direct substitution of the simple polymer polyglycine by serine and threonine residues to yield a somewhat variegated peptide (*23*).

Our earlier experiments began with the premise that prebiological chemistry is reflected in biological chemistry. Since organisms synthesize their protein from preformed amino acids, it was deemed most probable that prebiological protein was also generated from preformed amino acids. This recapitulationist outlook (*17*) is consistent with that of others, notably Brachet (*24*). This constitutes also a reason for preferring to experiment first with condensation of amino acids rather than to alter preformed peptide chains which lack highly variegated side chains, such as polyglycine. The same reasoning was also responsible for selecting amino acids as reactants instead of the easily polymerizable N-carboxy amino acid anhydrides (*25*), which do not to our knowledge have a close counterpart in nature. The involvement of phosphoric acid would, however, accord well with the findings of comparative biochemistry. A listing of examples of homopolymerization, copolymerization of pairs of amino acids, and multiple polymerization of 18 amino acids is found in Table 1. Most amino acids, when heated individually, yield tars and other unwanted products such as diketopiperazines. When two or more amino acids, one being aspartic acid, glutamic acid, or lysine, are heated together, mixtures of genuine peptides result, as well as some diketopiperazine. Since a large proportion of acidic amino acid effects reaction of neutral amino acid, excess acidic amino acid was utilized simultaneously with all of the neutral and basic amino acids common to proteins to give, in yields of 15 percent or more (some yields are much higher), polymers containing all of the reacted amino acids.

When the thermal polymers containing the 18 amino acids common to all organisms were first obtained, it was immediately apparent that such polymers resembled proteins in some respects. An exact appraisal of such resemblance requires extended scrutiny, and many special details of the structure of natural proteins are yet to be evaluated (*11*). It has, however, been possible to compare more than ten properties of natural protein and the synthetic polymers. In making these comparisons we are indebted to many chemists who volunteered aid (*26*).

Points of Comparison

Let us review briefly the principal points of comparison. Natural proteins of low molecular weight and the synthetic proteinoids have, in addition to the primary attribute of containing the same amino acids, other identical or overlapping properties as follows: elemental analysis, positive standard color reactions such as biuret, xanthoproteic, and Hopkins-Cole; similar infrared absorption spectra, range of molecular weights, solubility properties (including a tendency to be salted in and salted out), and electrophoretic mobility. In addition, they function as proteolytic substrates, they have nutritive value in replacing peptone for bacteria, their amino acid units show some degree of order which is the same in repeated polymerizations, and they possess morphogenicity—that is, a tendency to assume definite shapes. From the polymer are recovered D- as well as L-amino acids on hydrolysis. In this respect the polymer differs from mammalian protein. There is, however, some evidence of considerable proportions of D-amino acids in proteins of many bacteria (*26*). Tests for antigenicity in rabbits and guinea pigs have so far been negative. This is a principal way in which the synthetic polymer is distinguishable from most proteins. Among the explanations advanced for the non-antigenicity of the synthetic polymer, one of the most likely seems to follow from the fact that the mean molecular weights of the synthetic materials overlap only the lower end of the range of molecular weights of natural proteins as often defined. Antigenicity is found usually in proteins of molecular weight greater than 15,000. Ways of increasing molecular weight are being investigated, and additional tests for antigenicity are planned. Pirie and others have suggested that the proteins of highest molecular weight developed only after the first organism evolved (*27*). Similarly, since antigenicity is more characteristic of native than of denatured proteins, antigenicity might have developed only after organisms had evolved very subtly structured proteins.

Figure 1 is a chromatogram of a hydrolyzate of the synthetic polymer. The qualitative pattern for casein or any other protein is essentially the same. The synthetic polymer was prepared by heating at 170°C for 6 hours one part each of aspartic acid, glutamic acid, and an equimolar mixture of the 16 other amino acids. The product was aseptically dialyzed to free it of smaller molecules, and was dried and hydrolyzed (*28*). All the amino acids are present in considerable proportions, except for serine and threonine, of which there are only traces. These results were confirmed by microbial assay and column assay in two other laboratories.

The mean molecular weight increases from 3600 in a proteinoid made at 160°C to 8600 in one made at 190°C. These values were determined by end-group assay and may be compared with a value of 6000 for insulin, whereas the mean molecular weight of peptide per assayable end group of insulin is approximately 6000/2 (or 3000). Mean molecular weights of other thermal polymers containing all of the amino acids have been determined by the Archibald technique in the ultracentrifuge in John Edsall's laboratory, and

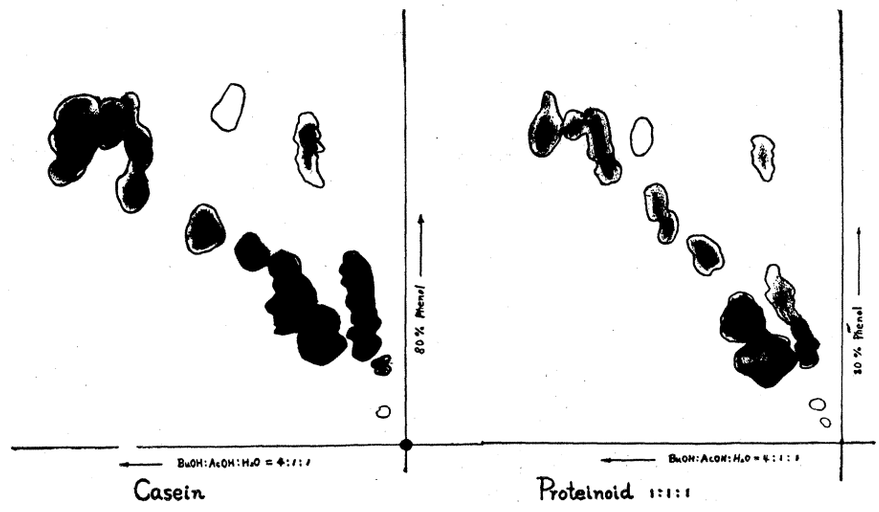


Fig. 1. Two-dimensional chromatogram of hydrolyzate of casein and of a proteinoid.

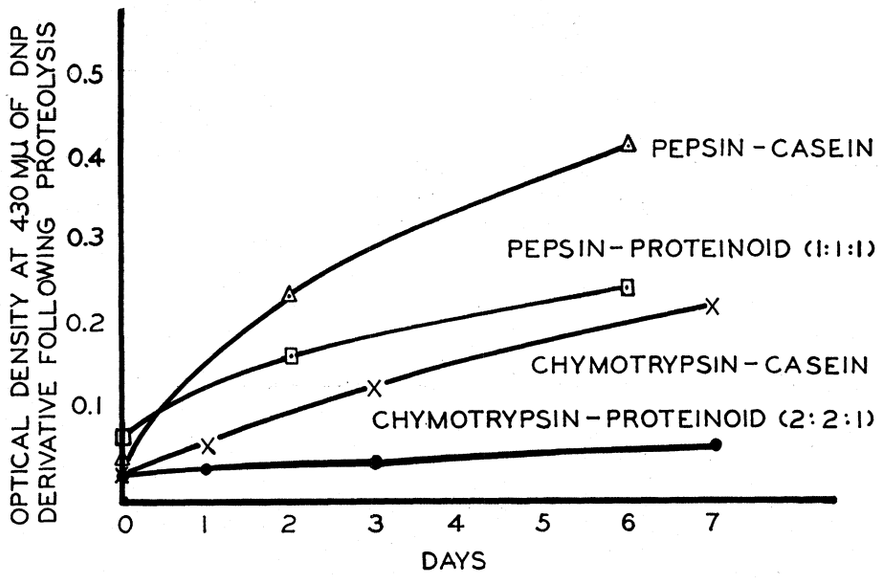


Fig. 2. Proteolysis of proteinoid by pepsin and chymotrypsin.

by the Craig dialytic method. Mean molecular weights for all these polymers fall in the range of many thousands.

The susceptibility to proteolysis of the thermal polymer is shown in Fig. 2. It is evident that proteolytic enzymes such as chymotrypsin and especially pepsin can act upon this material as upon the substrate to which they have been adapted in evolution. Essentially no splitting is observed in controls without enzyme. G. Krampitz has recently reported that thermal polymers of amino acids are also split by trypsin and papain (*29*). One can infer that the thermal treatment yields peptide bonds which behave normally in this respect and that the elevated temperature employed in synthesis does not produce secondary structures which mask all such bonds. The additional fact that bacteria can use these materials for protein nutrition is presumably a manifestation of the action of bacterial proteases.

Somewhere in the transition from the inanimate to the animate world, order such as is often ascribed to units in natural protein should have arisen. Table 2 presents data bearing on this point. Briefly, in each case, the proportions of aspartic acid, glutamic acid, and (collectively) the 16 other amino acids in the product differ markedly from those proportions in the reaction mixture. Also, the proportions in one position in the peptides, the N-terminal, differ markedly from those in the total composition. This result is typical of many which show the same kind of trend (*26*). The evidence thus indicates that the thermally reacting amino acids influence their own order. These results, in turn, suggest that nonrandom arrangements could have arisen in the first protein molecules before presumed templet or other mechanisms were sufficiently developed to govern, or perhaps largely to fix, the order of residues.

One can find individual proteins which have all of the properties possessed by the proteinoids except possibly the nonantigenicity, a point which requires qualification. Due to the variation in proteins, exact comparison presents some difficulties, but in essence these criteria do not permit distinguishing proteinoid from natural protein, or polypeptide, of molecular weight approximately 10,000.

Temperatures

The research to this point was based on our unwillingness to accept several commonly held assumptions, the most notable of which is the expectation that organic compounds are not sufficiently stable at the temperatures first employed for polymerization. This antithermal philosophy still persists (*30*), but the beneficial effect of starting with dry or, at the most, moist reactants in order to obtain the results reported here cannot be overemphasized (*31*). The conceptual difficulties for high temperature are aggravated by the unwarranted assumption that there were long geological periods in which unrenewed compounds were heated. A different assumption is the dynamic and continual generation of prebiological compounds, a picture consistent with the continual generation of compounds in organisms. It is of added interest that thermophilic organisms have been proposed as the most ancestral (*32*). It is also worthy of note that many of the organic compounds in nature, especially the polymeric proteins, polysaccharides, and nucleic acids, are schematically dehydration products (*33*). Although their results were not interpreted in the context of origins, Mora and Wood have recently shown that polyglucoses may be made from the monomeric glucose at temperatures of 140° to 170°C (*34*).

Despite the fact that temperatures above 150°C cannot be ruled out as one of the primordial conditions, it has been possible to lower substantially the necessary temperatures for polymerization in the laboratory. Yields have been enhanced by the addition of phosphoric acid, polyphosphoric acid, and various phosphates including adenosine triphosphate (*35*). One of the most active forms is a polyphosphoric acid which yields at temperatures as low as 70° significant amounts of polymer from 15 amino acids (*36*). This finding is of particular interest when it is compared with the known activating effect of phosphate in protein biosynthesis (*37*). The suggestion that there is a correlation is enhanced by the fact that experiments in which the phosphoric acid is replaced by sulfuric acid show that the latter fails to produce any polymer; sulfate is likewise not implicated in biosynthesis.

The finding that amino acids can be polymerized at a temperature well below 100°C underlines the futility of extended debate about experiments be-

Table 2. Distribution of amino acids.

Proportions in reaction mixture			Proportions in product			Proportions in N-terminus in product		
Aspartic acid	Glutamic acid	ΣBN*	Aspartic acid	Glutamic acid	ΣBN*	Aspartic acid	Glutamic acid	ΣBN*
33	33	33	55	13	32	7	30	63

* ΣBN, 16 basic and neutral amino acids determined collectively.

Table 3. Variation in spherules with variation in constitution of the polymer.

Polymer	Nature of unit
Proteinoid	Spherule
Aspartic acid-glutamic acid	Spherule
Aspartic acid-lysine	Spherule
Aspartic acid-leucine	Spherule
Aspartic acid-methionine	Spherule
Aspartic acid-glutamic acid-leucine	Spherule
Glutamic acid-glycine	Oblate spherule
Alanine-aspartic acid-glutamic acid-glycine-diaminopimelic acid-glucosamine	Nonuniform spherule
Polyglycine	No spherule
Polyaspartic acid	No spherule

fore there has been time to conduct and report them (*30*, *31*). The results deemphasize the alleged difficulties arising from laboratory temperatures of 150°C and emphasize instead the intellectual difficulties created by negative assumptions which, if viewed seriously, can paralyze experimentation.

The fact that it is possible to effect the polymerizations at an appreciable rate at temperatures below 100°C permits renewed investigation of experimental studies involving origin and retention of optical activity. It has, however, been evident for some time that several potential explanations, biological and chemical, exist for the origin of optical activity (*5*, *38*). The question is, rather, which mechanism or mechanisms were actually involved. The concept of spontaneous resolution in this context has gained some renewed favor with the demonstration that DL-glutamic acid can be resolved simply by seeding with one enantiomorph (*39*).

It is now clear that it is possible to produce by thermal polymerization many peptides which vary in molecular size, in composition, and in other physical properties. In addition, when compounds other than amino acids, of the nature of so-called prosthetic groups, are included in the reaction mixture, the characteristics of the products are altered. Polymers of each kind are being systematically surveyed for enzyme-like activity. Aside from conclusive demonstrations of catalytic activity, answers to related questions of interest are being sought: How many kinds and how much enzymic activity of each kind are present? Will the results suggest that much of the enzymic power of current organisms is itself the result of Darwinian evolution, a possibility that has been proposed by several workers, or was the most primitive organism highly self-sufficient in this respect?

The basic question of whether proteinoids have catalytic activity may be viewed against the known fact that peptides, such as polyhistidines, otherwise prepared, are hydrolytic catalysts (*40*), the known thermal stability of yeast invertase and other enzymes in the dry state (*41*), and the widely held notion that much evolution of enzymes occurred during the era of the beginning of life (*42*). When the questions about catalytic activity in proteinoids are answered, it may be apparent that the more basic question was that of the origin of large molecules combining a variety of amino acids through the peptide bond.

Morphogenicity

One of the properties of the synthetic material is its morphogenicity. A tendency to yield microspheres having diameters in a bacterial range is illustrated in Fig. 3. Typically, 1 billion of these units result from treating 15 milligrams of the proteinoid with 2.5 milliliters of hot water and allowing the clear solution to cool for a few minutes. The units shown are approximately 2 microns in diameter. These were formed in sea water and centrifuged; after centrifugation they were found to have retained their integrity (*43*).

Another property of the microspheres is the tendency to shrink in sodium chloride solution hypertonic to that in which they are produced. In such cases the spherules assume a volume much less than that of the original. Such experiments are carried out in solutions previously saturated with the thermal polymer, so that changes in size cannot be ascribed to solution. Accordingly, the behavior of the spherules suggests that they are osmotic. Of course, additional experiments must be performed to evaluate this.

The fusibility of the spherules prepared in the presence of certain substances, such as some lipids, suggests that useful biochemical abilities might have been combined in a similar fashion. This picture perhaps provides a mechanism for the lengthening of biosynthetic sequences envisioned in the Horowitz hypothesis (*44*), by the packaging of various groups of biochemical abilities in discrete units. Individual units which contained some synthetic capacities might then have combined with other cells which had other synthetic abilities to provide new cells with selective advantage. Other possibilities, however, such as an original autotroph, cannot yet be rigorously excluded from the larger conceptual framework.

The units described differ in many ways from most previous cell models, especially in being composed of polypeptide material. Oparin's coacervates, however, are made from gelatin and other materials (*1*). Here the properties are somewhat different. One salient difference is the fact that these spherules are the product of a continuum of phenomena suggesting successive biochemical and physiological origins. Secondly, inasmuch as our material is synthetic it is possible to vary its constitution over a wide range and thus to explore the variation of properties of spherules with that of the constitution of the polymer. Many variations have been examined; the property is evidently associated with many thermal copolymers of amino acids. We are thus not restricted to material of recent evolutionary origin, such as gelatin. It is, of course, of more interest to compare the thermal cell models with natural cells than with coacervates and to define, and experiment with, these differences.

Some of the variation is presented in Table 3. One salient feature is that polymers prepared with the inclusion of such special amino acids as diaminopimelic acid, found almost only in blue-green algae and some bacteria, yield also a sphere-forming polymer, but the units obtained are larger on the average, and not so uniform in size. A glutamic acid-glycine polymer gives units resembling erythrocytes in shape. Proteinoid synthesized in the presence of starch yields sickle-shaped units. Inclusion of ribonucleic acid or deoxyribonucleic acid in the hot water from which the spherules are made provides also some structural effects.

The tendency of varied polymers to form varied structures morphologically indicates that, despite the seemingly brutal thermal origin, fine differences in supramolecular and molecular structure are obtained (*45*).

Prebiological Origins

As stated, it becomes possible to visualize, on the basis of such experiments, physiological as well as biochemical origins. Probably prebiological evolution was gradual, as has been asserted by others, but events punctuating the organization of prebiological activities must have had a signal character. The first sudden production of osmotic boundaries in the primitive, presumably warm sea or in a perivolcanic spring must have been a momentous event for subsequent life. At this significant instant there appeared not only the form of the most luxuriant unit of life that we know but the first environment. Some of these first units could do prebiochemical business, and subsequently biochemical business, with that environment. The universal necessity for nutrition of all cells has been with us ever since (and I use "us" in the basic Darwinian sense of kinship with all living things). Also with us has been the eternal problem of understanding the relationship of the individual to his environment, a problem which has grown much more complex and vexing than it could have been at the moment of its remote evolutionary origin.

Another aspect of interest for future study is the relationship to optical activity. The finding that spherules can form from polymers composed of both D- and L-amino acid units was somewhat surprising. The conversion of polymers to spherules is typically one-third by weight. The possibility of optical enrichment in or by the spherules has not yet been investigated.

Another feature of such processes as the polymerization and spherule production in a continuum is their stark simplicity. These are processes which could have occurred on the primitive earth, before the establishment of scientific apparatus supply firms; only the natural geological crucible would have been required.

These considerations underline also another inferred feature in these processes—a repeatedly observed tendency toward self-organization (*46*).

The first experiment on thermal copolymerization of amino acids gave unexpected products and led to research which for 3 years also essentially organized itself. A principal part of the results is shown in Fig. 4. These are thermal pathways indicating the production of amino acids from Krebs-cycle acids (malic acid or fumaric acid), of one amino acid from another, and of the vitamin intermediate β-alanine from aspartic acid; the polymerization of amino acids; and the production of a nucleic acid biointermediate, ureidosuccinic acid (*38*). These compounds are all true biochemical substances except for some of the macromolecules and some DL forms that take the place of the natural L types. Most striking is the fact that the sequences of reactions are like those of a generalized biosynthesis. It was in fact possible to suggest in 1955 (*17*) a confluence of major biochemical cycles; in part this was also proposed in 1956, by Reichard and Hanshoff, on the basis of conventional biochemical investigation (*47*).

The finding of ureidosuccinic acid is essentially the only experimental demonstration yet published dealing with the prebiological origin of nucleic acid. Many thermal experiments are thus suggested, and these are being pursued. The results with proteinoid, of course, suggest experiments on a similar thermal route to nucleic acid-like materials. So far it appears more certain that polyribose has been produced than that polyribonucleotides have been (*48*). This goal is of interest because the gene, and therefore in some way nucleic acid, is believed to represent the apex of biochemical organization (*49*). Prebiochemical organization, however, probably proceeded from simplicity to complexity rather than the reverse, and the gene is molecularly most complex.

The notion that amino acids contributed to their own order in polymers before life began is not incompatible with the belief that nucleic acids govern this order in living cells. It may also now be possible to experiment with the

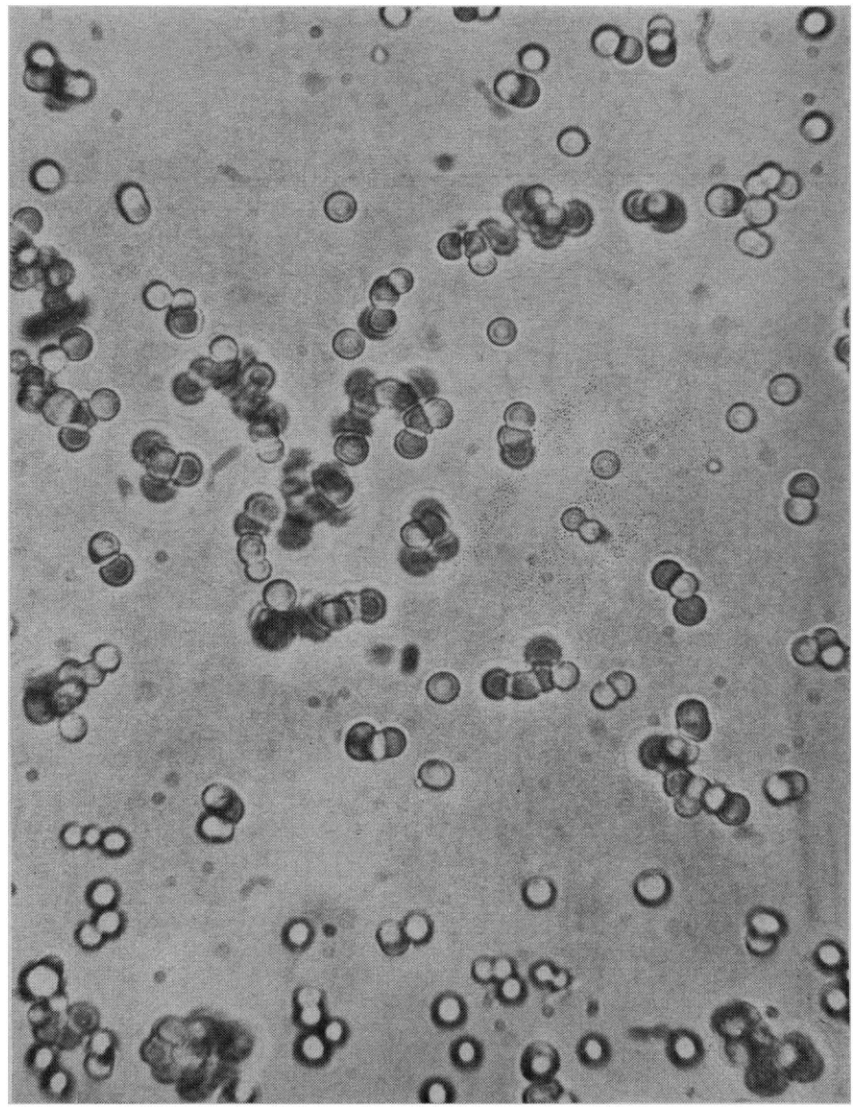


Fig. 3. Individual and aggregated microspheres from hot 0.1*N* potassium thiocyanate solution and 2:2:1 proteinoid (*43*) (× 1550).

effect of nucleic acids on ordering in thermal polymerization and to draw some inferences regarding the most likely stage at which nucleic acids may have entered the prebiological or biological picture in this function. A related point is that nucleic acid components arise biosynthetically through amino acids (*38*).

Summary of Research

In attempting to summarize research on how life began, we can see that for the first time, in the decade drawing to a close, a number of laboratories have supplied experimental demonstrations of the way in which isolated chemical aspects of life might have arisen spontaneously. This kind of research has gained much respectability which it formerly did not possess. The various contributions have involved one or two of the five stages of prelife. One experimental program and theory, that which is predominantly thermal, has connected parts of each of the five stages in a continuum which proposes many living phenomena as inexorable consequences of preceding phenomena. The fact that this mode is thermal primarily reflects the circumstance that thermal experiments continue to beget thermal experiments. It does not signify that other modes of energy were not involved in the emergence of life, particularly in the first stages in which the simplest organic compounds arose.

An integrated outline conceptualization of the way in which life might have begun in an orderly sequence of events, drawn largely from the thermal experiments, is presented in Fig. 5. The chemical reactions through the micromolecules might occur in any of many ways which have been demonstrated in many laboratories. The polymerization of amino acids on one hand or of pyrimidines, purines, and ribose on the other would occur in a hypohydrous—that is, a dry or at most moist—reaction mixture at moderately elevated temperature. The hypohydrous, dry, or phosphoric state would characterize steps *A*, *B*, and *C*. According to this concept, concentration of compounds in an oceanic soup would be unnecessary because the reactions occur with little water from the outset. The resultant mixtures of polymers and numerous reactants in a state of linked and continuous generation would, when subsequently discharged into a primitive aqueous pre-environment (step *D*), yield osmotic boundaries, with enzyme material and preanabolites in an intimate relationship. Prebiotic selection of proteins with catalytic activity would begin at step *C*. A first cell division would occur at step *E*, and dissociation of nucleoprotein, at step *F* to release or generate selected enzymes to catalyze the chemical steps which would constitute a repetition of the first cycle. The minimal cycle needed for repetition of itself would then be subject to deviations some of which would have selective advantage. Molecular evolution would proceed through step *G* (*17*).

This postulated cycle recognizes the interplay of experiment and theory not only for biochemistry as defined by substances but also for linked, continual, and dynamically related reactions, for the generation of protein to provide varied enzymes to catalyze the reactions, and for the development of a physiological organization to contain and repeat those processes. The last-mentioned phenomenon of replication is one that stands out among those phenomena for which no experimental model is at hand. An adequate cellular biosynthesis is another missing piece in the puzzle, and there can of course be others. The inferences arising most closely from the experimental model are (i) spontaneous generation of biochemical pathways, including production of amino acids and nucleic acid intermediates, (ii) origin of protein in intimate relationship in the same reaction mixtures, and (iii) spontaneous separation into preliving units and environment when the system became aqueous, all of these steps occurring in an evolutionary and physical continuum. Incidentally, we are justified in thinking of Darwinian selection of molecules in prelife if these molecules persist into, and confer advantage on, the first organism (see *50*).

Position of Viruses

Among the controversies which lace our subject matter are those that concern the definition of life and the position of viruses. Recently Pigman has discussed in a book review the postulate that viruses are a bridge between molecules and cells, and has referred to "the great difficulty that known viruses seem to be parasitic degenerate forms of cells incapable of surviving under the probable cultural conditions of the pre-life era, even if the seas were filled with synthetic amino acids and peptides."

Until someone succeeds in cultivating a virus in the absence of cells, many viewers of the scene will be unable to regard a virus as an evolutionary bridge between molecules and life, although virus molecules bridge the

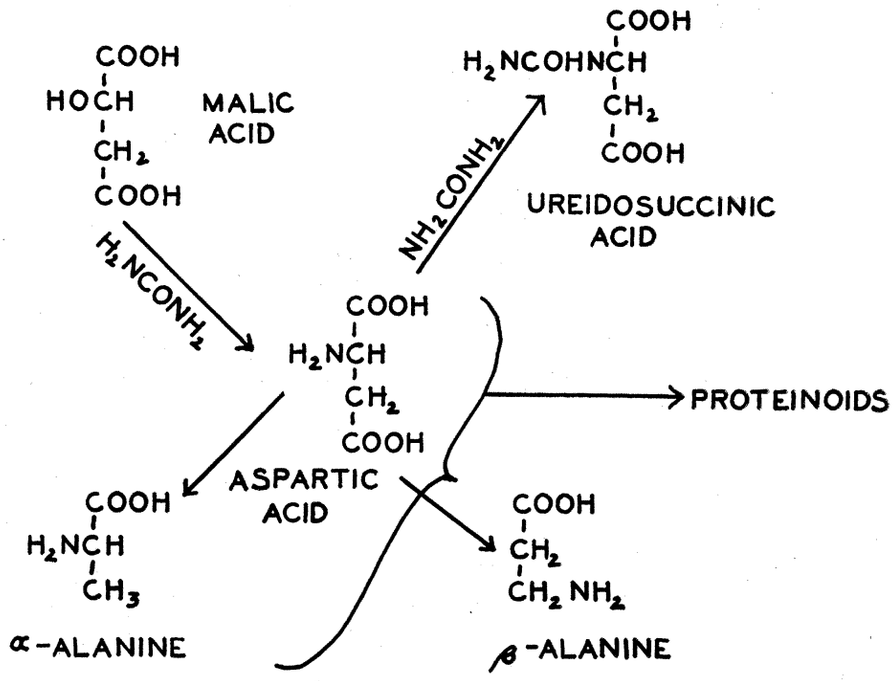


Fig. 4. Thermal pathways resembling biosynthetic pathways.

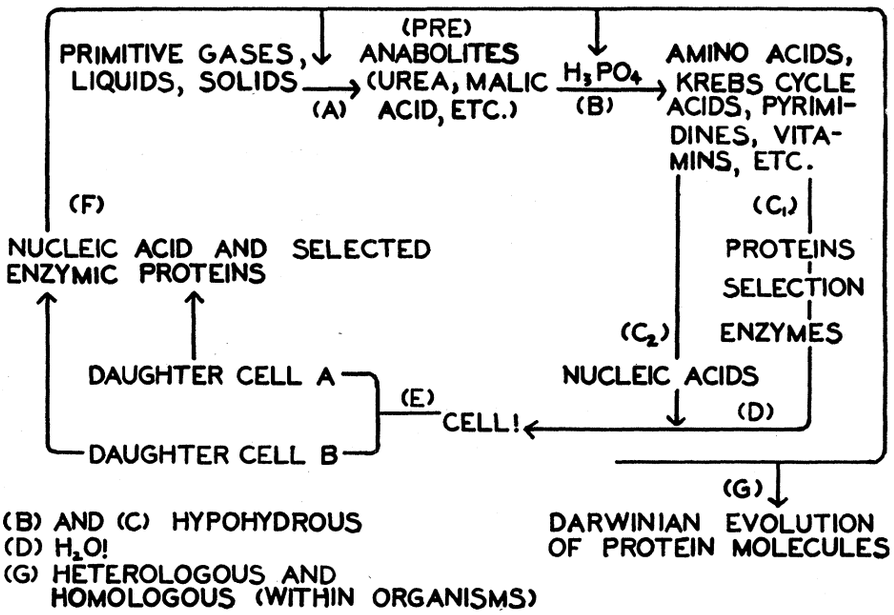


Fig. 5. Postulated first cycle leading to life, its reproduction, and mutation.

two realms from the standpoint of size (*51*). The controversy may actually disappear when considered in the rigorous context of life's beginning, by perception of the fact that a virus is not alive simply because it cannot be replicated in the absence of cells, and that cells would thus have had to appear first. One cannot, however, rule out the possibility that primitive viruses had more competence than their current lineal descendants, which may be more recently adapted to cells.

In the same general context, an enzymically synthesized, biologically active polynucleotide cannot be considered to be synthetic life until the enzyme is totally synthesized first, if it may be considered to be so even then.

Unified Outline Theories

At the present moment there is new reason to entertain seriously the germ theory of the origin of life. Kluyver and Van Niel, who did so much to help establish the concept of the "unity of biochemistry," developed one evolutionary picture of bacteria which began with the cocci (*52*). It is the cocci that the thermally produced spherules particularly resemble in range of diameter. Size and shape taken together are, of course, fundamental to taxonomic evaluations in the bacteria.

The significance in 1960 of a continuum embracing both molecular and cellular phenomena can be stated by a quotation from Michael Tswett, who said in 1908 (*53*): "It is too often forgotten, especially on the chemical side, that living tissues are not mere mixtures of compounds remaining in chemical equilibrium, but are organized structures, where, as a result indeed of osmotic boundries, the most varied reactive bodies stand next to each other. . . ."

The question "How did life begin?" focuses attention upon subtle but important differences between the verb *begin*, used intransitively, and the verb *start*, used transitively. We assume that someone some day will succeed in producing a cell which metabolizes and reproduces itself and its metabolic pattern in such a manner that no experts will disagree with the conclusion that the unit is alive. When that occurs, a chemical evolutionist will have *started* life, whereas that from which we are descended *began*. Will we be able, then, to say that the experimental demonstration reveals how life began? At first glance the answer seems to be negative. It should be possible, however, when life has been synthesized, to determine the latitude of each of the conditions required for synthetic life, and perhaps the latitude of some of them before life is started. As a hypothetical example, we now know that some synthetic polymers will not yield spherules, as reported in Table 3. When the full scope of conditions necessary for the synthesis of life is determined, it should be possible to judge whether these are conditions associated with the current earth, with what we believe to have been the prebiological earth, and with conditions prevailing on other planets.

A related thought is that life may be beginning now. Although we can with certainty say only that life arose at least once, there is increasing reason to believe that life can, or even must, arise in many places at many times. The common floral pattern of many hot springs areas and concepts of parallel evolution are consistent with this idea, and pose the possibility that we fail to recognize life beginning anew because it so resembles unevolved descendants of primitive forms already here. There is of course no assurance that life is beginning now on the earth; the point is that we have less reason to exclude this possibility than we had formerly. Perhaps we may evaluate this more precisely as we learn about the chemistry of the interior of the earth.

We must continue to entertain the possibility that one, many, or all experimental models of life's beginnings may prove to be no more than models. From the thermal model there is already a minimum gain in the discovery of methods for easily producing a variety of peptides (some of which are being studied in industry for potential utility), the discovery of convenient methods for synthesizing some amino acids, and contributions to the interesting process of microencapsulation. One of these advances was essentially an original objective in research that my co-workers and I directed. At the interpretative level, the research which has directed us has led in outline to a unified theory. This theory *at the least* indicates that a scientific answer to the question, "How did life begin?" need not be considered hopelessly incomprehensible. What can be said *at the most* is obviously subject to further experimental discipline, but experiments already performed may delineate the essential pathway of life's emergence.

However, judgments on this, or any other unified outline theory which may arise, need not wait entirely on the production of synthetic life and subsequent analyses. Darwin's theory of evolution has, for example, been judged and has proved to be intellectually useful on the basis of its consistency with much general knowledge, rather than on that of any single dramatic experiment. The same kind of evaluation is to some degree increasingly

possible with any theory of life's beginning. At the same time, we can anticipate much more rapid progress in this case from experiment than from the controlled observation employed by Darwin.

Having stirred the embers of Darwinian thinking, I should like to conclude with a popular quotation from a letter of Charles Darwin's, as recorded by Francis Darwin.

"It is often said that all the conditions for the first production of a living organism are now present, which could ever have been present. But if (and oh! what a big if!) we could conceive in some warm little pond, with all sorts of ammonia and phosphoric salts, light, heat, electricity, &c present, that a proteine compound was chemically formed ready to undergo still more complex changes. . . ."

One can again be amazed by the amount of insight expressed by Darwin, in this letter of 1871, before nucleic acid was identified, and before a simple phosphate-aided chemical route to polyamino acids ("proteine") yielding microscopic spheres could be demonstrated (*54*). In general historical perspective, we can see that, in 1960, the big *if* in Darwin's letter of 1871 is being replaced by a growing complex of more ramified *ifs*.

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54. My study was supported by grant No. G-4566 from the National Science Foundation, grants from the General Foods Corp., and grant No. C-3971 from the National Institutes of Health, United States Public Health Service. Contribution No. 130 of the Oceanographic Institute of Florida State University.