MOLECULAR EVOLUTION TO THE FIRST CELLS†

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ABSTRACT

The evolutionary sequence of galactic organic matter → amino acids → informational protoprotein → protocell → (nucleic acid-governed) contemporary cell is reviewed, and the supporting evidence is presented in outline.

The experiments suggest that thermal polymerization of diverse α-amino acids yielded partially-ordered protoproteins which possessed varied proto-enzymic activities. These protoproteins assembled, according to the experiments, on simple contact with water to yield protocells.

The model protocells contain enzyme-like activities, ultrastructure and selective membranes, and they have been found to reproduce in five modes, each of which is conceptually relatable as an evolutionary precursor to a contemporary mode of reproduction.

The processes have been shown to be geologically relevant, imparting much rigour to the model. The simplicity of the formation of the protocellular model and the terrestrial ubiquity of water support the concept of protocells having arisen easily and often as protoprotein formed. These experiments and the derived theory required constructionistic processes based on knowledge obtained by reductionistic methods.

INTRODUCTION

During the past few decades, the thesis that molecules underwent selection processes prior to the first reproducing organisms has gained wide acceptance[1]. In this paper experiments and interpretations are discussed that explain a continuous modulation of precellular chemistry to the first reproducing organisms—those first organisms which Darwin's theory of the origin of species left unexplained‡.

The results from the experiments indicate that total continuity was not achieved by a sudden modulation from chemical phenomena to contemporary biological structures. Rather, two key connecting links were a first informational protein[2] and an intermediate stage of a minimal cell[3], i.e. a protocell[2] or precontemporary cell. The sequence: amino acids → proto-

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‡ Darwin's views of the hypothetical first organism were from both directions. For example, Darwin wrote that a 'protein compound was chemically formed ready to undergo still more complex changes.'

protein → precontemporary cells (protocells = minimal cells) → contemporary (nucleic-acid coded) cells was possible because of the high geological likelihood of self-assembly of protein-like molecules (proteinoids), as demonstrated in the laboratory. No prior cell was necessary to produce such protein-like molecules; they could have arisen as the result of purely geophysical processes. Their assembly to cell-like units is triggered by water; the need for water to produce the first cells would have been as total as is the need for water to maintain contemporary cells.

In the construction of the theory, a knowledge of contemporary cells was necessary in order to identify associated properties for which the origin had to be explained. That knowledge was needed also in finding clues for the design of experiments to simulate geochemical occurrences. However, knowledge of the contemporary cell can be overwhelming and confusing. What we have known about the contemporary cell has come to us as the result of disassembling such systems. When disassembly is the mode of study, a number of cellular functions disappear simultaneously. A key question for the origin of cells, however, is that of the sequence of appearance of special properties in the cellular system. The order of emergence of these properties could not have been predicted solely from knowing the components of the cell. The experiments indicate that what was required was the assembly of micromolecules → macromolecules → cellular structure, in that order and in the same direction as the process of evolution and the synthetic processes themselves. In the course of such assembly in the laboratory, emergent properties and the order of their appearance can be identified.

REQUIREMENTS FOR THEORETICAL PROGRESS

Progress in understanding macromolecular and cellular origins has been possible especially because of attention to the favourable thermodynamics of open systems and to the unfavourable thermodynamics of dilute aqueous solution in the formation of polymers[4]. Contemporary organisms, like the geochemical realm, function as open systems[5]. They appear also to have evolved, and to have avoided the degradative effects of dilute aqueous solution on the molecules they contain[1]. These thermodynamic considerations, moreover, apply also to micromolecules[6]. The geological relevance, in fact, of many of the reported preparations of small molecules is open to serious question[1]. Review of the total set of experiments and principles in this area emphasizes that polymerization and the formation of spherules are high-yield processes (up to 60 per cent), whereas the production of small molecules, usually employing violent forms of energy[1], seldom exceeds 0·1 per cent.

The experimental studies that have yielded a model for the origin of protein and of the cell therefrom, had their roots in protein systematics[7]. Studies of composition in organismic protein provided a clue to circumstances necessary for the origin of the first protein, and for model experiments. That clue, consisting of a somewhat disproportionate content of non-neutral amino acids[8], was necessary to permit the visualization of a reversal of the

negative outlook left to us by the indications derived from the work by Carothers on the nylons[9].

Carothers[9] analyzed the valuable properties of high polymers in the living state as those of strength, elasticity, toughness, pliability, hardness, and the capacity to grow. He also, however, emphasized that the effect of heat upon α-amino acids was primarily that of forming cyclic molecules instead of linear polymers. Accordingly, in producing the silk-like nylons, he condensed ω-amino acids, not α-amino acids[10]. The fact that cyclization and decomposition in heated α-amino acids could be overcome by including sufficient non-neutral amino acid[1, 8] is the clue that emerged from studies of protein systematics[8]. When this clue became a working experimental hypothesis, the design of experiments became chemical except for a pervading undertone of biochemical recapitulation[7].

In understanding the origins of protein as a heteropoly-α-amino acid, we first consider how α-amino acids came into existence. Thirty-four years of experiments on chemical protobiogenesis have provided only one process that explains the origin of protein under geologically relevant conditions. Nevertheless, numerous models for the prebiotic production of amino acids have been advanced since 1953.

POLYATOMIC MOLECULES IN THE GALAXY

Nearly all the reports on the production of amino acids have assumed one or another prebiotic atmosphere, mostly highly reducing in character[1].

Table 1. Polyatomic molecules in the Galaxy

Molecule	Observers	Year found
H_2	Carruthers	1970
OH	Weinreb *et al.*	1963
SiO	Wilson, Penzias *et al.*	1971
H_2O	Cheung *et al.*	1969
NH_3	Cheung *et al.*	1968
CH^+	Dunham	1937
CH	Dunham	1937
CN	Adams	1938
CO	Wilson, Jefferts *et al.*	1970
CS	Penzias, Solomon *et al.*	1971
HCN	Snyder and Buhl	1971
OCS	Jefferts *et al.*	1971
H_2CO	Snyder *et al.*	1969
HNCO	Snyder and Buhl	1971
H_2CS	Sinclair *et al.*	1971
HCOOH	Zuckerman, Ball *et al.*	1971
$HC{\equiv}C{-}C{\equiv}N$	Turner	1971
CH_3OH	Ball *et al.*	1970
$CH_3C{\equiv}N$	Solomon *et al.*	1971
$HCONH_2$	Rubin *et al.*	1971
$CH_3C{\equiv}CH$	Snyder and Buhl	1971
CH_3CHO	Ball *et al.*	1971
HNC	Snyder and Buhl	1971

In the past few years, our understanding of the availability of raw materials has been revolutionized by the findings of the astrophysicists. The many organic polyatomic molecules shown to be present in the Galaxy are included in the list in *Table 1*[11].

Formaldehyde is believed to be the most abundant of these molecules, although the recognized cosmic raw material is mainly hydrogen. Under equilibrium conditions, reactions of the two would have converted formaldehyde to methane. But the Galaxy is not at equilibrium. During physical condensation, the more fugacious hydrogen would tend to escape. Its retention in laboratory experiments (up to 75 per cent H_2[12]) can be managed by the use of closed flasks which are not, however, matched by conditions in the Galaxy nor at the surface of the Earth. The new knowledge of the compounds abundant in the Galaxy plus awareness that the extraterrestrial realm is an open system provides a factual platform from which to refine concepts of origins of amino acids.

AMINO ACIDS FROM GALACTIC MOLECULES

After formaldehyde and ammonia were found to be abundant galactic compounds, we investigated their interaction and the hydrolysis of the product in open glassware[13]. The results are shown in *Table 2*. A family of amino acids was produced. Since members of the family are aspartic acid, glutamic acid, and glycine, all components of the mixture were capable of

Table 2. Amino acids from reaction of formaldehyde, ammonia, and water followed by concentration, heating, and hydrolysis

	Mole % of amino acid fraction Experiment			
Amino acid	1	3	4	5
Aspartic acid		1.3	8.3	2.3
Serine		4.0	6.3	1.7
Glutamic acid	9.9	1.6		1.9
Proline		5.0	trace	1.4
Glycine	90.1	39.5	45.8	81.3
Alanine		5.0	7.3	9.5
Valine		0.4	9.4	1.9
Isoleucine		9.4		
Leucine		0.5	3.1	
Phenylalanine		1.2		
Unknowns*		32.2	19.8	

*Equivalent to leucine.

In experiment 1, a 1:1 ratio of 37 per cent formaldehyde and 28 per cent ammonia was used; in experiments 3 to 5 a 3:1 ratio was used. The results are expressed as mole ratios in per cent, and were calculated without ammonia and without hexamethylenetetramine, which is the most abundant product.

being copolymerized[1]. Pyrocondensation of amino acids in such a mixture has, in fact, recently been reported by Saunders and Rohlfing[14]. The participation of formaldehyde and ammonia meets the requirement for open systems since these two intermediates are readily converted to solid hexa-

methylenetetramine, which is in turn convertible to solid amino acids by hydrolysis[13, 15]. Reactive mixtures of gases relying upon inclusion of diatomic hydrogen terrestrially[16] are not, however, conceptually favourable.

Although many questions remain to be answered, we observe that amino acids can be produced under widespread geophysical conditions from organic reactants which certainly exist in abundance and, with almost equal certainty, have existed in abundance. These amino acids are, however, produced partly from unknown chemical precursors (in addition to hexamethylenetetramine[13, 15]) by hydrolysis. The possibility of hydrolysis however poses no difficulty since water is geologically abundant.

AMINO ACIDS FROM LUNAR DUST

Evidence for direct precursors of amino acids on other bodies of the Solar System has been obtained since 1969. Hydrolysis of aqueous extracts of lunar dust from eight collections from Apollo 11, 12, 14 and 15 have yielded a common pattern of amino acids (*Table 3*). The amino acids are present in the hydrolyzates at levels of 20–70 ppb. Such findings conform to a model of a baked-out Moon, into the surface of which were implanted reactant compounds from the solar wind, interstellar matter, meteorites, or comets[17].

Larger amounts of almost the same set of amino acid precursors, measured as amino acids, have been found in meteorites by application of the methods of extraction which we first applied to lunar dust[18].

Although our understanding of the origin of amino acids is not on a rigorous basis, we can hardly doubt that the easily converted precursors of these compounds have existed in some abundance on Earth prior to living systems. The conversion of amino acids or their immediate molecular precursors to protein-like polymers and of the latter to cell-like microsystems is on a firm basis, if we assume the earlier availability of amino acids. The conditions for the conversions from amino acids in the laboratory are relatively widespread on the contemporary Earth.

One new emphasis that has emerged, in part from the lunar studies, is that amino acids are evolutionarily significant as intermediates, *transitory intermediates*. This view is related to the thermodynamic fact that stability of any one compound is relative to some other compound(s). Amino acids seem not to be as abundant as amino acid polymers. In living systems, this relationship is a consequence of nonequilibrium conditions, a situation that now appears to be partially analogous to what obtains geologically. We have long known that organisms are characterized by amino acid polymers (proteins) and by continuously generated biosynthetic precursors, whereas the concentration of free amino acids in organismic fluids is virtually always very low[19]. A corollary statement is that both the cosmochemical and organismic states are highly dynamic; they are not the static situations they are often assumed to be. Free amino acids are stable in bottles in the dry state, and at temperatures below those required for thermal activation. Outside bottles, amino acids are found predominantly as precursors or as polymers, in either the cosmochemical or the organismic realm.

An additional significance of the availability of amino acids as hydrolyzable precursors is the judgment that the early evolutionary compounds were

Table 3. Amino acid contents of hydrolyzates of extracts of lunar dust (% molar composition[a])

Amino acids in hydrolyzate	Apollo 11 analysis no. 1 No 10086	Apollo 11 analysis no. 2 No 10086	Apollo 12 Trench No 12033	Apollo 12 Surface No 12001	Apollo 14 Surface No 14003	Apollo 14 No 14163	Apollo 14 No 14298
Aspartic acid	5	5	< 1	2	2	2	7
Threonine	2	3	< 1		1	1	2
Serine	9	10	< 1	3	4	6	10
Glutamic acid	9	11	20	6	12	20	13
Glycine	50	52	37	70	62	47	57
Alanine	25	19	12	3	20	26	7
Valine							1
Isoleucine							3
Leucine			3				
Tyrosine				2			
Phenylalanine				2			
BAA[b]			25	10			
Total ppb ng amino acids / g lunar soil	53	37	19	69	19	30	37

[a] Calculated without ammonia.
[b] Unknown in the basic amino acid range.

protected, which would not have been the case for free amino acids exposed to destructive influences. The lunar findings may thus have proved to be crucial to our understanding of the molecular basis for the first proteins in the Solar System.

POLYMERIZATION OF α-AMINO ACIDS

As stated earlier, α-amino acids can be polymerized by heat if they are not the set of neutral α-amino acids alone. The neutral type does smoothly *co*polymerize with the acidic or basic α-amino acids. This is the finding that especially extends our understanding from where Carothers left it.

Among the polymers, natural or synthetic, the proteins stand out for the large number of types of monomer that they contain—typically twenty. The experiments in simulation of prebiotic events yield typically families of the proteinous amino acids. Twenty have not, so far, been demonstrated to be the result of any single laboratory synthesis, but ten or twelve α-amino acids have been obtained[1]. The resultant variation and pliability[20, 21] of proteins appear to have their evolutionary precursors in the polymers from such variegated sets of α-amino acids (See Reference 14). A special feature of the thermal condensation is that it is capable of accommodating simultaneously the twenty common contemporary α-amino acids[21], the polymers being known as proteinoids. In addition, temperatures that effect condensation of amino acids are the same as temperatures that would concentrate them from geological aqueous solutions[2].

NONRANDOMNESS IN THERMAL POLYAMINO ACIDS

In the laboratories that have reported performing such pyrocondensations, evidence has accumulated that the polymers are nonrandom, as judged by a number of criteria. A limited variety of sequences occur, presumably as a manifestation of the specific interaction rates of the individual amino acids in their initial reactions. According to our view of the situation, a further influence leading to ordering is the tendency for the polymer, while yet at elevated temperature, to undergo chemical transpeptidation toward the thermodynamically most stable sequence.

The evidence for such ordering is now of many kinds (*Table 4*). Compositions of the reaction mixtures differ from those of the corresponding polymers, sequences within the polymers are not random, and the polymers are sharply limited in their heterogeneity according to a number of criteria[2].

An especially striking example of near-homogeneity is found in the haemoproteinoid prepared by Dose and Zaki[22]. In this synthesis, Dose and Zaki heated haeme with twenty amino acids. They obtained a haemoproteinoid of mean molecular weight 18 000. The polymer had substantial peroxidatic activity, but less catalytic activity than the equivalent amount of haeme. The degree of heterogeneity is revealed in *Figure 1*. A disc-gel chromatogram of human serum albumin under the same conditions is also seen in the *Figure*. The heterogeneity of the haemoproteinoid, as determined

by this criterion, is thus much less than for blood albumins. One salient inference is that ordering at this stage did not require prior nucleic acid. The information for the ordering is in the diversity of the amino acids, that is, the amino acids contain a more primitive kind of information than in the polymer. The degree of ordering from this source was not predicted. The question has been raised whether the single band indicates a single macro-

Table 4. Evidence for self-ordering in the condensation of amino acids

Evidence	Date and authors
Thermal	
Nonrandom sequences by disparity between *N*-terminal and total analyses in thermal polymers	Fox and Harada (1958)
Amino acid contents in reaction mixture ≠ contents in polymer	Fox and Harada (1960)
Two peaks from proteinoids on electrophoresis	Vestling (1960)
Limited heterogeneity on ultracentrifugation	Vegotsky (1961)
Constant composition on repurification from water	Fox, Harada, Woods, Windsor (1963)
Single band on gel electrophoresis for acidic proteinoidamide	Fox and Nakashima (1966)
Nonrandom elution pattern from DEAE-cellulose	Fox and Nakashima (1967)
Symmetrical peaks from DEAE-cellulose; Almost uniform amino acid compositions in various fractions; Stoichiometric amino acid compositions; Uniform ultracentrifugal patterns of various fractions; Single spots on high-voltage electrophoresis fractions.	
Single species of 'active site' proteinoids	Usdin, Mitz and Killos (1968)
Single band for gel electrophoresis of basic haemoproteinoid	Dose and Zaki (1971)
Nonthermal	
Papain-controlled reactions of amino acid derivatives in aqueous solution	Fox, Winitz and Pettinga (1954)
Dicyandiamide-controlled reactions of amino acids in aqueous solution	Steinman (Calvin) (1967)

molecule, a circumstance which would have been terminal for evolution[23]. In Dose's study and in another one by Nakashima and Fox[24], the macromolecules which are operationally single on disc-gel electrophoresis can be fractionated into several peaks on DEAE-cellulose columns.

All the evidence (*Table 4*) emphasizes that a kind of non-Darwinian selection occurs, and undoubtedly occurred, at the molecular level.

ENZYMIC ACTIVITY IN PROTEINOIDS

The existence of enzymes has been believed to require nonrandom sequences, as well as other complexities[25]. How such order came into existence at the macromolecular level, without nucleoprotein organelles to

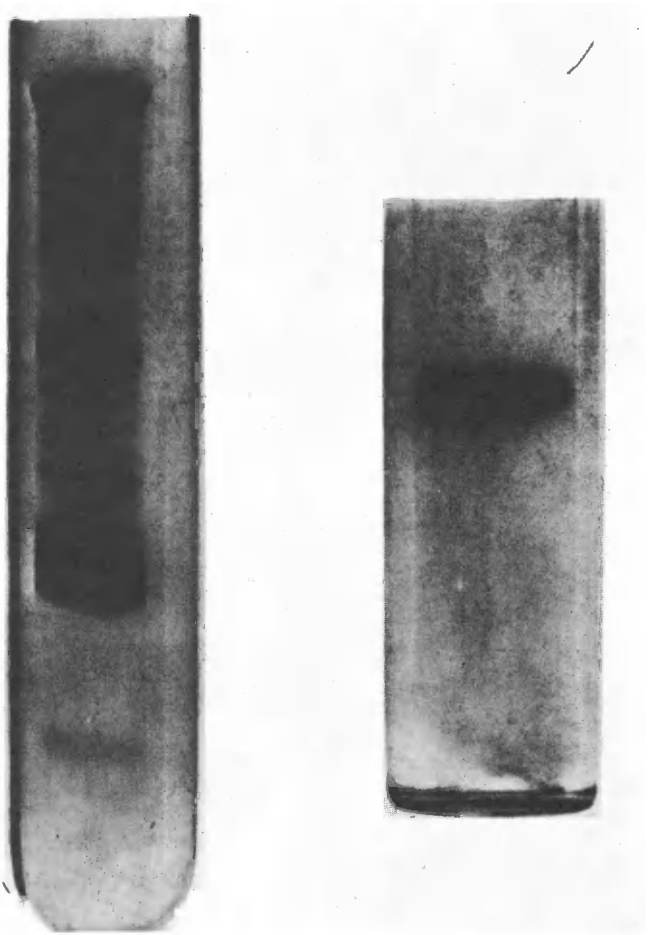

Figure 1. Disc-gel electrophoretogram of haemoproteinoid (single band) and of human serum albumins (many bands). Carried out at pH 8.6, staining with Amidoschwarz 10B.

guide it, has been a major question. This question had no disciplined answer until the informational input of the reactant amino acids was recognized experimentally[1, 2]. The question of the origin of the enzymic functions *per se* as central to that of the origin of life was already recognized by Pasteur[26]. The modern statement of this question is—if enzymes are formed only by enzymes, how were the first enzymes formed[25]?

The answer to this question is that what was necessary was diverse amino acids and appropriate geophysical conditions to polymerize them to proteinoids[2]. The studies of catalytic activity in proteinoid are now from a sufficient number of laboratories that the results are best presented in summary form (*Table 5*). The specificities observed are a direct kind of evidence that the proteinoid contains information†.

† Information in this paper connotes capacity for selective interaction of one molecule or system with others.

Table 5. Enzyme-like activities in thermal polyanhydro-α-amino acids (from References 21 and 24).

Reaction and substrate	Remarks	Authors and year
Hydrolysis		
p-Nitrophenyl acetate	Activity of proteinoid greater than of equivalent free histidine	Fox, Harada and Rohlfing (1962)
p-Nitrophenyl acetate	Thermal polymers most active	Noguchi and Saito (1962)
p-Nitrophenyl acetate	Inhibition by organic phosphates; reversal	Usdin, Mitz and Killos (1967)
p-Nitrophenyl acetate	General description	Rohlfing and Fox (1967)
p-Nitrophenyl acetate	Reactive site and inactivation	Rohlfing and Fox (1967)
ATP	Through Zn salt	Fox and Joseph (1965)
p-Nitrophenyl phosphate	A second phosphate hydrolysis	Oshima (1968)
Decarboxylation		
Glucuronic acid	From glucose, CO_2	Fox and Krampitz (1964)
Pyruvic acid	→ Acetic acid + CO_2; Michaelis–Menten kinetics	Krampitz and Hardebeck (1966); Hardebeck, Krampitz and Wulf (1968)
Oxaloacetic acid	Rapid, requires basic polymers	Rohlfing (1967)
Amination		
α-Ketoglutaric acid	Requires both Cu^{2+} and proteinoid	Krampitz, Diehl and Nakashima (1967)
Deamination		
Glutamic acid	Requires both Cu^{+} and proteinoid	Krampitz, Haas and Baars-Diehl (1968)
Oxido-reductions		
H_2O_2 (catalase reaction)	Activity of haemin lowered when incorporated into proteinoids	Dose and Zaki (1971)
H_2O_2 and hydrogen donors (guaicol, hydroquinone, NADH and others) (peroxidase reaction)	Activity of haemin increased up to 50 times in lysine-rich haemoproteinoids	Dose and Zaki (1971)

At least seven kinds of reaction have been shown to be catalyzed by proteinoid[2, 27–29]. While not all primordial metabolism has been explained by the specificities catalogued, and we cannot expect that they will or should be[30], the principle for the origin of the component reactions of metabolism is laid down.

The catalytic activities found are independent of the fact that the proteinoids are composed of mixed L and D amino acids (when made from L amino acids, although the L forms tend to predominate[27]). Such results reemphasize the significance of the three-dimensional juxtaposition of relevant amino-acid side chains, which can accordingly be independent of unique sequences or of a need for only L residues. This interpretation is consistent with the inferences from the finding that thermal polymers of amino acids possessing appropriate side chains have hormonal (MSH) activity[31, 32].

HORMONAL ACTIVITY IN PROTEINOIDS

Bagnara and Hadley[32] have shown that the MSH activity of the synthetic polymer is inhibited by norepinephrine as is the natural hormone; they are deinhibited by a common reagent. Thermal condensation could thus conceptually have yielded both enzymes and hormones, each significant to metabolism.

Understanding of the early evolution of metabolic activity of the protocell is aided by our recognition, through experiment, of the abilities of supramacromolecular proteinoid systems to join[33], to communicate[33], and to reproduce[34]. From these observations emerges the inference that daughter systems could have inherited lengthened metabolic pathways[33].

THE FORMATION OF MINIMAL CELLS

How microscopic cell-like structures first appeared is undoubtedly one of the two most fundamental contributions of the experiments to our understanding of the origin of life. The process producing cell-like replicating microunits (*Figure 2*) from self-ordered molecules is the simplest of operations, in contrast to general expectations[35]. All that is necessary is a triggering by water of assembly of heteropolyamino acids[3]. Mere contact suffices, although the cooling of a hot solution is experimentally preferable. The process must have occurred frequently as well as rapidly on this planet whenever protoprotein appeared—due to the geological ubiquity of water. This fact appears to be of crucial significance for inferring the order of events in the origin of life.

The problem of the organization of a unit as complex as a cell has received the attention of a number of investigators. Oparin, who is responsible for the modern naturalistic view on the origin of life, has devoted almost the whole of his experimental efforts to understanding the origin of the cell[36]. The model with which he has worked is the coacervate droplet (*Figure 2*). Oparin has essentially micro-encapsulated evolved enzymes in such units, and has demonstrated that the enzymic reactions proceed many times as fast within the boundaries as in the surrounding fluid. The coacervate droplet concen-

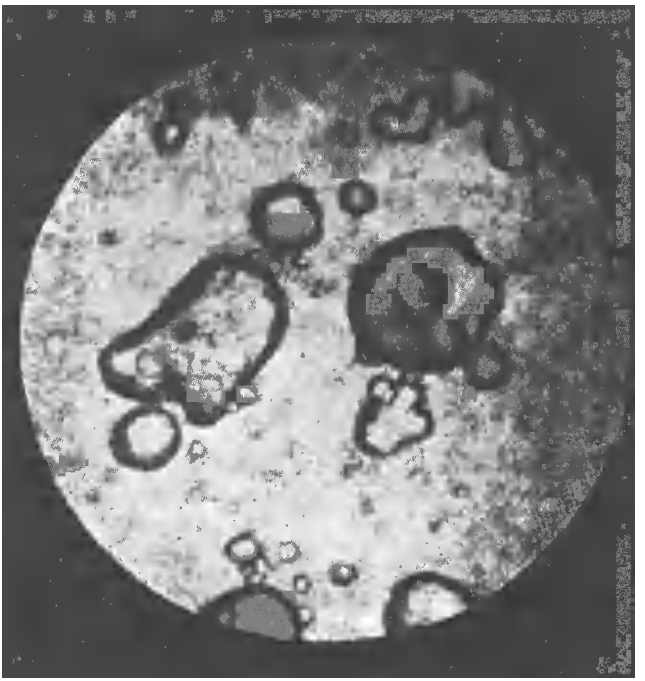

Figure 2. Coacervate droplets, as produced by A. I. Oparin and colleagues (T. Evreinova). Produced from gelatin, gum arabic, and RNA (Original × 320, reduced by $\frac{1}{2}$)

trates materials[36]. Defects of the coacervate droplet as a model are instability, a lack of uniformity (*Figure 2*), and especially the fact that these units are made from polymers obtained from evolved cells. Since they are produced from contemporary cells, they do not answer the basic question of how the first cells came into existence when there were no cells to produce the polymers from which they could have arisen[2].

This question is answered by the proteinoid microspheres. In addition, the microspheres are uniform in size, including those in the second generation (*Figure 3*). All that is or was necessary for production of such proteinoid microspheres was the triggering of a self-assembly of appropriate polymer by water. The appropriate type of polymer has proved to be a fairly wide range of thermal copolyamino acids[37]. The process is one of very high efficiency. It also provides insight into the order of events in the origin of the first living system, as earlier mentioned.

Since the process occurs with such great ease, and since water must have been ubiquitous on the early Earth, the opportunity for the appearance of a minimal cell must have been almost as great as the opportunity for the appearance of protoprotein, from which that minimal cell would have arisen easily and often. The molecular logic of this situation is such that other structures, such as nucleic acid, would have appeared at a later stage[38, 39].

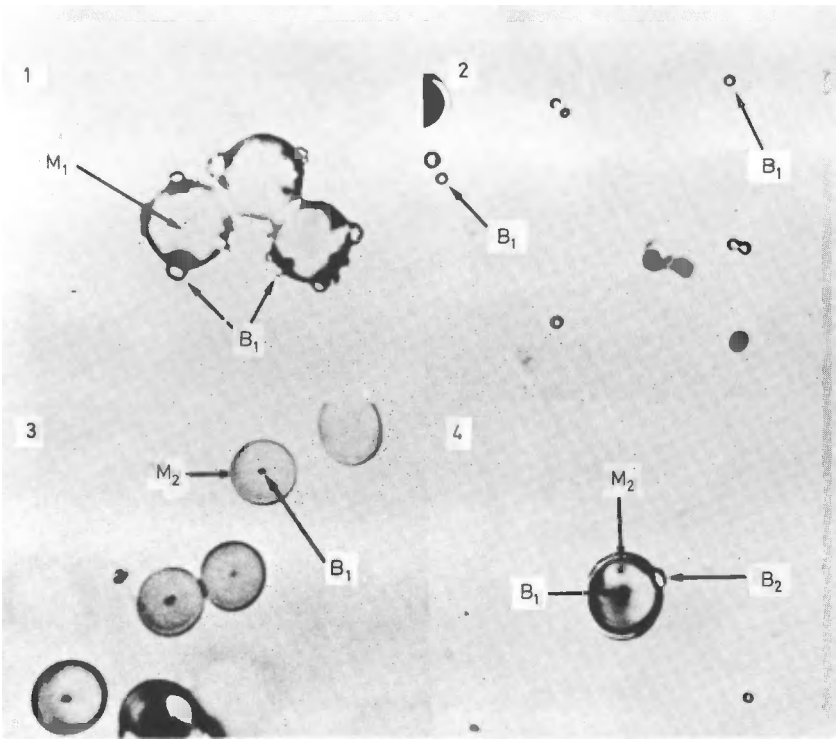

Figure 3. Replication of microspheres through budding and accretive growth. 1—Proteinoid microspheres (M_1) with buds (B_1). 2—Buds (B_1), separated by mechanical or thermal events. 3—Stained buds (B_1) around which has accreted proteinoid to yield second-generation microspheres (M_2). 4—Stained bud (B_1), second-generation microsphere (M_2), and second-generation bud (B_2). Microspheres are approximately 15 μm in diameter.

The significance of this view rests upon recognition of the logic that a minimal cell would have first arisen and then have evolved to a much more contemporary cell[3]. The initial appearance of a proteinoid microsystem was thus the first of a series of saltatory changes in cellular evolution.

The uniformity in size of the microspheres produced from proteinoid is illustrated in *Figure 4*. They can be made into a very stable form or into a considerably less stable type by the employment of crude proteinoid. The latter type appears to contain lipidlike material. Aside from the structure discernible in the optical microscope, the microspheres have an ultrastructure, revealed in the electron microscope. A relatively solid type of microsphere, typically one third water, is illustrated in *Figure 5*. The interior polymer diffuses through the boundary readily with very slight elevation of pH of suspensions of microparticles made from acidic proteinoid (*Figure 6*). Upon this treatment, one observes a double layer. The microspheres thus display ultrastructure of a kind which characterizes contemporary cells, although the double layer is not identical in size and detail to the present-day unit membrane[40].

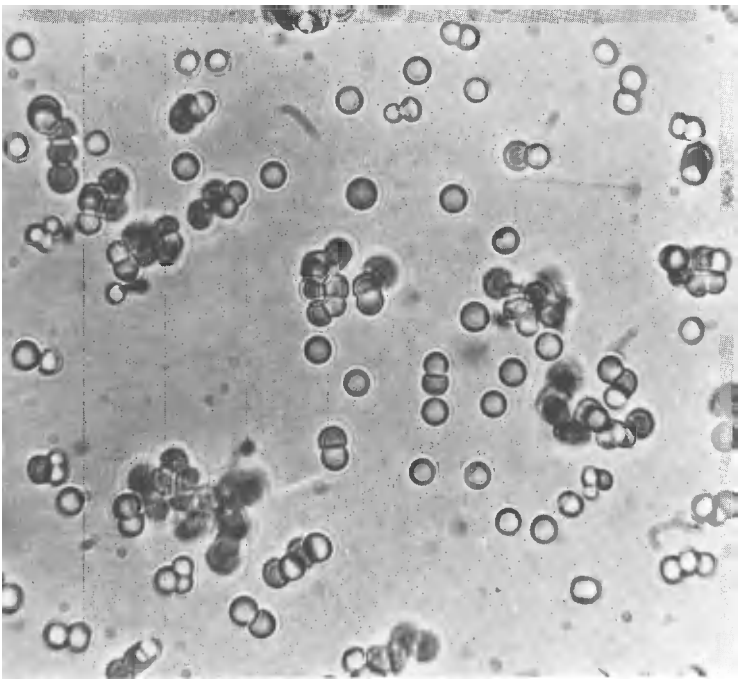

Figure 4. Proteinoid microspheres originally photographed at ×970. Uniformity in diameter is evident. (Reduced by $\frac{1}{2}$)

EMERGENT PROPERTIES

Some properties that are found in systems at a higher level of organization are properties which have first emerged at that higher level. These properties are the kind which make the living system, for example, appear to be the complex entity that it is. Other properties that are found in the unit at the higher level of organization are carried into it from a lower level. In the case of proteinoid microspheres, this is true of a number of the catalytic activities. The activities identified in proteinoid are in some cases found in the microspheres. The first cell was, accordingly, already equipped with some enzyme specificities. A third class of properties needs to be mentioned as well. These are the properties that appear upon evolution, rather than upon initial assembly, and which were not uniformly discernible in the unevolved entity. The catalogued properties of proteinoid microsystems are presented in *Table 6.*

In the present state of knowledge, the crucially significant emergent properties mentioned first could have been identified only by experiment.

ORIGIN AND EVOLUTION OF METABOLISM

Experiments suggest that an outstanding example of the kind of property appearing upon evolution is that of lengthened metabolic pathways. As

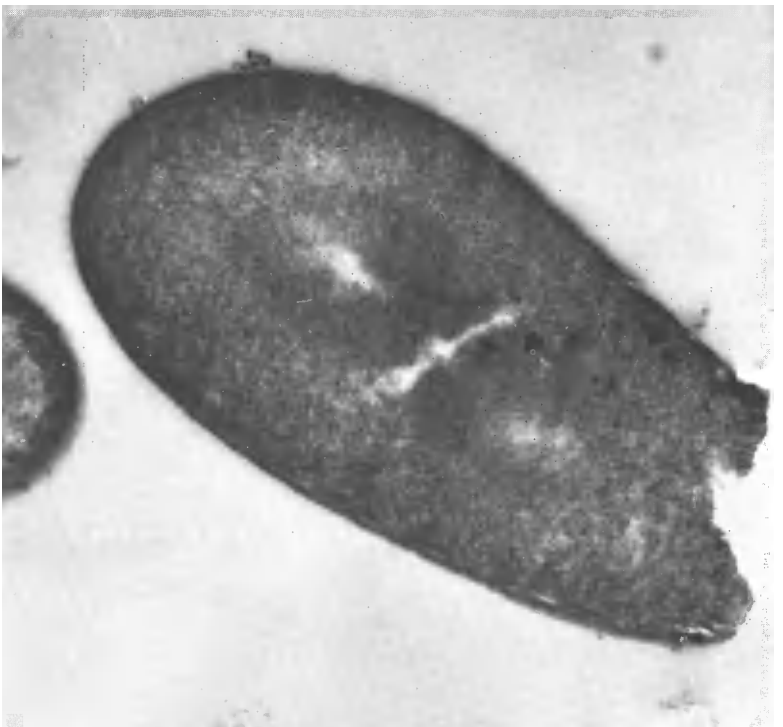

Figure 5. Relatively solid microsphere, approximately 3.5 μm in length. Stained with osmium tetroxide, embedded in methacrylate, and sectioned.

further explained from the modes of reproduction described later in this paper, two microspheres, each having its own metabolic capabilities, could have joined to pool metabolic capabilities. Those pooled, or lengthened, abilities could then have been bequeathed to offspring.

Table 6. Properties of proteinoid microsystems

Stability (on standing, during centrifugation and sectioning)
Microscopic size; variability of shape; uniformity of size
Numerousness
Stainability; producibility as Gram-positive or Gram-negative
Tendency to shrink or swell in atonic solutions
Boundaried structure; ultrastructure (visible under the electron microscope)
Selectivity of passage of molecules through boundary
Assembly from catalytically active polymer
Patterns of association
Budding and fission; growth by accretion; ability to propagate through budding and growth by accretion, etc.
Ability to form junctions; ability to transfer informational molecules

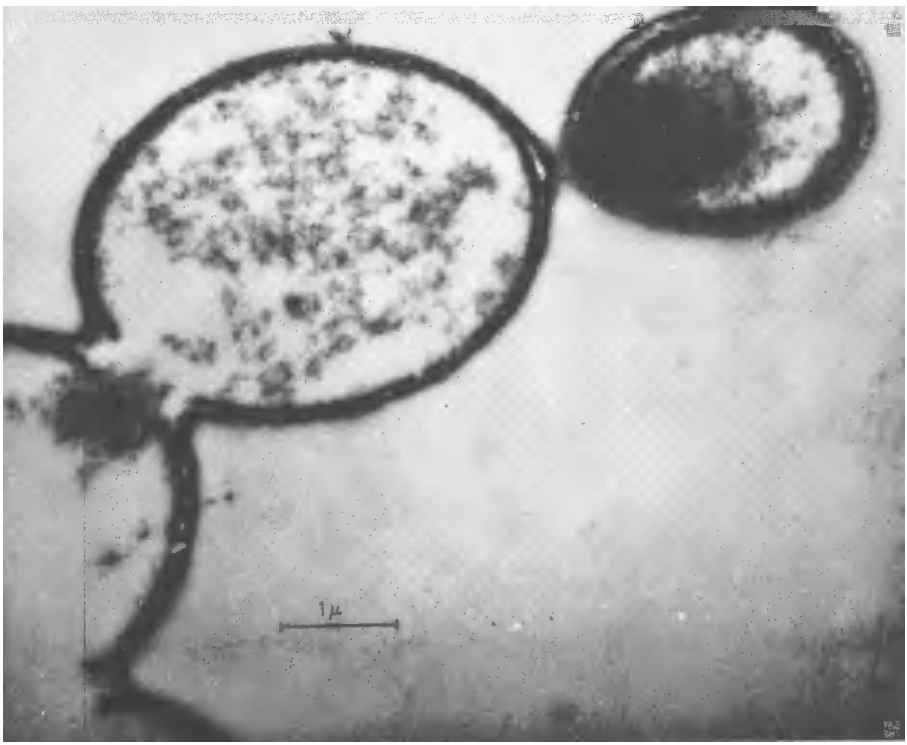

Figure 6. Proteinoid microsphere, after most of the interior polymer has diffused. Double layers are evident in the membrane.

We can thus visualize at least three kinds of appearance of enzyme reaction sequences. One was the intrinsic type (*Table 5*), another might have been a kind which resulted from the assembly of catalytically active or inactive proteinoid subunits (Ref. 3, p. 777), and a third kind was the metabolic reaction sequences which resulted from lengthening due to reproductive activities of systems each of which possessed its own activities.

ORIGIN OF MEMBRANES

The proteinoid microsphere contains within it membraneous layers. This has been demonstrated in at least two ways. In one of these, polymer from the interior diffuses through the membrane to the exterior, while the membrane itself remains as a double layer (*Figure 6*). In a second demonstration, the microspheres have been made in the presence of monosaccharides and polysaccharides. Upon standard washing with water the monosaccharides are removed, and the polysaccharides are found to be retained. A third kind of evidence is that in which a kind of osmotic behaviour has been demonstrated[41]. All these types of evidence point to the existence of membraneous functions in the microspheres.

The finding of membrane qualities in polymers has been familiar in industrial chemistry. A number of industrial polymers have 'permselective' qualities[42, 43]. More recently, Singer and Nicolson[44] have pointed out that a wide variety of biopolymers serve as membranes. Against this background the finding of membraneous qualities in the proteinoid microsphere is much less surprising than otherwise.

PROPERTIES OF MICROSPHERES OF A SEA-WATER TYPE

The properties identified have been noted and studied in microspheres which have almost all been made from acidic proteinoid, as earlier described. These properties, while sufficient to permit the visualization of an evolution toward more contemporary cells, have had one feature which is not characteristic of most contemporary cells. The microspheres made from acidic proteinoid tend to dissolve at pH 6 and above. In a number of respects microspheres which would be stable at higher pH, however, would have enhanced evolutionary potential. Dr. W. D. Snyder[45] has recently observed the production of such microspheres under conditions which make them attractive in other ways as well. He has heated all the common amino acids with the salts of sea-water in approximately equal amounts. The resultant sea-water microsphere from the sea-water proteinoid is stable to elevated pH including that of 0.1 N sodium hydroxide solution. The morphology is however altered in dilute alkali. Such microspheres, depicted in *Figure 7*,

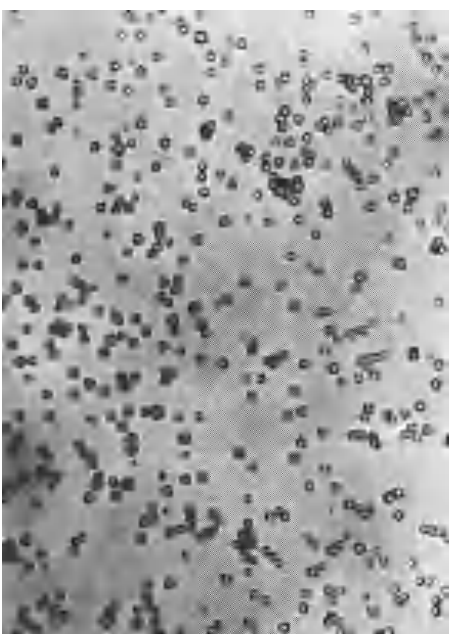

Figure 7. Sea-water microspheres. These are stable at pH 9. About 1 μm in diameter.

could have arisen in lagoons. Conceptually, these particles would easily have been stable in the primitive ocean at any inferred stage of its history in either an acid ocean or a basic ocean[46]. The results add weight to the long held belief that life began in a marine, rather than a freshwater, environment. A special feature is that one can now search for a simple model for the origin of internal synthesis of proteins and nucleic acids in microsystems which can tolerate a pH as high as 9. In contemporary cells such pH ranges represent values which are necessary for some steps in the enzyme-controlled synthesis of proteins[47] and nucleic acids[48].

The salient properties already discerned in microspheres from acidic proteinoid include ultrastructure, metabolic activity, membraneous functions, and the ability to reproduce (*Table 6*).

PRIMORDIAL MODES OF REPRODUCTION

The origin of five modes of reproduction in primordial cells has been suggested by experimental results from the proteinoid microsphere model. These five modes are summarized in *Table 7*. The first, most thoroughly developed, and best known of these is that which involves the formation of buds, their separation, and their growth to the size of the parent. This cycle was reported in 1967[34]; it has been treated in a biochemical context more recently[3]. The visualization of these phenomena through the optical microscope is presented in *Figure 3*. This method of reproduction, as for the others of *Table 7*, depends upon growth by accretion. Such a requirement

Table 7. Reproductive phenomena observed in proteinoid microspheres

Model for primordial process of:	Remarks
Budding—separation—growth	Published in 1967[34]
Binary fission—growth	Binary fission,[1] published 1964
Sporulation—growth	Aided by Ca^{2+}[53]
Partuitive replication	Repeated through three cycles
Partuition—recombination	Model of sexual[54] reproduction

All growth processes in these cycles are accretive (heterotrophic).

makes of the protocell a heterotrophic body, and heterotrophism for the protocell has long been proposed by students of protobiogenesis[49–51]. Of relevance also is the modern emphasis on reproduction, in contrast to 'self-reproduction.' As has been pointed out by Ashby[52], 'no organism reproduces itself.' An organism can, in conjunction with the environment, produce a likeness of itself. Moreover, the likeness begins as a smaller entity, which then grows to the full size of the parent. This analysis of repro-

duction fits either contemporary organisms or the proteinoid microsphere as a model for primordial cells, the latter reproducing in one of the ways presented here.

The process of binary fission is depicted for proteinoid microspheres by *Figure 8*. The carrying through of two or more cycles in binary fission has

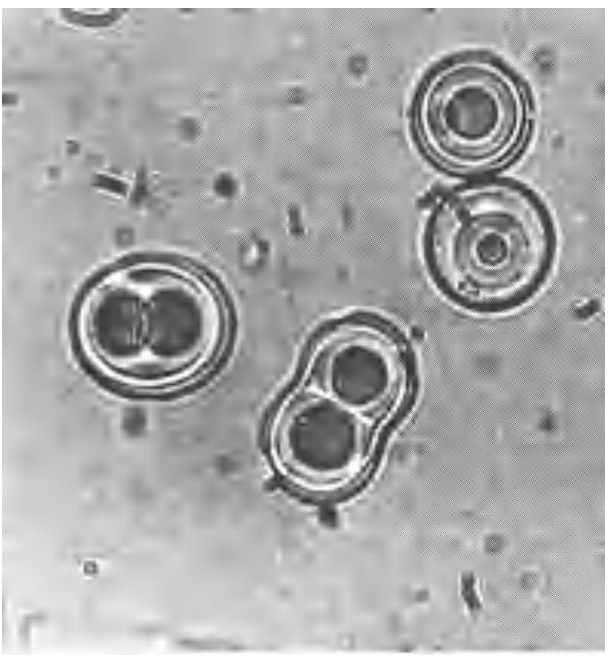

Figure 8. Early stages of binary fission in proteinoid microspheres, triggered by slight increase in pH. Approximately 10–15 μm in diameter.

not yet been developed for proteinoid microsystems as it has for the budding-and-growth cycle. In principle, however, the growth by accretion of the daughter particles of a microsphere which has undergone binary fission should apply as well for this mode as for the growth of the buds which are released from budded microspheres. In both these modes of reproduction and in others, the fact is critical that the microspheres tend to grow to a very precisely controlled parental size and then stop. Indeed, one can visualize the appearance of buds on the basis that they represent new entities which had to appear as discrete individuals because the deposition of proteinoid heterotrophically on the parent had attained its maximum permissible size. The fact that the parent and the bud, albeit of exogenous origin, are joined as firmly as they are would have been sufficient for primordial maternal–offspring relationships[33].

A third type of reproduction is represented by a model for sporulation. Models for spore-containing microspheres have been observed for a number of years; they are produced more easily in the presence of calcium[53]. As *Figure 9* shows, these are subject to disruption and the dissemination of the

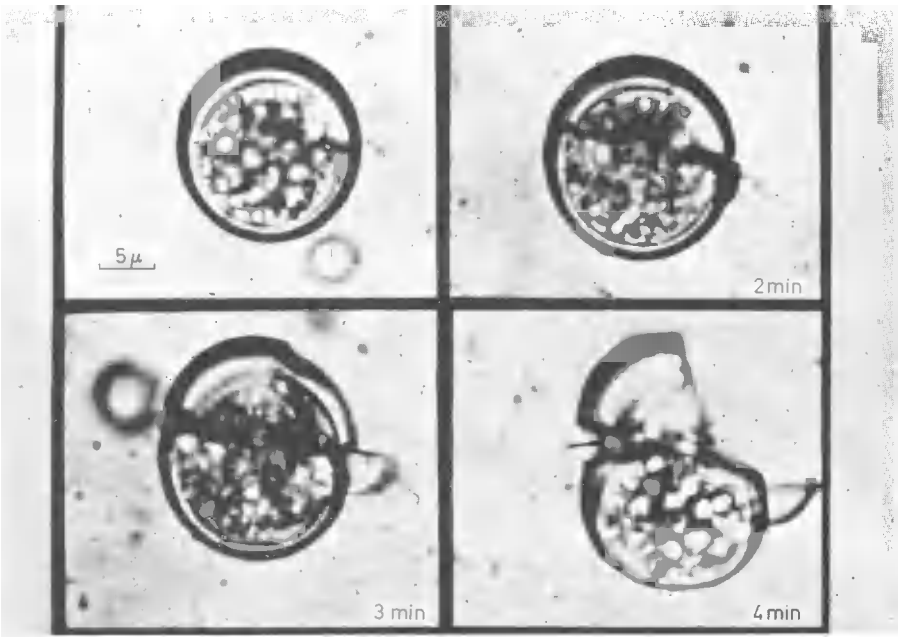

Figure 9. Time-lapse cinephotomicrographic sequence of a model for sporulation. These were produced from acidic proteinoid, basic proteinoid, and calcium salt. 1 min intervals.

spore-like particles which can then, presumably, as for the acidic type, accrete to the size of parents.

The fourth type of reproduction is that which we refer to as partuitive replication. This is illustrated in *Figure 10*. Again, growth by accretion would have been essential. This type has been carried through three cycles.

In the fifth kind of reproduction one can visualize the origin of sexual reproduction[54]. This phenomenon involves the combination of endoparticles from two microspheres and the growth by accretion around the combined endoparticles (*Figure 11*).

The third and fifth methods of reproduction have in common the process of ejection of an endoparticle. They also represent communication through a packet of information-containing material. Such phenomena, in fact, serve as a model for the origin of intercellular and intergenerational communication. This relationship has been discussed in some detail elsewhere[33]. The connection is possible through the formation of junctions, a high-efficiency process (*Figure 12*).

In principle, any of the types of cycle can be repeated indefinitely. Continuation of reproducibility† appears to be simple since the preparation can be held in a dormant stage indefinitely. One population prepared for budding experiments was maintained for several years before it became contaminated by wild growth on being exposed. As with cultures of contemporary organisms,

† Microspheres which have lost the ability to reproduce through aging can be rejuvenated by contact with fresh proteinoid solution.

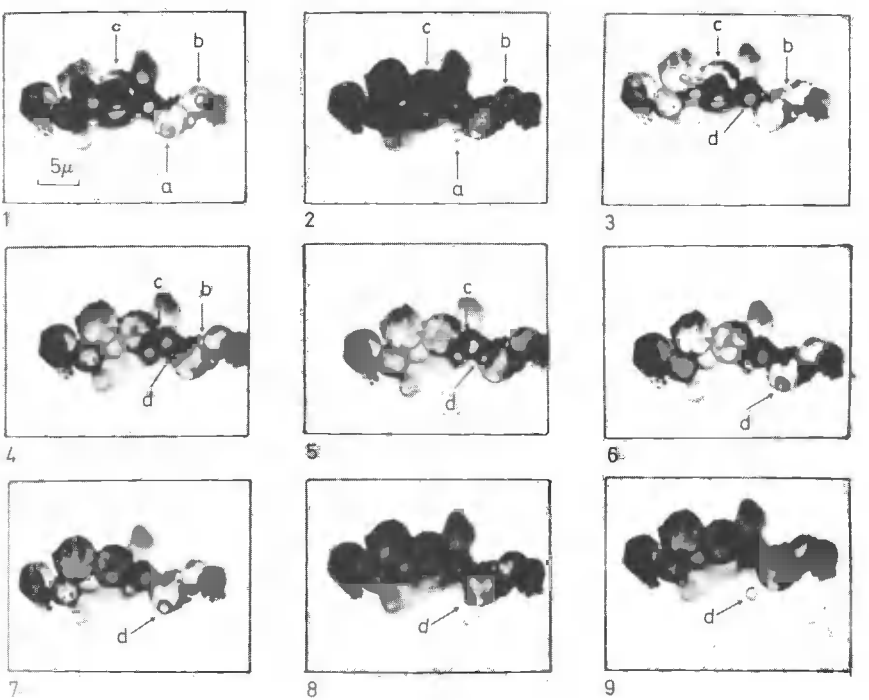

Figure 10. Model for partuitive replication. Endoparticle is ejected and subsequently allowed to grow by accretion, as in *Figure 3(3)*. Partuitive replication has thus been carried through three cycles. Note especially sequences 6–9.

the model systems are prepared and handled aseptically to minimize growth of contaminating organisms. The reproduction observed is less efficient than, and is different from, that of contemporary organisms, owing to the fact that internal synthesis of macromolecules is absent. In either the model for the primitive cell or in the contemporary cell, however, the parent participates with the environment in the reproduction of its likeness, through an offspring of lesser size.

ANSWERS TO QUESTIONS

The recognized characteristics of microspherical units assembled from proteinoid are now numerous. These systems are believed to possess a number of properties that have not yet been identified.

As a model, the systems and the fact that their emergence can occur under geologically relevant conditions, answers a number of fundamental questions for the first time[1, 55]. These include how: (a) enzymes arose before there were enzymes to make them; (b) how cells originated when there were no cells to produce them; (c) how membranes appeared when there existed no cellular systems with membranes; (d) how the process of reproduction first appeared; and (e) how informational macromolecules arose when there was no elaborate coding system to specify amino acid sequences. A non-nucleic

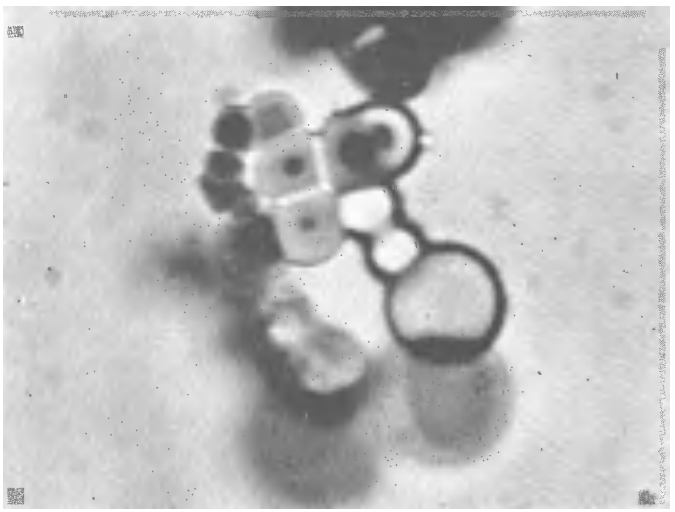

Figure 11. Model for origin of sexual recombination. Particle has grown by accretion around a combinant of two endoparticles.

acid pregenetic system was however present at the same moment that the protocells came into existence.

NATURAL SELECTION

The view that proteinoid microspheres could have evolved further may be examined by comparing their capacities to those required for natural selection. According to Lewontin[56] and others, natural selection requires (a) phenotypic variation, (b) correlation between parental phenotypes and phenotypes of offspring, and (c) different numbers of descendants from different parental phenotypes. The process of reproduction underlies (b) and (c). In a simple form, all the requirements are met in experiments in which two kinds of proteinoid microsphere undergo selection because one is soluble at pH 5 (acidic type) and the other (neutral type) is not. The one which remains stable under those conditions and can continue to reproduce by heterotrophic accretion is the surviving species. The dissolved type has suffered extinction[56]. A more subtle type of selection experiment has been performed in the laboratory by the use of proteinoids of different composition, as prepared and studied by Hsu and Mejido[57]. As *Figure 13* demonstrates, one kind of microparticle tends to accrete more efficiently with its own kind of proteinoid. In this way can be seen the faster generation of one type through differential rates of reproduction[58] and the origination of natural selection at an early period in evolution, before phenotype had evolved to a genotype–phenotype complex.

The evolution of the protocell would have been realized up to the point at which internal synthesis of macromolecules entered the total evolutionary development. In order to contemporize the model for a primordial cell, internal mechanisms for macromolecular synthesis are necessary. Investigation of a laboratory model for the development of internal synthesis and the

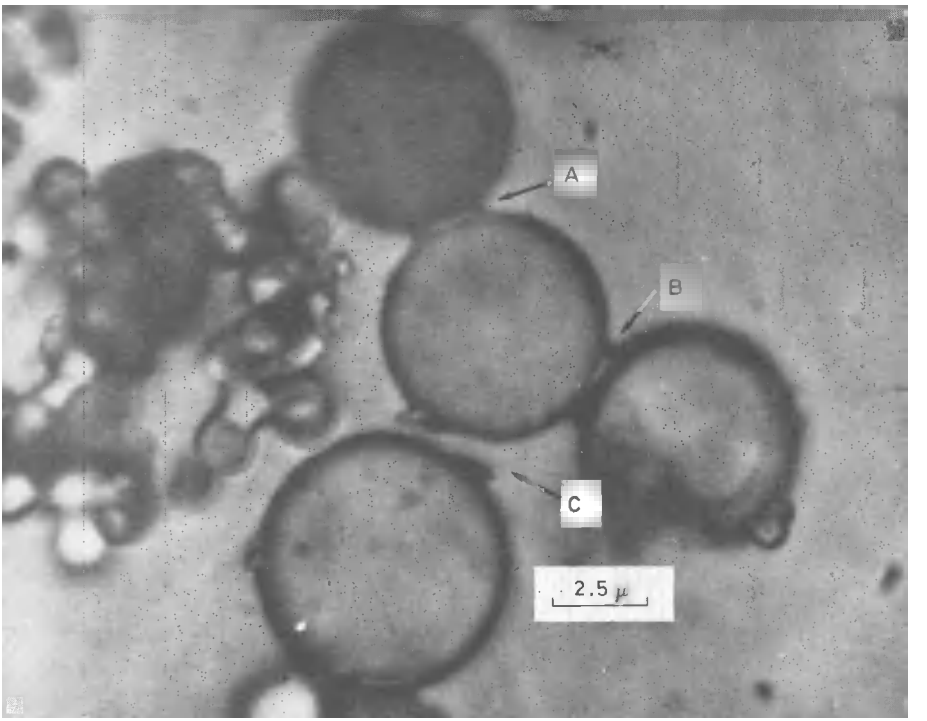

Figure 12. Evidence for formation of junctions between proteinoid microspheres. A—intact, B—cracked, C—separated.

attendant code is incomplete[59]. A significant development was attained empirically with microparticles composed of basic proteinoid and homopolynucleotide. The homopolynucleotide influences the incorporation of amino acids from their adenylates in a codonic manner[59] (*Figure 14*). These microparticles are thus models for protoribosomes.

SOME EXPERIMENTS RELATING CONTEMPORARY CELLS TO PROTOCELLS

The models for protoribosomes have a number of properties of nucleoprotein organelles (*Table 8*). One notable, unpublished, feature of these models for protoribosomes is that their ability to incorporate amino acids lasts for only a few hours. This behaviour is of interest to compare with the loss of initiating capacity in cell-free contemporary ribosomes[60]. In contrast to the models for protoribosomes, the proteinoid microspheres have been found to be stable for years. Since, at this stage of the research, we are interested in inferring which structures and functions preceded others, we can deduce that protoribosomes would not have preceded the cell as a whole. Indeed the experiments suggest that the original ribosomes were produced

Figure 13. Natural selection illustrated by accretive growth around compatible seeds, as a model for a budding cycle. 2:2:1 proteinoid (2:2:1) accretes well around 2:2:1 seeds or around phenylalanine-rich proteinoid (phe) seeds. Phe does not accrete around 2:2:1. Phe accretes poorly around phe. In this microscopic view, 2:2:1 has accreted around stained phe seeds. Phe has precipitated amorphously.

within a cell, which was of such a composition that the protocell could have existed and remained for the necessary further natural experiments for indefinite periods of time. In the modern synthesis of protein, ribosomes function as parts of polysomes which are aggregated along the inner surfaces of cellular membrane[61]. The physical instability of the unprotected nucleo-proteinoid microparticle and the relationship to the cell suggest that the cell had to precede the first ribosomes. Experiments employing both models are under way.

Incidentally, the appearance of the ribosome, as we know it, could have explained the origin of optical activity in one of the many plausible ways by which that question can now be answered[1].

THE ENLARGED OVERVIEW

A relatively comprehensive picture for the origin of contemporary living systems from galactic organics is presented in *Figure 15*. As one may see, the evolutionary progression here is one of simplicity to complexity. No discontinuities are discernible. The concept that a protoprotein preceded functionally a protonucleic acid is essential to this picture, and is consistent with views that have been expressed for many years regarding the later

Table 8. Properties of microparticles formed by the complexing of basic proteinoid with polynucleotides

Nucleoproteinoid composition
Morphology resembling that of nucleoprotein organelles
Stability greater than that of simple proteinoid microsystems
Microscopic size (0.5–1.5 microns); uniformity of size
Numerousness
Selectivity in formation dependent upon identity of polynucleotide and polyamino acid
Ability to form junctions
Selectivity in the promotion of condensation of aminoacyladenylates (related to the contemporary code)

appearance of a nucleic acid gene[38, 39, 62–64]. The opposite point of view, proposing that genes preceded the first proteins functionally, has however been entertained[65–67]. The 'proteins first' hypothesis explains the origin of ordered macromolecules, of enzymes, and of cellular structures without the need for nucleic acids. We do not visualize how a 'nucleic-acids first' hypothesis could answer such questions, or even how such hypotheses might be tested.

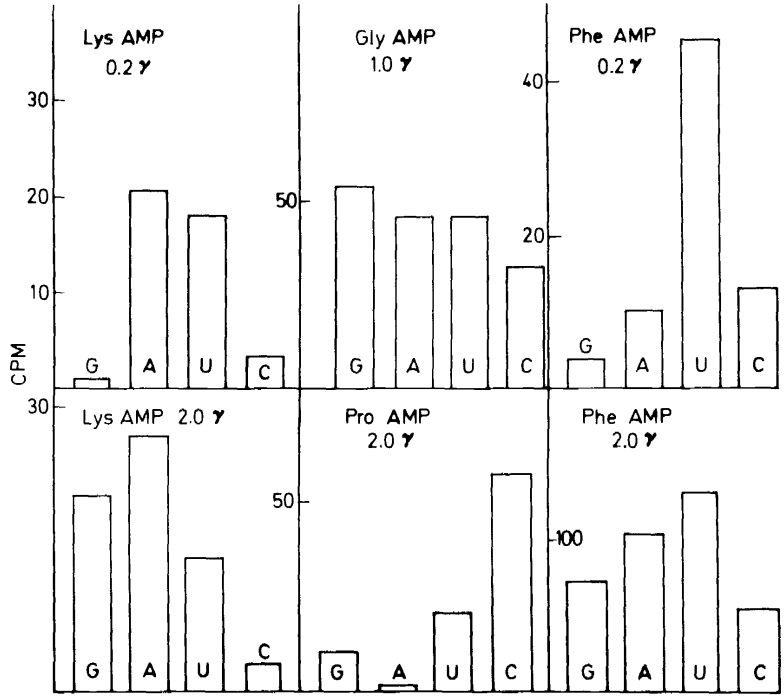

Figure 14. Codonic bias in incorporation of radioactive amino acids from adenylates into microparticles from basic proteinoid and homopolynucleotides. The conditions to yield such effects were identified empirically. Poly Ntd, 0.25 mg ml^{-1}.

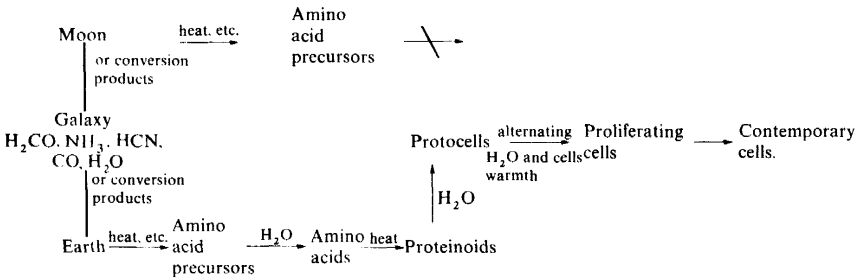

Figure 15. Increasing complexity of organic compounds, starting with galactic reactants. Termination of prebiotic molecular evolution on the Moon (above); continuation to replicating contemporary cells (on right). The principal difference in the extent of the two pathways is determined by water. Contemporary cells, which contain nucleic acid mechanisms, are the most complex.

The necessity for proteins first is consistent with a Darwinian view requiring that which is to be selected to have arisen first[64]. The notion that nucleic acids arose first is a kind of molecular Lamarckianism, which indeed has a superficial logical appeal to it. The more Darwinian point of view is perhaps better understood when we recognize that the building of models in the laboratory, a kind of constructionism, is in the same direction as evolutionary processes themselves[1]. When one pursues the reductionist approach of studying functions of the contemporary cell by dismantling the cell, one can easily develop the impression that the nucleic acids had primacy[65]. This approach to understanding the origin of life, or the origin of the first cell, presents an insuperable difficulty in that, as the system is destroyed, the total association of functions is destroyed. Since the destruction, as carried out in experiments at the present time, is not done in a stepwise fashion, no information is provided as to the order in which those individual functions appeared in the evolution of that contemporary unit. We infer, then, that we can obtain the desired knowledge only through a model-building approach.

The total number of functions that the constructionist experiments indicate were simultaneously introduced into the first cell was unanticipated. Some of these, such as motility and the ability to communicate, are open to classification as behaviour. As a consequence of this observation we may now entertain the thought that at the moment molecules had evolved to cellular systems, behaviour had appeared in its most primitive evolutionary form.

The theory which emerges emphasizes the role of water, in both absence and inclusion, each at appropriate stages. The remarkable rapidity with which appropriate copolyamino acid aggregates into a minimal cell and the near-ubiquitous nature of water suggest the past frequent occurrence of the sequence amino acids → protoprotein → minimal cell → (nucleic acid-governed) contemporary cell.

A theory of the origin of the first cells is necessary to supplement and support a view of chemistry in evolution and systematics. The need for such a theory appears to be, moreover, the most signal single question of the natural mechanism of evolution, a point stressed by the geologist, Rutten, in his posthumously published book, *The Origin of Life by Natural Causes*[68].

Darwin's *Theory of the Origin of Species* had to oppose at least six powerful ideas[69]; the theory of the origin of life has had to contend with these and, in addition, others such as an emphasis, derived from reductionism, on the original primacy of nucleic acid. We can anticipate that full awareness of any theory and of underlying evidence accumulated (by 1967 in this study) for the laboratory production of a primitive replicating cellular system[34] should require some time, as Pirie predicted in general terms for his eobionts[30].

We may be most prudent to eschew attempts to evaluate experiments on the basis of standard, varied, and reductionistically-derived concepts of the living state; we may benefit instead by allowing the experiments themselves to construct a definition of life, much as Pirie has proposed that experiments could redefine the problem. A satisfactory definition of life may well be an inevitable consequence of understanding the origin of life. The experiments have already made clear that we should not simply define life, but rather define it at various stages of its development, which occurred both vertically (simplicity → complexity) and laterally (variation).

In our view, the experiments performed have uniquely limited the possibilities for the interpretation of the results. Such limitation in thinking has, however, led to initially unexpected inferences, such as the primacy of a functional protoprotein molecule[70]. It is this kind of interpretation, however, that appears to open the door to understanding reproduction, selection, and other biological phenomena at the molecular and biological levels simultaneously. Selection at the molecular level, according to this view, led into selection at the cellular or systemic level[71], simply because the first cells emerged from the first protobiomacromolecules. But we do not reduce biology to chemistry from the outside in; we let chemistry become biology from the inside out.

ACKNOWLEDGEMENT

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