Radiation and the First Biopolymers*

Sidney W. Fox

Institute of Molecular Evolution,

University of Miami

Coral Gables, Florida, U.S.A.

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Attempts to discipline a theory of the origin of life have focussed attention especially on the origin of the cell as the unit of life, and on the polymer of which the first cell was composed. Answers to questions of sources of energy for the synthesis of such materials and systems, and knowledge of the effects of radiation on such polymers must of course be compatible with any relevant laboratory demonstrations [1].

In this paper I intend briefly first to review a coherent model of the origin of the stuff and structure of the protocell; second, to discuss briefly other models of some of the steps; and, third, to consider the effects of radiation in the formation and durability of the polymers implicated.

In discussing the first life-related macromolecules, we have reason to be most concerned with polyanhydro-α-amino acids and with polynucleotides. A general feeling that highly organized cells are necessary for the synthesis of such complex polymers has prevailed for decades [2]. While this feeling may be most justified for the synthesis of <u>exactly</u> the polymers which are produced by contemporary cells, some explanation for the synthesis of a first protein-like material to yield a cellular type of structure has been needed. Without such understanding, we may be intellectually immobilized

by basic chicken-egg dilemmas which stand in the way of a comprehensive theory. These dilemmas have been defined by Wald [3], Tatum [4], Blum [5], and others. Stated more broadly, the basic problem has been one of explaining how sufficient quantities of polyanhydro-α-amino acids having adequate molecular size and other properties required by the first cells could arise to form those cells in the absence of any previous cells [6].

Assuming the availability of free α-amino acids on the primitive Earth [7-9, cf. 10], one may view the essential problem as that of the synthesis of the peptide bond. In identifying the conditions by which precellular protein-like polymers might have arisen, experiments have been performed in the laboratory, many in closed flasks. The geochemical environment is however predominantly an open one. While one might perhaps imagine the equivalent of a steel bomb in nature, reactions that could occur on the open surface of the Earth can more easily be regarded as spontaneous. Also, gases confined beneath the surface of the Earth would not be subject to activation by ultraviolet radiation or electrical discharge, although they could receive high energy bombardment from radioactive materials.

Not only is open system chemistry the appropriate type of organic chemistry, the meaningful thermodynamics

is also the irreversible thermodynamics of the open system. This point has been made eloquently by Oparin [11], and by Prigogine [12], who has said, " - - - the steady flow of energy which originates in the sun and the stars prevents the atmosphere of the earth or stars from reaching a state of thermodynamic equilibrium.

"Obviously then, the majority of the phenomena studied in biology, meteorology, astrophysics and other subjects are irreversible processes which take place outside the equilibrium state."

The relative meaninglessness of closed system thermodynamics extends also to compounds other than peptides. Similarly, experiments yielding amino acids in open vapor phase systems [9] have for the geochemical situation relavance which is lacking when the amino acids are produced in closed flasks [10]. Relating the thermodynamics of the formation of the peptide bond to closed and to open systems has been crucial in our own line of study.

Of the various laboratory demonstrations of synthesis of polyamino acids, we should however first mention Akabori's process [13] in which polyglycine is progressively substituted. The first compound by Akabori's scheme is not an α-amino acid. Other laboratories have reported from amino acids small yields of small peptides

in aqueous solution [14,15, cf. also 16]. Another has reported larger yields by carrying out reactions of glycine in ammoniacal solution in sealed tubes or in steel bombs [17].

In considering the thermodynamics of peptide bond formation in an open system, we may focus on the equation:

$$H_2NCHRCOOH + H_2NCHR'COOH =$$
$$H_2NCHRCONHCHR'COOH + H_2O$$
$$\Delta F = 2000 \text{ to } 4000 \text{ cal.}$$

The energetic requirement was determined first by Huffman [18]. Whereas the free energy values were determined on solid amino acids and peptides, Borsook and Huffman [19] pointed out that the values in aqueous solution would not be significantly different. Such values of ΔF give equilibrium constants of 0.01-0.02 per mole of peptide bond in a dipeptide.

For a tripeptide the ΔF value of the second peptide bond would be smaller. The derived figure for a tripeptide is not simply the square of the figure for a single peptide bond, inasmuch as the energy requirement should theoretically decrease with larger peptides [20]. Nevertheless, one must anticipate that only small yields of quite small peptides can result unless the peptide bond reaction is somehow properly coupled in aqueous solution

with an energy-yielding substance.

In an open system, the thermodynamic barrier can however be easily overcome by volatilization of water.

$$H_2NCHRCOOH + H_2NCHR'COOH =$$

$$H_2NCHRCONHCHR'COOH + H_2O \uparrow$$

By starting with almost dry α-amino acids in appropriate mixtures and raising the temperature above 100°, experiments in our laboratory have shown that one can obtain 10-40% yields of polymers of molecular weights of many thousands. Sufficient proportions of aspartic acid, glutamic acid, or lysine are required to avoid pyrolysis [21]. Furthermore, any number of types of amino acid can be simultaneously incorporated. Such polymers are found to have many of the salient properties of proteins. These polymers are referred to as proteinoids.

Consistent with the thermodynamic considerations in open systems, all samples of volcanic material taken above 100° have been shown to contain α-amino acids in almost entirely polymerized form [22]. We shall return to other detailed questions of geological locale later.

Time does not permit reviewing here in detail the many properties of the thermal polyanhydro-α-amino acids [23]. Of most significance is the ease with which such polymers form microscopic units which function as models

of the protocell. Recently, evidence from a number of laboratories has accumulated for the ability of the polymers to catalyze a number of reactions of natural substrates. In our laboratory much evidence has appeared to lead to the inference that such macromolecular preparations are relatively highly uniform and ordered; they are clearly not the disordered polymers one might anticipate from what was earlier regarded as a pyrolytic thermal process acting on a mixture of eighteen amino acids.

The catalytic activities which have so far been reported are listed in Table 1.

Some of the evidence for the limited heterogeneity of a 2:2:3-proteinoid is shown in Table 2 [31]. Despite repeated fractionation, compositions are almost identical.

In Table 3 is some of the evidence, in a 2:2:1-proteinoid (2 parts aspartic acid, 2 parts glutamic acid, 1 part all 16 other amino acids in the reaction), for nonrandom arrangements of amino acid residues.

In Fig. 1 is evidence of limited heterogeneity of a 1:1:1-proteinoidamide fractionated on DEAE-cellulose. The material in all peaks have similar amino acid compositions [32].

When this and much other evidence is reviewed, one is led to conclude that the interactions of individual

TABLE 1

Indications of "Catalytic" Activity in

Thermal Polyanhydro-α-Amino Acids

Activity in splitting p-nitrophenyl acetate	[24]
Loss of activity with opening of imide linkage	[24]
Inhibition by choline esterase inhibitors	[25]
Glucuronic acid → CO_2	[26]
Pyruvic acid → CH_3COOH + CO_2	[27,28]
Oxaloacetic acid → pyruvic acid	[29]
ATP → ADP → AMP → adenosine	[28]
ATP → ADP	[30]

TABLE 2

Composition of Hydrolyzates of 2:2:3-Proteinoid Following One and Two Purifications

Amino acid	Unpurified %	Purified %	Repurified %
Lysine	5.1[a]	5.4	5.4
Histidine	1.8	2.0	2.0
Ammonia	8.6	8.1	6.9
Arginine	2.0	2.3	2.4
Aspartic acid	51.7	50.2	51.1
Glutamic acid	10.7	11.6	12.0
Proline	0.7	0.6	0.6
Glycine	2.7	3.1	2.8
Alanine	4.0	4.3	5.5
Half-cystine[b]	4.5	3.5	3.4
Valine	1.2	1.2	1.2
Methionine	1.8	1.9	1.7
Isoleucine[c]	1.2	1.3	0.9
Leucine	1.3	1.2	1.1
Tyrosine	2.0	1.9	1.7
Phenylalanine	1.8	1.7	1.5
Total recovery[d]	84.8	97.5	100.0

[a]Values are given in gram residues of amino acid/total gram residues.

[b]Half-cystine values may be partly other material.

[c]Isoleucine includes alloisoleucine.

[d]Total recovery = total residues of amino acid/wt. of polymer, after 4 da. at 110° with 6 N HCl.

TABLE 3

Distribution of Three Kinds of Assayable Amino Acid in Two Proteinoids

	N-Terminal	Total	C-Terminal
		2:2:1-Proteinoid	
Aspartic acid	6%[a]	71%[a]	1% [a]
Glutamic acid	46	11	8
Basic-neutral amino acids (BN)	48	17	91
		2:2:3-Proteinoid	
Aspartic acid	[b]	50	28
Glutamic acid	[b]	12	1
BN	[b]	38	70

[a] Mole %

[b] Not det'd.

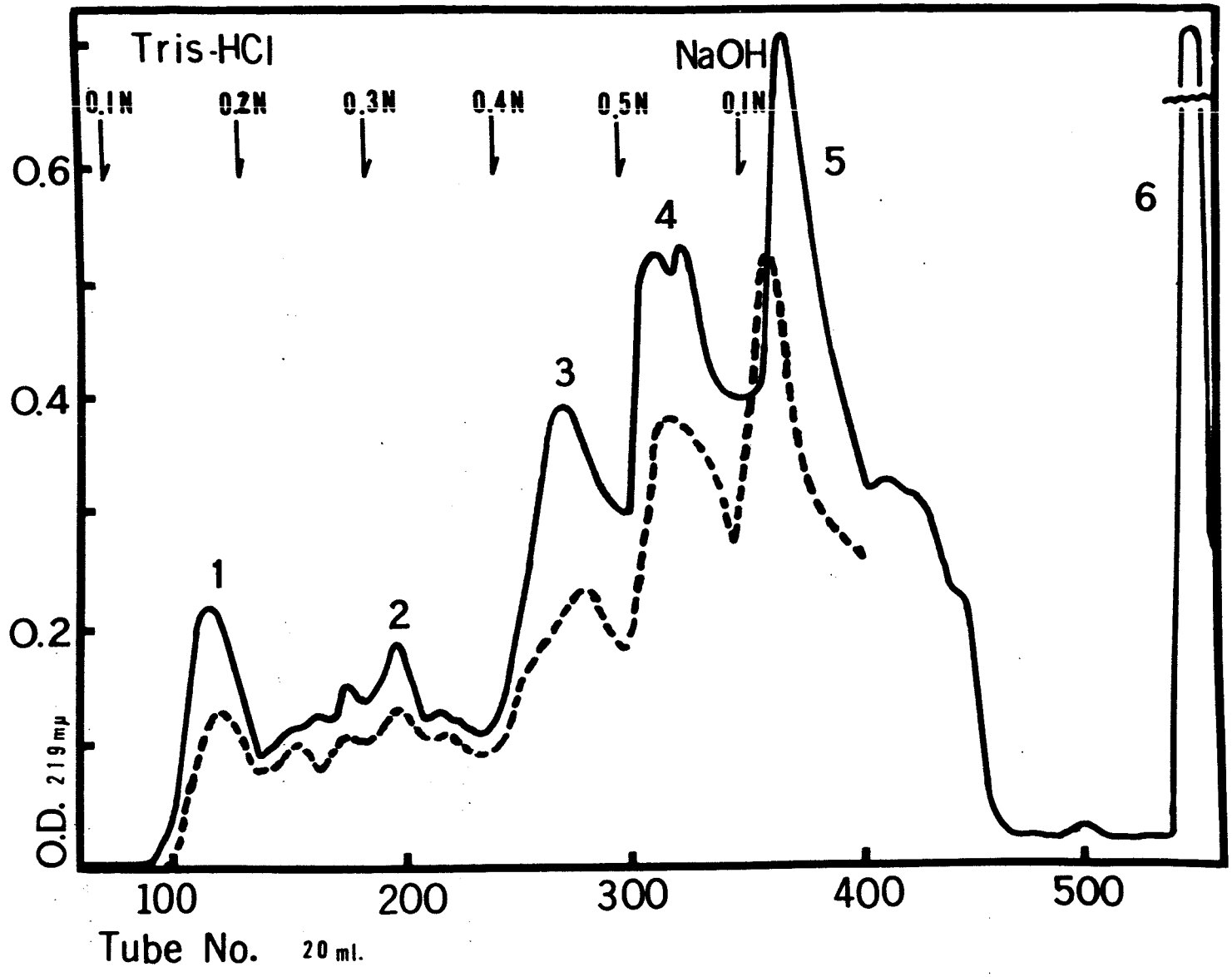


Fig. 1. Elution pattern of 1:1:1-proteinoidamide from DEAE-cellulose with tris buffer.

amino acids lead to a high degree of order in the resultant polymers. This result can be at least partly explained by the steric and electronic individuality of each kind of amino acid, resulting in a selectivity of coupling at each stage of polymerization.

The other properties which the thermal polymers possess in common with proteins are listed in Table 4.

The property of crucial significance to a theory of abiogenesis is the propensity of proteinoids and similar polymers to form, in the presence of water, organized units possessing many of the properties of cells. The result of heating proteinoids with water and allowing the hot solution to cool is shown in Fig. 2. The formation of bud-like appendages may also be noted in this and other photographs. The units obtained are microscopic, these microspheres being less than 2 μ in diameter.

These units are approximately as stable as true cells. They can be sedimented in the clinical centrifuge without disruption and they do not break down on standing. They fulfill the requirement set forth by Prof. Oparin, who stated, "Thus, all the evidence now available agrees in indicating that the organic polymers which were originally formed, and in particular the protein-like polypeptides of high molecular weight, must, at some stage in the evolution of carbon compounds,

TABLE 4

Properties of Thermal Proteinoids

Limited heterogeneity
Qualitative composition
Quantitative composition
Range of molecular weight
Color tests
Solubilities
Inclusion of nonamino acid groups
Optical activity
Salting-in and salting-out properties
Precipitability by protein reagents
Hypochromicity
Infrared absorption maxima
Recoverability of amino acids on hydrolysis
Susceptibility to proteolytic enzymes
Catalytic activity
Inactivatability of catalysis by heating in aqueous solution
"Nonrandom" (nonuniform) sequential distribution of residues
Nutritive quality
Morphogenicity

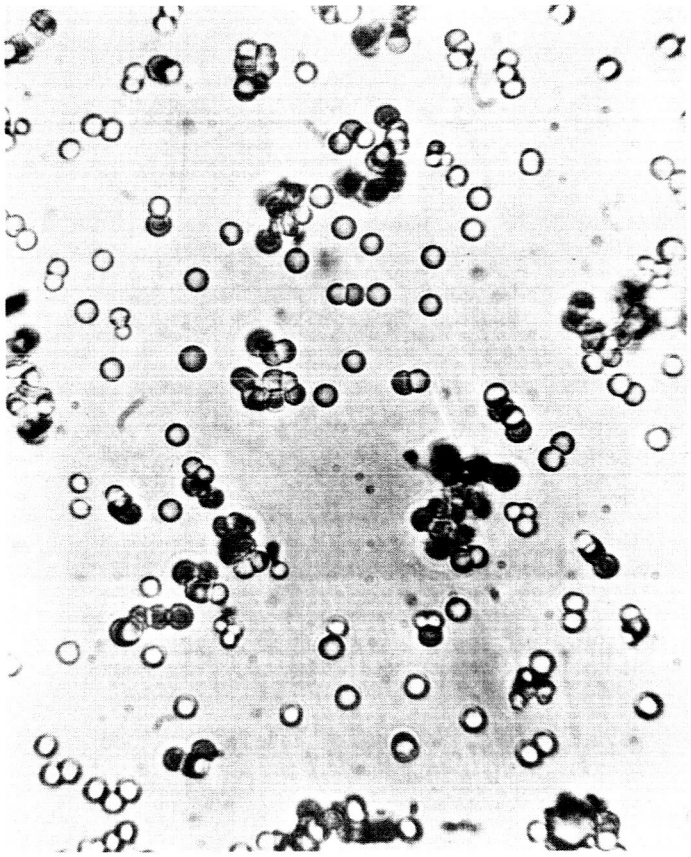


Fig. 2. Microspheres of proteinoid. Approximately 2 μ in diameter.

have separated out from a homogeneous solution in the form of multimolecular aggregates similar to the drops of coacervate which are obtained under laboratory conditions." [2, p. 320] As Oparin has also indicated, those aggregates which would have the kind of stability displayed by cells would have a selective advantage [2, p. 354]. This stability is found in proteinoid microspheres.

The proteinoid microspheres are the result of phase separation, rather than of coacervation in the usual sense, and they do fulfill Oparin's operational requirement. Of most significance is the fact that they arise from polymers formed abiotically.

The other properties which the microspheres have, in common with cellular systems, are listed in Table 5. A few of these attributes will be described here in detail. One of these is the Gram stain, shown in Fig. 3. The microspheres accept many stains, including the Gram stain. As the units are usually prepared, the stain is Gram-negative. When, however, sufficient basic amino acid is included, the units stain Gram-positive.

Fig. 4 illustrates the fact that the microsphere can be induced easily to initiate a type of septate fission. This effect is brought about by raising the pH of the suspension by one to two pH units.

TABLE 5

Properties of Microparticles Organized from Thermal Polyanhydro-α-Amino Acids and Water

Property	References
Stability (to standing, centrifugation, sectioning)	[33,34]
Microscopic size	[23,33,34]
Variability in shape Spheres, "buds", filaments	[35]
Uniformity of size	[34]
Numerousness	[36]
Stainability	[37]
Producibility as Gram-positive or Gram-negative particles	[37]
Solubility in dilute alkali parallel to that of bacteria	[37]
Shrinkability in hypertonic solution	[34]
Swellability in hypotonic solution	[34]
Simulation of cell division	[35]
Electron micrographability	[38]
Presence of boundary	[35,38]
Selectivity of boundary	[35]
Bilamellarity of boundary	[38]
ATP-Splitting activity (by suitable inclusion of Zn)	[30]
Structured associations (algal-like)	[36]
Simulation of motility	[39]
Growth in size of "bud"-like appendages	[39]
Internal streaming	[39]

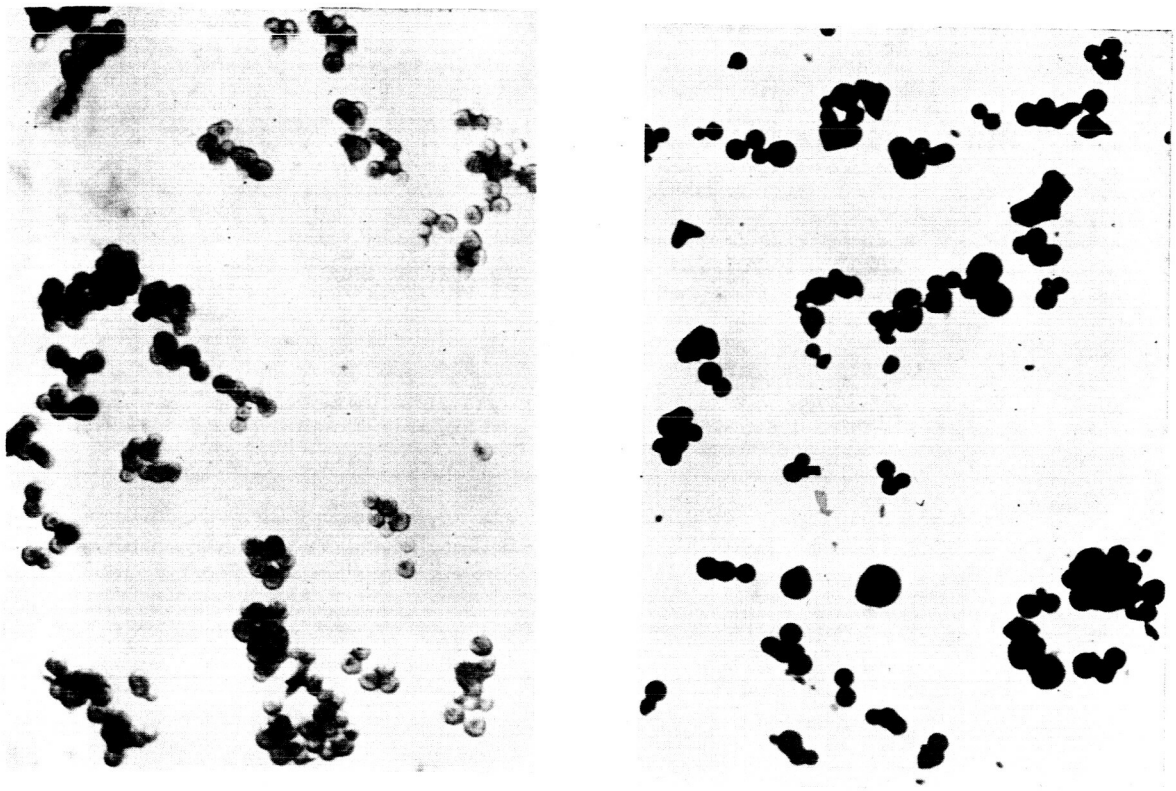


Fig. 3. Proteinoid microspheres stained with Gram stain. Microspheres on left are Gram-negative. Microspheres on right, containing sufficient basic lysine-rich polymer, are Gram-positive.

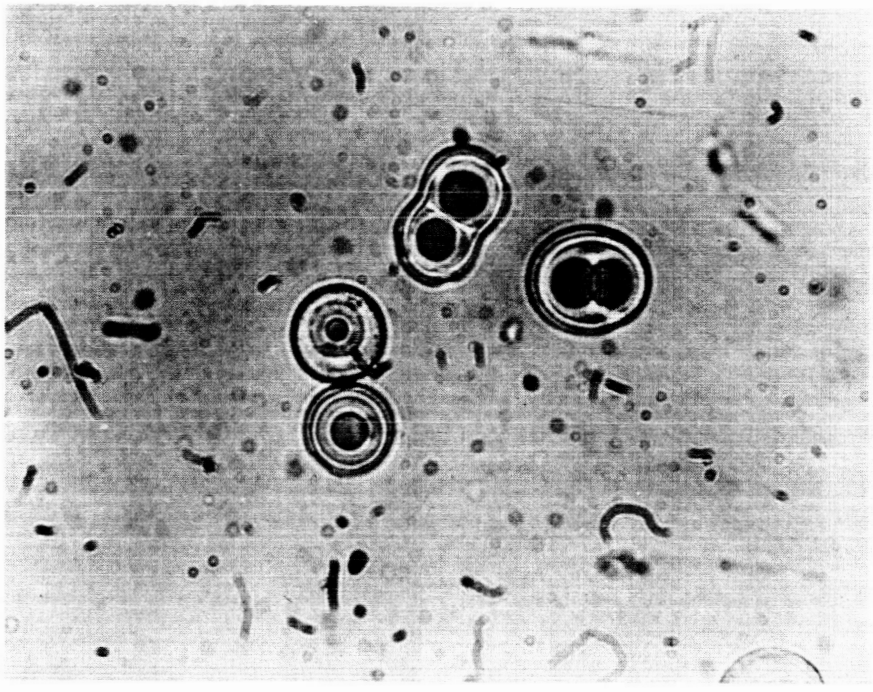
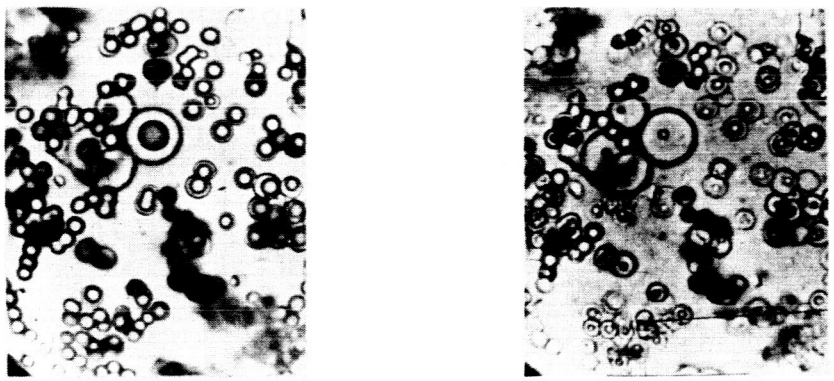


Fig. 4. Septate formation in proteinoid microspheres induced by raised pH.

That the effect of raised pH is one simulating fission rather than fusion is revealed by time-lapse studies. In Fig. 5 are two frames of such a series. These two frames are 74 minutes apart. This pair of frames shows also an effect of diffusion of polymer through the boundary. Analyses of the boundary material reveal only small differences in amino acid composition from the remaining polymer. This study and others indicate that the boundary has selective qualities.

The structure of such boundaries is revealed by electron microscopy. In Fig. 6 is seen such an electron micrograph. The boundary left after the diffusion is clearly a double layer structure.

The overall inferred mechanism derived from these and many other related demonstrations is that of how the large molecules of which life is composed could first have come into existence on the surface of the primitive Earth. Could abiotic protein have emerged by some other mechanism, using a less gentle type of energy? Despite many other attempts, no polyamino acids of demonstrated high molecular weight with a complete roster of proteinogenous amino acids have been obtained under geophysically plausible conditions. The question can therefore be asked. The answer may well reside in more information, on the effect of radiation, than we now have.



NO. 2
(1 1/2 MINUTES)

NO. 14
(75 1/2 MINUTES)

Fig. 5. Two frames from a time-lapse sequence showing that septate effect is the result of fusion rather than fission.

Fig. 6. Electron micrograph of section of osmium tetroxide-stained proteinoid microsphere.

The experiments also provide some sense of the variety of polymers of amino acids which could first yield a cellular type of organization regardless of which processes preceded them.

The question of feasibility can be analyzed. One component question is that of the effect of radiation on synthesis. The other is the effect of radiation on the stability of already synthesized polymers. Another set of questions relates to the effect of various kinds of radiation, thermal or nonthermal. Other superposed questions concern the effect of water, as on equilibrium. Another fundamental kind of question concerns the effect of the conditions studied in open and in closed systems.

The known effect of radiation on the amino acids in ovalbumin in aqueous solution is illustrated in Table 6. Other studies of the same sort on free and on combined amino acids give a similar pattern. Of relevance to the geological concepts involving thermal polymers is the finding by Proctor and Bhatia [40] that dry protein, in contrast to protein in solution, withstands well cathode radiation with no significant destruction of amino acids. This result indicates that primitive protein synthesized abiotically (and spontaneously) in dry, hot regions according to our laboratory conditions would be relatively resistant to decomposition by nonthermal radiation. Such

TABLE 6

Amino Acids Remaining After Radiation of Ovalbumin

Amino acid	Amino acids remaining[a] after radiation dose (rads) of:			
	10^5	10^6	10^7	5×10^7
Histidine	0.0	0.0	0.0	0.0
Cystine	67	0.0	0.0	0.0
Methionine	73	56	0.0	0.0
Phenylalanine	73	58	0.0	0.0
Threonine	81	63	0.0	0.0
Leucine	95	69	35	0.0
Isoleucine	89	72	38	0.0
Tyrosine	97	78	38	0.0
Serine	94	79	65.5	0.0
Arginine	87	75	68	0.0
Lysine	90	85	71	7.4
Glutamic acid	105	97	97	34
Aspartic acid	100	95	102	44
Glycine	81	73	87	46
Valine	95	84	76	16
Alanine	130	126	128	38

[a]Calculated as per cent of 0 rad.

From Shimasu and Tappel [41]

polymers could therefore survive for some time until rain, tides, etc. converted them to organized units under layers of water. The overlying layers would then, in some cases, be sufficiently deep to protect against radiation.

McLaren and Shugar [42] have expanded an analysis of Hull [10] who has raised queries about the lifetime of amino acids formed in the atmosphere and dissolved in the ocean. They calculate that 97% of the glycine formed in the upper atmosphere would be decomposed by ultraviolet radiation before it reached the sea by fallout. Hull claims that the concentration of glycine in equilibrium with methane, water, and ammonia would be uselessly minute. The quantitative results obtained by electric discharge are of no help in answering these criticisms because the use of a closed flask in the laboratory may well have favored the formation of glycine. Glycine is the dominant amino acid of the four which were produced by electrical discharge.

As McLaren and Shugar further emphasize, such considerations become more complicated when an attempt to apply them to polymers of amino acids is made. In general, the formation by ionizing radiation of high molecular weight polymers containing common amino acids is difficult to visualize because ionizing radiation is

likely to break carbon-carbon bonds in protein [38]. The breaking of carbon-carbon bonds can be helpful in yielding amino acids from gases such as methane, water, and ammonia. Once the amino acids are joined, however, and radiation then decomposes any amino acid residue in a polymer, the entire macromolecule may well suffer irreversible decomposition.

We may turn our attention to the state of understanding of the origin of prebiotic and nonenzymic polynucleotides. The state of this art is not as advanced as for polyamino acids. Both Schramm's laboratory [43] and ours [44] have demonstrated how small polynucleotides might have been formed in anhydrous phosphate reaction mixtures. While not relating their studies to the origin of life, Aguilera et al. [45] have shown that, with γ-radiation, uridylic acid can be induced to polymerize in aqueous solution to molecules of weight 5,000. They have also shown that the radiation is absorbed by the phosphate groups of the mononucleoside phosphate. This helps to explain how the process could circumvent the difficult problem of easy decomposition of the carbonaceous portions of the nucleotide by γ-radiation.

Such results suggest carrying out analogous experiments with amino acids, phosphates, and radiation in

aqueous solution. This type of approach does not, however, solve the problem of the need for imparting energy to the compounds. Instead it moves the problem elsewhere, as for example to the hypohydrous reaction of phosphate and nucleoside.

Abiotic polymerization may thus have occurred in a number of ways. The urgent problem now is how such polymerizations evolved to cellular types of polymerization. We can on the basis of experiments visualize, however, at least one sequence of abiotic preprotein yielding organized units in which natural experiments resulting in cellular polymerizations [30] could begin. In such experiments, microspheres containing zinc have been shown to catalyze the splitting of organic phosphates.

The film strip to be projected will demonstrate one way in which the sequence of processes yielding primitive abiotic protein and protocells could occur terrestrially. The open reaction system would be hot lava cooled by rain. Splashing of the sea onto such a reaction surface can also be visualized. The necessary amino acids can be understood as having arisen by open system vapor phase reaction [9], rather than by production in the atmosphere and precipitation into the ocean, as explained earlier.

An even more relevant geochemical matrix than hot lava beds and rain is that described by the Sicilian volcanologist, Rittmann [46]. Rittmann visualizes what happened when the temperature of the Earth's surface first fell below 374°C, the critical temperature of water, as follows, "An intense rain of hydrothermal solutions showered down upon the still hot crust of the earth, where they were first volatilized again, and then recondensed, so that a very vigorous circulation was initiated." In this kind of dynamic cycle one can visualize that amino acids could polymerize in hot dry regions, and the polymers would then organize into spherules when the water recondensed and cooled. The opportunity thus provided for reactions as described in the laboratory must have been almost limitless.

REFERENCES

1. S. W. Fox (ed.), "The Origins of Prebiological Systems and of their Molecular Matrices," Academic Press, New York, 1965.

2. A. I. Oparin, "The Origin of Life on the Earth," Academic Press, New York, 1957, p. 217.

3. G. Wald, Sc. Amer., 44-53, Aug. 1954.

4. E. L. Tatum in H. J. Vogel and V. L. Bryson (eds.), "Evolving Genes and Proteins," Academic Press, New York, 1965, p. 10.

5. H. Blum, "Time's Arrow and Evolution," Princeton Univ. Press, 2nd ed., 1955, p. 170.

6. S. W. Fox in P. Gavaudan (ed.), "Elementary Biological Systems and Abiogenesis," in press.

7. S. W. Fox in I. A. Breger (ed.), "Organic Geochemistry," Pergamon Press, 1963, pp. 36-50.

8. C. Palm and M. Calvin, J. Am. Chem. Soc., 84, 2115 (1961).

9. K. Harada and S. W. Fox, Nature, 210, 335 (1964).

10. D. E. Hull, Nature, 186, 693 (1960).

11. A. I. Oparin, ref. 2, p. 323.

12. I. Prigogine, "Thermodynamics of Irreversible Processes," 2nd edition, Interscience Publishers, New York, 1961, p. v.

13. S. Akabori, K. Okawa, and M. Sata, Bull. Chem. Soc. Japan, 29, 608 (1956).

14. C. Ponnamperuma and E. Peterson, Science, 147, 1573 (1965).

15. G. Steinman, R. M. Lemmon, and M. Calvin, Science, 147, 1574 (1965).

16. J. Kovacs and H. Nagy, Nature, 190, 531 (1961).

17. J. Oró and C. L. Guidry, Arch. Biochem. Biophys., 93, 166 (1961).

18. H. M. Huffman, J. Phys. Chem., 46, 885 (1942).

19. H. Borsook and H. M. Huffman in C. L. A. Schmidt (ed.), "Chemistry of the Amino Acids and Proteins," Charles C. Thomas, Springfield, Ill., 1944, pp. 822-870.

20. J. S. Fruton and S. Simmonds, "General Biochemistry," John Wiley and Sons, Inc., New York, 1958, p. 711.

21. S. W. Fox and K. Harada, J. Am. Chem. Soc., 82, 3745 (1960).

22. C. R. Windsor and S. W. Fox, unpublished analyses.

23. S. W. Fox, Nature, 205, 328 (1965).

24. D. L. Rohlfing and S. W. Fox, Arch. Biochem. Biophys., 00, 000 (1966).

25. V. R. Usdin, M. A. Mitz, and P. J. Killos, Abstr. 150th Mtg. Amer. Chem. Soc., 21 C (1965).

26. S. W. Fox and G. Krampitz, Nature, 203, 1362 (1964).

27. G. Krampitz and H. Hardebeck, Naturwiss., 53, 81 (1966).

28. D. H. Durant and S. W. Fox, Federation Proc., 25, 342 (1966).

29. D. L. Rohlfing, Federation Proc., 25, 524 (1966).

30. S. W. Fox in S. W. Fox (ed.), "The Origins of Prebiological Systems and of their Molecular Matrices," Academic Press, New York, 1965, p. 367.

31. S. W. Fox, K. Harada, K. R. Woods, and C. R. Windsor, Arch. Biochem. Biophys., 102, 439 (1963).

32. S. W. Fox and T. Nakashima, Abstracts 150th Mtg. Amer. Chem. Soc., 1965, p. 21 C.

33. S. W. Fox, K. Harada, and J. Kendrick, Science, 129, 1221 (1959).

34. S. W. Fox, K. Harada, and J. Kendrick in M. Sears (ed.), International Oceanographic Congress preprints, 1959, p. 80.

35. S. W. Fox and S. Yuyama, Comp. Biochem. Physiol., 11, 317 (1964).

36. S. W. Fox and S. Yuyama, Ann. N.Y. Acad. Sci., 108, 487 (1963).

37. S. W. Fox and S. Yuyama, J. Bacteriol., 85, 279 (1963).

38. S. W. Fox and S. Fukushima in V. L. Kretovich, T. E. Pavlovskaya, and G. A. Deborin (eds.), "Problems of Evolutionary and Industrial Biochemistry," U.S.S.R. Publishing House, Moscow, 1964, p. 93.

39. S. W. Fox, R. J. McCauley, D. Joseph, and S. Yuyama, COSPAR Abstr., 127 (1965).

40. B. E. Proctor and D. S. Bhatia, Food Technol., 4, 357 (1950).

41. F. Shimazu and A. L. Tappel, Radiation Res., 23, 203 (1964).

42. A. D. McLaren and D. Shugar, "Photochemistry of Proteins and Nucleic Acids," Macmillan Co., New York, 1964.

43. G. Schramm, H. Groetsch, and W. Pollmann, Angew Chem., 73, 619 (1961).

44. A. Schwartz and S. W. Fox, Biochim. Biophys. Acta, 87, 696 (1964).

45. A. Aguilera, E. Colombara, R. Jimenez, and J. Toha, Biochim. Biophys. Acta, 95, 569 (1965).

46. A. Rittmann, "Volcanoes and their Activity," Interscience, New York, 1962, p. 274.