The Terrestrial Origins of Macromolecules and of Cells

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Approximately 140 years ago, investigation in the laboratory disclosed that the organic compounds which are found in living systems do not require those systems for their production. In 1828 Wöhler demonstrated that a chemist could synthesize an organic compound, urea, from inorganic reactants. Since 1950, experiments have been performed and interpreted to indicate that the synthesis of organic compounds not only did not require a cell; they did not require a chemist either. All that was necessary was the appropriate reactants (Fox, 1957) and suitable geophysical conditions.

The realization that the problem of spontaneous generation is primarily a detailed problem in organic chemistry was the essential background for a true understanding of the origin of life. This point of view, the advancement of which we owe to Professor Oparin (1924; 1965) preceded the first chemical experiments conducted in this framework.

In the nineteenth century, however, experiments in the context of spontaneous generation were of a biological nature consistent with the techniques available at the time. Many historical treatments tell us that Louis Pasteur's classical experiments disproved the concept of spontaneous generation. Indeed, in describing his work in 1864, Pasteur stated that "Never will the doctrine of spontaneous generation recover from the blow of this simple experiment" (Vallery-Radot, 1920). Even so, evidence can be found for mental reservations by Pasteur about this conclusion and in 1878 he wrote (Nicolle, 1961) "Spontaneous generation? I have been looking for it for 20 years, but I have not yet found it, although I do not think that is an impossibility".

Moreover, Pasteur displayed some awareness of what may well prove to be the crucial concept in abiogenesis (Wald, 1954) when he said (Descour, 1922):

"If as a result of my experimental work, I happened to demonstrate that matter can become organized of itself into a cell or living being, I should announce it in these precincts - - -" (underline added).

The difference between an experiment conducted by a chemist and one set up to carry itself out or to yield self-organizing material is perhaps more subtle than real. The subtle experiments which, to my knowledge, were first performed and interpreted in the primordial geochemical context, were those of Calvin and coworkers (Garrison et al., 1951). In these experiments the inorganic compounds, carbon dioxide and water, were converted to the organic compounds, formaldehyde and formic acid. Subsequently Miller (1953), under Urey's sponsorship and stimulus (Miller and Urey, 1959), reported the production of four proteinogenous amino acids by electric

discharge in an Oparin-Urey "reducing" atmosphere (Oparin, 1938; Urey, 1952). Oró has explained the origin of adenine, other nitrogen bases, and deoxyribose (Oró, 1965). Ponnamperuma and coworkers have demonstrated other routes to nitrogen bases and to ATP (Ponnamperuma, 1965). Harada and Fox (1964; 1965) have shown how most of the amino acids common to protein, and no others, are producible under terrestrial conditions in a volcanic zone.

The studies to which our laboratory has devoted major attention are those which can explain the origin of biopolymers in the absence of cells and in the absence of the chemist, and the spontaneous conversion of the polyamino acid type of such polymers to the first cells. These experiments began as a somewhat typical chemical investigation, but they have turned increasingly to studies of polymer morphology, or to use a more biological term, to studies of cell models. A number of more purely chemical questions have as a result so far remained unanswered. Other workers, however, including Professor Gerard Biserte of Lille, are providing for some of these questions illuminative answers (Biserte et Finot, 1963; Finot et al., 1963; Germain et al., 1963).

Many methods of production of amino acids, nitrogen bases, and other small molecules under terrestrial conditions have been demonstrated in the laboratory. Several modes of production of peptide bonds or of homopoly-α-amino acids have also been modelled. So far, however, only one terrestrial method yielding material closely resembling protein in size and variety of anhydroamino acids has appeared. The possibilities for this kind of spontaneous generation may be, basically, quite limited.

The essential conditions for thermal polycondensation of some proportion of all of the twenty amino acids common to protein is a temperature of 170° acting, for several hours, on a mixture of α-amino acids containing a sufficient proportion of aspartic acid and glutamic acid or a sufficient proportion of lysine (Fox and Harada, 1960; Fox et al., 1962).

Most of the thermal condansations on which our knowledge is based have been carried out in the usual way in glassware. The condensation polymerization can occur, however, on lava (Fox, 1964) and in the presence of siliceous sand when the mass of sand is ten times the weight of the reactant amino acids (Gordon et al., 1965).

Analyses (Fox, 1965a) of lapilli from 160° zones of the cinder cone at Kilaeua-Iki and of pumice collected during eruptions on Hawaii reveal many amino acids. These amino acids are found to be largely in the polymerized form. While contamination by bacteria or by bacterial remnants cannot be ruled out, these samples have been examined by aseptically crushing the rock after the first extraction with hot water. The crushed

material yielded a qualitatively similar amino acid profile following hydrolysis.

One of the principal questions about the nature of the thermal copoly-α-amino acids has been a determination of the degree of heterogeneity of the polymers composed chemically from eighteen amino acids. One question of evolutionary significance is that of whether such a model of primordial protein would be wildly disordered or not.

Table I describes the variation in content of individual amino acids in thermal proteinoids (heteropoly-α-amino acids). Information of this sort is necessary to judge the degree of variation to be found in compositional data of various fractions. This table demonstrates that each amino acid tends to be incorporated in the condensation polymer to an extent greater or less than its proportion in the reaction mixture. These results are in repetitions highly reproducible for one kind of polymer, such as the 2:2:1- [a] or the 2:2:3- [a], but they vary with the proportions in the reaction mixture. The amino acids which are most easily condensed in one kind of polymer (e.g. 2:2:1-proteinoid) tend also to be most easily condensed in other kinds of polymer (e.g. 2:2:3-proteinoid).

Indications of a low degree of heterogeneity in the proteinoids first appeared from electrophoretic studies carried out by Dr. Carl Vestling (Fox and Harada, 1960). Vegotsky has found a low degree of heterogeneity of proteinoid in the ultracentrifuge (Vegotsky, 1961). More recently, repurification of the polymer from water has been found to yield almost the same analysis in the crude, the once purified, and the twice purified polymer (Table II; Fox et al., 1963). The simple method of purification employed allows recoveries of approximately 50%. Under these conditions fractionation of significantly varied polymer molecules would be considerable, but little or none is found.

Most recently, Dr. Tadayoshi Nakashima in our laboratory has verified this analysis in a different way (Fox and Nakashima, 1965). He has fractionated amidated proteinoid on DEAE-cellulose columns. Although sufficiently varied in charge to permit fractionation, the individual fractions are quite similar in amino acid composition. Following partial hydrolysis, the "fingerprint" peptide patterns of individual fractions are also similar. These results indicate that the total polymer is

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[a] 2:2:1-indicates that the polymer was produced from 2 parts of aspartic acid, 2 parts of glutamic acid, and 1 part of the 16 other amino acids in equimolar proportions. 2:2:3-, etc. is analogous.

Table I

Proportion of α-Amino Acids in Reaction Mixtures and in Thermal Polyanhydro-α-Amino Acids

	2:2:1		2:2:3	
	Reaction Mixture	Reaction Product	Reaction Mixture	Reaction Product
Aspartic acid	42.0 %	66.0 %	30.0 %	51.1 %
Glutamic acid	38.0	15.8	27.0	12.0
Alanine	1.25	2.36	2.72	5.46
Lysine	1.25	1.64	2.72	5.38
Half-cystine	1.25	1.32	2.72	3.37
Glycine	1.25	1.32	2.72	2.79
Arginine	1.25	0.94	2.72	2.44
Histidine	1.25	0.95	2.72	2.03
Methionine	1.25	0.94	2.72	1.73
Tyrosine	1.25	0.94	2.72	1.66
Phenylalanine	1.25	1.84	2.72	1.48
Valine	1.25	0.85	2.72	1.16
Leucine	1.25	0.88	2.72	1.06
Isoleucine	1.25	0.86	2.72	0.90
Proline	1.25	0.28	2.72	0.59
Serine	1.25	0.6		
Threonine	1.25	0.1		

Table II

Amino Acid Contents of Reaction Mixture

and 2:2:3-Proteinoids in Three Stages of Purity

	Proportion in Reaction Mixture	Crude	Purified	Repurified
Aspartic acid	30.0%	52 %	50 %	51 %
Glutamic acid	27.0	11	12	12
Alanine	2.7	4.0	4.3	5.5
Lysine	2.7	5.1	5.4	5.4
Half-cystine	2.7	4.5	3.5	3.4
Glycine	2.7	2.7	3.1	2.8
Arginine	2.7	2.0	2.3	2.4
Histidine	2.7	1.8	2.0	2.0
Methionine	2.7	1.8	1.9	1.7
Tyrosine	2.7	2.0	1.9	1.7
Phenylalanine	2.7	1.8	1.7	1.5
Valine	2.7	1.2	1.2	1.2
Leucine	2.7	1.3	1.2	1.1
Isoleucine	2.7	1.2	1.3	0.9
Proline	2.7	0.7	0.6	0.6

highly uniform in composition and probably also in total sequence of amino acid residues.

These and other studies on terminal residue compositions (Fox and Harada, 1960) lead to the conclusion that the proteinoids are far from "wild"; they are, in fact, highly ordered. We can thus extend the inferences on the assumption that thermal proteinoid is a valid model of primitive protein. Protein, viewed in the entirety of its sequences of residues throughout phylogeny, has been judged by Gamow et al. (1956), Williams et al. (1961), Sorm and Keil (1962), and Vegotsky and Fox (1962) to be almost random in its nature. In contrast, the primitive type of protein modelled by the thermal polymers is nonrandom, or ordered. The evolution of protein is thus one, during organismic evolution, of randomization tempered by selection, as has been suggested for organisms at the biological level (Ross, 1962). The problem of life as a "pocket of entropy" is, in this view, a phantom problem.

Other protein-like properties of the proteinoids are indicated in Table III. We will consider especially catalytic activities, which are most relevant to the properties which we believe the first living material would need to possess.

The activity of proteinoids in enhancing the rate of splitting of p-nitrophenyl acetate has been described (Fox et al., 1962; Rohlfing, 1964; Usdin et al., 1965). This activity requires histidine in the polymer, and the activity is enhanced by imide linkages from aspartoyl residues (Rohlfing, 1964). The activity is inhibited by the organic phosphates which function also as choline esterase inhibitors (Usdin et al., 1965).

The zinc salt of proteinoid has been shown to possess, in particulate form, the ability to split ATP (Fox, 1965b).

Proteinoid without combination with metal ion has been demonstrated, moreover, to catalyze conversion of glucose to carbon dioxide through glucuronic acid (Fox and Krampitz, 1964). Such activity is weak, orders of magnitude weaker than that of an enzyme, but the activity is absent from the amino acids used for the polymer, and absent from the hydrolyzate of the proteinoid. These weak activities have been demonstrated in rigorously aseptic preparations. The first product observed was radiocarbon dioxide when radioglucose was used as substrate. This observation was made first with uniformly labelled glucose. When glucose labelled in the 1- position or 2- position was tested, no radiocarbon dioxide was obtained. When 6-labelled glucose was tested, active carbon dioxide was obtained (Fig. 1). These results suggested that the 6-position of glucose was changed to a carboxyl group by the proteinoid. Glucuronic acid was isolated also as the p-bromophenylhydrazone. Radioglucuronic acid was found also to be decarboxylated by proteinoid.

Table III

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

(Fox, 1965c)

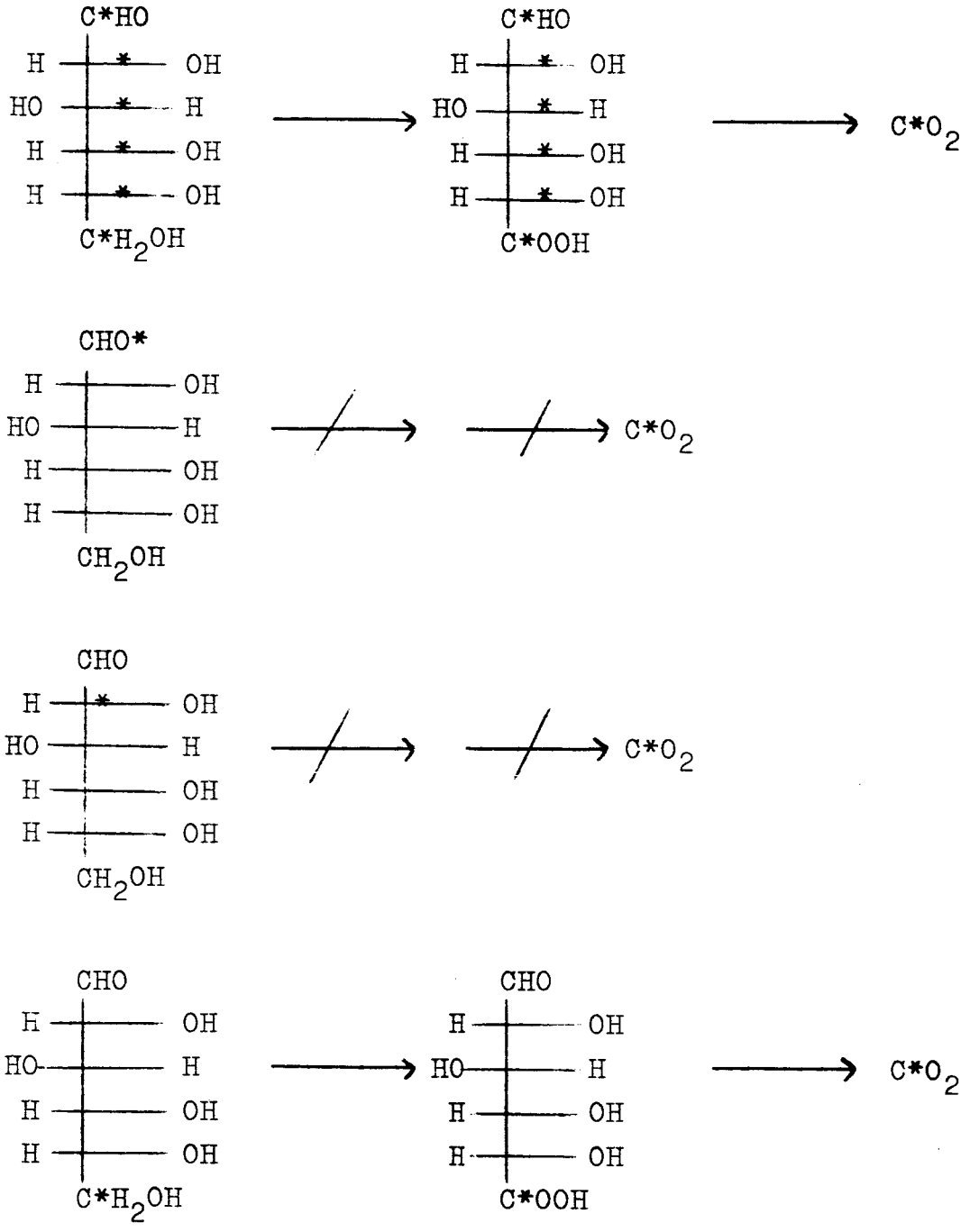


Figure 1. Conversion of radioglucose to radiocarbon dioxide.

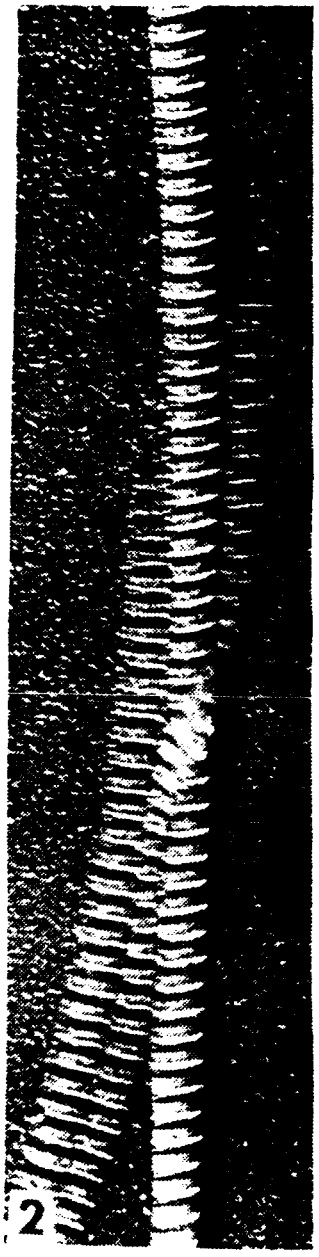
The possibility of catalysis of reactions of other natural substrates by thermal proteinoid is being investigated. While primitive preorganismic protein need not have had all of the enzymic activities which are found in contemporary organisms, one should expect a moderate number of such activities. Proteinoid, like protein, has the variety of intramacromolecular structure which conceptually could serve as a structural basis for a spectrum of somewhat specific activities.

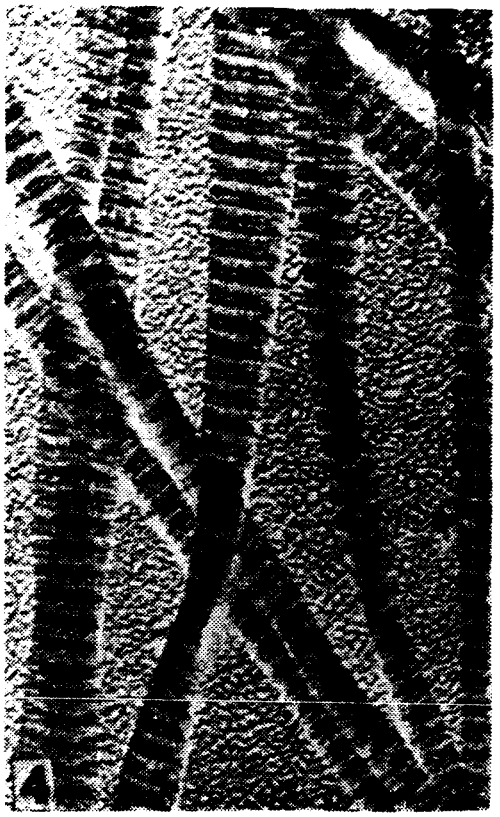
The thermal model has provided us with one experimentally based answer to the fundamental chicken-egg question of which came first — cell or protein? Contemporary cells carry out the synthesis of protein and are in turn made of protein in particular. But cells are larger units, on the molecular scale, than are units of protein. Among modern scientists, Wald (1954) originally suggested the answer to this dilemma (Blum, 1951) when he proposed that molecules might assemble themselves into cells. Wald made this proposal at a time when molecules of organismic protein, such as collagen, had recently been shown to have self-organizing properties (Fig. 2). Since those first observations (Schmitt, 1956) many other examples have come into view (e.g. Anfinsen, 1963; Fernandez-Moran et al., 1964).

The overview of self-organizing properties suggests that even in the contemporary cell, the genic control of morphology may be indirect, through the coding mechanism, the self-organizing properties of biomacromolecules being the direct determinants of morphology (Fox and Yuyama, 1964).

The thermal poly-α-amino acids have been found to have self-organizing properties which are probably unique and many of which we will review here. The key features of the interpretations which follow are: a) the fact that any polymer prepared under as presumably brutal conditions as heating of α-amino acids could have so many static and dynamic properties of the cell and b) the fact that this polymer arises not from organisms, but under acellular conditions that could (we believe, must) have occurred in innumerable locales on innumerable occasions on the Earth during its long history (Fox and Yuyama, 1963a).

The self-organization of such structures is surpassingly simple. It results from contact of preparations of almost any thermal polyanhydro-α-amino acid with water or with aqueous solution. This process occurs most easily with heated aqueous solution, after the hot, clear solution cools (Fig. 3). The simplicity of these conditions is even more pronounced than that of the condensation polymerization. This simplicity also falls easily into the range of geological phenomena. The requirements for formation of polymer and microscopic units are no more special than a condition of rain falling on volcanic zones (Fox, 1964).





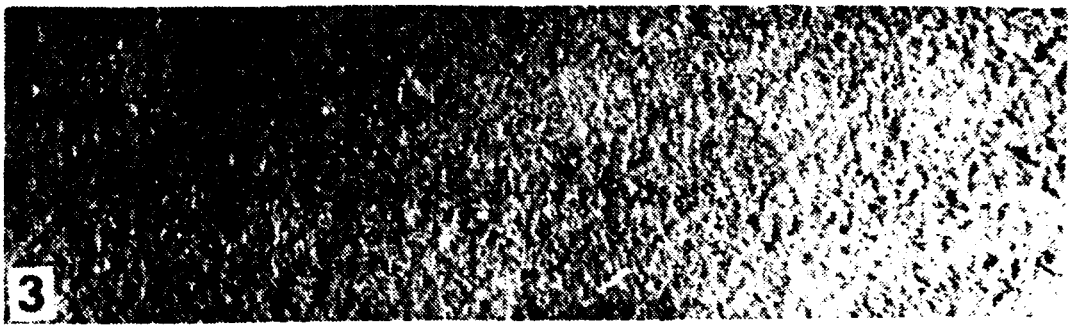


Figure 2. Self-organization of collagen into fibrils. 3) clear filtrate, X 35,000, 4) and 5) fibrils after dialysis (Schmitt, 1956).

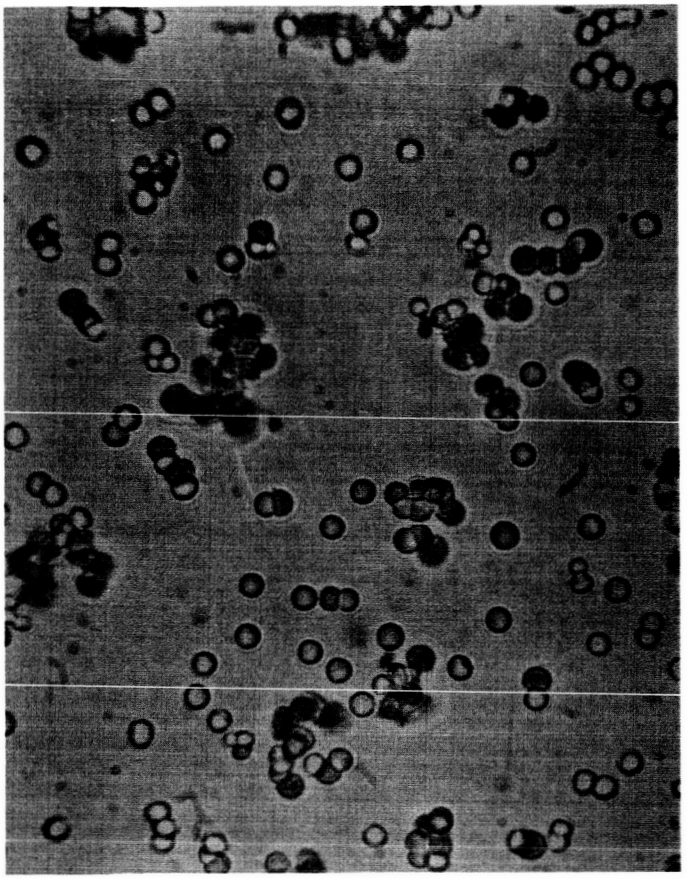


Figure 3. Proteinoid microspheres. Some "buds" are visible.

The microscopic spheres which are formed are found to be in the shape, range of size, and mode of association of the coccoid bacteria (Fig. 3). These units have a degree of stability which overlaps the degree of stability of biological cells. They can be similarly centrifuged, washed with salt solutions, or sectioned. The size, which depends upon the conditions of preparation, varies between 0.5 μ or less in diameter, to 80 μ. Under many conditions of preparation, the units obtained are highly uniform in size. This uniformity has permitted quantitative experiments, which showed that the microspheres would shrink when transferred to solutions hypertonic to those in which they were prepared, and that they would swell when transferred to hypotonic solutions (Fox et al., 1959).

The microparticles are however not solely spherical. Some tend to be rod-like (Fig. 4) or filamentous; "buds" are also seen (Fig. 3). Aggregation of units without coalescence can be readily induced (Fig. 5; Young, 1965).

Another aspect of the proteinoid microspheres is the very large number which result. One gram typically yields 10 billion units. When one sees these vast numbers, their general uniformity, and their superposed variety he can more easily visualize these as models of natural experiments on an evolutionary highway.

The constitution of these microspheres is sufficiently understood that experiments in which they serve as models of bacteria, on a compositional basis, can be performed. In 1924, Stearn and Stearn attributed the Gram stain of bacteria to the protein content of those microbes. Since the microspheres are composed of proteinoid which resembles protein, the question of whether microspheres would accept the Gram stain arose. Performance of the Gram stain on proteinoid microspheres showed that these accepted one of the dyes (Fox and Yuyama, 1963b), and thereby stained Gram-negative. In a closer examination stimulated by the controversial literature on the constitutional difference between Gram-negative and Gram-positive cells, microspheres having a sufficient proportion of the basic amino acid, lysine, in their makeup were shown to be Gram-positive.

A closer examination of the structure of these units was possible because of the fact that they could be sectioned for electron microscopy. The first electron micrographs were made with osmium tetroxide in the same way as has been done for bacteria. An electron micrograph of such a section of a proteinoid microsphere (Fox and Fukushima, 1964) is shown in Fig. 6. A section of Bacillus cereus may be found in the treatise of Gunsalus and Stanier (Murray, 1960). Some bacterial sections in high resolution electron micrographs display more structure than does the electron micrograph of the section of microsphere in Fig. 6. However, the similarity in appearance

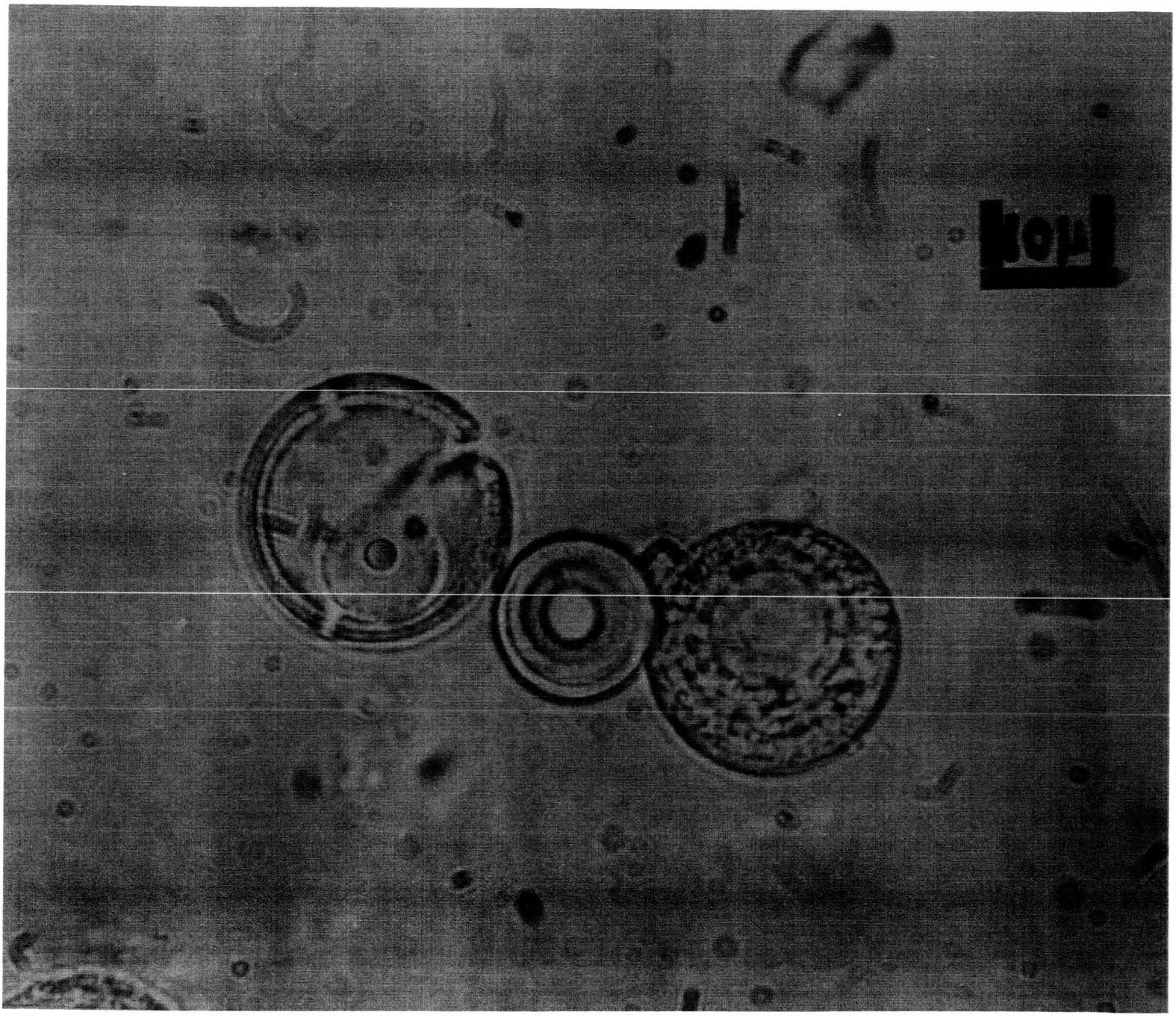


Figure 4. Proteinoid microparticles. Some filaments are visible.

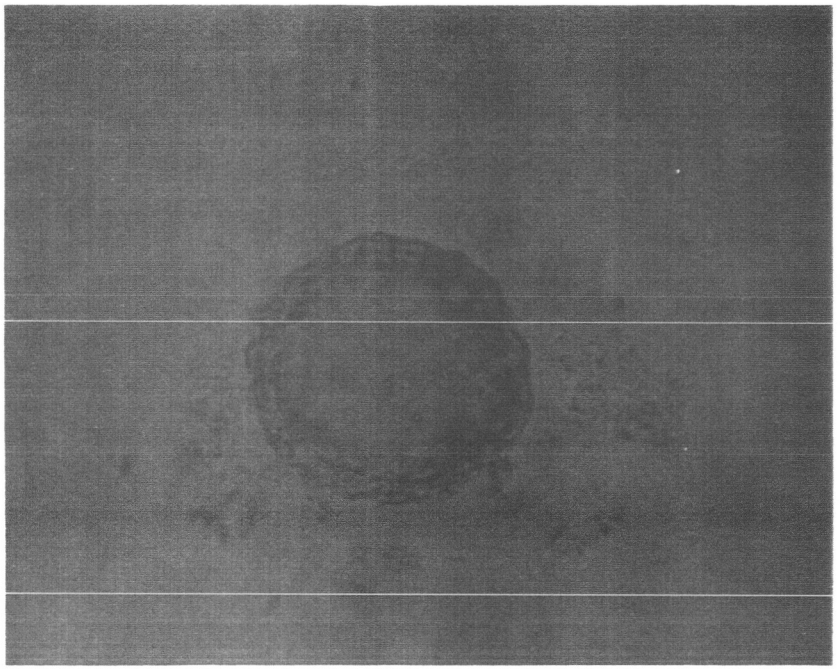


Figure 5. Aggregated proteinoid microspheres, without coalescence (Young, 1965).

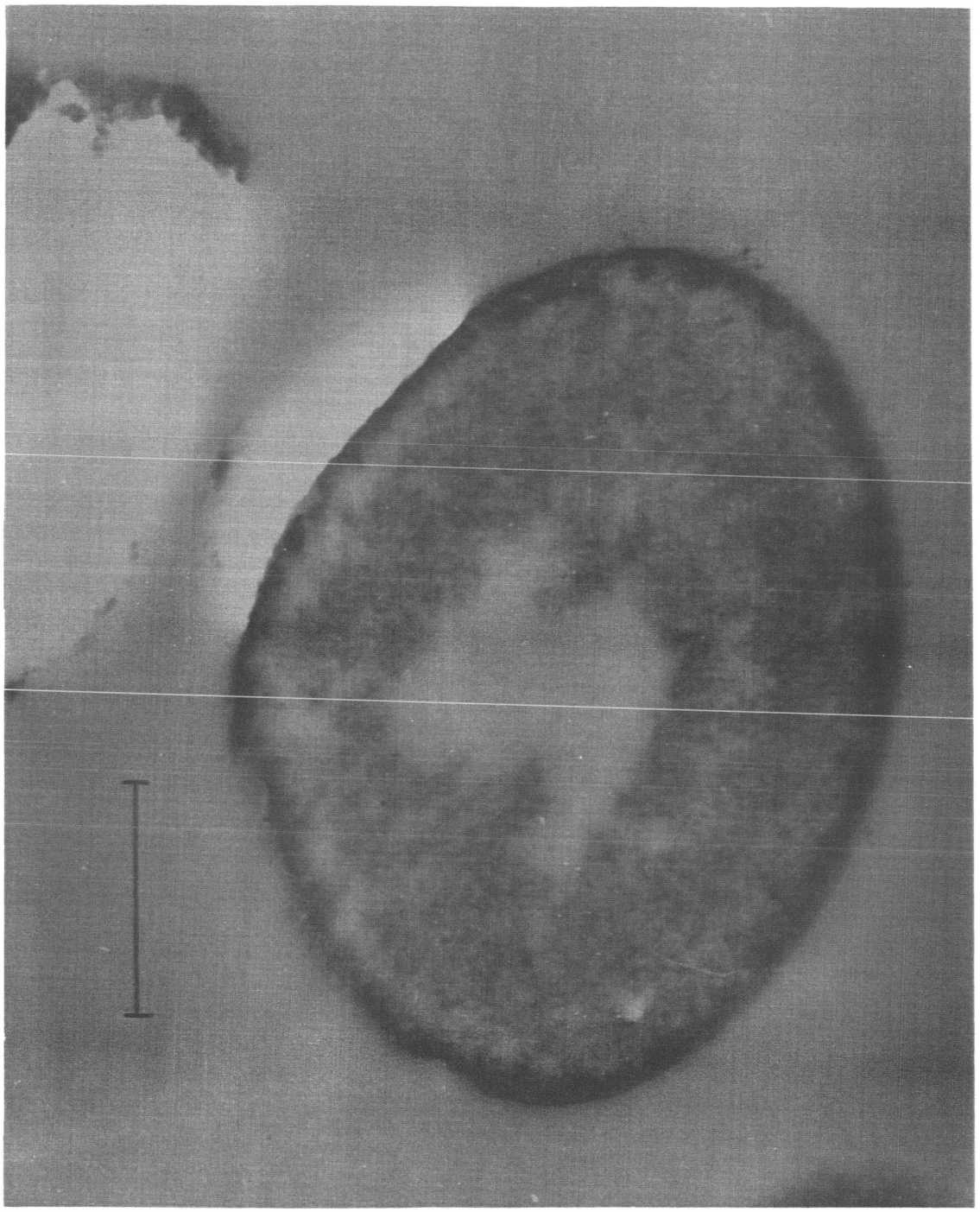


Figure 6. Electron micrograph of section of osmium tetroxide-stained proteinoid microsphere.

of boundary and granular substance to that in the simplest bacteria is "really striking" (Oparin, 1965). One interpretation of these results is that self-organizing properties of a primitive type of protein-like polymer can be expected to yield units resembling in appearance the most primitive cells known, but not to yield those which have developed specialization through biological evolution.

When the pH of a suspension of microspheres is raised 1-3 units, a double layer becomes visible in electron micrographs (Fig. 7). This is, rather than an expression of self-organizing properties, an expression of self-reorganizing properties. In either mode, the propensity of a particular kind of synthetic polymer to assume a complex structural organization is clear.

In Fig. 8, selective diffusion is illustrated. The large microsphere near the center of the photograph shows that the polymer in the interior diffuses out through the boundary when the pH is raised. The boundary itself is composed of polymer similar to that which diffuses out (Fox et al., 1965) but this polymer is retained. Such results are confirmed by the electron micrographs and also by ultraviolet time-lapse cinemicrography. They agree also with the observation that microspheres tend to shrink or swell in hypertonic or hypotonic solutions respectively (Fox et al., 1959).

A more active kind of dynamic behavior is found in a considerable tendency of the individual microspheres to divide into two. In Fig. 8, several microspheres can be seen to be undergoing septate division. In other sequences, the daughter halves can in some cases be seen to separate entirely. Some diminution in size accompanies such separations.

Growth in size, however, has been separately observed. One kind of growth in size which has been captured on film is that involving growth of "buds". One of these is shown in the next sequence (Fig. 9; Fox et al., 1965). These buds can be separated from the larger spherule, and they are being studied separately.

The material within the microsphere undergoes streaming (Fig. 10; Fox et al., 1965). When it contains zinc, is asymmetric, and ATP is added to the suspension, clearly nonrandom movement of the asymmetric particle results (Fig. 11; Fox et al., 1965). The spherical or nearly spherical particles have been observed only to exhibit what appears to be Brownian motion.

This last effect is observed with particles which have been shown to accelerate the splitting of ATP (Fox, 1965b). One explanation for the propulsive power, in fact, is the splitting of ATP.

This incorporation of metal ion illustrates one way in which a primitive metabolic activity might appear in a primordial

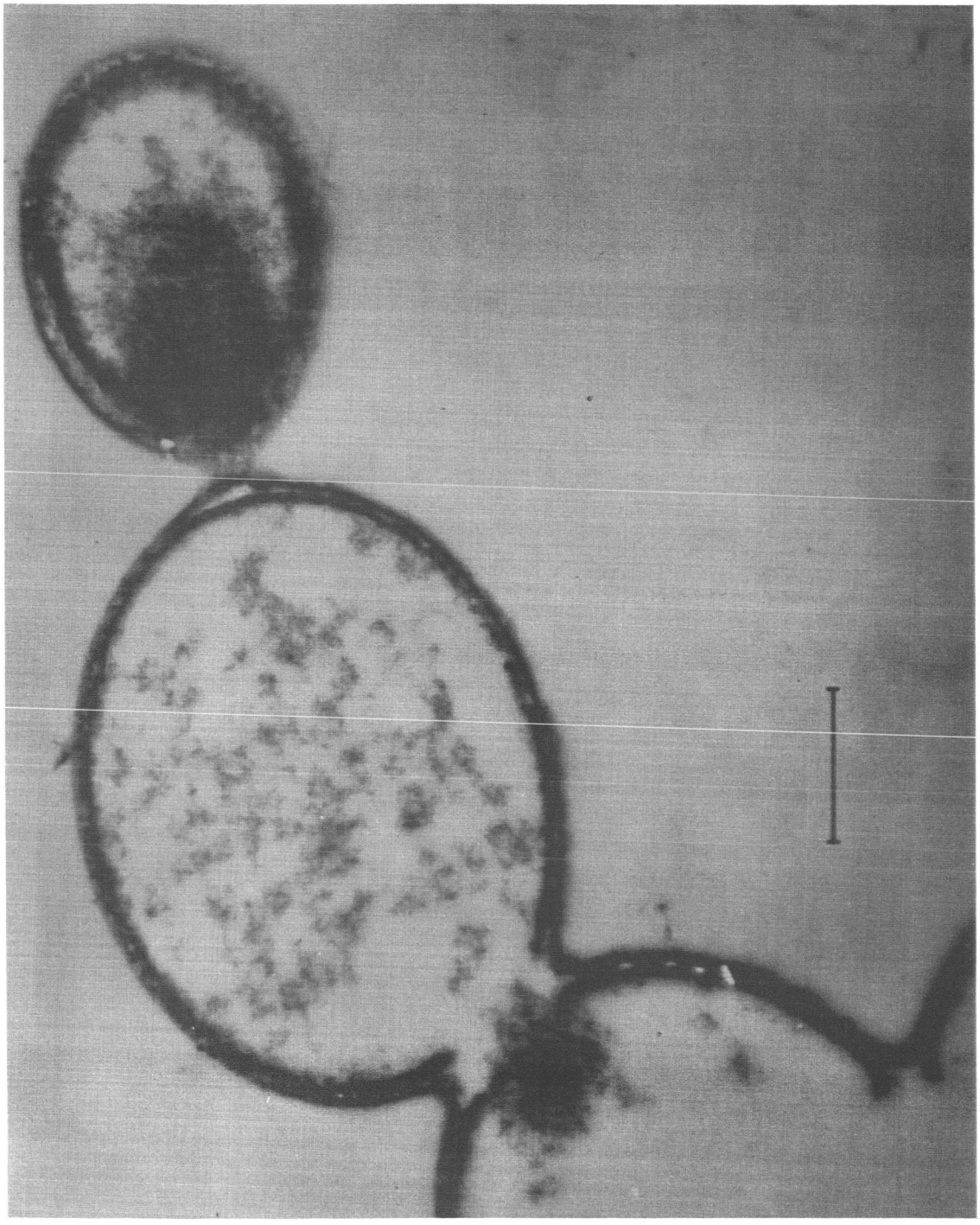


Figure 7. Double layer in osmium tetroxide-stained proteinoid microsphere subjected to increase in pH.

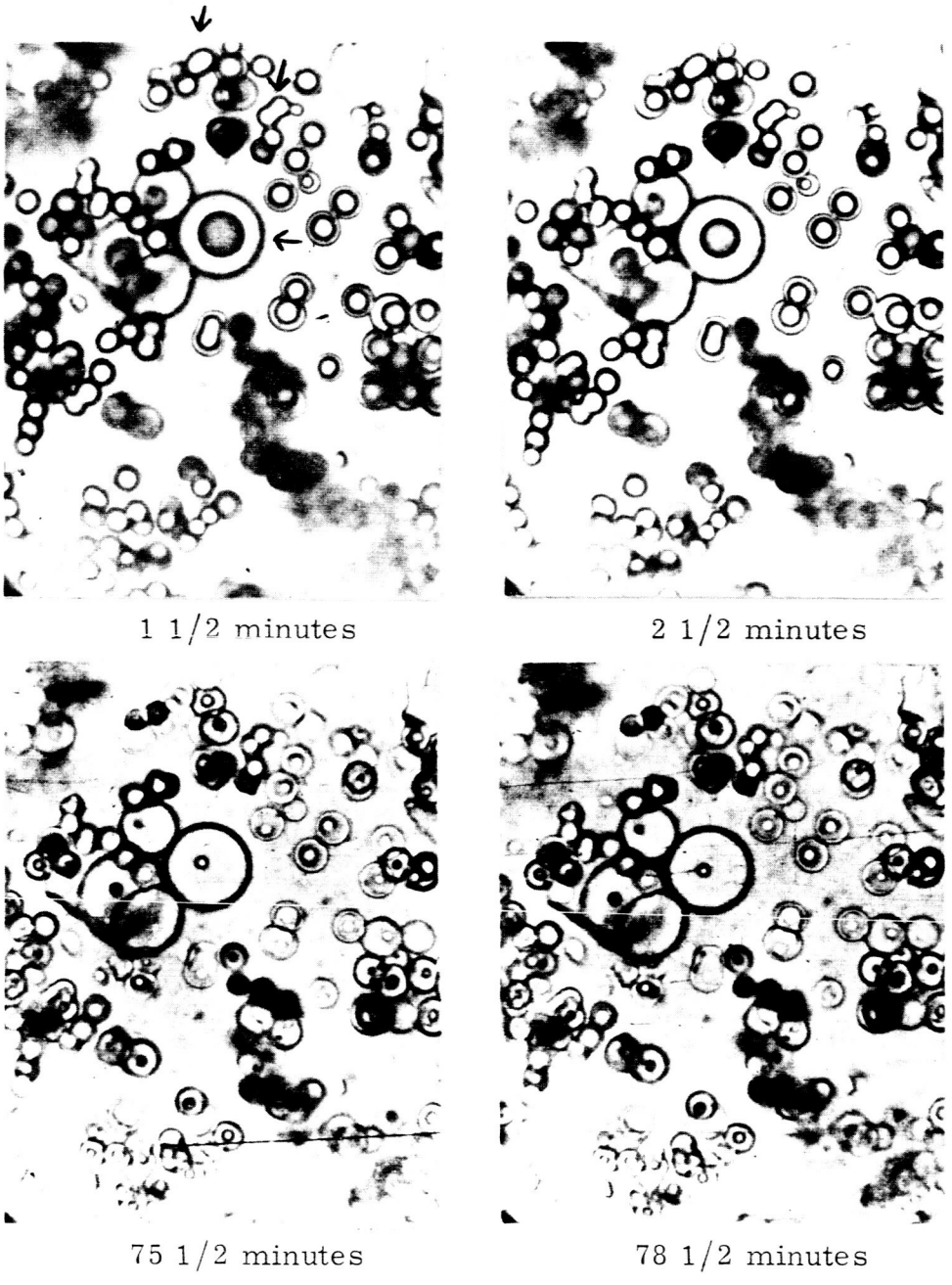
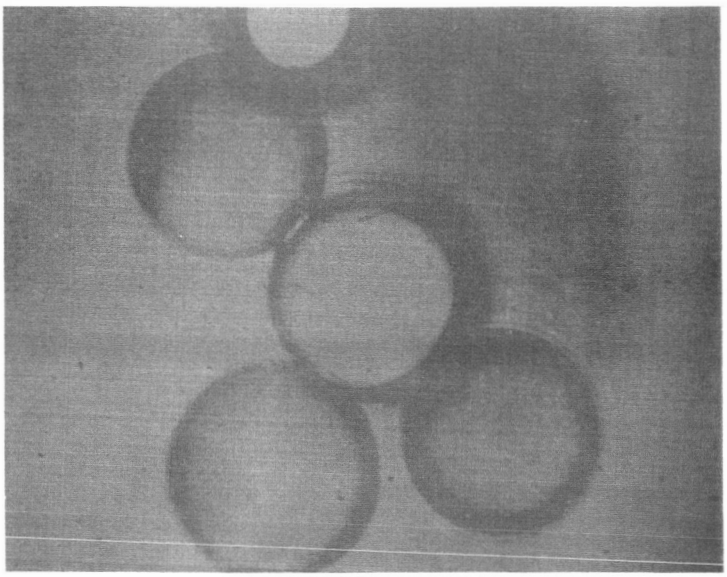
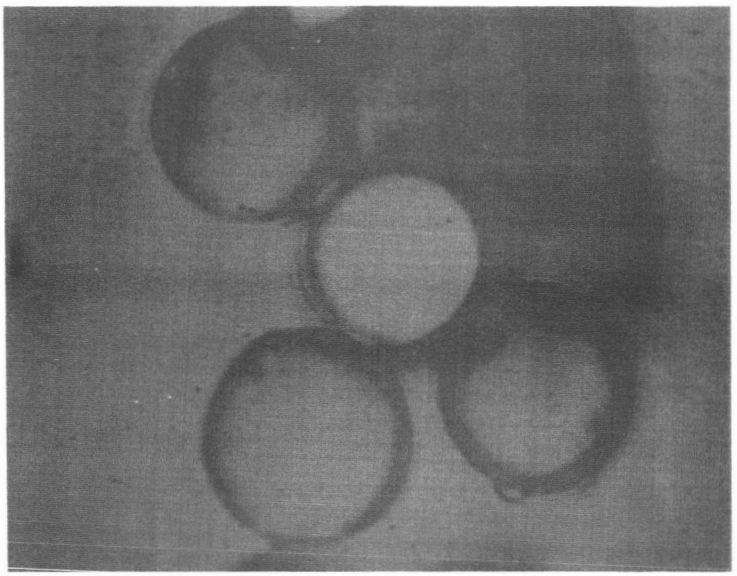


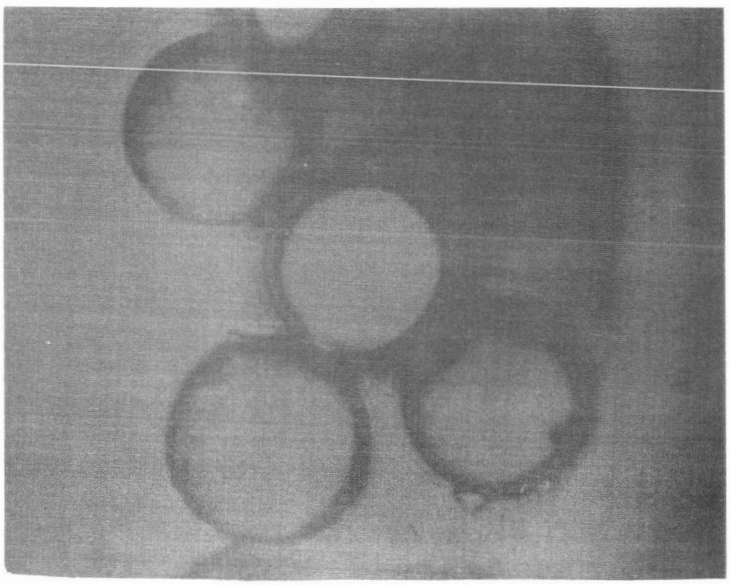
Figure 8. Time-lapse sequence showing septate division, and selective diffusion through the boundary, of proteinoid microspheres by increase in pH.



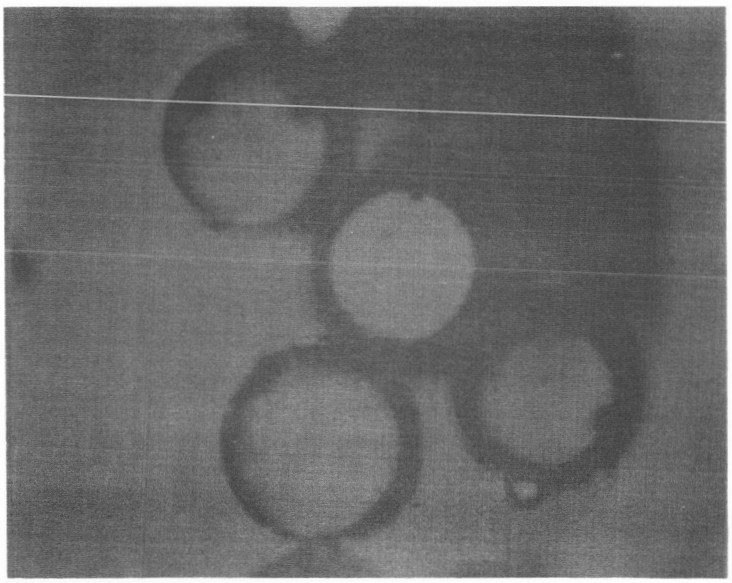
2 HOURS



25 HOURS

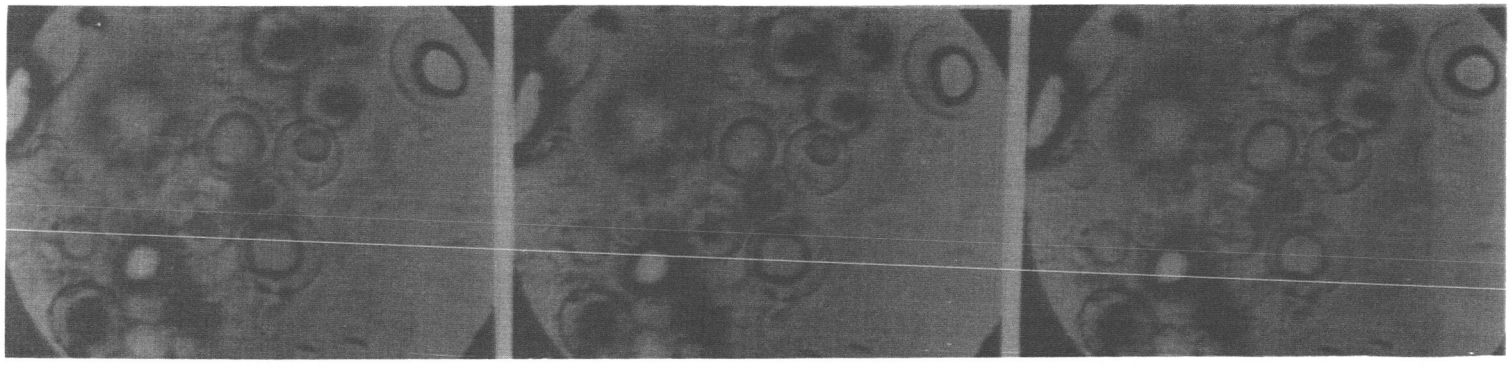


42 HOURS

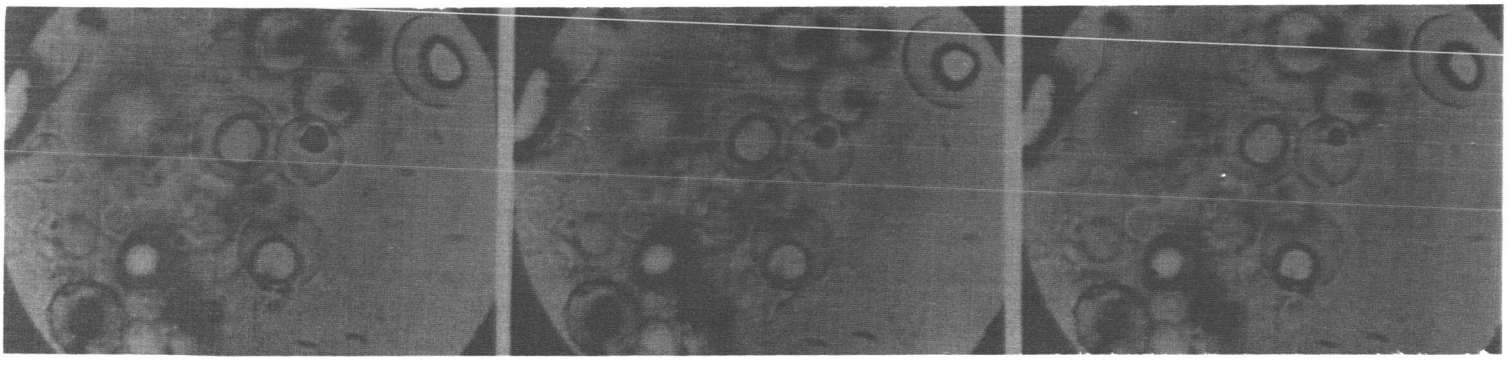


68 HOURS

Figure 9. Growth in size of buds on microspheres.



START 2 SECONDS 4 SECONDS



6 SECONDS 8 SECONDS 10 SECONDS

Figure 10. Rotation of particle within proteinoid microsphere, explainable as a kind of internal streaming (Fox et al., 1965).

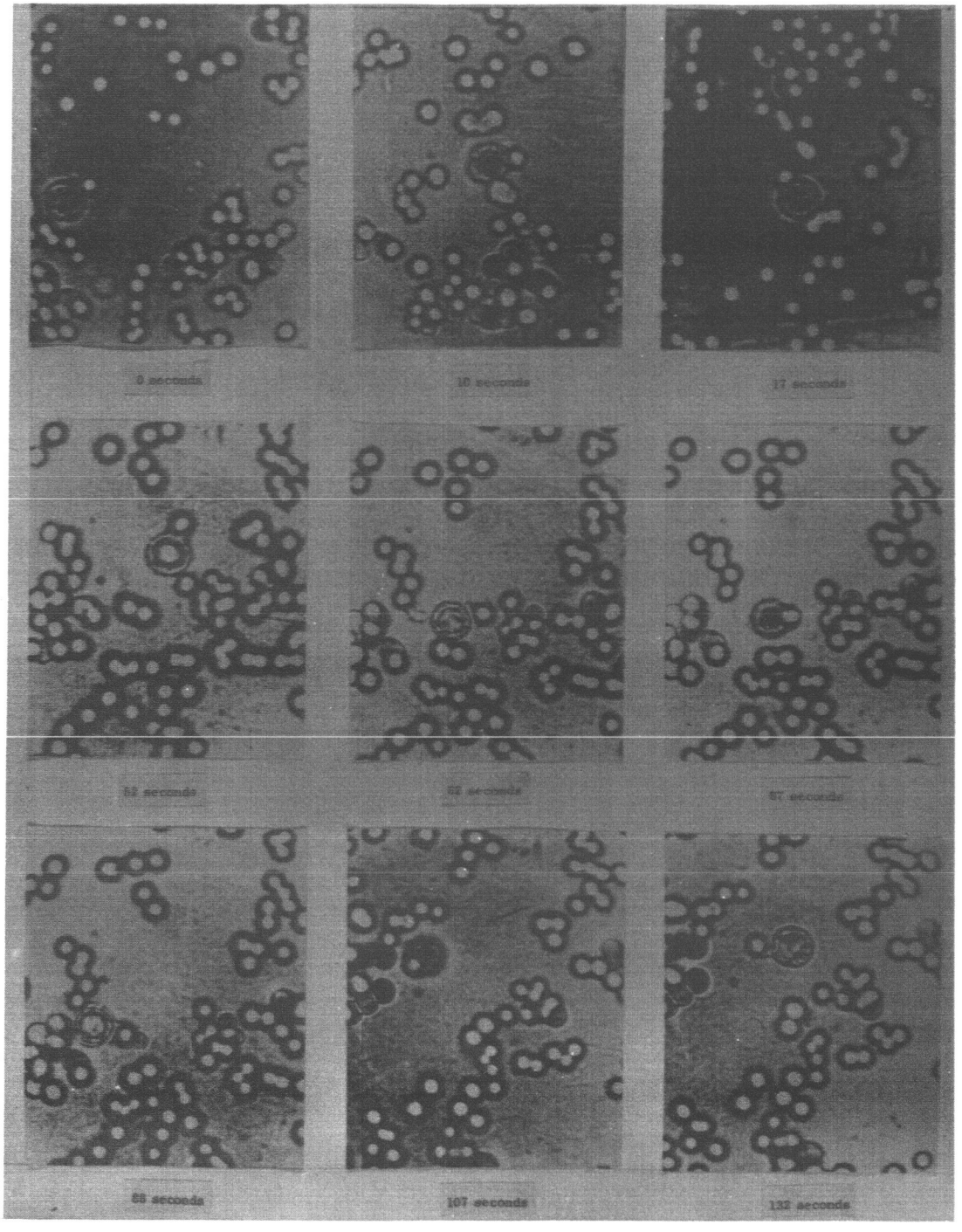


Figure 11. Nonrandom movement of an asymmetric zinc-containing proteinoid microparticle in a suspension to which ATP has been added.

cell. The polymer also carries into the microspheres which form therefrom the ability to convert glucose to glucuronic acid and to decarboxylate the latter (Fox and Krampitz, 1964). According to this model, primitive catalytic activities need not have found their way into primordial cells; they were present as preformed attributes of the polymers which yielded the primordial cells.

The ability of such units to transfer phosphate from ATP opens conceptual answers to some of the fundamental questions of primordial priority of macromolecules. We have seen already how the most primitive protein may have preceded the most primitive cell. This kind of primitive protein could have arisen in an acellular mode. Sooner or later, however, such synthesis had to be superseded by a cellular ATP-transfer mechanism (Meister, 1965). The experiments with the zincous particles suggest in a general way how a first step in such a direction might have occurred.

The other metabolic activities which are found in the microsphere, and are attributable to the material of which they are made, gives us an explanation of the origin of metabolism. The large number of reactions and intermediates which characterize contemporary cells has raised the question of their origin. One speculation on this problem was that of the mutational development of synthetic pathways in initial heterotrophs living in the multitudinous nutrients of a "primordial soup" (Horowitz, 1945). The experimental thermal model, however, suggests attention to intrinsic chemical abilities of primitive protein and of primordial prosthetic groups, which obviate the need for an initial storehouse of all the organic compounds found in organisms. Metabolism, according to the experimental model, could have begun with organisms which possessed an intrinsic set of metabolic pathways, perhaps minimal in number compared to those of contemporary metabolic networks. A microglobule of proteinoid which, like polyfunctional protein, had much versatility in chemical reaction plus the ability to form membranes, is consistent with emphases, from evolutionary theory, on the individual rather than on its environment. A unit self-organized from poly-α-amino acid would thus be incompatible with the concept of an initial total heterotroph; the unit would necessarily have some metabolic activities. A proliferation of organic intermediates would then result from the exercise of those activities.

To return to consideration of the first synthesis of protein having minimal abilities, our theory needs not only a primeval mechanism but some understanding of how synthesis of protein could be highly reproducible in kind of polymer produced. The thermal model provides several suggestions.

One such suggestion, which became clearest in recent studies, is the possibility that specific sequence would be determined entirely by the reactant amino acids. The thermal proteinoid, for example, has sharply limited heterogeneity, as

indicated earlier in this paper. The possibility that early organisms, without templates, might produce proteins has been suggested more or less directly by Lederberg (1961), Thimann (1963), Lipmann (1965), and Tatum (1965). This inference is based partly on demonstration of the biosynthesis of polypeptides without template control (Ito and Strominger, 1960; Mach and Tatum, 1964; Friedman and Weinstein, 1964; compare the zymosequential hypothesis, also derived from experiments-Haurowitz, 1963). The concept is also consistent with the evolutionary premise of stepwise development, from simpler systems, of the complex control and regulation of synthesis of contemporary protein (Jacob and Monod, 1961: Monod et al., 1963).

The role of code-active nucleic acid can be used as an argument that it appeared after protein (Fox, 1959; Thimann, 1963) in the sequence of events. The fact that phenomena of growth and division can be observed in units formed of thermal poly-α-amino acids is consistent with the concept that true growth and division are properties of the whole organized cell (Oparin, 1938: Lanham, 1952) rather than of molecules alone.

Sooner or later in the evolution before, or within, organisms, a coding mechanism would, however, have to appear. At least two distinct sequences for the emergence of protein, cell, and polynucleotide can be visualized and have been modelled by experiments. One such concept is the prior appearance of a polynucleotide, as suggested by Muller (1961). That a kind of polynucleotide might emerge spontaneously has been shown in Schramm's laboratory (Schramm, 1961, 1965), in our laboratory (Schwartz, et al., 1965) and by Aguilera et al. (1965). Mononucleotides have been polymerized in the dry state with ethyl metaphosphate in Schramm's study, with polyphosphoric acid in our studies, and in aqueous solution by γ-radiation. The last mode has not been interpreted as prebiological. Schramm's ethyl metaphosphate is not at all a geological type of material (Schramm, 1965), but wherever phosphate existed near moderate heat, polyphosphate would surely result (Ponnamperuma, 1965; Schramm, 1965). In this way have been obtained in the laboratory oligonucleotides, of 5-6 units and having a large proportion of natural linkages (Schwartz and Fox, 1964; Schwartz et al., 1965; Schwartz, 1965). The proportions of such linkages are indicated in Table IV. Thermal condensation of adenylic acid has been accomplished in the presence of cytidylic acid but not in its absence. Such cocondensation effects are comparable to those observed with aspartic acid and other amino acids.

However, more progress has so far been made in the laboratory on the model of the alternative sequence in which essentially a cell first appeared, as illustrated earlier in this paper. The development of a cellular synthesis of polynucleotides, and indeed, of proteins would then have to be explained as later stages for this model, inasmuch as organisms do not rely on heat but on the energy available from phosphate transfer, specifically from ATP. Contemporary phosphate transfer

Table IV

Digestion of Oligocytidylic Acid with Ribonuclease and with Venom Phosphodiesterase After Treatment with Alkaline Phosphatase

Residues Liberated by RNAase (% of total)	Residues Liberated by Venom Phosphodiesterase (% of total)
33	63

enzymes are often magnesium-protein compounds, but evidence exists for occasional zinc-protein compounds (Mathies, 1958; Ploche and Vallee, 1962). David Joseph has been able to incorporate zinc into proteinoid microspheres, as indicated earlier. Such zinc-containing microspheres then split ATP (Fig. 12; Fox, 1965b). In this way we can visualize how ATP-dependent syntheses could occur in cells formed from primitive abiotic protein. The dilemma of whether the cell or an ordered protein came first (Blum, 1951; Oparin, 1957; Jirgensons, 1962) is in principle resolved, and the origin of biosynthesis of protein is suggested. The dilemma posed by the need for polymerase protein for the first polynucleotide, or some other sequence involving nucleic acid and protein (Lederberg, 1961; Tatum, 1965; Lipmann, 1965; Thimann, 1963) is also resolved in principle. The experiments based on self-organizing properties of thermal poly-α-amino acids suggest in principle that a minimal kind of cell did not need a prior nucleic acid for its formation. Even so, as stated earlier, all theoretically possible sequences involving protein, cell, and gene are being investigated in model experiments. Of particular relevance to the proteinoid → cell sequence described in the laboratory, however, is the high degree of repeated sequence found in the proteinoids, that sequence emerging without the agency of any other macromolecules.

The theory presented has been tested against a number of tentative points of view. The data and interpretations in this paper reveal that preclusive assumptions based on the historical priority of the gene are not justified and, in fact, such negative premises may have discouraged or delayed experimental approaches to the facts.

Neither Pasteur nor others could have hoped in "twenty years" of experiments in single flasks to identify the key processes of abiogenesis. Changes of state in the geological situation are, and undoubtedly were, as frequent and sporadic as rain and its evaporation (Fox, 1965c). The thermal experiments, consistent with geological processes, suggest that ordered polymers formed abundantly in relatively dry, hot locales and that the resultant polymers organized themselves into primordial cells when water came into contact with the material. In contrast to most laboratory experiments in synthesis these processes constitute a connected sequence with intervening change of state.

The problem of spontaneous generation can also be seen in the 1950s and 1960s to be otherwise a different problem than it was in 1859, when the French Academy of Science formally requested, and stimulated attempts by Pasteur and his contemporaries, to illuminate the subject. The problem is now recognized as chemical in its detailed structure (Oparin, 1957; Fox, 1957) rather than grossly biological. The problem is also different in the biochemical dimension. No one could have been expected to foresee in 1860 the overwhelming chemical complexity of the

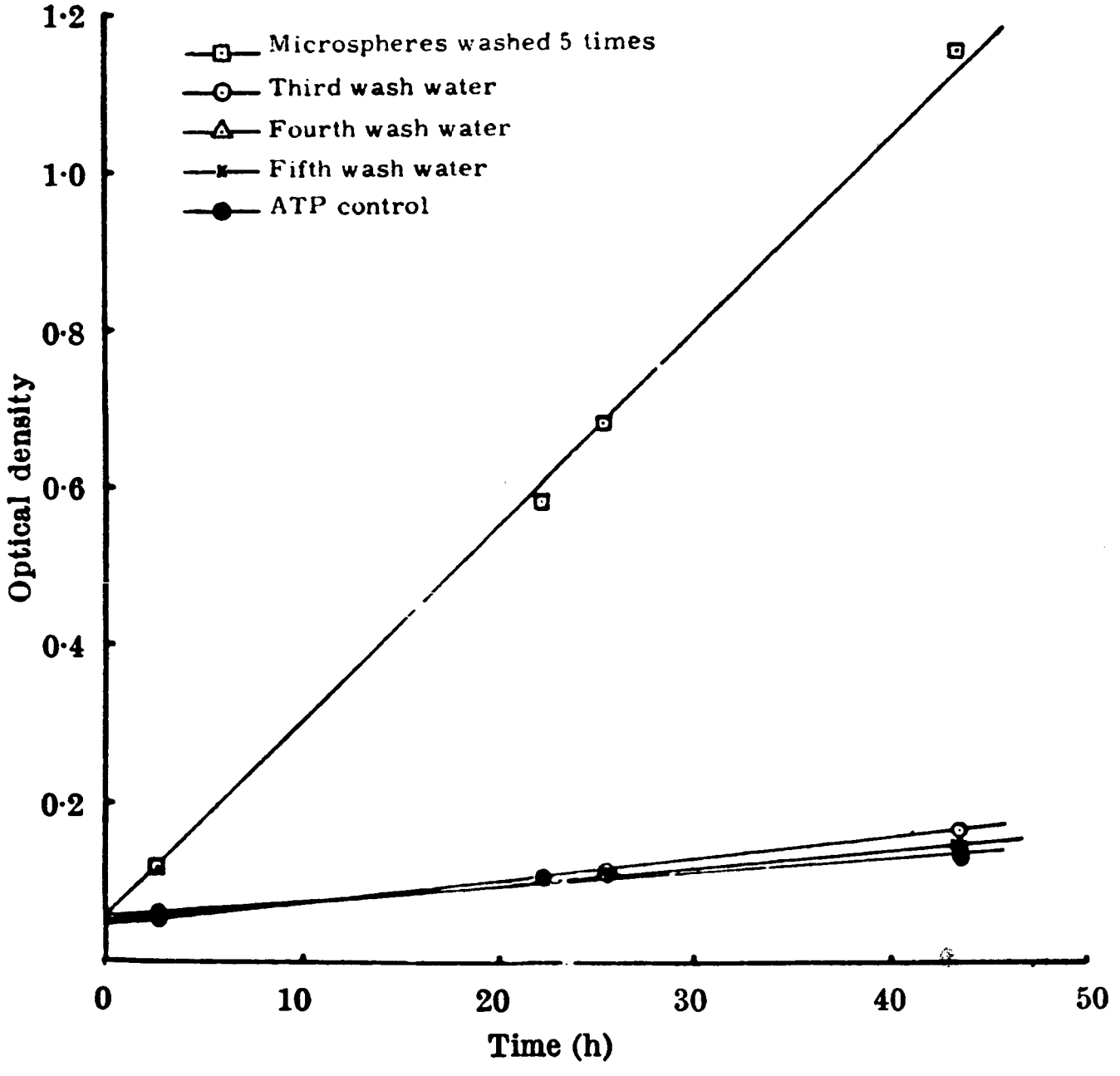


Figure 12. Adenosine triphosphate-splitting activity in Zn-containing microspheres. Optical density is molybdate color intensity measuring release of phosphate (Fox, 1965b).

cell as biochemists and others have catalogued it within the past few decades. Whereas official French science encouraged investigation of spontaneous generation in the last century, the complexity of biochemistry has in this century been a deterrent to a disciplining of the problem in the laboratory. This complexity can perhaps help explain the fact that so few traditionally oriented biochemists have chosen to do the necessarily heuristic research required in this field.

A brief summary of the principal advance which has been reported here would accordingly emphasize that the experiments have shown how moderately controlled heating of α-amino acids, either in the laboratory or in the geological matrix, could readily yield a polymer which, by simple contact with water, would easily organize itself into a minimal kind of membranous cell, having catalytic, fissile, and other activities imitating those of the true biological unit. At the interpretative level, these findings indicate some of the particular, internally limited, ways in which the now recognized and vexing biochemical and cytological complexity could begin to arise by simple processes. Consistent with Louis Pasteur's analysis we can say, at the experimental level, that studies since 1959 have demonstrated how abiotic "matter can become organized of itself into a cell", if that matter is also of the appropriate origin and molecular structure.

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