BioSystems, 9 (1977) 1—22

COMPARTMENTALIZATION IN PROTEINOID MICROSPHERES

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(Received June 10th, 1976)
(Revision received November 18th, 1976)

Proteinoid microspheres with stable internal compartments and internal structure are made from acidic proteinoid and basic proteinoid with calcium. The populations of microspheres are characterized by a wide diversity of structure. A model of primitive intracellular communication is suggested by the observed movement of internal particles between compartments of a multicompartmentalized unit. Differential response to pH change and to temperature change has been demonstrated within one population and suggests one mode of adaptive selection among primordial cell populations.

1. Introduction

Microspheres having a number of properties of contemporary cells (Fox and Dose, 1972; Ambrose and Easty, 1970; Knight, 1974; Lehninger, 1975; Florkin, 1975) have been assembled by treating thermally synthesized acidic proteinoid with water, usually by heating and cooling. These units provide a basis for a theory of the origins of primordial and contemporary cells from simple reactants (Fox, 1974) since the component self-ordered polymers are synthesized under geologically relevant conditions *.

Microsystems of greater morphological complexity (Smith and Bellware, 1966) have been produced by interacting both acidic proteinoid and basic lysine-rich proteinoid (Fox and Yuyama, 1963) in the presence of either water or calcium chloride solution (Miquel et al., 1971; Fox and Dose, 1972).

This paper reports further results in the assembly of complex microsystems containing more than one type of proteinoid and calcium. The new properties that emerge include the formation of compartments and a movement of particles between these compartments. This movement of particles suggests a model of primordial intracellular communication akin to the previously reported model of intercellular communication (Hsu et al., 1971).

* The amino acids available were probably fewer in numbers of type than those combined in proteinoids. Eighteen types are typically included in proteinoids to cover the range of potential activities.

2. Materials and methods

The proteinoids used in these experiments were made according to previously reported methods (Fox and Harada, 1966). Free-base forms of lysine, arginine, and histidine were used in the initial reaction mixture yielding basic proteinoids.

2.1. Formation of microstructures

A suspension of acidic 2 : 2 : 1- (two parts glutamic acid, two parts aspartic acid, and one part of an equimolar mixture of eighteen amino acids in the reaction mixture) proteinoid is gently heated to boiling for 5—15 sec with continued stirring, and then allowed to cool slowly. In other preparations two proteinoids are mixed together, and heated as for the unmixed type. The inclusion of calcium is described under the appropriate experiments.

One of these latter products was analyzed for calcium content by the method of Pierce and Haenisch (1937). The content was 6.7%.

2.2. Gram staining

Experiments involving the Gram stain followed the procedure used in staining acidic 2 : 2 : 1-proteinoid microsospheres described earlier (Fox and Yuyama, 1963).

2.3. Amino acid analysis

Proteinoids were analyzed following 72-h hydrolysis of 1 part of sample with 250 parts of 6 N HCl at 100°C in sealed tubes under nitrogen. Analyses were conducted on a Phoenix amino acid analyzer K-5000. Results are summarized in Table 1.

TABLE 1

Amino acid composition [1] of proteinoids.

Amino acid	Lysine-rich basic proteinoid SB V 60	Lysine-rich basic proteinoid DJ VI 81	2:2:1-acidic proteinoid July 1967	2:2:1-acidic proteinoid AM XII 57
Lys	61.1	57.8	1.6	1.4
His	1.7	1.2	0.5	0.5
Arg	1.0	1.3	0.6	0.7
Asp	2.7	2.5	65.7	68.1
Thr	0.8	0.6	0.1	0.0
Ser	0.7	0.3	0.4	0.1
Glu	5.5	8.3	16.5	15.3
Pro	2.4	1.9	0.6	0.9
Gly	4.9	6.0	1.6	1.4
Ala	4.9	4.4	3.8	3.2
Val	1.4	1.9	1.1	1.1
Met	1.9	2.3	1.2	1.1
Ile	0.9	1.0	0.6	0.7
Leu	1.8	2.1	1.3	1.2
Tyr	1.9	2.3	1.3	1.4
Phe	2.6	2.6	1.4	1.6
Alloile	1.3	1.1	0.6	0.6

[1] Calculated in mol. ratios, ammonia omitted.

3. Experiments and results

Fig. 1 shows the results of various concentrations of acidic 2 : 2 : 1-proteinoid during the preparation, and the effects of separate and added lysine-rich proteinoid.

The preparations are seen in Figs. 1A—F. Those in Fig. 1A are not typical of a "1 mg/ml" population; at this concentration most units which form are 0.5 μm or less in diameter. Microspheres from acidic 2 : 2 : 1-proteinoid are typically 1—3 μm in diameter, the size depending upon the concentration used. Some differences in diameter are observed from one acidic proteinoid preparation to another, but they are usually of uniform size within any one preparation. Basic lysine-rich proteinoid does not form microspheres at low concentrations, but yields particles in the 1—30 μm range at 100 mg/ml. (Lysine-rich proteinoids are known to form microparticles under other conditions (Rohlfing, 1975).) The units formed by acidic and basic proteinoid together are less than 1.0 μm in diameter and are produced in abundance (Fig. 1F). Colonies or clusters, as seen with acidic proteinoids alone (Fox and Yuyama, 1963) are not common.

Except for Fig. 1A, the forms in each figure in this paper are typical of numerous others seen in extensive microscopic examination.

3.1. Microstructures made with calcium

Acidic proteinoid and a combination of acidic and basic proteinoids were interacted in the presence of varying calcium chloride concentrations. Figs. 2A—C show uniform microparticles from various calcium chloride concentrations. Junctions of a type reported elsewhere (Hsu et al., 1971) can be seen in each of the fields.

Figs. 2D—F show the types of microparticle obtained when acidic and basic proteinoids are mixed. The microparticles in Fig. 2D are small and generally less than 1.0 μm in diameter. An occasional unit is 5—10

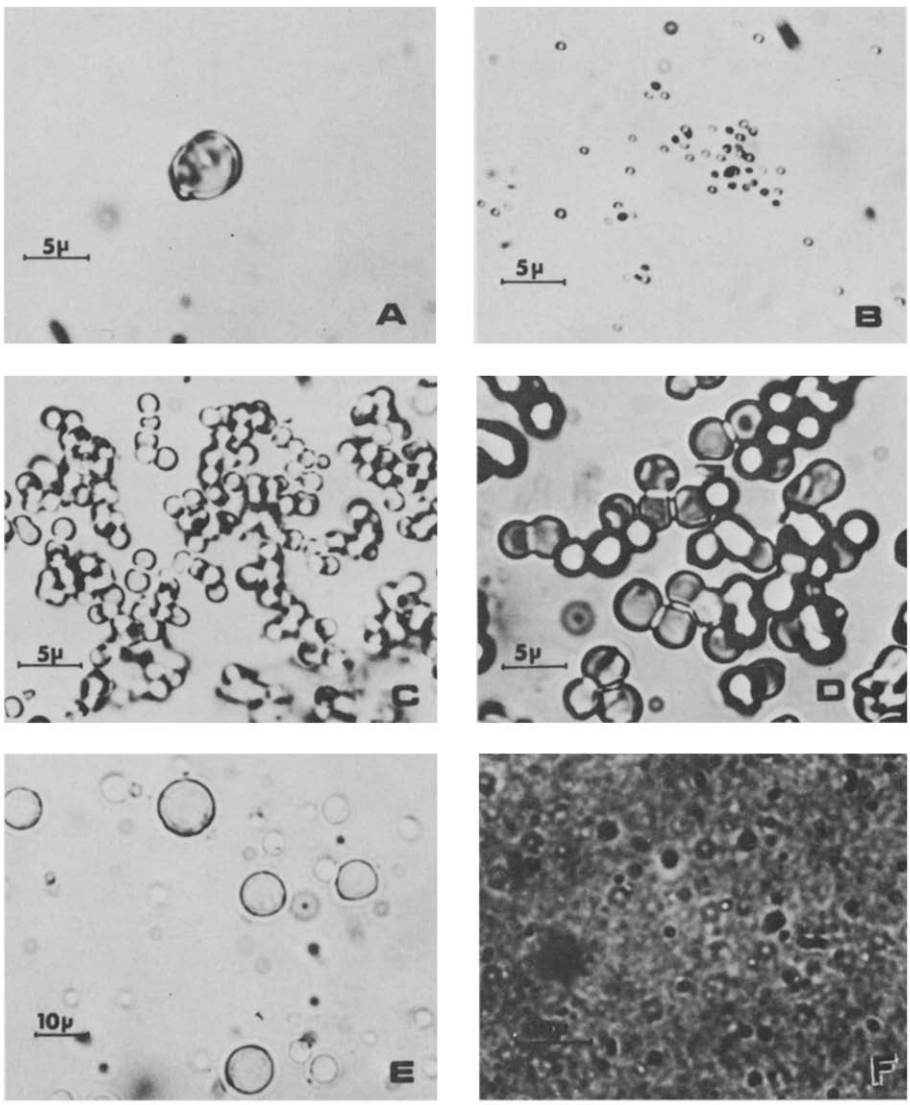


Fig. 1. Microparticles from acidic 2 : 2 : 1-proteinoid and water. Proteinoid concentrations are A: 1 mg proteinoid/ml, B: 2 mg/ml, C: 10 mg/ml, D: 25 mg/ml, E: Lysine-rich basic proteinoid microparticles formed in water, 100 mg/ml, F: 80 mg of acidic 2 : 2 : 1-proteinoid and 20 mg of basic lysine-rich proteinoid heated in 4 ml of water.

μm in diameter and contains an internal compartment. As the concentration of calcium increases (Figs. 2E and F), the number of these compartmentalized units increases along with the number of other morphological types.

The effects on compartmentalization of varying ratio of acidic and basic proteinoid components are shown in Figs. 3A—D. Microparticles formed in the 9 : 1 ratio are seen in Fig. 3A. Units of varying type, including compartmentalized microparticles, are seen. Similar results were obtained at a ratio of 4 : 1. This ratio yields compartmentalized microparticles in the greatest abundance. A representative field is seen in Fig. 3B. Several large

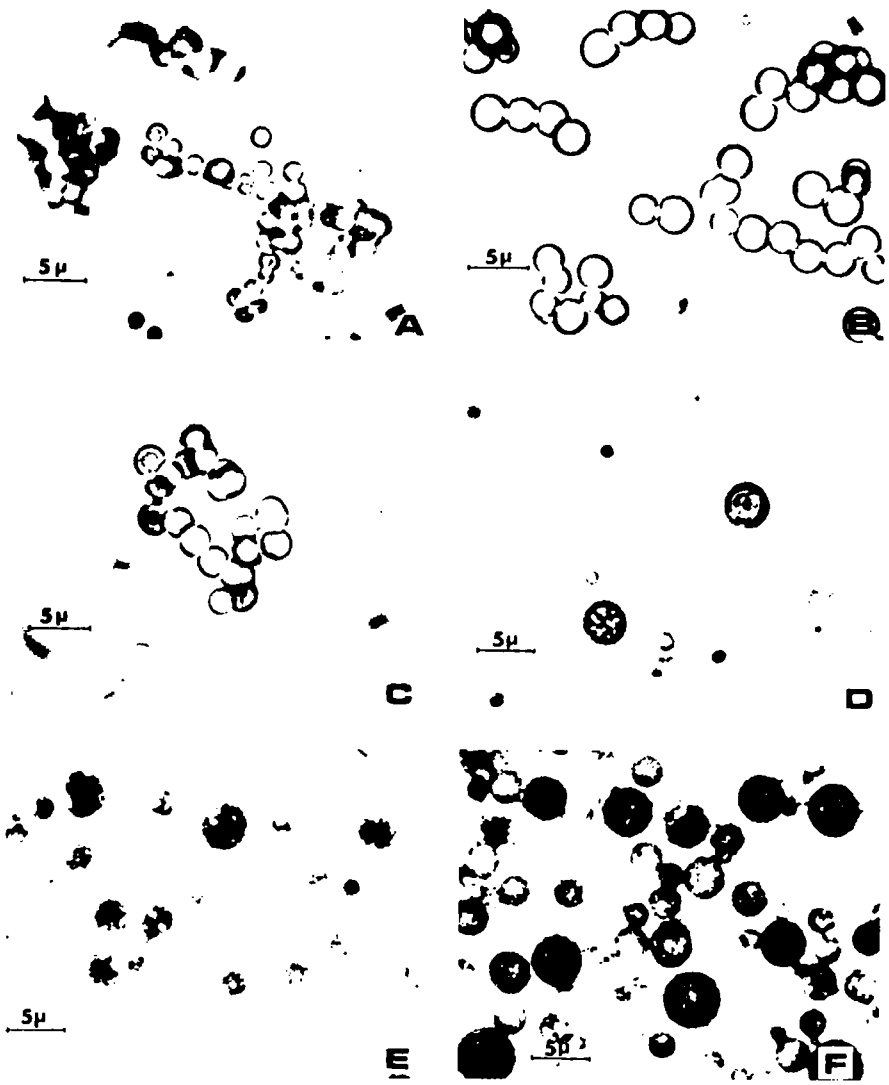


Fig. 2. Microparticles formed in the presence of calcium. A—C: acidic 2 : 2 : 1-proteinoid microparticles formed in 0.01 M, 0.1 M and 1.0 M $CaCl_2$ respectively, D—F: acidic 2 : 2 : 1-proteinoid/basic lysine-rich proteinoid microparticles formed in 0.01 M, 0.1 M and 1.0 M $CaCl_2$ respectively. D—F have been published (Miquel et al., 1971).

compartmentalized units were observed in the 1 : 1 ratio preparation. However, very few of the 1—3 μm acidic-type units were seen. In Figs. 3C and D, large sheets of amorphous material are present and can be compared with the formed particles.

The following set of experiments was run to determine the effect of changing the order of addition of components on the self-assembly of microparticles from two proteinoids and calcium. (a) Basic lysine-rich proteinoid added to preformed acidic 2 : 2 : 1-proteinoid microspheres made in the presence of water failed to produce compartmentalized units. Most units were less than 0.75 μm (Fig. 4A). (2) When the basic lysine-rich proteinoid was added with calcium salt to preformed acidic 2 : 2 : 1-proteinoid microspheres, the results

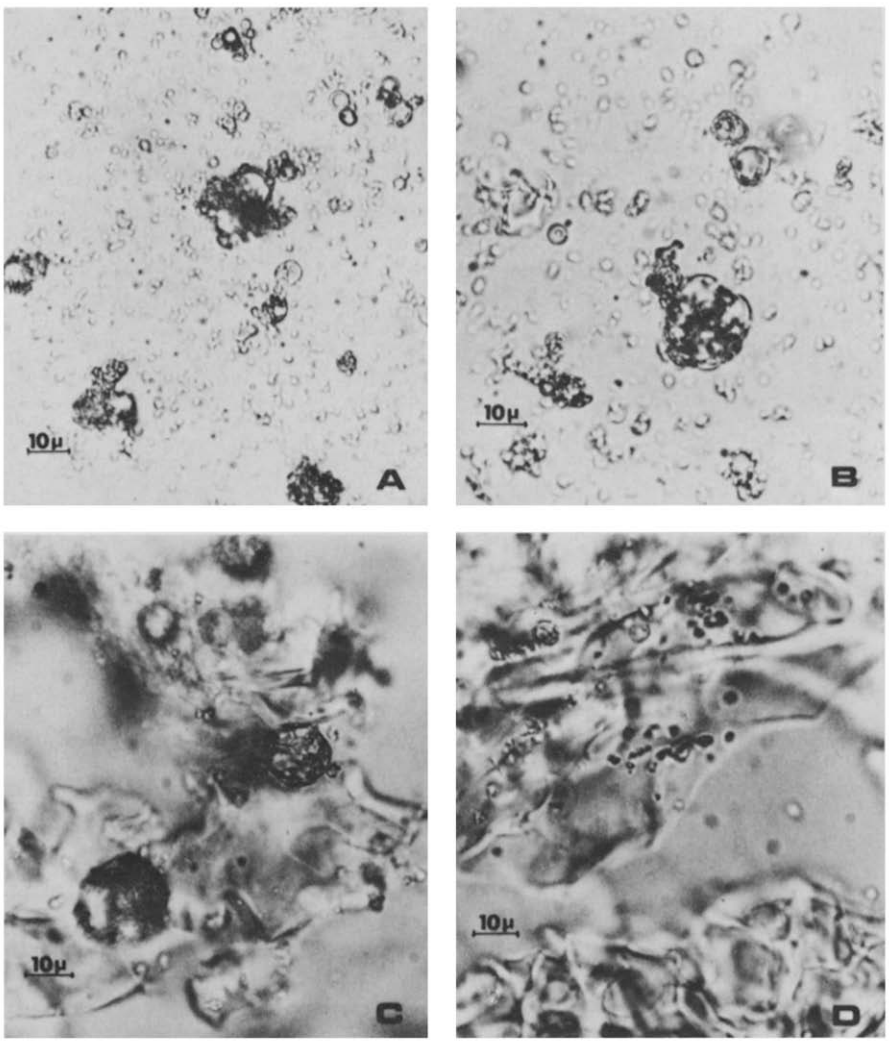


Fig. 3. Microparticles formed from varying acidic 2 : 2 : 1-proteinoid/lysine-rich proteinoid weight ratios. All particles formed in the presence of 0.1 M $CaCl_2$. A: 9 : 1 acidic proteinoid : basic proteinoid, B: 4 : 1 acidic proteinoid : basic proteinoid, C: 1 : 1 acidic proteinoid : basic proteinoid, D: 1 : 3 acidic proteinoid : basic proteinoid.

were similar to those described in Fig. 4A, except for an overall higher density (Fig. 4B). (3) Addition of the lysine-rich proteinoid with calcium salt to preformed acidic 2 : 2 : 1-microspheres, made in the presence of calcium initially, produced several compartmentalized microparticles. These units were a small fraction of the entire population (Fig. 4C), most units being in a 1.0 μm range. (4) When the lysine-rich proteinoid was added to preformed acidic 2 : 2 : 1-proteinoid microspheres made initially with calcium, but with retention of the original mother liquor, compartmentalized units were formed, as seen in Fig. 4D.

Fig. 5A shows a large, noncompartmentalized unit. Figs. 5B, C and D show microparticles containing one, two and three compart-

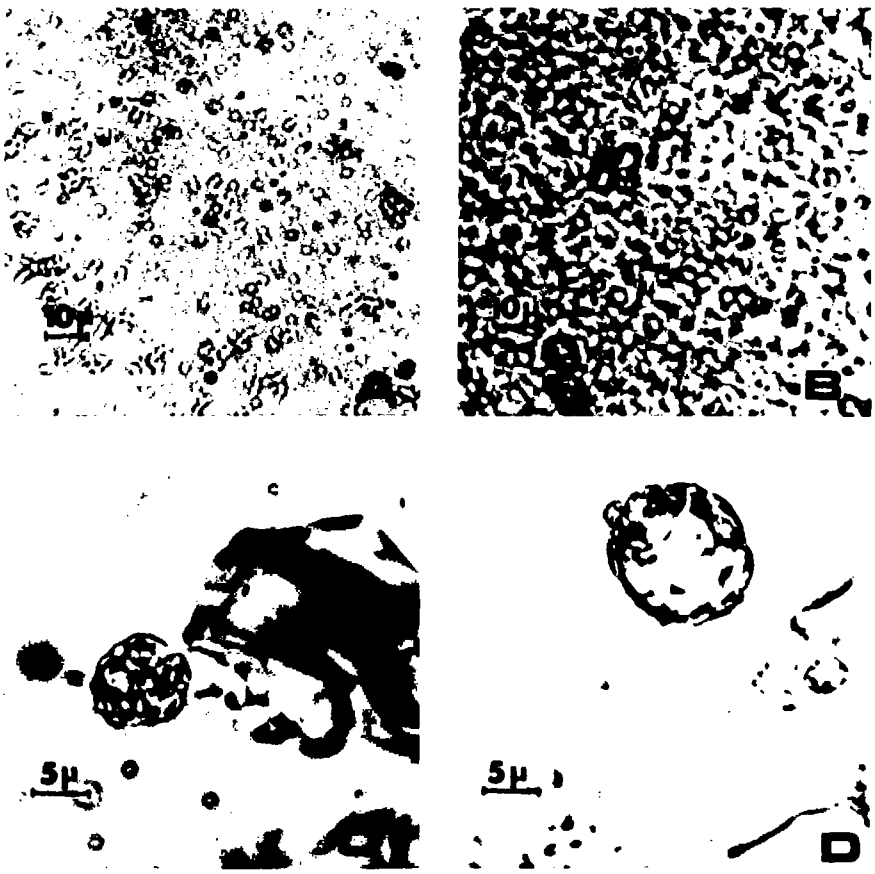


Fig. 4. Effect on compartmentalization of varying the order of addition of the components in acidic proteinoid-basic proteinoid-calcium microparticle preparations. A: basic lysine-rich proteinoid added to preformed acidic 2 : 2 : 1-proteinoid microspheres. B: basic lysine-rich proteinoid and calcium added to preformed acidic 2 : 2 : 1-proteinoid microspheres. C: basic lysine-rich proteinoid added to preformed acidic 2 : 2 : 1-proteinoid microspheres made initially with calcium. D: basic lysine-rich proteinoid added to preformed acidic 2 : 2 : 1-proteinoid microspheres made with calcium and including the original mother liquor.

ments respectively. Microparticles having compartments are seen in Figs. 5E and F. Whether the compartmentalized units are formed initially by a kind of aggregation is undetermined.

Compartmentalized microparticles are often seen to contain smaller particles within their chambers. The formation of such particles, called endoparticles, has been reported for 2 : 2 : 1-proteinoid microspheres (Hsu et al., 1971). The endoparticles in the populations of mixed proteinoid units all appeared after formation of the microspheres, and with no experimental manipulation.

In Fig. 6A is seen a microparticle with one large compartment containing endoparticles in the 0.5—1.0 μm range. These are in constant motion within the boundary of the large unit. Microparticles having more than one compartment have been observed to contain endoparticles in one, some, or all of the compartments. In Fig. 6B, endoparticles are observed in one of the three compartments. This is in contrast with the unit in Fig. 5D. The fact that endoparticles are seen in only one compartment suggests that the compartments exist separate from one another in this particular particle, or that they have connections smaller in diameter than endoparticles.

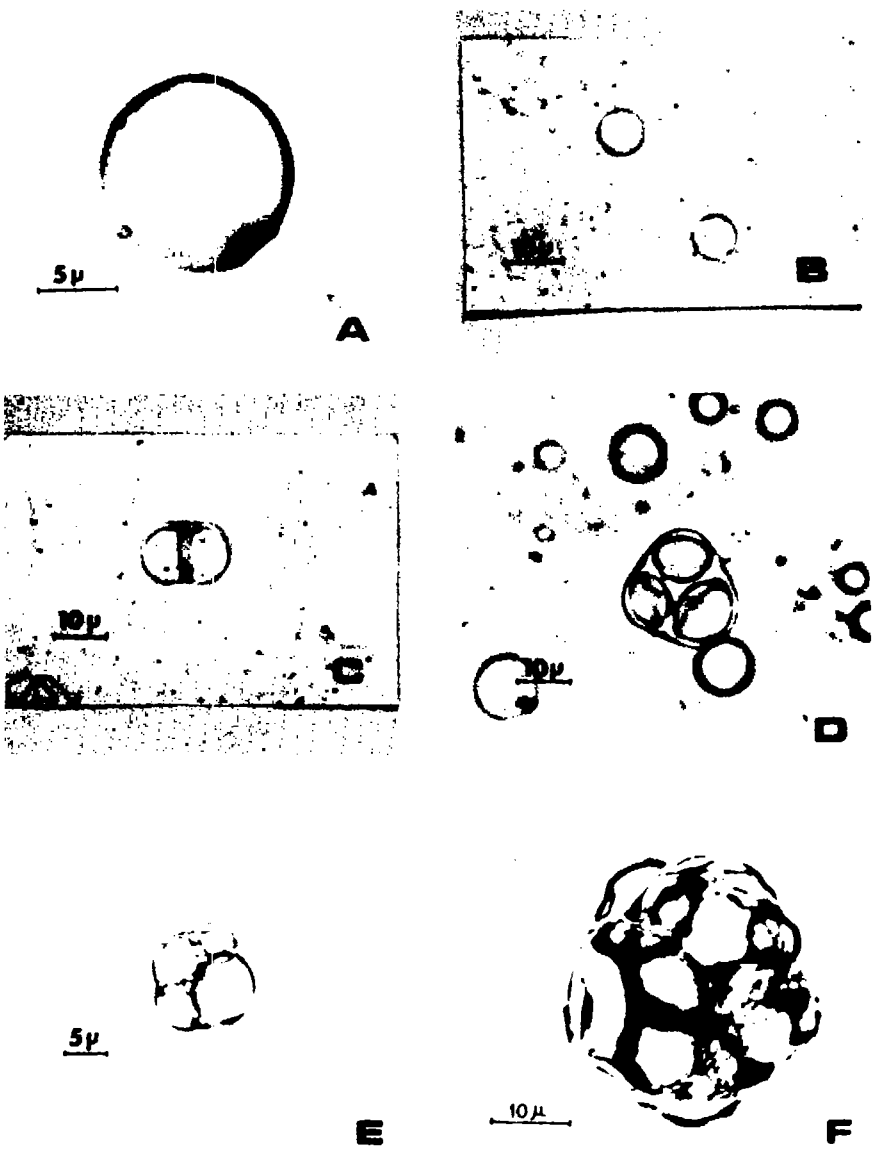


Fig. 5. Variation in compartment number of acidic proteinoid-basic proteinoid microparticles made in the presence of 0.1 M CaC_2. A: a noncompartmentalized unit. B: units with one central compartment. C: unit with two compartments. D: unit with three compartments. E and F: multicompartmentalized units.

Fig. 6C shows a multicompartmentalized unit with granular endoparticles in all compartments.

Variety in size of endoparticles within one compartment has been observed in several units. Fig. 6D shows endoparticles ranging from 1.1 to 3.8 μm, but without visible internal structure.

The variety in morphology of endoparticle is further illustrated in Fig. 6E, wherein endoparticles with internal compartments of their own are contained within the boundary of a larger compartmentalized unit. In Fig. 6F the compartmentalized endoparticle itself is seen to contain smaller particles.

An example of the amount of diversity

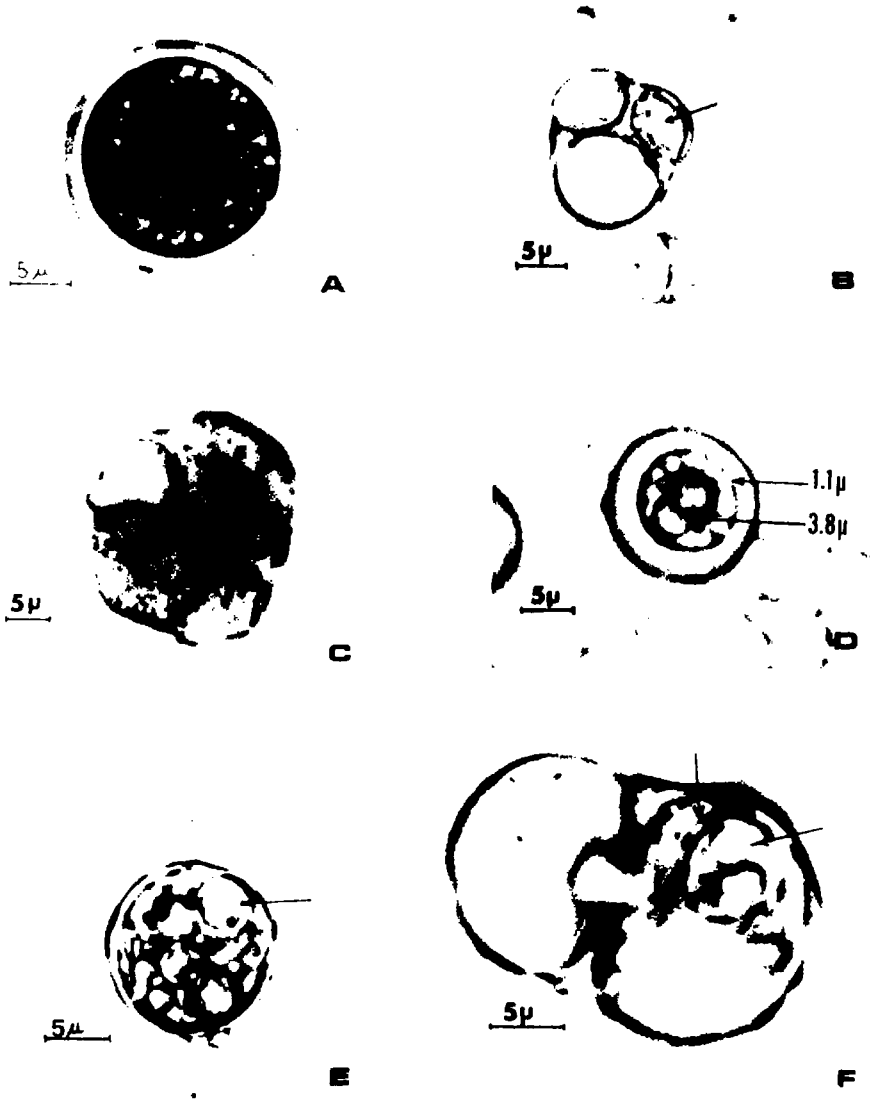


Fig. 6. Compartmentalized microparticles containing endoparticles. All units shown are from acidic 2 : 2 : 1-proteinoid/lysine-rich basic proteinoid (4 : 1 by weight) preparations made in the presence of 0.1 M $CaCl_2$. A: a unit with granular endoparticles in the 0.5—1.0 μm range. B: endoparticles contained in one of the three visible compartments. C: granular endoparticles in all compartments. D: endoparticles of varying sizes. E: compartmentalized microparticle containing a compartmentalized endoparticle. F: compartmentalized endoparticles containing smaller units.

which exists in one field of microparticles from mixed proteinoid and calcium is seen in Fig. 7. Particles of varying sizes with various sized compartments, with and without endoparticles, are seen. The diversity also illustrates the fact that individual types are not rare.

The microparticles in Fig. 8 illustrate examples of differential compartment volume/total volume ratio within one population.

3.2. Results of scanning electron microscopy

A sample of 4 : 1 (acidic proteinoid : basic proteinoid) microspheres made in 0.1 M

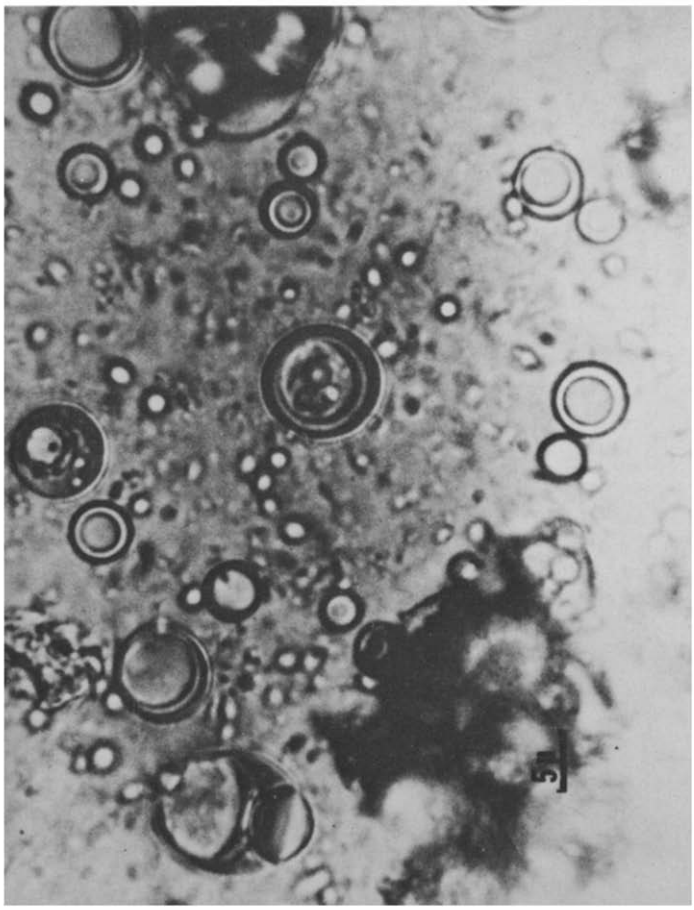


Fig. 7. Diversity in mixed proteinoid microsphere populations. All units are from a population made from 80 mg of 2 : 2 : 1-proteinoid, 20 mg of lysine-rich proteinoid dissolved and heated in 5 ml of 0.1 M calcium chloride.

$CaCl_2$ was prepared for scanning electron microscopy by air-drying and shadowing the air-dried sample with gold-palladium dust. These survived the air-drying and vacuum treatment with gold-palladium dusting. The wide distribution of sizes observed was in contrast to the relatively uniform size of proline-rich 2 : 2 : 1-proteinoid microspheres prepared in the same manner (Figs. 9A and B).

3.3. Surface and boundary vacuoles

The variety of morphological types found in acidic proteinoid-basic proteinoid-calcium populations is further enriched by the presence of units with surface vacuoles and other boundary structures as seen in Figs. 10A and B. Wide diversity in the size and shape of the surface and boundary vacuoles is seen. These units are very stable, in contrast to foam bodies and coacervate droplets, which they resemble.

By removing water from the coverslip edge, the pressure of the coverslip on the surface of a large unit is increased. Careful manipulation can cause a break in the outer boundary. Fig. 11 shows cracks through several small surface vacuoles, most of which appear at the outer boundary layer.

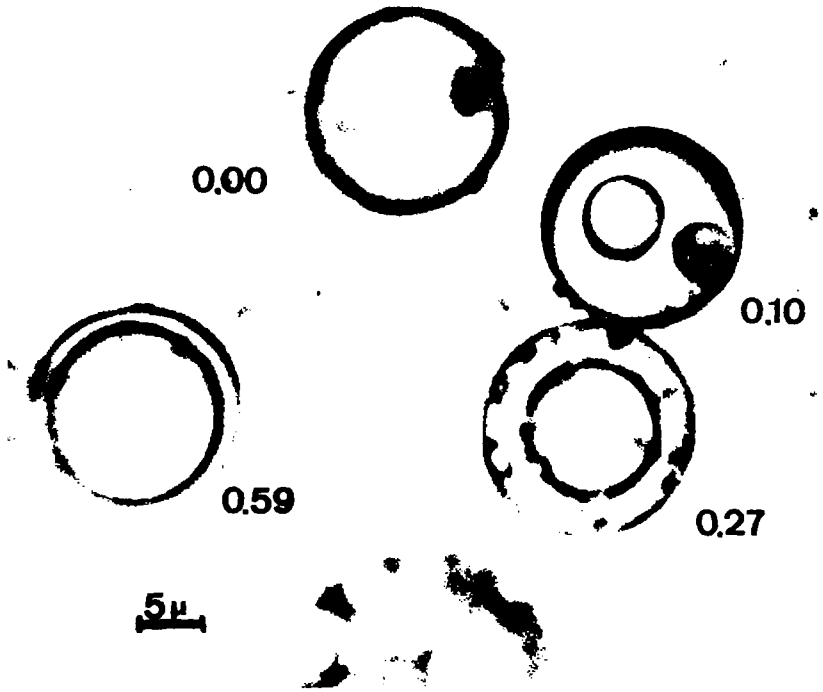


Fig. 8. Ratio of compartment volume to total volume in mixed proteinoid microparticle populations made in the presence of 0.1 M $CaCl_2$.

3.4. Gram stains

Microparticles that were Gram-stained vividly show complex structures in the boundary regions. In Fig. 12A, coil-like structures are seen in the dark regions near the outer boundary layer. In many Gram-stained particles from mixed proteinoid preparations, the outer boundary layer is stained a light red while the interior is stained a darker blue. An example of this is seen in Fig. 12B. A central vacuity, endoparticles and boundary vacuoles are seen in Fig. 13, which is an electron micrograph of an acidic-basic-calcium-microparticle that has undergone freeze-fracturing.

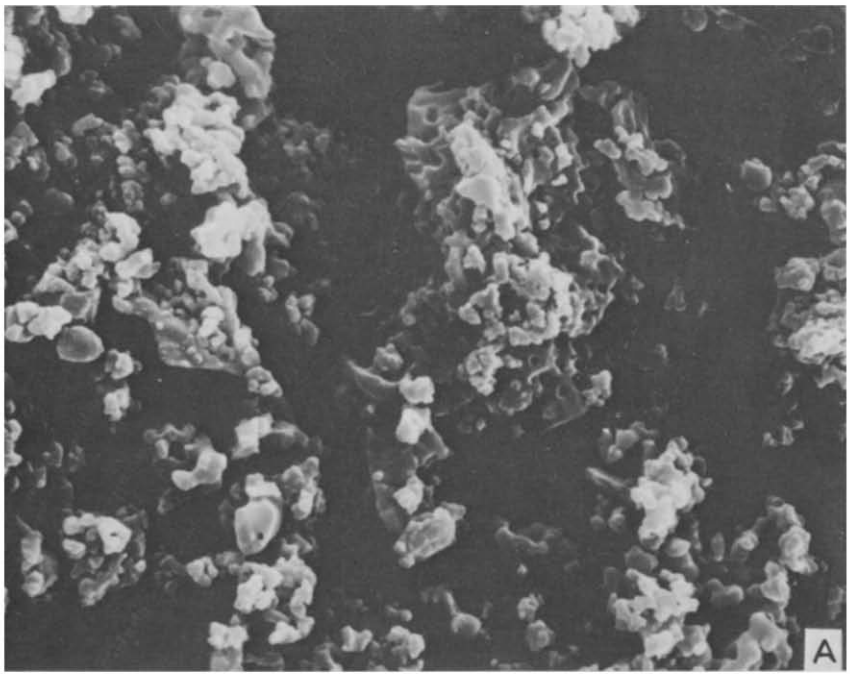
3.5. Other structural features

Although it is undetermined whether mixed proteinoid microspheres proliferate through the budding reported for 2 : 2 : 1-proteinoid microspheres (Fox et al., 1967), the presence of budlike protrusions within the former has been observed. Fig. 14 illustrates such a protrusion.

Close junctions or extended bridges have been observed between individual compartmentalized units. These junctions exist between units of similar morphology and between units of differing morphology.

As indicated previously, compartments within one particulate may exist isolated from each other. On the other hand, junctions or spaces between compartments are also found. Junctions between boundary vacuoles and a central compartment are seen in Fig. 15. Fig. 15B illustrates the similarity between the unit of Fig. 15A and a precambrian microfossil from the Gunflint chert reported by Barghoorn and Tyler (1965).

A field of microparticles containing a compartmentalized unit having endoparticles



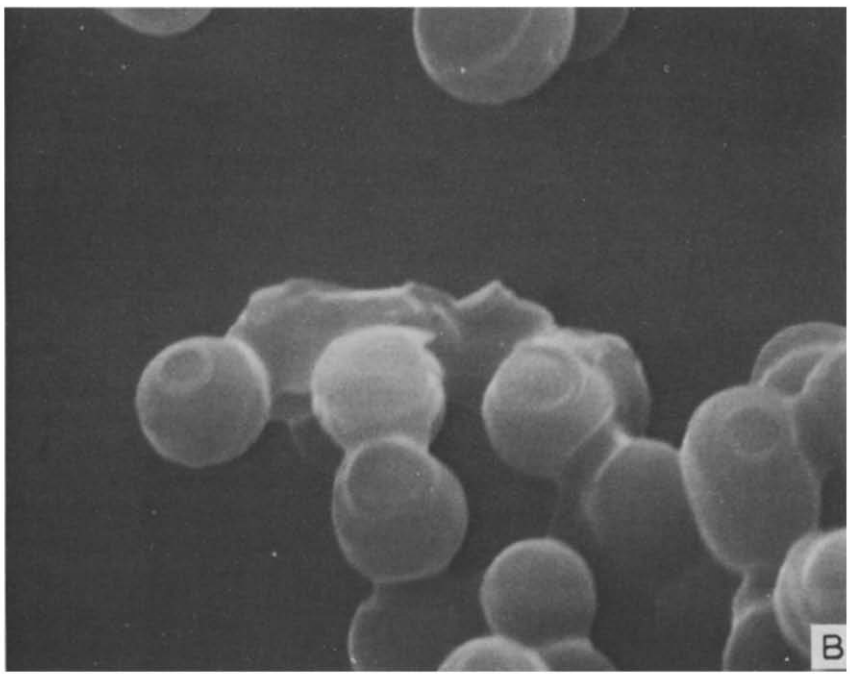
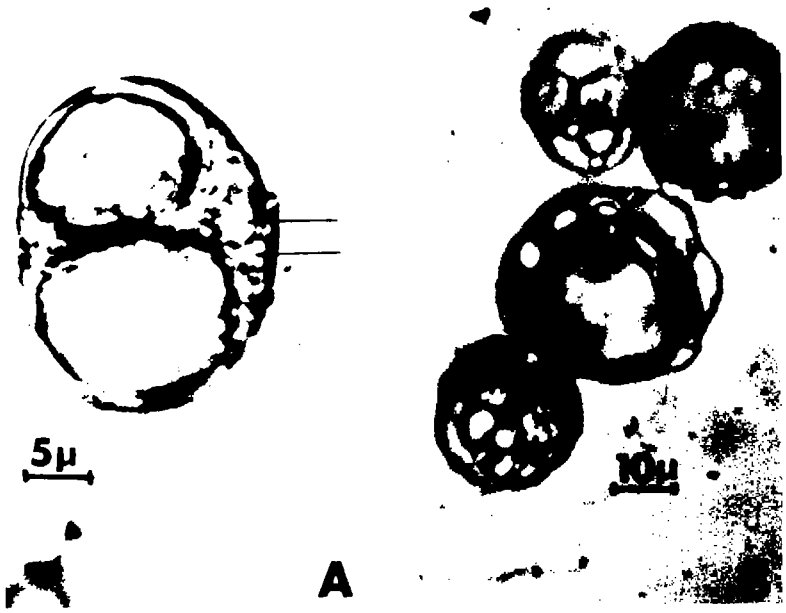


Fig. 9A. Scanning electron micrograph of powdered proline-rich acidic 2 : 2 : 1-proteinoid.
Fig. 9B. Scanning electron micrograph of a proline-rich 2 : 2 : 1-proteinoid microparticle population. Scars from previous attachment of other units can be observed.



Figs. 10A and B. Stable surface and boundary vacuoles in mixed proteinoid microparticles made in the presence of 0.1 M $CaCl_2$, as indicated by arrows.

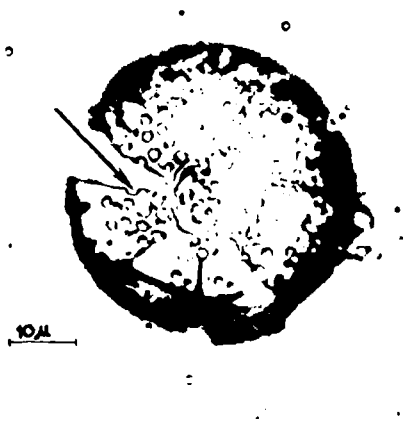


Fig. 11. Results of applying pressure to outer boundary of a microparticle. Cracks through small surface vacuoles can be seen. This particle was in a population of mixed proteinoid microparticles made in the presence of 0.1 M $CaCl_2$.

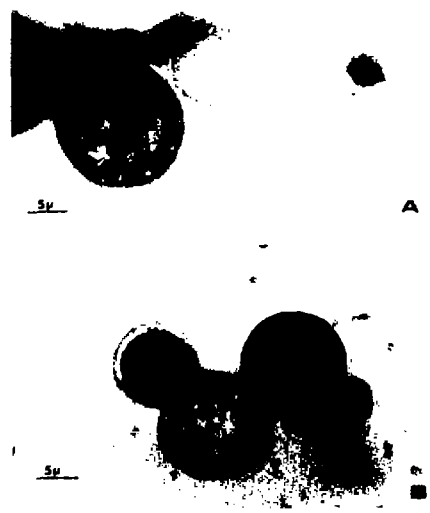


Fig. 12. Gram-stained mixed proteinoid microparticles made in the presence of 0.1 M $CaCl_2$. A: coil-like structure is visible in the outer layer. B: differential staining in the inner and outer regions is seen.

Fig. 13. Freeze fracture of a mixed proteinoid microparticle made in the presence of 0.1 M $CaCl_2$. A central vacuole containing endoparticles as well as several budlike units are seen.

was selected. By applying bibulous paper to the edge of the coverslip, it was possible to dry the slide slowly. In so doing, the pressure of the coverslip on the surface of the microparticle was increased; this cracked the outer boundary, thus liberating the endoparticles. This sequence is shown in Fig. 16. It illustrates a model for protosporulation referred to earlier (Fox, 1973).

Time-lapse frames of compartmentalized microparticles containing endoparticles showing the passage of endoparticles from one compartment to another are presented in Fig. 17.

Fig. 14. A budlike protrusion from within a mixed proteinoid microparticle made in the presence of 0.1 M $CaCl_2$.

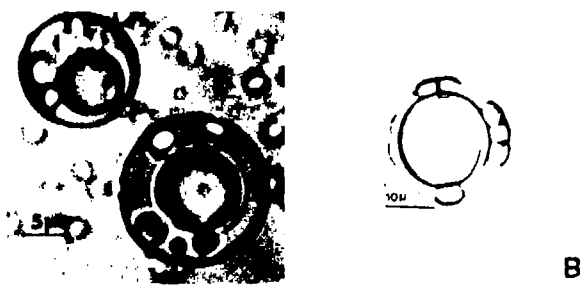


Fig. 15. Comparison of a laboratory mixed proteinoid microparticle made in the presence of 0.1 M $CaCl_2$ with a microfossil. A: a microparticle with a large central compartment and boundary vacuoles and junctions between the two. B: a microparticle from the Gunflint chert (Barghoorn and Tyler, 1965).

Fig. 16. Release of an endoparticle following cracking of the outer boundary. The unit is from a population made from 80 mg of 2 : 2 : 1-proteinoid and 20 mg of lysine-rich proteinoid dissolved and heated in the presence of 5 ml of 0.1 M $CaCl_2$.

3.6. Differential pH stability

A sample of 4 : 1 (acidic : basic) proteinoid microparticles made in 0.1 M $CaCl_2$ was placed on a slide. A drop of 0.5 N NaOH was added to the edge of the coverslip. Photographs were taken as the NaOH flowed. Fig. 18A shows a population prior to pH increase. The larger compartmentalized units survived while the smaller units dissolved (Figs. 18B—D).

3.7. Differential thermal stability

A population of compartmentalized microparticles was made by mixing 80 mg of 2 : 1 : 1-proteinoid and 20 mg of lysine-rich proteinoid in the presence of 4 ml of 0.1 M $CaCl_2$, and heating and cooling in the usual manner. Fig. 19A shows a field prior to an increase in temperature. Noncompartmentalized units and larger compartmentalized units can be seen. During slow increase in temperature, a majority of the small noncompartmentalized units began to dissolve until most of the remaining units were of the compartmentalized type (Fig. 19B).

In the sequence shown in Figs. 20A—D, smaller units are dissolved first. Several of the large compartmentalized units then fuse into one larger unit. This unit and the internal vacuolar structure remain stable upon cooling.

In continued heating/cooling cycles, there exists a class of units which quickly dissolves upon heating, and which reforms upon cooling. This has been observed in the same field of microparticles through four heating/cooling cycles (Figs. 21A—E).

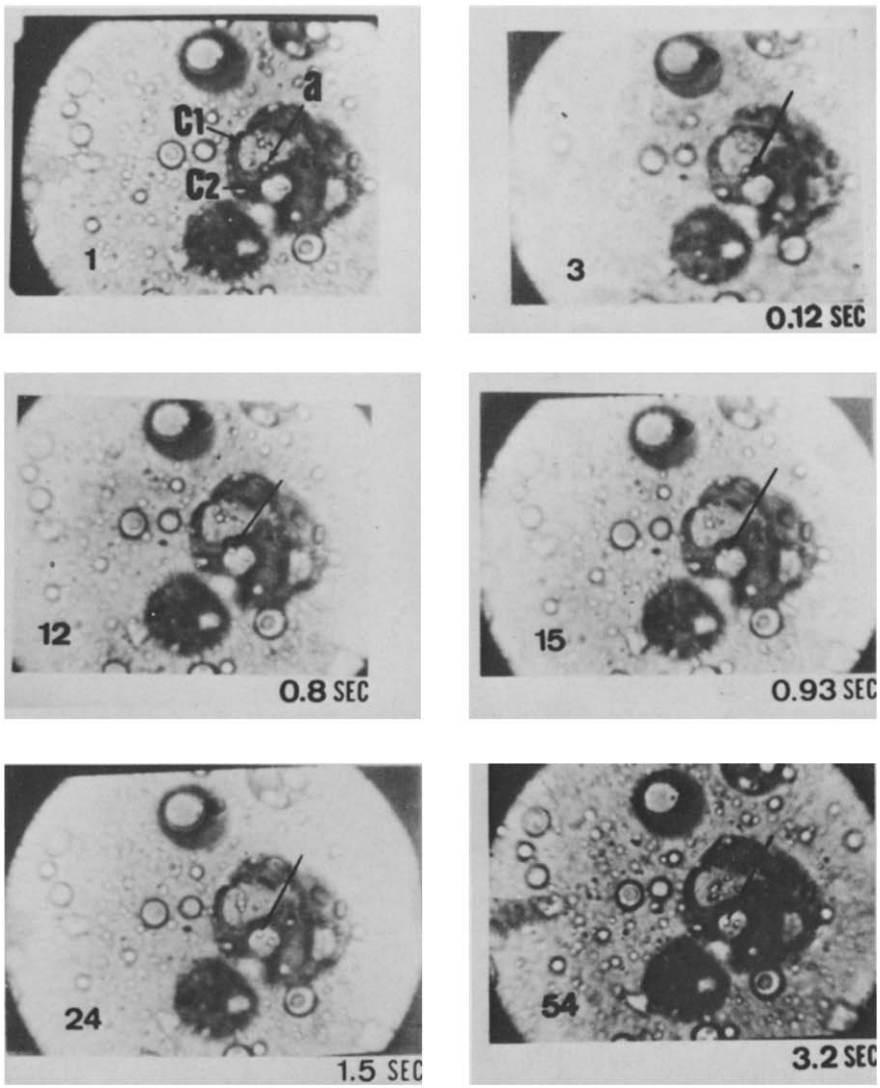


Fig. 17. Transfer of an endoparticle between compartments of a mixed proteinoid microparticle.

4. Discussion

The properties of the proteinoid microspheres are emergent; they were not predicted. Most of those recorded earlier (Fox and Dose, 1972) have been observed in microspheres from acidic 2 : 2 : 1-proteinoid. The descriptions recorded here are mostly for another type of microparticle, that which is made of acidic proteinoid, basic proteinoid and calcium ions.

4.1. Size and shape

Acidic 2 : 2 : 1-proteinoid microspheres are relatively uniform in size and shape. Departures from the spherical shape are found in these populations, as are units which may exceed the typical 1—3 μm range of size. By the addition of only one more structural component, i.e., a basic proteinoid, and the geologically ubiquitous cation, calcium, diversity of form results. This variation is the basis for

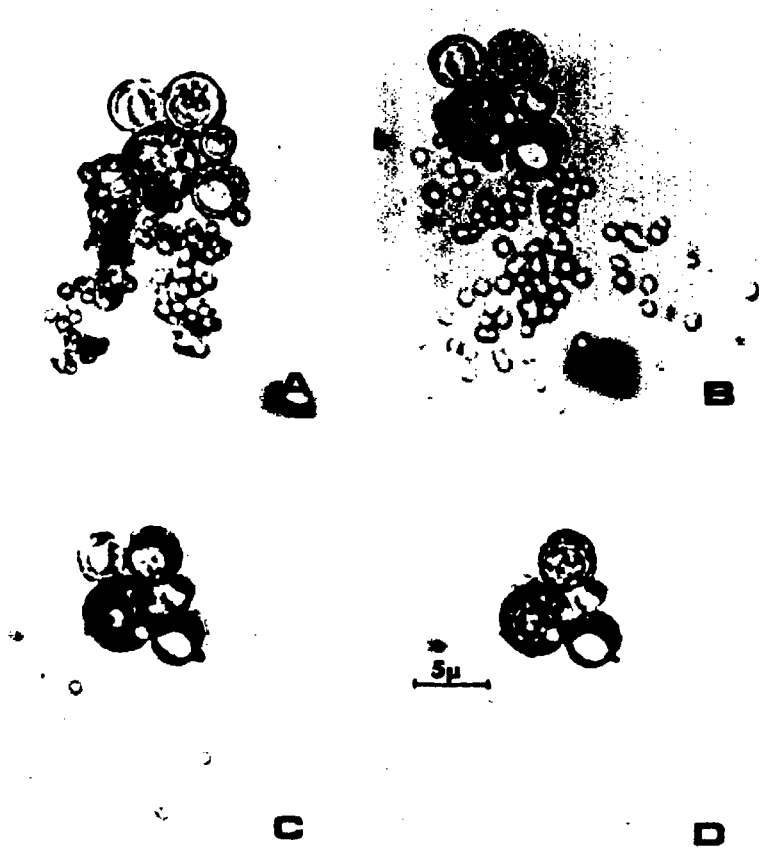


Fig. 18. A sequence showing the effect of pH increase on a population of mixed proteinoid microparticles made in the presence of 0.1 M $CaCl_2$. A: the population prior to pH increase. B—D: the gradual dissolution of the non-compartmentalized types. Approximate time between photomicrographs is 30 sec.

differential survival demonstrated over a range of temperature of pH. The photomicrographs in this paper have shown only a fraction of the morphologies obtained with a mixed proteinoid preparation.

4.2. Compartments

Compartments have been reported in populations of acidic proteinoid microspheres (Fox and Dose, 1972) under three different conditions: (1) by elevation of pH, or by subjecting acidic proteinoid microspheres to a flow of water (which also raises the pH), (2) by cyclic dehydration and rehydration (Smith and Bellware, 1966) resulting in vacuolization and (3) by standing in suspension for several months. In this latter situation, the interiors show a tendency to recede from the outer boundary, which creates a compartment.

In mixed proteinoid-calcium populations compartmentalization occurs naturally during the self-assembly process, and is not limited to one vacuole per cell.

4.3. Structural stability

Acidic 2 : 2 : 1-proteinoid microspheres are stable under a variety of conditions including centrifugation and sectioning for electron microscopy. Microparticles from mixed proteinoid calcium preparation also show remark-

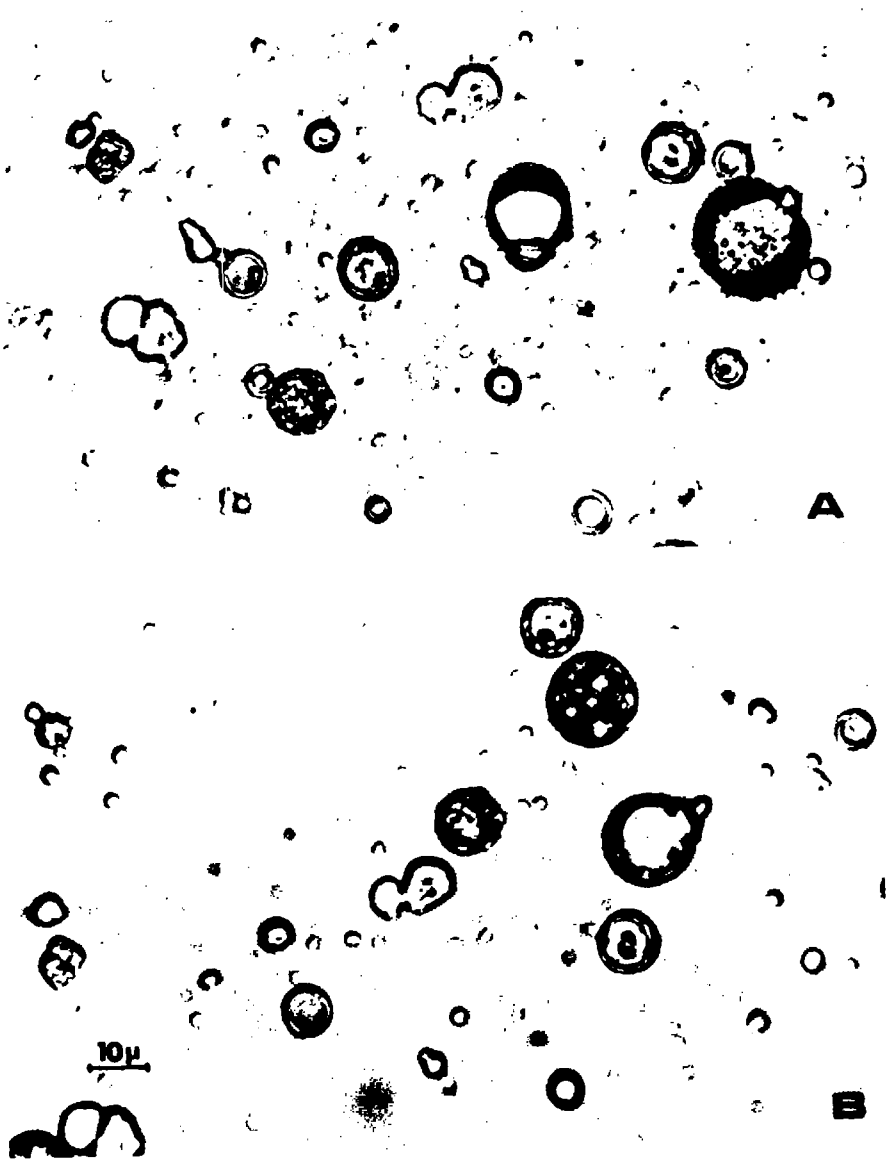


Fig. 19. Effect of temperature increase on a population of mixed proteinoid microparticles made in the presence of 0.1 M $CaCl_2$. A: the population prior to temperature increase. B: the dissolution of the smaller noncompartmentalized units after warming of the preparation with the heating stage.

able stability. They retain their structural integrity for months after repeated centrifugation and resuspension, and after being subjected to air-drying and vacuum. They retain their structure also under conditions of pH and temperature increase. This increased stability is probably in part due to the presence of calcium. Heilbrunn (1956) suggested that calcium is not only responsible for cell cortex rigidity in amoeba, but for vacuolization in general.

The coacervate droplets studied by Oparin (1968) have been made from materials such as gum arabic, gelatin, and other organic polymers. These coacervate droplets often show a tendency to vacuolize. Oparin (1953) has

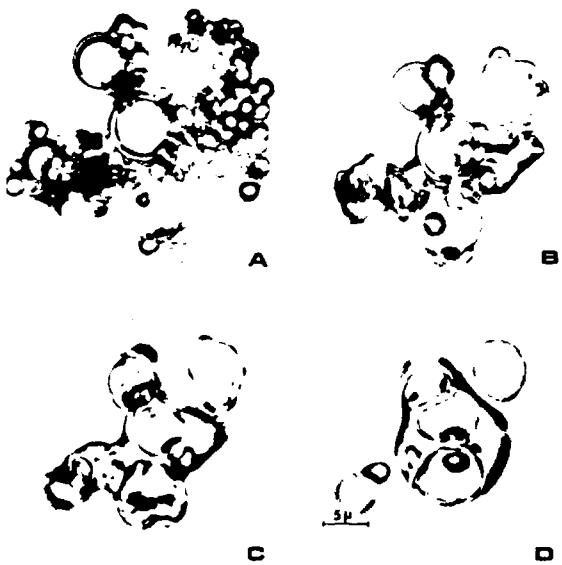


Fig. 20. Effects of temperature increase on mixed proteinoid microparticle populations made in the presence of 0.1 M $CaCl_2$. A shows the population prior to increase in temperature. B–D show the gradual dissolution of the smaller noncompartmentalized units and the fusion of others into a larger particle. D shows the final resulting unit which remained stable after cooling of the preparation.

stated that vacuolization is a characteristic that distinguishes protoplasm from most other colloidal systems. Bungenberg de Jong (1949) has further noted that coacervates show this tendency to vacuolate; he attributed the vacuolization to the coacervate becoming less hydrated. He noted, however, that "when the cause of the vacuolization of the coacervate is removed (i.e., rehydration), the vacuoles disappear and the coacervate looks as before" (Bungenberg de Jong, 1966). The vacuoles which arise in mixed proteinoid-calcium microparticles are quite stable once formed, and appear as lasting structures in the particle. This difference in vacuole stability is a significant difference between the coacervate droplet and the proteinoid microsphere as a model for the primitive cell.

Microspheres made from 2 : 2 : 1-proteinoid stain Gram-negative (6). Addition of lysine-rich basic proteinoid above 35% of the total proteinoid concentration results in a Gram-positive particle. The acidic proteinoid-basic proteinoid-calcium microparticles show a wide variety of acceptance of stain. Generally, the compartmentalized microparticles are seen to stain Gram-negative on the surface and Gram-positive in the interior regions. In individual fields, some units are either totally Gram-negative or totally Gram-positive. The findings are also experimentally supported by staining with the FAN technique (Miquel et al., 1971), which indicates an irregular distribution of acidophilic and basophilic components in units of similar composition.

Gram-positiveness has been attributed to the presence of a basic type of polymer (Stearn and Stearn, 1924; Fox and Yuyama, 1963). The results of the Gram-staining of mixed proteinoid microparticles would indicate that the more basic polymer is found in the interior regions of the compartmentalized units while the acidic components are found at the exterior. This parallels the early observations of Heilbrunn (1956) and others that the exterior regions of cells are negatively charged colloids while the main mass of protoplasm in cells are positively charged.

Mixed proteinoid microparticles show a tolerance to a wide range of pH (Fox and Yuyama, 1963; Snyder and Fox, 1975). In some experiments in this study, the pH of a suspension of mixed-proteinoid microspheres was raised from 3.8 to 9.3 with NaOH. Many of the compartmentalized units survived the increase while others did not. This type of differential survival suggests a rudimentary aspect of the primordial selection process, in addition to others reported earlier (Hsu and Fox, 1976). The differential survival resembles the mechanism of extinction, as discussed for more contemporary organisms by Lewontin (1970).

Differential heat-stability, also, is demonstrable in mixed proteinoid populations, with compartmentalized units showing a greater resistance to changes in temperature than the noncompartmentalized ones. For developing populations on the primitive Earth, such

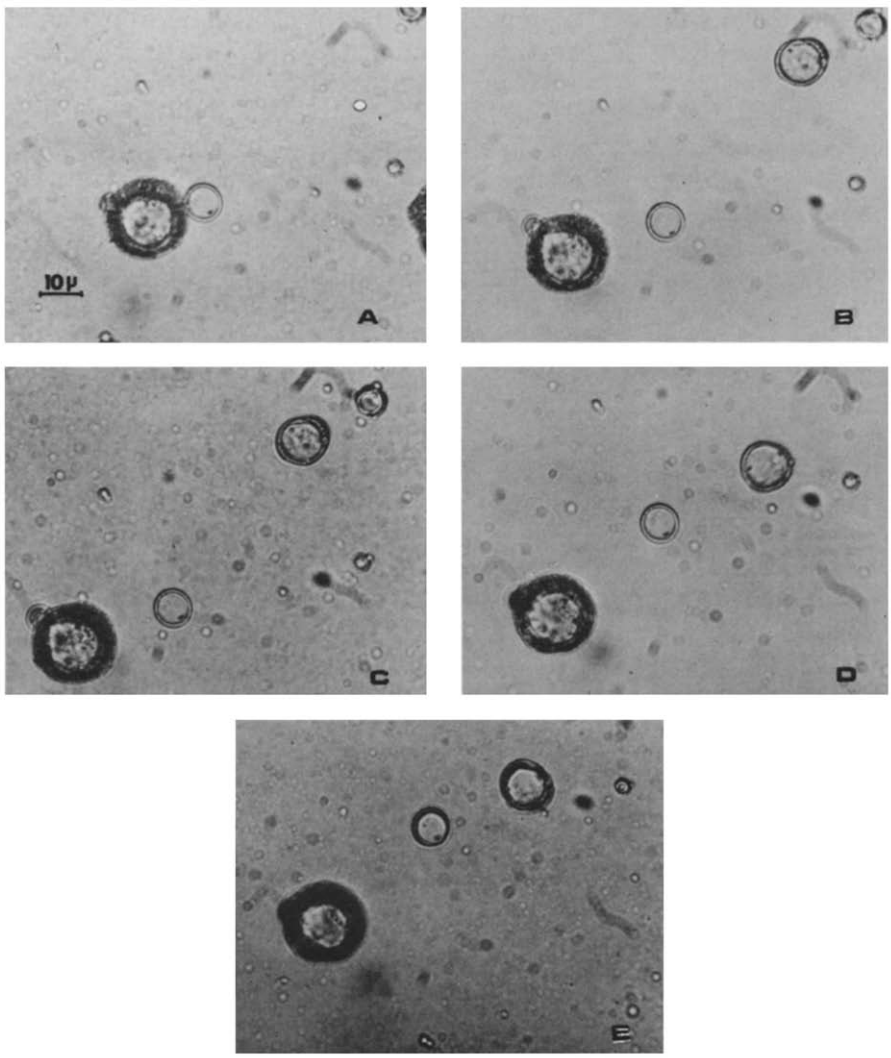


Fig. 21. Effects of heating/cooling cycles on a mixed proteinoid microparticle population made in the presence of 0.1 M $CaCl_2$. A: the population prior to temperature increase. B: effect of temperature increase with small noncompartmentalized particles dissolving. C: results of cooling to room temperature with the reformation of the smaller particles. D: results of reheating the same field with the dissolution of the small particles for the second time. E: results of allowing the preparation to again cool to room temperature showing the reformation of the small noncompartmentalized units. No apparent changes are seen in the compartmentalized units throughout the heating and cooling cycles.

resistance to changes in temperature would also have provided a selection mechanism (Snyder and Fox, 1975).

4.4. Morphodynamic properties

Discrete junctions have been reported to form between individual 2 : 2 : 1-proteinoid microspheres (Hsu et al., 1971). Smaller internal particles pass through these junctions. These so-called endoparticles are viewed as informational because of the specific structural and catalytic activities of the microspheres (Hsu and Fox, 1976) and of the component polymer (Fox, 1974). (Basic proteinoids also show selective interactions with

homopolyribonucleotides.) Thus, the passage of endoparticles between individual units serves as a model for primordial *inter*cellular communication.

A model for *intra*cellular communication is seen in mixed proteinoid microspheres. Many compartmentalized microparticles show a spontaneous tendency to form endoparticles. As indicated, these may vary in size and shape within one individual unit. Endoparticle formation, whether spontaneous or induced by change in temperature, may involve a rearrangement of material inside the compartment or may involve structural alterations in the outer boundary. Such outer-boundary changes may subsequently allow the intrusion of soluble proteinoid from the external environment. This new proteinoid may then combine and recombine with preexisting proteinoid in the interior to form the endoparticle.

Junctions have been observed between compartments within one multicompartmentalized unit. Endoparticles have occasionally been seen to pass through these junctions or through spaces between compartments. This movement, like the motion of endoparticles in 2 : 2 : 1-proteinoid microspheres (Hsu et al., 1971), owes its impulse to Brownian motion. In each case, the Brownian motion is of a constrained nature due to the confines of the compartments and the portals between them.

The presence of compartmentalized endoparticles within larger compartmentalized microparticles (Fig. 6E) suggests that these compartmentalized microparticles may also participate in the proliferation of their likeness. Although a complete cycle has not been photographed, the subsequent release of often numerous endoparticles may serve as the first step in a primitive proliferative sequence similar to contemporary sporulation. The endoparticles may, conceptually, serve as seeds around which proteinoid from the environment may accrete (Fox et al., 1967). The chief feature of the newly described process is that, unlike budding *, the new endoparticles are protected inside the mother particle until they are released by mechanical shock or otherwise. Microparticles with internal particles have been observed intact at pHs above 9.0. Other units not so protected do not survive. When not released to the environment, units as shown in Fig. 6F result. The similarities of this type to the contemporary *Echinococcus* (Wardle and McLeod, 1952) are striking. The endoparticles seen in the freeze-fracture micrograph (Fig. 13) have withstood fixation as well as freezing.

All of these phenomena point to the flexibility in these microsystems — a flexibility possible in the presence of structural integrity. Presumably, a balance between these two conditions had to have been established in order for more complex units to develop.

A limit is found to apply to the proportion of lysine-rich basic proteinoid which can be present and yet not inhibit compartmentalization. Indeed, when the weight-to-weight ratio of acidic proteinoid to basic proteinoid is less than one, the formation of any other particles as well as the formation of compartmentalized microparticles is precluded. The exact ratios whereby one can make compartmentalized particles depends upon the particular proteinoid used.

Units obtained from mixed proteinoid and calcium bear strong resemblance to some of the microfossils reported by Barghoorn and Tyler (1965). Earlier papers compared microspheres with meteoritic particles (Fox, 1964; Fox, 1966) and indicated limitations in recognition of extraterrestrial life forms. The units from mixed proteinoid-calcium populations add significantly to the types of structure that might be encountered in searches of extrater-

* Some terms in this paper, such as budding, connote processes that are more complicated in contemporary systems than in simulated protosystems. However, terms like budding, growth, behavior, etc. are used by chemists for chemical systems; they are used here also in the broader context, with the meaning defined fully only by the accumulated associated properties, in an evolutionary context.

restrial planets having substantial hydrospheres.

The proteinoid microspheres may be relevant to the compartmentalization called for by Eigen (1971) in his analysis of self-organizing phenomena. In Eigen's theory, compartmentalization and individualization characterized those systems that survived in evolution. The microspheres may provide a kind of physical representation of early compartmentalization.

5. Summary

The 2 : 2 : 1-proteinoid microspheres have an array of physical, chemical and morphodynamic properties which, when viewed alongside the catalytic and other chemical properties of its component polymer, provide a geologically plausible model of the origin of the first cells (Lehninger, 1975; Florkin, 1975; Zuckerkandl, 1975). Microparticles from two such thermally synthesized polymers in the presence of calcium chloride have properties which parallel and extend those of the acidic proteinoid microspheres. This occurs at both individual and population levels.

The most striking properties of populations of mixed proteinoid (acidic and basic) microparticles are (1) an extremely wide diversity of morphological types within any one population, (2) stable compartmentalization in individual units, (3) transfer of particles between compartments and (4) differential response to environmental changes within the members of a single population.

The populations of compartmentalized microspheres are characterized by a wide diversity of form based upon (a) different sized internal compartments, (b) the number of internal compartments, (c) the presence of smaller internal particles (endoparticles) within the compartments and (d) the presence of boundary vacuoles.

The movement of endoparticles between compartments suggests a model for a primitive intracellular communication.

Acidic proteinoid-basic proteinoid-calcium microspheres tend to stain Gram-negative on the exterior and Gram-positive on the inside when the standard Gram-stain is applied.

Compartmentalized microspheres are more resistant to increases in pH and temperature than the non-compartmentalized members of the same population, for at least several hours. This fact suggests a simple mode of selection among members of a primitive cell population.

Many of the compartmentalized microspheres have been found to resemble microfossils reported by Barghoorn and Tyler (1965).

As has been the case with much constructionistic experimentation, the degree of diversity of form obtained was not predictable from knowledge of the nature of the components alone.

Acknowledgments

From the M.S. thesis of Steven Brooke, Department of Biology, University of Miami, 1975. Research aided by Grant No. NGR 10-007-008 of the National Aeronautics and Space Administration. Contribution No. 298 of the Institute for Molecular and Cellular Evolution. We thank Mr. C.R. Windsor for amino acid analyses, and Ms. Mary Ann Hart for the freeze-fracture preparations.

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