CURRENTS IN MODERN BIOLOGY 4 (1971) 12–25. NORTH-HOLLAND PUBLISHING COMPANY

CONJUGATION OF PROTEINOID MICROSPHERES: A MODEL OF PRIMORDIAL COMMUNICATION

Laura Ling HSU, Steven BROOKE, and Sidney W. FOX
Institute of Molecular Evolution, University of Miami, Coral Gables, Florida 33134, USA

Received 4 November 1970

Proteinoid microspheres, produced under geologically relevant conditions, have been found to form junctions. Some of these junctions are hollow; internal particles much smaller than the original microspheres transfer between microspheres. Since these particles contain macromolecular information, they represent a model of primordial communication and of inheritance. The phenomena observed have been viewed as an imposition of constraints on Brownian motion.

1. Introduction

This paper reports the formation of junctions between microsystems which constitute a physical model of the protocell; it also reports the transmission of internal, smaller endoparticles made possible by such relationship. The model of the protocell assembles from a polymer of self-ordered amino acids. The polymer and microsystem each arise under conditions which are geologically relevant now and are interpreted as having been relevant on the primitive Earth (Fox et al., 1970; Fox and Windsor, 1970). Many of the properties of contemporary protein and of lipoproteinaceous cells have also been demonstrated in thermal proteinoid and in proteinoid microspheres respectively (Fox et al., 1970). Behavioral phenomena such as nonrandom motility have been documented (Fox et al., 1966).

The proteinoid microsphere has been shown to participate in the proliferation of its own likeness through processes resembling budding and heterotrophic growth, by accretion, of the separated budlike units (Fox et al., 1967). Demonstration of the formation of junctions and communication therethrough possesses one set of significances. Such demonstration in association with a budding-and-growth cycle suggests additional evolutionary possibilities. Models for each of these sets are described and interpreted in this paper.

2. Methods

The production of the proteinoids has been described often (Fox and Harada, 1960; Fox and Harada, 1966) and is simple (Fromer, 1970). The production of the microspheres requires only contact of the polymer with water. The cooling of a briefly heated solution is often employed to yield uniform microparticles, although chilling of a solution at room temperature will also produce particles (Young, 1965).

In these studies, 25 to 30 mg of 2:2:1-proteinoid per ml of distilled water were mixed, and the mixture in a test tube was heated briefly to boiling in a hot water bath, and then again brought quickly to boiling twice over an open flame. The temperature remained at the boiling point for typically less than one minute (prolonged heating tends to increase heterogeneity of size). The hot clear solution was decanted into a test tube kept in a boiling water bath. The tube was allowed to cool to room temperature either in the water bath or by wrapping the tube in foam rubber sheeting. Microspheres made by this method are

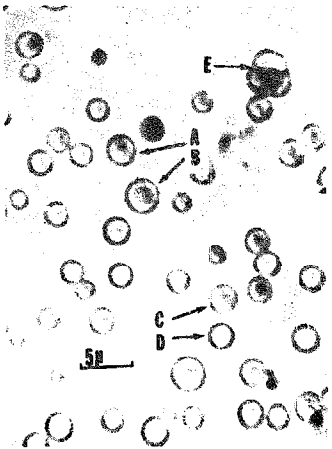

Fig. 1. Microspheres of 2:2:1-proteinoid.

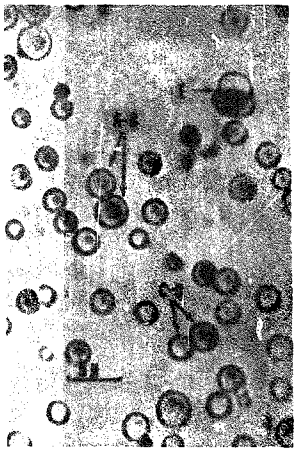

Fig. 2. Same field as fig. 1, 10 sec later. A and B have formed junctions, as have C and D. E represents a separated structure.

essentially colorless and are slowly soluble in distilled water. Crystal violet stain serves to fix somewhat the boundary of the microsphere, making it less water-soluble and more conspicuous due to the purple-blue color, while the interior of the microsphere remains unstained and more soluble in water. The staining is accomplished by shaking, for example, 10 ml of suspension with one drop of Gram's crystal violet solution. Staining of microspheres differs according to a variety of factors such as the morphological type or age of the microspheres, mode of application of the stain, density of microspheres in the suspension, pH and temperature, time of incubation with the stain, and other factors. In many of the experiments reported the microspheres were further fixed after staining by application of iodine as in the Gram stain. A number of experiments indicate that the iodine treatment can be omitted without affecting the phenomena; its use enhances the stability of the relevant structures.

Experiments such as those from figs. 1 and 2 are from a time-lapse series; the two frames shown represent a 10 second interval. In experiments such as those of figs. 11–19, 20–23, and 24–27, a drop of the suspension of stained microspheres was placed on the slide and was covered with a slip. One or two drops of water were then applied at one edge of the cover slip. In order to induce the water to flow, a strip of filter paper was carefully applied to the opposite edge of the cover slip. Specific experimental details are given in the legends beneath the figures.

3. Results

The microspheres obtained in this study ranged from 3 to 7 μm in diameter in separate preparations. The particles tend to be uniform in diameter within each preparation. Sometimes populations of two uniform sizes are observed. The larger units are more brittle and stain more readily. The outer layer of the stained particles is less soluble than the internal material. Either smaller or larger particles display the phenomena described in this paper.

The observation of bridge formation, such as has been observed in this laboratory over a period of twelve years, is in view in figs. 1 and 2. A number of pairs of microspheres have moved toward each other in the 10 second interval. On contact, they form bridging junctions. Two of these pairs are designated by arrows. These represent the typical formation of junctions. That they are not optical artifacts is indicated by experiments described later. Another kind of junction, also often seen (Fox and Yuyama, 1964), is designated E. A septum between the two lumens is discernible.

An exact morphological correlation to the occluding and septate junctions and perijunctional insulation as defined by Bullivant and Loewenstein (1968) is not evident. With endoparticles, the microspheres do exhibit a functional relationship to occluding and septate junctions, the septate type appearing to change to the occluding type during some experiments.

Fig. 3 indicates the nature of the collar junctions in these systems. Collar A is intact, collar B is cracked, and collar C has some asunder. Fig. 4 shows an elongated junction, considerably more extended than the collars which typically form on contact of microspheres. Fig. 5 shows a collar attached to a single microsphere. This resulted when a member of the pair of microspheres broke off. Figs. 6 and 7 depict a plug-and-socket type of association.

In fig. 8 can be seen the socket type of orifice left when the plug has pulled away. This orifice leads into the interior of the microsphere, as observed by escape of endoparticles.

Figs. 9 and 10 show bud-like projections differing in some detail from those described earlier (Fox and Yuyama, 1964; Fox et al., 1967). In fig. 9 the large microsphere possesses a pre-budlike structure. Fig. 10

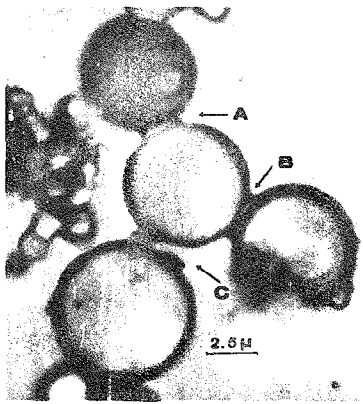

Fig. 3. The connecting structures between proteinoid microspheres are shown in various stages of breakage. The microspheres were prepared by heating 25 mg of 2:2:1-proteinoid per ml of distilled water, and decanting. The solution was allowed to cool slowly and was then aged 3 months at room temperature. A is intact, B shows fracture, C has come asunder.

shows another bud-like appendage in a microsphere preparation which has aged three months. The interior of the mother microsphere has spontaneously shrunk from the walls.

Mention should be made also of the tendency of nucleoproteinoid microparticles to form junctions (fig. 2 in Waehneldt and Fox, 1968).

As water flows through the microsphere, the interior dissolves inward from the persisting boundary, to yield enclosed smaller particles. These smaller particles are referred to as endoparticles. The entire process can be recorded in time-lapse or cinematomicrographic studies. Somewhat similar observations, resulting from dissolution caused by increase of pH in

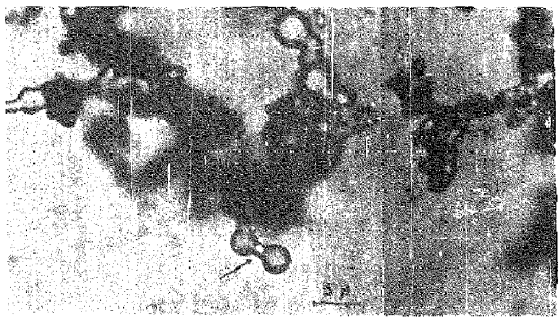

Fig. 4. An elongated junction between two proteinoid microspheres, aged 2 weeks.

Fig. 5. The collar-like structure remained on this microsphere when the latter was severed from its partner. Separation may also occur by fracturing of collar-like structures, as shown in fig. 3.

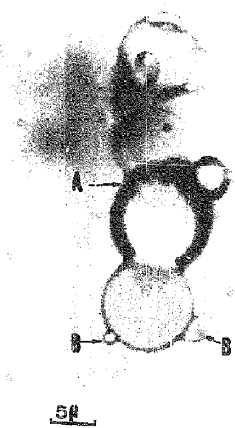

Fig. 6. (A) One example of a plug-and-socket connection between two microspheres. (B) Two budlike structures can also be seen.

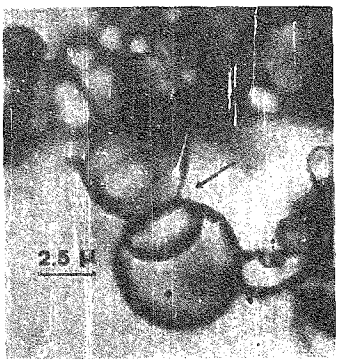

Fig. 7. An alternative form of connection is seen in this plug-and-socket pair. This type of junction occurs less frequently than the collar like connections shown in figs. 2–4.

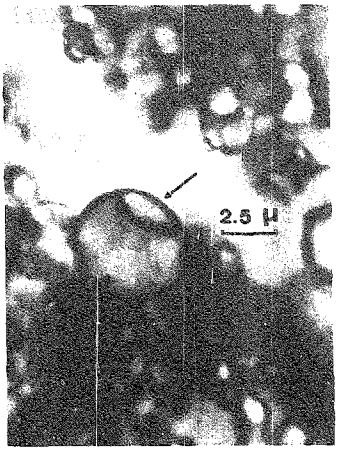

Fig. 8. The orifice on this microsphere remained when two connected microspheres separated. Similar openings can be induced on free surfaces of microspheres by subjecting them to controlled solution with distilled water (figs. 11–12). The contents of such microspheres can pass freely into the surrounding environment (figs. 11–12, 18–19).

the suspension, have been recorded earlier (Fox and Yuyama, 1964). The salient feature resulting from the flow of water is the transfer of endoparticles through connecting junctions. In addition, orifices which appear in the boundary of the microsphere permit some endoparticles to pass into the environment (figs. 11–12, 17–19).

The endoparticles are found within the microspheres, and are neither above nor beneath them in the field of the microscope. This fact has been established by watching the process of their appearance as residues in the microspheres resulting from partial solution of the contents (e.g. figs. 11–13). Additional evidence is the fact that these endoparticles (a) undergo Brownian motion and (b) are made to move with a flow of water within the confinement of their mother microspheres until they happen to reach an orifice which connects to another microsphere or to the surrounding medium (figs. 11, 12, 17–19).

Figs. 20–23 present additional evidence of transfer of endoparticles. The phenomena of three series, figs. 11–19, 20–23, and 24–27 have features in common, when the experiments are performed as indicated. The endoparticles are observed without experimental manipulation (figs. 10, 28), but the movements are most easily recorded when the flow is induced.

In addition to migrating from one microsphere to another, the endoparticles can transfer successively through two or more microspheres (figs. 24–27).

4. Discussion

An extensive literature on contemporary modes of cellular communication (cf. Loewenstein, 1967) is available for comparison and contrast with models of

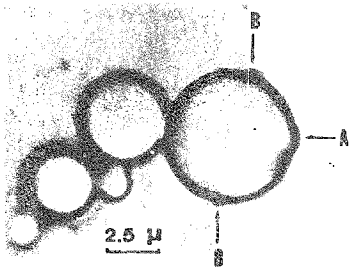

Fig. 9. (A) An extrusion from the mother microsphere is precursor to a bud, analogous in some respects to those produced by contemporary micro-organisms such as yeasts. (B) Bud-like appendages as depicted occur frequently in microsphere preparations aged 1 week or more. These buds may have had their origin as independent small microspheres which subsequently became intimately attached to the larger microsphere by the process of approach of figs. 1–2.

the primitive. The junctions which are seen in proteinoid microspheres may be considered as evolutionary precursors of structures involved in intercellular communication.

The ease with which the collars form and become firm seems to be a property of proteinoid (perhaps polyamino acid in general) microparticles. This plasticity is remarkable; it was not predicted nor would it have been predictable. We might have anticipated such structure-forming ability from particles having the capacity for internal synthesis of polyamino acids, as has been sought (Krampitz and Fox, 1969). The fact that these microsystems, composed of the kind of preformed polymer of which they are constituted, have such properties deserves further investigation. Is this a property of the material alone, of the size range of the microsystems (Went, 1968), or both? Do particles just above the colloid range of size have unique properties as microsystems? The ages of the polymer and of the microspheres are evidently a factor. The unpredicted nature of the phenomena, however, is a consequence common to much of the related constructionistic research (Fox et al., 1970).

Fig. 10. The interior of this microsphere, aged 3 months, has receded from its walls without experimental manipulation, showing the place and nature of attachment of the two bud-like appendages.

Of particular interest is the intrinsic tendency of proteinoid microspheres to communicate. A basis for the origin of communication can be visualized through these microsystems as a model. The significance of this interpretation is most meaningfully examined in association with the other properties of the microsystems, the material of which they are composed, and the geological relevance of the processes used for their preparation (Fox et al., 1970).

The essential material for the phenomena described in this paper and other phenomena reported elsewhere (Fox, 1969) is a heteropolyamino acid. This material has been shown to form as a highly ordered linear polymer (Fox and Nakashima, 1967; Usdin et al., 1968; Dose and Zaki, 1971). A number of laboratories have shown that diverse proteinoids each has enzymelike activities (Rohlfing and Fox, 1969).

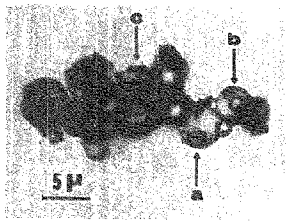

Fig. 11.

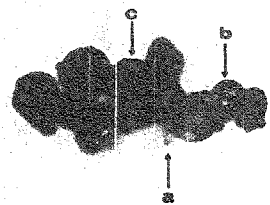

Fig. 12.

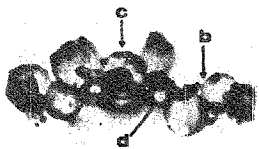

Fig. 13.

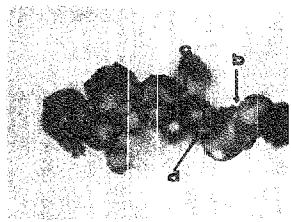

Fig. 14.

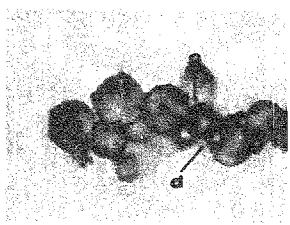

Fig. 15.

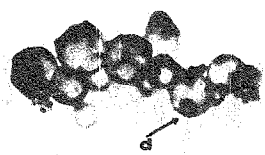

Fig. 16.

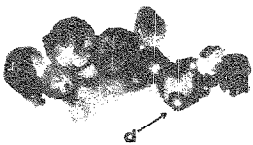

Fig. 17.

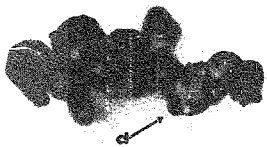

Fig. 18.

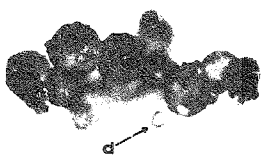

Fig. 19.

Figs. 11–19. Microspheres in this series of photomicrographs were prepared by boiling a mixture of 25 mg of 2:2:1-proteinoid per ml of distilled water, decanting, and allowing the solution to cool slowly to room temperature. Incubation at room temperature for 2 weeks followed. Microspheres were then stained with crystal violet and stabilized with iodine solution. Controlled solution of the interior was accomplished by allowing distilled water to stream through a sample on a microscopic slide. This series of photomicrographs was taken at 2 min intervals approximately 20 min after the onset of solution.
Endoparticle (a) escapes into the environment (figs. 11–12) while endoparticle (d), originated from a microsphere immediately below, is seen to exit through the same route and portal as used by endoparticle (a) (figs. 13–15). The process of formation of endoparticle (c) (figs. 11–13) shows the existence of a communicative tunnel prior to solution and the subsequent formation of discrete endoparticles. Endoparticle (c) is shown entering a neighboring microsphere (figs. 14–15). The movement of endoparticle (b) can be traced in figs. 11–14.

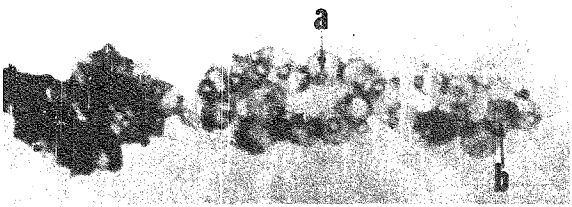

Fig. 20.

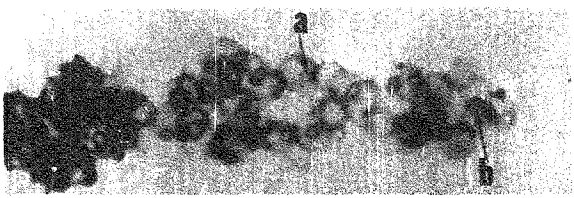

Fig. 21.

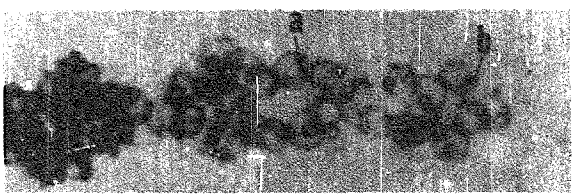

Fig. 22.

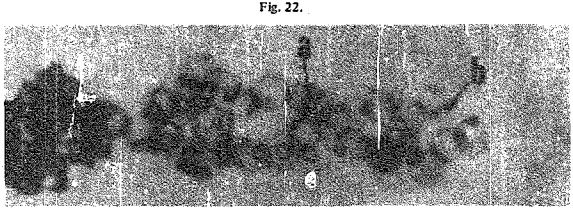

Fig. 23.

Figs. 20–23. Time-lapse series showing transfer of endoparticles. (a) and (b) at 10 sec intervals.

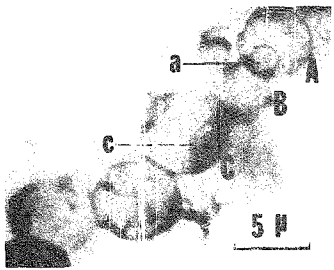

Fig. 24.

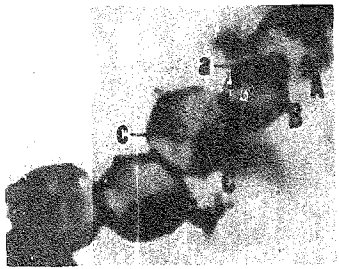

Fig. 25.

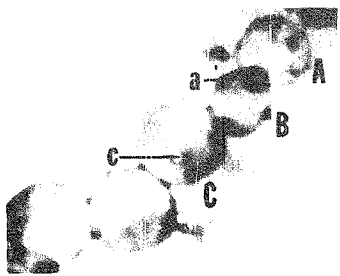

Fig. 26.

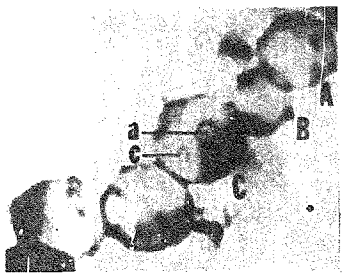

Fig. 27.

Figs. 24–27. Microspheres in this series of photomicrographs were prepared and treated in the same manner as those in figs. 11–19. The photomicrographs were taken approximately 20 min after the onset of solution with distilled water at 5 min intervals. Endoparticle (a), which originated in microsphere A, is shown to enter into microsphere C after having traversed microsphere B.

The heteropolyamino acids, including proteinoids prepared thermally, have a variety of other properties in common with contemporary proteins (Fox, 1969). Contemporary proteins, indeed, can be regarded as evolved and highly specialized members of the class of heteropolyamino acids. The much higher activities of some contemporary enzymic proteins are explained by their having evolved through processes of Darwinian selection (Calvin, 1962; Fox, 1953).

The proteinoid microspheres have already been recognized as dynamic in their properties (Fox and Yuyama, 1964; Fox et al., 1966; Fox, 1969). They permit selective passage of micromolecules as contrasted to macromolecules (Fox et al., 1969), they

display a mode of proliferation suggesting a primitive precursor process for replication by budding (Fox et al., 1967), cleavage into daughter particles can be elicited (Fox and Yuyama, 1964), and they shrink or swell in response to atonicity (Fox et al., 1969).

While they are dynamic, they also possess stability comparable to that of contemporary cells. This structural integrity, for example, permitted their being embedded in methacrylate blocks which were sectioned for electron microscopy such that a double layered membrane was identified (Fox, 1968).

The production of polymer and microsystem occurs under experimental conditions which exist on the contemporary Earth: temperatures above the boiling point of water, and entrance of water in that order. Those conditions, as well as the reactant amino acids, are inferred for the primitive Earth (Fox, 1969). Such phenomena as are described in this paper are intrinsic to the heteropolyamino acid and to the microsystems formed under these conditions.

The connections between microspheres, such as depicted in figs. 2 and 3, have been observed over a period of years in this laboratory, by a number of investigators. These connections have often resulted from contact during (Brownian) movement of two or more microspheres. A minor doubt has existed in that what has been seen might have been optical artifacts. The studies reported here have eliminated that doubt. Moreover, close examination indicates that the connections are tubular and are present prior to solution (figs. 11–14); they permit the passage of the particles smaller than their diameter and endoparticles are formed spontaneously (figs. 11–19, 20–27). The development of this understanding parallels in some respects the studies of bridges between contemporary cells (Fawcett, 1960).

The phenomena in general are reminiscent of the reproductive behavior of *Spirogyra* (Plunkett, 1934) which conjugate in a morphologically simple manner. *Spirogyra* exist in a colonial conformation, and transfer material through a conjugation tube during a reproductive phase, in a somewhat analogous manner.

Other well known biological phenomena are suggested by these structures and behavior, for which they serve as models of evolutionary precursors. Such phenomena include mating in *Paramecia* (Storer,

Fig. 28. Proteinoid microspheres prepared by boiling a mixture of 25 mg of 2:2:1-proteinoid per ml of distilled water, decanting, allowing to cool to room temperature, and then aging for 4 weeks. The interiors of two microspheres have receded from the walls upon aging, with no experimental manipulations (fig. 10). The microspheres are connected in cluster, and are potentially communicative, as illustrated in earlier figures.

1943), transfer of cytoplasmic factors (Sonneborn, 1948-1949), and transmission of vesicles in axons (Schmitt, 1969). All of the structures mentioned, including the endoparticles, are in size near the limits of visibility in the optical microscope.

An especially suggestive observation is that of the ease with which nucleoproteinoid microparticles also form "bridges" (fig. 7b in Fox, 1969). Nucleic acids are, however, not required for the kind of information transfer modelled here by endoparticles of proteinoid. An evolutionary intermediate without nucleic acids is, however, consistent with the proteins-first or the cells-first hypothesis (Moody, 1970; Lehninger, 1970).

The informational content of proteinoid has been demonstrated and studied in four contexts. Proteinoids are self-ordered; the arrangements of their monomeric residues are highly nonrandom (Fox and Nakashima, 1967). Secondly, varied proteinoids show a variety of relatively specific enzymelike activities (Rohlfing and Fox, 1969). Thirdly, basic proteinoids of varied constitution show selective reactions with homopolyribonucleotides in the formation of nucleoproteinoid microparticles (Yuki and Fox, 1969; Lacey et al., 1970). Lastly, the acidic or neutral proteinoid molecules assemble themselves into proteinoid microparticles (Fox, 1969).

The proteinoid microsphere thus represents a simply produced system for the transmission of information. This information existed in a latent form in diverse mixtures of diverse amino acids and was converted to macromolecules of limited heterogeneity by virtue of this diversity. The informational content of the polyamino acids was then transferred into the individual microspheres comprised of such polymer. The information that resided within microspheres could transfer from one to another, as demonstrated in the experiments reported. Physical transfer of information could then occur during eons of proliferating generations of proteinoid microspheres. A later step in evolution would have been the emergence of an internal synthesis of polyamino acid and a nucleic acid templet mechanism based on a code (Fox, 1969).

The evidence that Brownian motion contributed to the processes recorded in this paper raises questions about Brownian motion in biological phenomena in general. Brownian motion (Finstein, 1908) is often thought of as being "random". The kinds of order which are relevant here can exist at various levels, e.g., in monomeric composition of polymers, in sequential arrangement of monomers in polymers, in conformation of polymers, in polymer-polymer assembly in particles, and in motion of the particles (Fox et al., 1966). An assumed randomness in motion of endoparticles would thus have to be explained relative to order in the macromolecular composition of the endoparticles.

The proteinoid microsphere demonstrates that apparent Brownian motion can contribute to a model of primordial communication by providing the impulses from which a transfer of intramicrospheric particles emerged in evolution. The motion of the particle appears to be Brownian and may have been random within the microsphere but the fixed portals within the constrained structural system were directive, thus eliciting increased nonrandom motion. Similarly, in a model of motility (Fox et al., 1966), symmetrical particles appeared to be undergoing Brownian motion. One asymmetric particle, however, described paths of observably nonrandom "motility". This behavior can be understood also as a derivative of Brownian motion in that the asymmetry of one particle provides constraints which render motion increasingly nonrandom. At the more evolved level, flagellated cells represent highly asymmetric organisms.

The depiction of the ejection of an endoparticle from a microsphere (figs. 11, 12, 17–19) bears on earlier studies of proliferation of proteinoid microspheres through simulation of budding, and growth of what appeared as separated buds (Fox et al., 1967). Evidence has shown two possible sources of the buds: (a) initiation within the mother particle (fig. 9) and (b) independent formation of particles of varying sizes, followed by prompt attachment to one another. The ejection of a particle is a kind of phenomenon demonstrating that budded particles could have formed, and separated from, the mother microsphere. Figs. 6, 9, and 10 show bud-like projections from a proteinoid microsphere.

These phenomena of alteration of structure are occasionally recorded (fig. 9) and are seen far less often than are the microspheres bearing bud-like appendages of undetermined origin. Even if one assumes that the only true primitive budding is of the kind shown in fig. 9, followed by separation and heterotrophic growth by accretion, the efficiency of the proliferation processes observable now appears to be low. A proliferation process of low efficiency is nevertheless a process of proliferation. In the evolutionary context, processes of continually higher efficiency would come to dominate the generational repetitions.

Since the experiments demonstrate that particles composed of informational macromolecules can transfer between microspheres, the necessity for a primitive mechanism close to that of contemporary budding is alleviated. The more meaningful phenomenon was a transfer of material from one protocell to another, as a means for transferring in-

formation to a later generation. This kind of descendant information could, for example, have built up metabolic pathways by combining in new microspheres and recombining supplementary enzymelike activities found in varied proteinoids (Rohlfing and Fox, 1969).

While communication between microspheres of the same generation can serve as a model for the origin of informational transmission, communication between microspheres of succeeding generations serves as a model for genetic inheritance. Perhaps of most interest is the suggestion, emerging from the experiments, that information transfer and genetic inheritance had the same physical origin in evolution.

Acknowledgement

Discussions on Brownian motion in biological phenomena, with Dr. Behram Kursunoglu of the Center for Theoretical Studies, are gratefully acknowledged. The research in this paper has been aided by Grant no. 10-007-008 of the National Aeronautics and Space Administration. Contribution no. 177 of the Institute of Molecular Evolution.

References

Bullivant, S. and W.R.Loewenstein, 1968, Structure of coupled and uncoupled cell junctions, J. Cell Biol. 37, 621.

Calvin, M., 1962, Communication: from molecules to Mars, Bull. Am. Inst. Biol. Sciences 12, 29.

Dose, K. and L.Zaki, in: The origin of life, eds.: R.Buvet and C.Ponnamperuma (North-Holland Publishing Co., Amsterdam) in press.

Einstein, A., 1908, Elementary theory of Brownian motion, Z. Elektrochem. 14, 235.

Fawcett, D.W., 1961, Intercellular bridges, in: Experimental Cell Research, Supplement 8, 174.

Fox, S.W., 1953, A correlation of observations suggesting a familial mode of molecular evolution as a concomitant of biological evolution, Am. Naturalist 87, 253.

Fox, S.W., 1968, Natural polymers: abiotic polymerization and self-organization, in: Encyclopedia of polymer science and technology, vol. 9, eds.: H.F.Mark, N.G.Gaylord, and N.M.Bikales (Interscience, New York) p. 284.

Fox, S.W., 1969, Self-ordered polymers and propagative cell-like systems, Naturwissenschaften 56, 1.

Fox, S.W.and K.Harada, 1960, Thermal copolymerization of amino acids common to protein, J. Am. Chem. Soc. 82, 3745.

Fox, S.W. and K.Harada, 1966, Thermal polymerization of α-aminoacids, in: A laboratory manual of analytical methods of protein chemistry, vol. IV, eds.: P.Alexander and H.P.Lundgren (Pergamon Press, Oxford) pp. 127–151.

Fox, S.W. and T.Nakashima, 1967, Fractionation and characterization of an amidated thermal 1:1:1-proteinoid, Biochim. Biophys. Acta 140, 155.

Fox, S.W. and C.R. Windsor, 1970, Synthesis of amino acids by the heating of formaldehyde and ammonia, Science 170, 984.

Fox, S.W. and S.Yuyama, 1964, Dynamic phenomena in microspheres from thermal proteinoid, Comp. Biochem. Physiol. 11, 317.

Fox, S.W., R. McCauley, D. Joseph, C.R. Windsor, and S.Yuyama, 1966, Simulation of organismic morphology and behavior by synthetic poly-α-amino acids, in: Life sciences and space research, vol. IV, eds.: A.H.Brown and M.Florkin (Spartan Books, Washington, D.C.) p. 111.

Fox, S.W., R.J.McCauley, and A.Wood, 1967, A model of primitive heterotrophic proliferation, Comp. Biochem. Physiol. 20, 773.

Fox, S.W., R.J.McCauley, P.O'B.Montgomery, T.Fukushima, K.Harada, and C.R.Windsor, 1969, Membrane-like properties in microsystems assembled from synthetic protein-like polymer, in: Physical principles of biological membranes, eds.: F.Snell, J.Wolken, G.J.Iverson, and J.Lam (Gordon and Breach, New York) p. 417.

Fox, S.W., K.Harada, G.Krampitz, and G.Mueller, 22 June 1970, Chemical origins of cells, Chem. Eng. New 48, 80.

Fromer, C., in: Stong, C.L., 1970, Experiments in generating the constituents of living matter from inorganic substances, Sci. American 222, 130.

Krampitz, G. and S.W.Fox, 1969, The condensation of the adenylates of the amino acids common to protein, Proc. Nat. Acad. Sci. U.S. 62, 399.

Lacey, J.C., Jr., T. Nakashima, J.R. Jungck, and S.W. Fox, 1970, Influences of polynucleotides in the chemical condensation of mixed anhydrides of adenylic acid and amino acids, Federation Proc. 29, 897 abs.

Lehninger, A., 1970, Biochemistry (Worth Publishers, New York) p. 776.

Loewenstein, W.R., 1967, On the genesis of cellular communication, Develop. Biol. 15, 503.

Moody, P.A., 1970, Introduction to Evolution, 3rd ed. (Harper and Row, New York) p. 119.

Plunkett, C.R., 1934, Elements of modern biology (Henry Holt & Co., New York) p. 295.

Rohlfing, D.L. and S.W.Fox, 1969, Catalytic activities in thermal polyanhydro-α-amino acids, Advances in Catalysis 20, 373.

Schmitt, F.O., 1969, Fibrous proteins – neuronal organelles, in: Neurosciences research symposium summaries, Vol. 3, eds.: F.O.Schmitt, T.Melnechuk, G.C.Quarton, G.Adelman (MIT Press, Cambridge, Mass) p. 56ff.

Sonneborn, T.M., 1948–1949, Cellular transformations, Harvey Lectures, Ser. 44, 145.

Storer, T.I., 1943, General Zoology (McGraw-Hill, New York) p. 283.

Usdin, V.R., M.A.Mitz, and P.J.Killos, 1967, Inhibition and reactivation of the catalytic activity of a thermal α-amino acid copolymer, Arch. Biochem. Biophys. 122, 258.

Waehneldt, T.V. and S.W.Fox, 1968, The binding of basic proteinoids with organismic or thermally synthesized polynucleotides, Biochim. Biophys. Acta 160, 239.

Went, F.W., 1968, The size of man, Amer. Scientist 56, 400.

Young, R.S., 1965, Morphology and chemistry of microspheres from proteinoid, in: The origins of prebiological systems, ed.: S.W.Fox (Academic Press, New York) 347 - 356.

Yuki, A. and S.W.Fox, 1969, Selective formation of particles by binding of pyrimidine polyribonucleotides or purine polyribonucleotides with lysine-rich or arginine-rich proteinoids, Biochem. Biophys. Res. Communs. 36, 657.