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ANCIENT MICROSPHERES: ABIOGENIC, PROTOBIOGENIC, OR BIOGENIC?

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ABSTRACT

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Criteria of biogenicity of microspheroidal objects, which have been interpreted as microfossils, are here reviewed in the light of additional data. Much weight has been placed by some commentators on constrained heterogeneity as a primary criterion of biogenicity. The data from the field and laboratory suggest the need for continuing reservation in the interpretation of these objects. On the basis of these and other data, reasons are given for the alternative explanation that the objects are lithified relics of protobiotic assemblages. The question remains open as to whether the early Archean spheroidal objects are abiotic, protobiotic, or biotic in origin.

INTRODUCTION

The probability that the first organisms on Earth were unicellular forms in the micron range of size has developed from: (1) the logical consideration that unicells are simpler than multicellular forms; (2) protocell models constructed from simpler components (Herrera, 1942; Fox et al., 1959); and (3) studies of objects interpreted as microfossils (Barghoorn and Tyler, 1963; Schopf, 1976). The difficulty of, and need for, avoiding overinterpretation and underinterpretation of the evidence for (3) has been articulated by Schopf (1975). The interpretations in general have, however, leaned toward acceptance of the objects as fossils of ancient biotic microstructures. This tendency has been expressed by such statements as "the most reasonable explanation for this population of microstructures is that it consists of fossil microorganisms arrested and preserved in the midst of biological activity"

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(Knoll and Barghoorn, 1977) to "almost unquestionably biogenic" (Doolittle, 1980). The evidence regularly invoked in such studies includes: morphology, size, sedimentary context (Ford, 1980), appearance of division (Knoll and Barghoorn, 1977), and unimodality of size distribution (Schopf, 1975, 1976; Ford, 1980; Lenk et al., 1982). All of these criteria, especially the last, and except by necessity sedimentary context, are examined in this paper from tests used for relating 'microfossils' to modern biogenic organisms. As the evidence in this paper indicates, the 'microfossils', now also referred to as microspheres (Strother and Barghoorn, 1980), may also be related to model protobiogenic microstructures* (Fox and Yuyama, 1963; Keosian, 1974; Francis et al., 1978; Cloud and Morrison, 1979; Corliss et al., 1981).

DIVISIONAL PHENOMENA

The finding of objects appearing to have been microfossils of organisms undergoing binary fission (Knoll and Barghoorn, 1977) is comparable to that which has been recorded for proteinoid microspheres in three figures in one paper (Fox and Yuyama, 1963). (Proteinoid constitutes one kind of thermal copolyamino acid.) Such paired structures may be the result of junction formation and coalescence (Hsu et al., 1971), internal division (Fox and Yuyama, 1964), or external pressure (Fox and Yuyama, 1963). A comparison of reported microfossils with the divided artificial cells is presented in Fig. 1A and B. Since proteinoid microspheres made in the laboratory provide with ease the kind of paired appearance seen in the 'dividing microfossils', this criterion is especially indicative of the possibility that the terrestrially lithified objects were originally proteinoid microspheres.

ARTIFICIAL SILICIFICATION

Since the structure of proteinoid microspheres from the laboratory and microstructures of unknown origin from Archean strata are both seen to be based on copolyamino acids (Fox and Dose, 1977) prior to lithification, similar silicification and diagenesis can be expected to have occurred for each. The fact that normally cellular structure is rooted in proteins is long known, e.g., Giese (1962) stated, "Proteins are responsible for the characteristic structure of the cell." The similarity to proteins of thermal copolyamino acids (proteinoids) is illustrated by the fact that the latter have been listed in Chemical Abstracts as "proteins, thermal", since 1972. Much of the detailed supporting data for such evaluation can also be found in a single reference (Fox and Dose, 1977). By nearly all protein tests, thermal polymers of amino acids and proteins from modern organisms are indistinguishable.

*A reviewer of this paper has proposed that the 'microfossils' be referred to as carbonaceous microspheroids.

Moreover, both proteinoid and organismic protein can be lithified. Francis et al. (1978) have applied a new method of artificial fossilization in the laboratory to proteinoid microspheres and to algae by using tetraethyl orthosilicate as a lithifying agent. They have thus documented that silicified proteinoid microspheres and algae are comparable morphologically.

The similarity in morphology and texture between proteinoid microspheres, spheroidal Archean fossils, and artificially fossilized proteinoid microspheres and artificially fossilized algae are illustrated in Fig. 1. Figure 1A shows dividing proteinoid microspheres (Fox and Yuyama, 1964), Fig. 1B divided or paired microfossils (Knoll and Barghoorn, 1977), Fig. 1C artificially fossilized proteinoid microspheres (Francis et al., 1978), and Fig. 1D artificially fossilized algae (Francis et al., 1978). Common to all four types are cell-like assemblies of proteins, organismic or artificial.

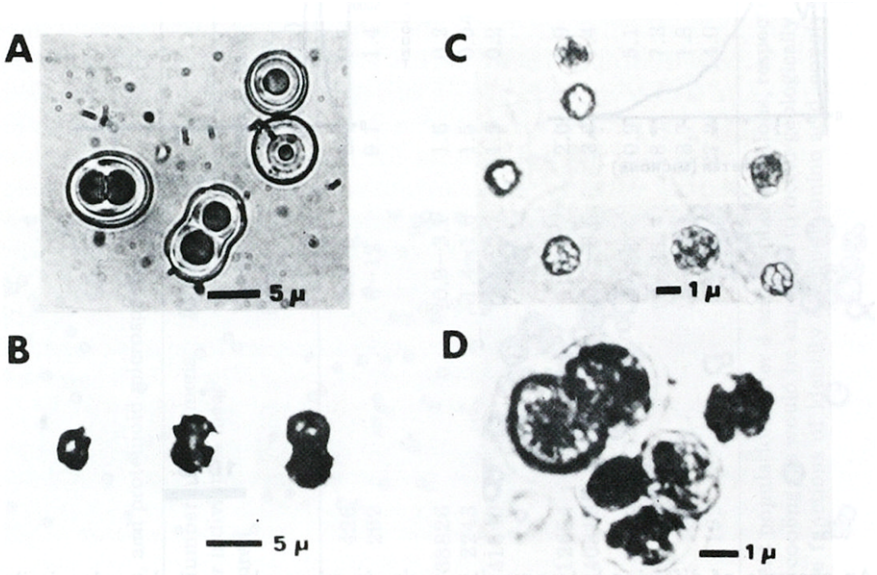

Fig. 1. (A) Stages of twinned proteinoid microspheres (Fox and Yuyama, 1964). These were observed to be undergoing at least partial fission. (B) Stages of twinned microfossils reported as undergoing division (Knoll and Barghoorn, 1977). (C) Artificially fossilized proteinoid microspheres (Francis et al., 1978). (D) Artificially fossilized *Chlorogloea microcystoides* (Francis et al., 1978).

DIVERSITY IN SIZE

The experiments that are possible with copolyamino microspheres, but not possible with fossils, have singled out several parameters that determine the degree of heterogeneity in size. The parameters include: identity of copolyamino acid, ratio of solid to solution in the spherulization, mode (rate) of cooling of the heated solution, and added substances. As an example of

one of these, a thermal polymer from mixed amino acids from a modified Fischer–Tropsch synthesis (Hayatsu et al., 1971; Yoshino et al., 1971) was treated with a given amount of water, and also with six times as much water; the two were heated simultaneously in the same hot water bath. The degree of heterogeneity in size of the microspheres that separated on cooling was assessed by measurements of the diameter of several thousand microspheres in a calibrated flow cytometer. The distributions of diameter measurements are shown in the upper portion of Fig. 2.

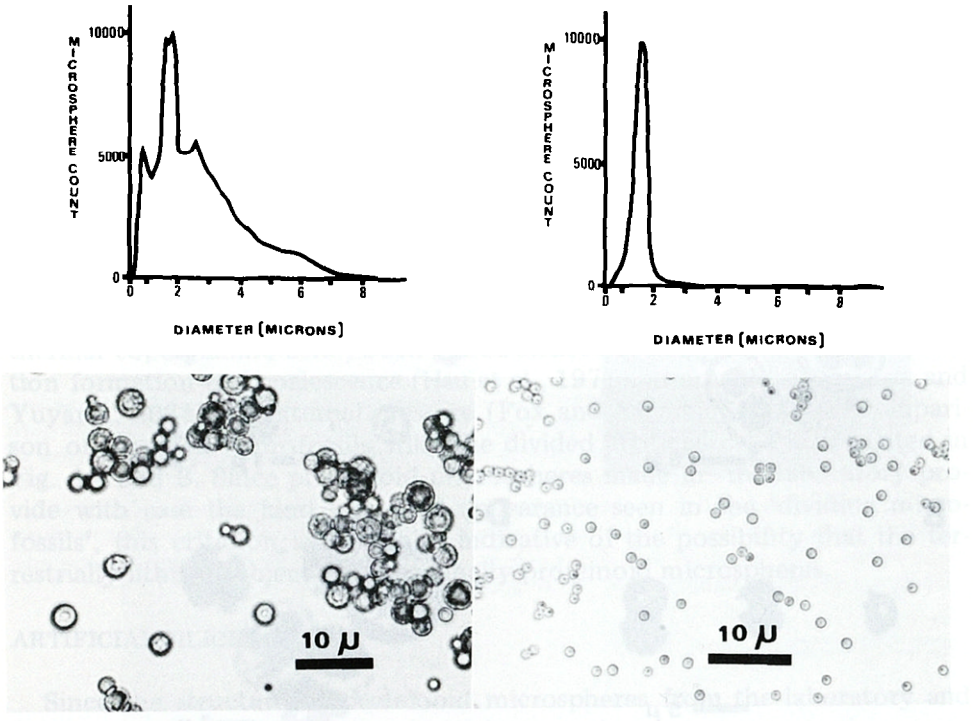

Fig. 2. An example of differing heterogeneity in size in microspheres made under similar but differing conditions. The polymer was produced from an amino acid mixture such as reported in run No. 21 of the Anders' group (Yoshino et al., 1971). That mixture was heated at 225°C under nitrogen for 18 h. Polymer from 91 g of mixture was heated with 500 ml of H_2O (left). An aliquot was heated with 6 volumes of hot water and also cooled (right). The individual diameters of several thousand microspheres from each preparation were measured by forward angle light scatter on a calibrated EPICS IV Cell Sorter (Coulter Electronics, Inc., Hialeah, FL 33010). The histograms of distribution of diameter in these samples are shown in the upper half of the figure. The lower half illustrates by photomicrographs the different degrees of heterogeneity in these two samples.

The diversity in size of microspheres was also examined by flow cytometry on artificial particles from thermal copoly (aspartic acid, glutamic acid, glycine, alanine) aggregated into spherules four times, and the same process repeated once to total five times (Table I). Also used was the thermal poly-

TABLE I

Modern unicellular algae, fossil unicells, and proteinoid microspheres

Modern unicellular alga	Number of specimens or individuals measured	Diameter				
		Range (μm)	Mean (μm)	Std. dev. (μm)	Coefficient of variation (%)	Divisional dispersion index (DDI)
Chlorella sp.[a]	426	1.5—5	2.7	0.7	24	5
Tetraspora sp.[a]	292	6—12	9.1	1.4	15	3
Microspheres of small dispersity						
RS Copoly (asp, glu, his)	38826	0.9—3.1	1.6	0.2	13	5
RS 94-4 Copoly (asp, glu, gly, ala)	2243	1.4—1.6	1.5	0.04	3.2	1
RS 6-106B (Fig. 2, Right)	41540	1.1—3	1.9	0.2	9.1	4
Microspheres of large dispersity						
RS 94-5 Copoly (asp, glu, gly, ala)	12654	1.2—3	2.0	0.9	18	4
RS 6-106 A (Fig. 2, Left)	240816	0.7—8.5	3.0	1.4	56	11
Orgueil elements[a]	268	2—36	10.9	5.1	46	13
Precambrian spherules[a]	19	10—36	18.7	7.3	39	—
Swartkoppie Fm[a]	28	15—24	18.7	1.8	10	—
Swaziland-3[a]	192	6—74	27.2	14.0	51	—

Microspheres RS 94-4 and 94-5 represent populations after 4 and 5 spherulizations, respectively, of poly (asp,glu,gly,ala) by solution in hot water followed by cooling, as would be expected to occur geologically. Experiments have shown that both size and heterogeneity of size are functions of identity of copolyamino acid, copolyamino acid/water ratio, and of rate of cooling.

[a]Data from Schopf (1976).

mer from an amino acid mixture resulting from a modified Fischer—Tropsch synthesis (Fig. 2 photomicrographs). These samples were selected from a wide range of results to illustrate extremes in distribution of size. The polymer of aspartic acid, glutamic acid, glycine, and alanine was made because those four amino acids are dominant in hydrolyzates of extracts of lunar samples and meteorites (Saunders and Rohlfing, 1972; Harada and Hare, 1980; Fox et al., 1981). Table I shows statistical parameters from each of these samples in comparison with those calculated for modern unicellular algae and 'fossil' unicells (see Schopf (1976) for a discussion of these statistical parameters).

All of the microspheres of Table I except those with the RS designation were obtained from the geological realm in the locales indicated by citation; the RS series was produced in the laboratory.

The principal inference drawn from this table is that aggregation products of thermal polymers of amino acids, of the kinds that plausibly would have arisen on the Earth (Rohlfing, 1976; Corliss et al., 1981; Follmann, 1982), can be either more heterogeneous in size or less heterogeneous in size than are algae and other forms used for assessment of biogenicity. Thus, unimodality or dispersion of size are not criteria for inferring biogenic or nonbiogenic history, especially of early Archean assemblages. Related to this judgment is the possibility that replicating cells devoid of templated protein synthesis arose very early in evolution (Fox and Dose, 1977; cf. Eigen and Schuster, 1978; Fox, 1981; Follmann, 1982).

In a theoretical analysis, Dyson (1982) infers for the earliest cells several of the same interpretations that have been earlier derived from experimental model-building (Fox and Dose, 1977; Fox, 1981) — e.g., that "cells came before enzymes, enzymes before genes." Both experiment and theory negate a frequent assumption, as Dyson states, "there is no evidence that the earliest cells which are preserved as microfossils contained a modern genetic apparatus."

CONCLUSIONS

In summary, many of the laboratory copolyamino acid microspheres produced under simulated geological conditions (Fox et al., 1959; Mueller, 1972; Fox and Dose, 1977; Corliss et al., 1981) and many of the microfossils have a common morphology, the size ranges of the two overlap considerably, comparable pairing is seen in both series, and unimodality of size distribution can occur for each. These criteria, especially the last, have been shown not to be limited to 'biogenic' microspheres.

In 1979, Cloud and Morrison stated, "In fact, the proposed Swaziland fossils look enough like the non-biological microspheres of Fox and Yuyama (1963), and the cause of their crude pairing is so uncertain, that we cannot in any objective way eliminate the possibility that they might be the remains of pre-living chemical assemblages or even spheroidal psuedofossils." The

studies reported here on unimodality of size distribution and on pairing indicate that the ambiguity highlighted in 1978 (Francis et al.), and 1979 (Cloud and Morrison) remains unresolved.

Because of the availability of amino acid mixtures from natural sites (Fox et al., 1981), the ease with which amino acids copolymerize, and the concomitant ease with which the resultant polyamino acids aggregate to cellular structures (Fox and Dose, 1977) of constrained heterogeneity of size, the possibility that the 'microfossils' are lithified proteinoid microspheres must, as Cloud and Morrison (1979) indicate, be kept in purview. The question remains open as to whether the early Archean objects were abiotic, protobiotic, or biotic.

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