Metabolic Microspheres

Origins and Evolution

Sidney W. Fox
Institute for Molecular and Cellular Evolution, University of Miami, Coral Gables, Florida

A systematic review of catalytic activities in thermal proteinoids and microspheres aggregated therefrom yields some new inferences on the origins and evolution of metabolism. Experiments suggest that, instead of being inert, protocells were already biochemically and cytophysically competent. The emergence and refinement of metabolism *ab initio* is thus partly traced conceptually. When the principle of molecular self-instruction, as of amino acids in peptide synthesis, is taken into account as a concomitant of natural selection, an expanded theory of organismic evolution, including saltations, emerges.

In the stepwise evolutionary emergence of life on this planet, new microstructures arose spontaneously. Experiments in the production of proteinoid microspheres suggest that the transition from appropriate inanimate matter to organized cell-like structures, i.e., protocells, occurred easily and often [1–3].

This view brings into focus the question of which came first: cellular structures or cellular functions. Prior to collection of data from proteinoids and proteinoid microspheres, popular assumption placed the emphases on structure first; the protocell or its equivalent was thought of as a static entity composed of disorderly polymers (e.g., [4]). Such a view is related to the yet earlier vitalistic concept that only living things could synthesize organic compounds [5]. It is in the era of organic chemistry that we have learned that many biological activities are rooted in carbon compounds that can be separated from cells.

The functions that are intrinsic to proteinoid microspheres are now known to be numerous. The reports are somewhat scattered in the literature; they are partly assembled in textbooks (e.g., [1, 2, 6–8]). The catalytic functions of microspheres are reviewed here in a systematic fashion. In addition to catalytic activities, the proteinoid microspheres have proliferative activity, selective membranes, infrastructure, what appears to be some protobehavioral properties [9, 10], etc. The accumulated data indicate that the microspheres are dynamic, not static. Accordingly, the protocells on Earth were already dynamic.

The assumption that the first organisms were static was prevalent at the time that the principal structural models for protocells were coacervate droplets made from polymers such as gum arabic and gelatin, themselves inactive. Studies of coacervate droplets made from biopolymers were instructive by providing insight into principles of organization of cells as we know them, and into related phenomena. This was not, however, relevant to the protobiogenetic question; catalytic activity in such droplets was studied only when it, too, was introduced by inclusion of *contemporary* enzymes in the droplets [11]. A more relevant approach was that of Herrera [12], who assembled models of protocells from small compounds rather than from biopolymers. Herrera's work was however done at a time that was mostly too early for a systematic technological examination of the question of enzymic activity. This question could be approached with proteinoids and microspheres because the proteinoids arise from smaller molecules, amino acids, under geologically relevant conditions [13]. The conversion to polymers of sets of amino acids obtained from lunar samples or from meteorites [14], and then to microspheres from those polymers, has been shown by Rohlfing [15].

Catalytic Activities in Proteinoids

The various kinds of catalytic activity that have been found in various preparations of proteinoid are listed in Table 1. These activities have been recorded from various laboratories. Activities are both catabolic and anabolic, the latter with ATP. The catabolic activities include esterolysis, phosphatolysis, decarboxylation,

Naturwissenschaften 67, 378–383 (1980)

Table 1. Enzymic and related activities in thermal copolyamino acids

Activity	Ref.
Esterolysis	[16–19]
Phosphatolysis	[20, 21]
Decarboxylation	[22–24]
Amination	[25]
Deamination	[26]
Oxidation	[22, 27]
Photochemical decarboxylation	[28, 29]
Hormonal (MSH)	[30, 31]
Peptide synthesis	[9, 32–34]
Internucleotide synthesis	[35]
Loss of activities by heating, DFP	[17, 19]
Enzyme inhibition by polymers	[36]
Effects on growth	[37–39]

amination, deamination, and oxidoreduction. The anabolic activities include syntheses of peptide and internucleotide bonds. In addition, proteinoids have been shown to possess hormonal [30, 31], photochemical [28, 29], and enzyme inhibitory activities [36], and effects on growth of bacteria [37], plants [38] and animals [39], as well as simulated protobehavioral functions [10].

Although many proteinoids have been found, in a number of laboratories, to have catalytic activities for conversion of substrates, these activities can be only a minor fraction of those known to occur in modern cells. The results to date suggest that the more basic proteinoids are more often catalytic than are the acidic proteinoids.

Meaning of Self-Instruction of Amino Acids to Catalytic Activities in Proteinoids

The presence of measurable catalytic and other activities in proteinoids is rooted in the self-instructing power of amino acids [6]. On the basis of self-instruction, it is possible to rationalize the existence of many copies of a single catalytic macromolecule in any one generation of macromolecules. We thereby understand much more catalytic activity for a given substrate than one could expect in a "random polymer" (cf. [40, 41]).

The mechanism of self-instructing has been partly explained by the function of pyroglutamic acid as an $N \rightarrow C$ polymerization initiator [42]. The principle of self-instruction of amino acids is evident also in Lipmann's nonribosomal synthesis of antibiotic polypeptides [43, 44], and in an earlier report of selective synthesis of peptide anilides by enzymes [45], when the determinant effect of the amino acids on protease specificity [46] is recognized.

Inhibitors of Enzymes

A number of thermal polyamino acids have been found to be inhibitors of modern enzymes [36]; specificities have been observed. This kind of result has led to a detailed study of requirements for copolyamino acid inhibitors of glyoxalase I, because of its proposed relationship to the cancer process [47]. Inasmuch as inhibitors have been found for a number of modern enzymes, the concept of proteinoid inhibitors for protoenzymes is a plausible one. On this basis, control mechanisms were present at the outset of cellular evolution.

The proteinoids provide the variegation and variety necessary for enzymes, protoenzymes, or inhibitors [1, 36]. One or more of the proteinoids has been found to meet the salient requirements of enzymes such as Michaelis-Menten kinetics, pH-activity curves, etc. [48].

Assembly Mechanisms

Proteinoid microspheres (simulated protocells) form with great ease from almost all of the wide variety of thermal copolyamino acids [1] that have been studied.

The mechanism of assembly has been proposed as partly one of intermeshing of branched macromolecules [9]. Intermeshing is visualized because acidic proteinoids have been shown to have one branch for each eleven residues in the mainchain [49]. This degree of branching is comparable to that of amylopectin [50] or gelatin (collagen) [51]. Amylopectin yields gels [50] while acidic proteinoid and gelatin each yield microspherical structures [52].

In a number of ways, the most interesting microspheres are those composed of both acidic proteinoid and more basic (lysine-rich) proteinoid. In this kind of assembly, interaction between polyanion and polycation, as well as intermeshing, must be involved. The presence of both kinds of proteinoid in the same proteinoid microsphere has been established [53]. The production of polycationic-polyanionic microspheres by mixing the two types of polymer was first described in 1963 [54]. The simultaneous production of both polymers from a single matrix was later discovered and described in 1975 [55]. However prepared, the mixed microspheres are stable at high pH or high temperature in aqueous solution [54, 55]

A key question in assembly of microspheres of any type is that of whether the catalytic functions of the proteinoid are incorporated into microspheres assembled therefrom. Theoretically, incorporation of activity should be anticipated unless conditions of warm-

ing were to inactivate proteinoid. Thermal inactivation of catalytically active proteinoids has been observed [17], but it need not be total. Moreover, many microspheres, especially the mixed polycationic-polyanionic type, are made from highly undersaturated solutions without warming. Of special interest is the finding of enhanced activity in mixed microspheres [9].

Catalytically Active Microspheres

All of the catalytically active proteinoids that have been assembled into microspheres, and were then tested for catalytic activity, have been found to have that activity (Table 2). The inclusion of two catalytically active proteinoids in one microsphere preparation can yield synergistic effects. For example, when peroxidatic proteinoid was aggregated around unequilibrated, or "immature", seeds of phosphatatic proteinoid, the resultant suspension displayed more peroxidatic power than found in a suspension of the same mass of proteinoid of the peroxidatic type present alone in microspheres. Even though this could be a consequence of greater relative surface in smaller microspheres, the difference is a real one. In the inverse case in which phosphatatic proteinoid coated peroxidatic seeds, no enhancement was observed [56].

Another kind of mutualism is observed when acidic proteinoid is complexed with lysine-rich proteinoid [9] into phase-separated microparticles. The ability of proteinoid to catalyze the formation of peptides from free amino acids and ATP is a property of the lysine-rich type. While acidic proteinoid is inactive in this respect, it enhances the activity of lysine-rich proteinoid. Acidic proteinoid has other catalytic properties [57]. Lysine-rich proteinoid forms microspheres of itself in salt water [58] and together the two proteinoids interact to form stable, dynamic microspheres able to withstand a wide range of pH and to catalyze peptide synthesis [9].

Table 2. Enzymelike and related activities incorporated into proteinoid microparticles

Activities	Ref.
Catabolic catalyses	
Decarboxylative	[22]
Peroxidatic	[56]
Phosphatatic	[56]
Anabolic catalyses	
Polynucleotide-synthetic	[35]
Peptide-synthetic	[9, 32, 34]
Cooperative catalyses	[56]

It is the mixed microspheres that have been shown able to synthesize internucleotide as well as peptide bonds [32]. Since the modern genetic process requires synthesis of both types of bond, internucleotide (nucleic acids) and peptide (proteins), such microspheres may have provided an ideal environment for the development of the genetic code. It is also the mixed microspheres that have been shown to resemble primitive algae upon fossilization [59]. The possibility that microfossils are indeed silicified microspheres has received renewed attention recently [60–62].

Interpretations

The number of phenomena that are plausible as primordial functions in the absence of prior nucleic acids is especially worthy of note. The explanation for this fact is visualized as manifest by a stage of evolution in which the self-instructing of amino acids could have played a role subsequently assumed by genetics coded in nucleic acids. Polynucleotides would, instead, have appeared in such a protocellular matrix (cf. [63]). Since the polyamino acids have order transmitted by their own instructions, the chicken-egg question of nucleic acids-or-proteins first is resolved [6].

In this context, several aspects of the theory of evolution of metabolism come into purview:

1. *The most fundamental matrix for metabolism is that of variegated copolyamino acids:* proteins contemporaneously, and proteinoids in the primordial context. The various sidechains: carboxyl, amino, guanidino, imidazolyl, sulfhydryl, disulfide, alcoholic, phenolic, alkyl, and benzenoid provide the raw groups for contemporary enzymic function. That simple list does not represent the variety possible, however. The full scope is represented by the plethora of interactions of different assemblages of these groups within individual macromolecules. Evolutionary fine-tuning would have been possible with different numbers of reactive groups and even with varied intergroup distances within individual copolyamino acids.

The above roster of individual reactive groups of different amino acids is one we can assign to proteins with much certainty. We of course do not know what the roster was for the original terrestrial proteinoids. While it seems likely that they were fewer in number, they may have been more than ten [64]. The principle of geometrical magnification of types of macromolecular bioactivity should in any event have applied to yield a very large number of types of whichever groups were present.

2. As a consequence of the astronomical number of variations in copolyamino acids and their deploy-

Naturwissenschaften 67, 378–383 (1980)

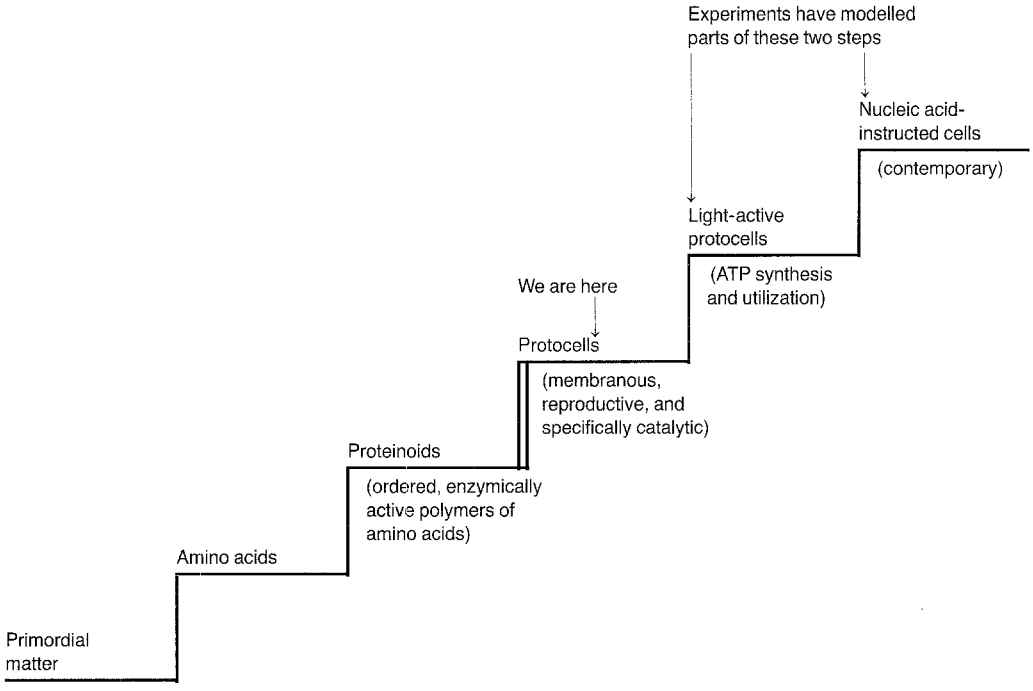

Fig. 1. Stepwise emergence of living organisms, as modelled in the laboratory

ments of reactive sidechains, *a plethora of protoenzymes would have existed.* It is not necessary to answer a question of how a specific enzyme structure would have arisen for a specific function. A more appropriate question is how and why there occurred natural selection from so many candidates for the functions that we study in contemporary organisms.

3. *The fact that* thermally copolymerized amino acids yield polymers of which *some (lysine-rich) proteinoids combine with others to yield cellular structures that can make their own peptides helps to provide a connected flowsheet of organic evolution* (Fig. 1). The evolutionary transition from a geothermal synthesis of peptides to a physiological-type of peptide synthesis using ATP in the presence of water thus would have occurred especially through a lysine-rich proteinoid. This result helps to provide inferences on the evolutionary development of protein biosynthesis prior to an era of coding.

The question of how large are the peptides that arise in this fashion is being explored. As experiments on origins become more constitutionally biochemical and less environmentally geochemical, however, our thinking must itself become more biochemical than that which has characterized most publications in the "origin-of-life" area. We undoubtedly need now to think in biochemical terms such as priming and pumping. Proteinoids participating in evolution could also function as polyamino acid primers. In this context, pumping means repeated availability of intermediates such as ATP. It is through such biochemical devices that we can visualize how the concentrated materials of prebiotic simulation experiments evolved to the dilute concentrations on which the modern cell's biochemical processes are constructed [65].

4. *The first cells already had a high level of biochemical competence.* The popular view is exemplified by the 1957 statement of A.I. Oparin [4], who said, "All that we can expect (are) .... organic polymers in the shape of polypeptides and polynucleotides, assemblages having, as yet, no orderly arrangement of amino acid and nucleotide residues adapted to the performance of particular functions.

These polymers were, nevertheless, able to form multimolecular systems .... only by the prolonged evolution of these systems .... (arose): metabolism, proteins, nucleic acids and other substances .... which characterise the contemporary living organism".

Although Oparin has shared [11] some of the newer view from experiments performed since 1957, the above quotation represents rather widespread persistent thinking as well as Oparin's earlier perspective. The newer view emerged, as it only could, from experiments performed with polymers produced under prebiotic geothermal conditions. It is these experiments that showed that proteinoids already had arrays of biological activities. This perspective could not have been derived from experiments employing evolved polymers.

None of these comments are to deny that much of metabolism developed during evolution. That evolution could proceed, however, because protometabo-

lism existed. Pathways could have lengthened when two proteinoids were in juxtaposition in solution, e.g.,
oxaloacetic acid $\xrightarrow{\text{basic proteinoid}}$ pyruvic acid
$\xrightarrow{\text{acid proteinoid}}$ acetic acid $+ CO_2$ [57].
A more lasting form of evolution would have been combinations of proteinoids within a single evolving protocell [56].

5. *Proteinoids of different catalytic activities can link in the same population of proteinoid microspheres* [56]. The microspheres made from combined proteinoids have enhanced catalytic activities and a larger number of activities. We can consequently visualize that, by this process buttressed by phenomena of microsphere conjugation and microsphere division [1], metabolic pathways could have been lengthened. Lengthened pathways could have been inherited through reproduction of the microspheres having linked catalytic abilities. The lengthened pathways, however, could have become fixed in the evolutionary development only when they were rooted in polyamino acids synthesized by the evolving protocells themselves. The onset of genetics coded through nucleic acids synthesized, transcribed, translated, etc. through polyamino acids [66], and in turn made by an ATP:lysine-rich polyamino acid mechanism [9], would have provided a more secure, more efficient inheritance of biochemical pathways.

This explanation differs from that of the reverse evolution of biochemical steps [67]. That explanation requires mutation in the context of already coded genetics; it leaves unspecified the origin of the coding mechanism. The present explanations derived from experiments and the earlier suggestion [67] are not mutually exclusive. The present one is to be understood as a mechanism of lengthening of metabolism in a stage of evolution prior to the onset of coding of protein biosynthesis in nucleic acids. The proteinoid answers, which emerged from the special kind of constructionistic investigation demanded by the basic question [68], thus permits an explanation for the evolution of biochemical pathways *ab initio*.

6. *The assembly of diverse active proteinoids illustrates the potential for variation in evolution.* Visualization of the opportunities for variation in protocells dependent upon the assembly of proteinoids of diverse composition [56] has contributed to amplified thinking about Darwin's theory of natural selection. The fact that each copolyamino acid is ordered by its matrix of amino acids signifies that natural selection from proteinoid microspheres on the primitive Earth did not have to occur from units assembled from statistically random arrays of precursor macromolecules. An amplified definition of evolution of organisms is a conceptual consequence. It states that *the evolution to and of organisms has consisted of natural internally directed assembly processes interdigitated with natural selection of the products* [69]. The to-and-of proviso relates to the assumption that processes yielding the first organism would not be greatly different from those by which more evolved organisms are generated.

The concept that nature experimented in the assembly of several interacting components into single structures furthermore provides the possibility for deeper understanding of the processes of macroevolution. The origin of the protocell, as modelled by the proteinoid microsphere, can be viewed as a prime example of saltative assembly [69]; the product appears to be "discontinuously" different from the components of the amino acid matrix (cf. [70], p. 176). When extrapolated into the origin of assembly of organelles [69], the selective quality of stereomolecular interactions eventuating in the prime example of assembly of a protocell [6, 71] helps to explain what has looked to some like design and direction in Darwinian evolution [70].

Aided by Grant No. NGR 10-007-008 of the National Aeronautics and Space Administration. Contribution No. 337 of the Institute for Molecular and Cellular Evolution.

1. Fox, S.W., Dose, K.: Molecular Evolution and the Origin of Life. New York: Dekker 1977
2. Dose, K., Rauchfuss, H.: Chemische Evolution und der Ursprung Lebender Systeme. Stuttgart: Wiss. Verlagsges. 1975
3. Fox, S.W.: The Sciences *20* (1), 18 (1980)
4. Oparin, A.I.: The Origin of Life on the Earth. New York: Academic Press 1957
5. Keosian, J., in: The Origin of Life (Noda, H., ed.). Tokyo: Japan Sci. Soc. Press 1978
6. Fox, S.W., in: The Nature of Life (Heidcamp, W.H., ed.). Baltimore: Univ. Park Press 1978
7. Rohlfing, D.L., in: Life, the Individual, the Species (Lane, T.R., ed.). St. Louis: Mosby 1976
8. Lehninger, D.L.: Biochemistry. New York: Worth 1975
9. Fox, S.W., Nakashima, T.: BioSystems (in press)
10. Fox, S.W.: Comp. Biochem. Physiol. (in press)
11. Oparin, A.I.: Sub-Cell Biochem. *1*, 75 (1971)
12. Herrera, A.L.: Science *96*, 14 (1942)
13. Rohlfing, D.L.: ibid. *193*, 68 (1976)
14. Saunders, M.A., Rohlfing, D.L.: ibid. *176*, 172 (1972)
15. Rohlfing, D.L.: Bull. So. Carol. Acad. Sci. *37*, 186 (1975)
16. Rohlfing, D.L., Fox, S.W.: Arch. Biochem. Biophys. *118*, 122 (1967)
17. Rohlfing, D.L., Fox, S.W.: ibid. *118*, 127 (1967)
18. Noguchi, J., Saito, T., in: Polyamino Acids, Polypeptides, and Proteins (Stahmann, M.A., ed.). Madison: Univ. Wisconsin Press 1962
19. Usdin, V.R., Mitz, M.A., Killos, P.J.: Arch. Biochem. Biophys. *122*, 258 (1967)
20. Oshima, T.: ibid. *126*, 478 (1968)
21. Fox, S.W., Wiggert, E., Joseph, D., in: The Origins of Prebiological Systems, p. 368 (Fox, S.W., ed.). New York: Academic Press 1965

22. Fox, S.W., Krampitz, G.: Nature *203*, 1362 (1964)
23. Hardebeck, H.G., Krampitz, G., Wulf, L.: Arch. Biochem. Biophys. *123*, 72 (1968)
24. Rohlfing, D.L.: ibid. *118*, 468 (1967)
25. Krampitz, G., et al.: Experientia *24*, 140 (1968)
26. Krampitz, G., Haas, W., Baars-Diehl, S.: Naturwissenschaften *55*, 345 (1968)
27. Dose, K., Zaki, L.: Z. Naturforsch. *26b*, 144 (1971)
28. Wood, A., Hardebeck, H.G., in: Molecular Evolution, p. 233 (Rohlfing, D.L., Oparin, A.I., eds.). New York: Plenum Press 1972
29. Hardebeck, H.G., Haas, W.: Z. Naturforsch. *26b*, 862 (1971)
30. Fox, S.W., Wang, C.-T.: Science *160*, 547 (1968)
31. Bagnara, J.T., Hadley, M.E.: Experientia *26*, 167 (1970)
32. Fox, S.W., Jungck, J.R., Nakashima, T.: Origins Life *5*, 227 (1974)
33. Fox, S.W., et al.: Polymer Preprints *20*, 16 (1979)
34. Nakashima, T., Fox, S.W.: Proc. Nat. Acad. Sci. USA *69*, 106 (1972)
35. Jungck, J.R., Fox, S.W.: Naturwissenschaften *60*, 425 (1973)
36. Fox, S.W., Syren, R.M., Windsor, C.R., in: Submolecular Biology and Cancer (Wolstenholme, G., Fitzsimons, D.W., Whelan, J., eds.). London: Ciba Found. Ser. 67, 1979
37. Fox, S.W., Harada, K.: J. Am. Chem. Soc. *82*, 3745 (1960)
38. Haas, W., Hardebeck, H.G.: Naturwissenschaften *56*, 220 (1969)
39. Krampitz, G., Knappen, F., in: Nutrition in Space and Related Waste Problems, p. 339 (NASA Publ.). Washington: US Govt. Printing Office 1964
40. Dose, K., in: Theory and Experiment in Exobiology *1*, 41 (1971)
41. Fox, S.W.: Naturwissenschaften *60*, 359 (1973)
42. Fox, S.W., Melius, P., Nakashima, T., in: Evolution of Protein Molecules (Matsubara, H., Yamanaka, T., eds.). Tokyo: Japan Sci. Soc. Press 1978
43. Gevers, W., Kleinkauf, H., Lipmann, F.: Proc. Nat. Acad. Sci. USA *60*, 269 (1968)
44. Lipmann, F., in: Molecular Evolution (Rohlfing, D.L., Oparin, A.I., eds.). New York: Plenum Press 1972
45. Fox, S.W., Winitz, M., Pettinga, C.W.: J. Am. Chem. Soc. *75*, 5539 (1953)
46. Hurst, T.L., Fox, S.W.: Arch. Biochem. Biophys. *63*, 352 (1956)
47. Szent-Györgyi, A.: Proc. Nat. Acad. Sci. USA *74*, 2844 (1977)
48. Fox, S.W.: Mol. Cell. Biochem. *3*, 129 (1974)
49. Harada, K., Fox, S.W.: BioSystems *7*, 213 (1975)
50. Meyer, K.H.: Natural and Synthetic High Polymers. New York: Interscience 1962
51. Stimler, N.P., Tanzer, M.L.: Adv. Exp. Med. Biol. *86B*, 675 (1977)
52. Fox, S.W., Brooke, S., in: Microencapsulation (Kondo, T., ed.). Tokyo: Techno 1979
53. Brooke, S., Fox, S.W.: BioSystems *9*, 1 (1977)
54. Fox, S.W., Yuyama, S.: J. Bact. *85*, 279 (1963)
55. Snyder, W.D., Fox, S.W.: BioSystems *7*, 222 (1975)
56. Hsu, L.L., Fox, S.W.: ibid. *8*, 89 (1976)
57. Rohlfing, D.L., Fox, S.W.: Adv. Catal. *20*, 373 (1969)
58. Rohlfing, D.L.: Origins Life *6*, 203 (1975)
59. Francis, S., Margulis, L., Barghoorn, E.S.: Precambr. Res. *6*, 65 (1978)
60. Keosian, J., in: The Origin of Life and Evolutionary Biochemistry (Dose, K., et al., eds.). New York: Plenum Press 1974
61. Pflug, H.D., Jaeschke-Boyer, H.: Nature *280*, 483 (1979)
62. Cloud, P., Morrison, K.: Precambr. Res. *9*, 81 (1979)
63. Ehrensvärd, G.: Life: Origin and Development (transl.). Univ. of Chicago Press 1962
64. Rohlfing, D.L., Saunders, M.A.: J. Theor. Biol. *71*, 487 (1978) (1978)
65. Atkinson, D.E.: Cellular Energy Metabolism and its Regulation. New York: Academic Press 1977
66. Dillon, L.S.: The Genetic Mechanism and the Origin of Life, p. 208. New York: Plenum Press 1978
67. Horowitz, N.H.: Proc. Nat. Acad. Sci. USA *31*, 153 (1945)
68. Fox, S.W., in: Bioorganic Chemistry III (van Tamelen, E.E., ed.). New York: Academic Press 1977
69. Fox, S.W.: BioSystems (in press)
70. Gillespie, N.C.: Charles Darwin and the Problem of Creation. Univ. of Chicago Press 1979
71. Fox, S.W., in: Mathematical Challenges to the Neo-Darwinian Interpretation of Evolution, p. 17 (Moorhead, P.S., Kaplan, M.M., eds.). Philadelphia: Wistar Institute Press 1967

Received January 22, 1980