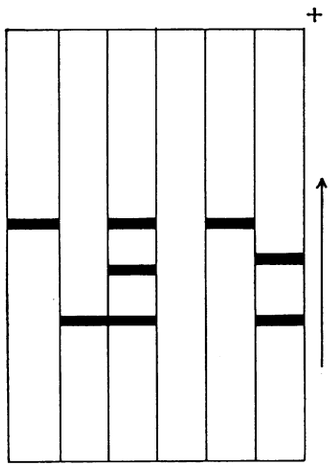

Fig. 1. Diagrammatic comparison of larval APH phenotypes in phosphate buffer, 0.01*M*, *p*H 6.5. The genotype is indicated below each pattern by the superscript of the appropriate *Aph* alleles.

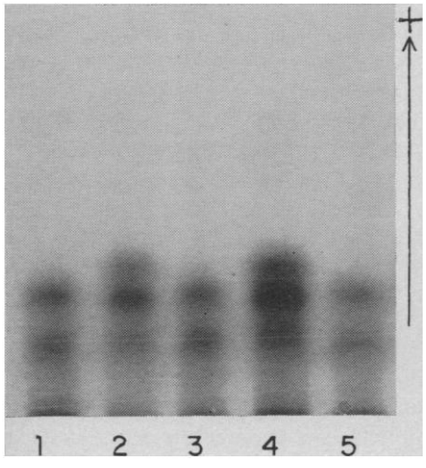

Fig. 2. Photograph of starch gel showing alternate SO (*1*, *3*, and *5*) and FS (*2* and *4*) phenotypes.

acrylamide slabs at *p*H 8.5 and 7.0; and in disc electrophoresis (*2*) at *p*H 9.5 and 6.6. This finding can be interpreted as indicating a structural difference between fast and slow APH, which prevents the fast and the silent protein subunits from combining into active enzyme.

F. M. JOHNSON*
Department of Genetics, University of Wisconsin, Madison

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* Present address: Department of Zoology, University of Texas, Austin 78712.

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Dehydration and Rehydration in a Prebiological System

Abstract. *A proteinoid microsphere suspension system was subjected to cyclic dehydration and rehydration. Particles having somewhat coacervate properties were observed, suggesting a relation between the coacervate and proteinoid origin of cells.*

In an attempt to see how simple environmental changes could affect a prebiological system, we subjected the proteinoid microsphere suspension of Fox (*1*) to periods of alternate dehydration and rehydration. Hinton and Blum (*2*) speculated that such treatment could be a factor in molecular evolution, suggesting "that the chemical complexity required for the origin of the organisms was achieved by a series of reactions that occurred in the alternately wet and dry niches so numerous on the land." Their reasons for this statement were that in small niches, there would be a relatively high concentration of macromolecular substances compared to that in the free sea and also high pressures generated by surface tension forces. These conditions could lead to the formation of complex systems.

Our prebiological system was prepared by the thermal copolymerization of the 18 amino acids common to protein. The resulting protein-like polymer has been named proteinoid by Fox and Harada (*3*) who have also described its chemical properties. A hot aqueous solution of the purified polymer (15 mg/2.5 ml) was cooled, with resultant production of microspheres (*1*), which were approximately 2 μ in diameter (Fig. 1). Drying and rehydration experiments were then carried out with this suspension.

A drop of the suspension was placed on a microscope slide and allowed to air dry at room temperature. This treatment yielded a hard transparent matrix in which the microspheres were embedded. The stability of the microspheres under dehydration has been reported by Young (*4*).

We then rehydrated the preparation with one or two drops of McIlvaine's buffer (citric acid–sodium phosphate), *p*H 8.0, placed on the edge of the dried matrix. The buffer streamed into the matrix forming a rehydration front. As the front advanced, the matrix and microspheres were quickly dissolved, and after about 5 minutes, larger spheres, 10 to 30 μ in diameter (Fig. 2) appeared. Unlike the original microspheres, they showed a great deal of coalescence and plasticity, as evidenced by changes in shape. As these large spheres moved off into the rehydration medium, they would swell and eventually disappear presumably due to dissolution. As the rehydrating front slowed down, the spheres no longer coalesced and showed increased stability. If at this time water was added, the coalescence phenomena

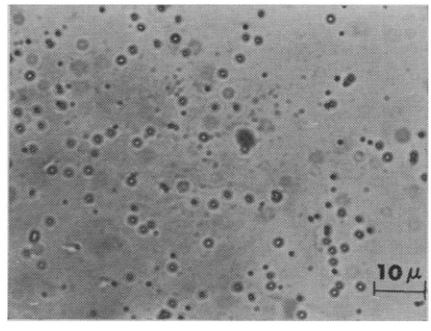

Fig. 1. Microspheres prepared by cooling hot aqueous solution of proteinoid polymer.

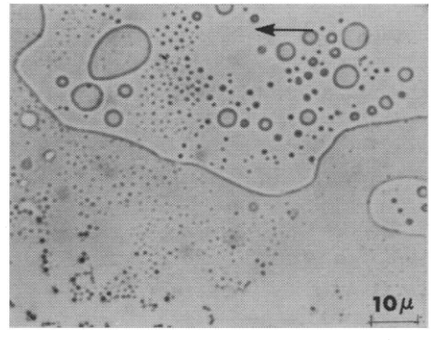

Fig. 2. Formation of new spherical bodies at rehydration front. Arrow indicates rehydration direction. The newly formed bodies become spherical very rapidly. In this first rehydration, little complexity is observed.

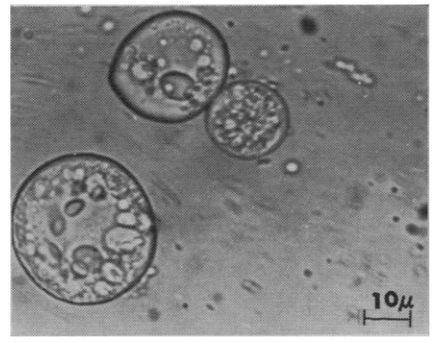

Fig. 3. Coacervate-like spheres showing complex morphology.

was noted again, and the spheres became complex (Fig. 3). The preparation was then allowed to dry again. We carried out another rehydration with water, and the same phenomena were observed except that the spheres obtained seemed to be even more complex in regard to internal structure. After three or four more cycles of dehydration and rehydration the degree of complexity in the spheres seemed to have reached a maximum. When the foregoing steps were repeated in the presence of methylene blue, the spheres concentrated the stain.

The factors which may cause the development of complex morphological forms in the system are numerous. During the drying process, there are localized high concentrations of proteinoid material. The first rehydration with buffer seems to be an important factor in causing development of complexity. This change is probably due to interaction of the buffer ions with the proteinoid.

Thus simple environmental changes of dehydration and rehydration can indeed cause the development of complexity in an experimental prebiological system; this finding seems to support the suggestion of Hinton and Blum (*2*). We believe that we have developed, from thermal proteinoid, a system which behaves like coacervates (*5, 6*). The large spheres described above have several properties in common with coacervates: complex morphology, selective adsorption as evidenced by concentration of methylene blue, and ability to coalesce. Our experiments may possibly link the seemingly different concepts of the origin of life, the coacervates of Oparin (*6*) and the proteinoids of Fox (*1*).

ADOLPH E. SMITH
FREDERICK T. BELLWARE
Physics Department,
Sir George Williams University,
Montreal 25, Quebec

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2 February 1966

Evolution of the Structure of Ferredoxin Based on Living Relics of Primitive Amino Acid Sequences

Abstract. *The structure of present-day ferredoxin, with its simple, inorganic active site and its functions basic to photon-energy utilization, suggests the incorporation of its prototype into metabolism very early during biochemical evolution, even before complex proteins and the complete modern genetic code existed. The information in the amino acid sequence of ferredoxin enables us to propose a detailed reconstruction of its evolutionary history. Ferredoxin has evolved by doubling a shorter protein, which may have contained only eight of the simplest amino acids. This shorter ancestor in turn developed from a repeating sequence of the amino acids alanine, aspartic acid or proline, serine, and glycine. We explain the persistence of living relics of this primordial structure by invoking a conservative principle in evolutionary biochemistry: The processes of natural selection severely inhibit any change in a well-adapted system on which several other essential components depend.*

Many of the principles of organic evolution have long been known and are productively used in the organization of biological concepts, but are seldom used to full advantage in biochemistry. In nature, biochemistry is included in biology. An organism is a functioning system composed of the structures, organs, tissues, and organelles of classical biology. These in turn are composed of metabolites, macromolecules, enzyme aggregates, and biochemical feedback systems. Biochemical details concerning these components have only recently become accessible. Potentially, a much greater amount of information relevant to evolution is available in biochemistry than in classical biology.

According to evolutionary theory, each structure or function of an organism is subject to occasional changes or mutations, but the infrequency of these mutations necessitates that they will almost always occur, and be selected for, one at a time. Each change or addition must be an improvement, or at least not too severe a disadvantage, in order that the processes of natural selection permit its survival. This limitation has a very conservative effect. If its ecological niche stays the same, a well-adapted organism strongly resists change. Thus we find familiar-looking fossil shells a third of a billion years old. If its niche changes, new functions evolve, but the most primitive structures tend to remain unchanged, since these older components have already come to be relied upon by several later additions. Any change in a very old component, even though it might be advantageous in some way, would coincidentally disturb so many other things that it would almost always be extremely disadvantageous to the organism. This conservatism is well illustrated in the amino acid sequences of proteins. For example, we can compare the amino acid sequences of cytochrome *c* from yeast (*1*) and from horse (*2*), position by position. In 64 of the 104 positions the amino acids in the two chains are identical. Between horse and human cytochrome *c* (*3*) there are only 12 amino acid differences.

When we consider evolution retrospectively, the constraints are even more severe. One basic evolutionary principle is that every living organism or structure or function had ancestors very similar to itself, but simpler. (This is true even if it had more complex *immediate* ancestors.) In a particular case there are generally only a few plausible slightly simpler ancestors. As we trace the changes in a structure or function back through time, we must bear in mind that *all* of the structures and functions of the cell may be simpler. We are then dealing with primitive components ancestral to those seen today.

The amino acid sequence of ferredoxin from *Clostridium pasteurianum*, a nonphotosynthetic anaerobic bacterium, has been reported (*4*). This protein seems to have arisen at an earlier times than many others which have been studied. We draw this inference from the following considerations.

1) Ferredoxin occurs in primitive anaerobic organisms, both photosynthetic and nonphotosynthetic (*5*). It must have been present in simpler organisms, the extinct common ancestors of these.

2) Ferredoxin contains iron and sulfur, bonded to the protein at its active site (*6*). Ferrous sulfide, FeS, is a widely dispersed mineral, a catalyst which would have been readily available to the most primitive organism.