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IS LIFE ORIGINATING DE NOVO?

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IS LIFE ORIGINATING DE NOVO ?

Recent evidence suggests that the possibility of neobiogenesis should be seriously reconsidered.

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I. Introduction

In 1864 Louis Pasteur declared that living organisms can arise only from parental organisms like themselves (1). He believed that he had conclusively demonstrated that microbes could not arise spontaneously from non-living matter. For over seventy years Pasteur's conclusion was accepted by the overwhelming majority of scientists. However, two decades ago an effort started which has shown that Pasteur's doctrine is not valid in the case of the original appearance of life on the primitive earth. Various mixtures of hypothetical primitive earth gases and liquids produced most of the simpler biochemicals used by life today. Plausible means for the spontaneous appearance of biopolymers and primitive cell-like forms have also been demonstrated (2). Most textbooks which describe these origin of life experiments are careful to point out that spontaneous generation of life was shown to be possible only in the case of the formation of the first life on the prebiotic earth.

In 1960 John Keosian raised the possibility of contemporary spontaneous generation of life, that is, the origin of life under present conditions. He proposed the term "neobiogenesis" for such phenomena and answered objections which are usually raised against the idea (3). Since biology and medicine accept the Pasteur doctrine that living organisms at the present time can come only from parental forms like themselves, we believe it would be beneficial to reexamine this fundamental premise. We review here advances made during the last decade and show how they relate to the question.

II. The Appearance of Organized Structures in Sterile Media

We have already mentioned that origin of life experiments have shown that simple biochemicals, biopolymers, and microspheres are easily formed under primitive earth conditons. We may ask the question of what would happen if we started with complicated biochemicals. A long line of observations exists on the appearance of organized structure in sterile media containing sera from various sources (4). On agar plates such structures assume the form of small growths which bear a remarkable resemblance to colonies of Mycoplasma, bacterial L-forms, and actinomycetes (5). These structures were shown to result from the components of the sera and were not due to contamination. Although many investigators have dismissed these structures as misleading crystallization artifacts (6), considerable uncertainty remains about their actual nature. Bonifas gave the name "Stegasma" to the organized forms, concluding they were actually organisms, but later he had doubts that they were alive (7).

In a recent series of papers, Činátl has described similar observations on the so-called "calcareous structures" (8,9). He killed various mammalian cells by heating them at 56° for one hour. The dead cells were then placed on sterile PPLO agar supplemented with calf sera. Filamentous colony-like forms grew from the nuclei of the dead cells. Similar, but less complex structures were observed in tissue culture media and even in inorganic salt solutions.

In what ways do Stegasma (and the similar or identical structures seen by other workers) resemble oganisms? They can be passed from one agar plate to another without loss of colony-forming ability. The time required for a given amount of new organized material to appear on a fresh plate is shortened

with increasing number of transfers (9). The ultrastructure of the colony-like formations consists of stacks and whorls of closely spaced lamellae reminiescent of myelin figures (7). The structures contain a variety of biological materials including proteins, polysaccharides, and phospholipids. During their formation the structures take up calcium from the medium (9). In view of the great chemical complexity of sera, it seems likely, though the pertinent data on this point are very scanty, that a variety of chemical reactions takes place within the forming structures.

We do not wish to make any judgment on whether the forms are alive or not, but agree with the conclusion that they certainly do possess life-like and inorganic properties at the same time, just as in the case of viruses. But we want to emphasize that the serum-agar mixtures were not supplemented with nucleic acids initially.

If sterile media containing proteins exhibit such organizing properties, what about nucleic acid alone? Nucleic acid was found to form cell-like structures under the very simple conditions of drying and rehydrating a drop of aqueous solution on a microscope slide (10). These coacervates formed over a range of nearly eight pH units, using a wide variety of rehydrating solutions. Nucleic acid has been known for a long time to form complicated coacervates with protein and these formations were studied in detail by Bungenberg de Jong (11).

Since nucleic acid and protein form cell-like structures so readily, we may wonder what would occur if we combined them with lipids under physiological conditions. This experiment was reported in 1932 by George Crile and his associates (12). Crile thought that if the protein and lipid fractions of tissue were combined in electrolyte solution the resulting

structures might be able to perform some of the primitive functions of life. At that time the great importance of nucleic acid was unknown, but we may assume from the descriptions that nucleic acid was also present. When the ether-soluble lipid fraction of brain tissue was mixed with the water soluble fraction (containing proteins and nucleic acids), cell-like forms of remarkable architecture appeared within minutes in the originally clear solution. The globular structures were 50-150 μ in diameter and each contained a central structure resembling a nucleus. Fine filaments resembling cilia rapidly grew from the surfaces of the structures. These cell-like forms, which Crile called "autosynthetic cells," even exhibited a high rate of oxygen consumption as well as a high respiratory quotient as measured on a Warburg apparatus. Similar control measurements on protein and lipid fractions alone showed much lower rates. The structures grew slowly, multiplied at times by budding and sometimes by direct division, and in some cases exhibited dynamic activity resembling ameboid motion. When the lipoid material which was used in these experiments was irradiated with X-rays, the organized forms did not appear. In spite of the remarkably life-like properties of the autosynthetic cells, Crile's report has been largely ignored.

The experiments of Crile and Činátl are basically similar. They differ in the degree to which the cells were broken down. Crile used dilute (0.5-1.0% w/v) clear extracts from the cells, whereas Činátl used intact dead cells and serum agar. All these experiments demonstrate a tremendous inherent power of organization in cell fragments, products of cell death, blood sera, and by implication body fluids in general.

III. The Plasticity of Simple Forms of Life

Let us now examine some recent lines of evidence which suggest a possible relationship between the organized forms appearing in sterile media and *in vivo* transformation of form and function among various microbial units of life. In the past few years rapid evolution of cells has been demonstrated and shown to be of practical importance. Drug resistant strains of microbes have been shown to develop very rapidly. Until several years ago, such processes were believed to occur because of survival of drug-resistant mutants through the relatively slow process of natural selection. However, the process is not believed to occur largely through the rapid spreading of a resistance factor through the bacterial population by direct cell-to-cell contacts. Evolutions by accretion of bits of genomes (plasmids) occurs much more rapidly than evolution by natural selection. This process has been reviewed by Pollock (13). Our main interest in this pnenomenon is to point out the fact that life at the level of microbes can change its form very rapidly in an attempt to survive. It is probable that a great variety of such bacterial transformations takes place *in vivo* involving the movement of episomes (including the lysogenic phages) from one cell to another. We can imagine that dying bacteria release a variety of "genetic packages" into the tissue fluids of the host organism and that some of these packages avoid enzymatic breakdown by entry into other intact cells.

Other rapid changes of form are seen in the transformation of bacteria into the L-phase in the presence of a stress condition such as an antibiotic (14). In the standard methods used to induce the L-phase some stress is applied to the medium. In the case of penicillin, for example,

the bacteria near the applied strip of antibiotic are dead. In a zone far away, the bacteria are normal, but in the intermediate zone between the dead and normal bacteria, the L-phase is seen. Very often, on release of the stress, the L-phase bacteria revert to the normal form, but the mechanism of the reversion is largely unknown. Here too, the drastic transformation in the bacteria represents a temporary means of survival.

The L-forms derived from bacteria are very similar in morphology to the Mycoplasma. Despite the similarities, however, the relationships of the two groups is unclear since the Mycoplasma contain cholesterol and differ significantly in their guanosine-cytosine content from bacteria. Both L-forms and Mycoplasma are known to produce smaller granules in the size range of myxoviruses and other animal viruses under certain conditions (15). It is unknown whether any of these or smaller granules are virus-like entities or whether they can ever revert back to the higher form of organization from which they are derived. In this connection it is of great interest that rod-shaped bacteria-like structures, Mycoplasma, and myxovirus particles are all consistently recoverable from tissues infected with Rous sarcome virus but not from normal tissues (16). such an experimental system would seem to be ideal for investigating relationships between the three different levels of organization.

Another experiments which shows the extreme plasticity of Mycoplasma was reported by Razin and Kahane (17). These workers found that the solubilized membrane components of one species will reaggregate with those of another to form a hybrid reaggregate. Their results indicated that the reaggregation process is not species specific and that solubilized

proteins and lipids from membranes of two different species may reassemble and form a hybrid membrane possessing the serological properties of both organisms. We suggest that similar processes may be occurring *in vivo*.

The fusion of eucaryotic cells in tissue culture has been demonstrated in recent years. Here a hybrid cell is formed by the union of two or more somatic cells from the same or even different species (18). The current interest in cell fusion derives mainly from its utility in genetic studies. Giant cells containing several nuclei are known to occur in tumors (19). It has also been observed that chromosomes from one cell can be incorporated into the nucleus of another cell. Chromosome incorporation and the well-known transformations of mammalian cells by myxoviruses (20) (*i.e.*, influenza and mumps) are the eukaryotic analogs of bacterial transformations by episomes (plasmids). In the case of the myxoviruses, the membrane of the virus particle is formed from the plasma membrane of the host cell and thus contains cholesterol (21). Although the *in vivo* importance of all these processes has not yet been demonstrated, the increased survival ability which cell fusion and chromosome incorporation could bring should not be overlooked.

Mycoplasma are often closely associated with eukaryotic cells in tissue culture (22) and *in vivo* in a great many diseases. In the case of He-La cells, electron microscopy shows an intimate surface-to-surface bonding between mycoplasma and the infected cells. It has been suggested that this is the reason that it is so difficult to eliminate them from tissue culture lines (23). Plant mycoplasma do not grow on the usual serum agar used for cultivation of mycoplasma from animal cells, again indicating a very close relationship between the mycoplasma and the host.

To date it has not been possible to culture plant mycoplasma. We suggest that it will be possible to culture plant mycoplasma on a medium supplemented with plant sap.

The observations discussed in this section suggest that many interactions and transformations are occurring among the simplest forms of contemporary life. Indeed, one is tempted to conclude that within the body fluids of host organisms rapid back and forth movement is occurring between the states of organization of living matter represented by bacteria, L-forms, Mycoplasma, and certain viruses. Furthermore, it seems likely that a variety of different kinds of genetic packages consisting of nucleic acids and possibly enzymes and fragments of membranes would "cascade down" into the medium following death of the microbial units and even the cells of the host. The fate of these genetic packages in the fluids of diseased organisms is largely unknown, although it is generally presumed that they are picked up and chemically degraded by macrophages along with other forms of debris.

IV. A Model for Neobiogenesis In Vivo

We are now in a position to suggest a relationship between the organized structures which arise spontaneously in sterile media and the *in vivo* activities of microbial entities near the threshold of life. We have already mentioned the remarkable degree to which the calcareous forms resemble the colonies of Mycoplasma and bacterial L-forms. So close is this correspondence that elaborate criteria have been devised for distinguishing the "pseudoorganisms" from authentic microorganisms (24). These pseudoorganisms are capable of multiplication and even incorporating DNA (25). Nevertheless, a distinct gap remains between the most complicated forms observed on agar plates and fluid media and the simplest

microbes. The latter have the proper genetic information for insuring propagation of cell units while the former seem to be devoid of nucleic acid. Therefore, it seems most unlikely that calcareous forms arising *in vivo* could ever spontaneously reach the living state on their own.

Animal sera do contain appreciable amounts of nucleotides (26), but their spontaneous polymerization into the proper sequences within the forming calcareous structures is not a serious possibility according to our present state of knowledge. But what would be the result if some minimal package of genetic material derived from L-forms or mycoplasma could somehow get incorporated into developing calcareous forms? Such a process would be a lower level analog of chromosome incorporation by eukaryotic cells and transfer of episomes among bacterial cells. We suggest that some of these associations could be or could become minimally alive. In such a case, the genetic package would pull a previously sub-vital structure over the threshold to the living state and the result would be a mycoplasma-like organism.

Several recent observations lend additional support to our proposed model for neobiogenesis. In the growth of mycoplasma and L-form organisms on serum agar, "large bodies", 5-10 μ in diameter and rich in lipids, appear in the colonies. Their nature is poorly understood and there is controversy over whether they are alive or not. A recent study suggests that the large bodies are "structures resulting from accumulations of metabolic by-products and medium products within or on which minimal reproductive units had become entrapped" (26). If such large bodies also arise *in vivo* the minimal reproductive units could play the role of the minimal packages of genetic material proposed above.

Acetone extracts of liver cells and egg yolk form structures on agar which are practically identical with mycoplasma when viewed with the phase contrast microscope (27). This result would appear to be a kind of lower-level analog to Crile's observations on autosynthetic cells.

If such partial de novo origin of microbes is occurring in nature generally, then we can propose a whole continuum of states of organization between the subvital and the minimally alive. The only forms now generally recognized as subvital are the viruses (28). The Stregasma-like forms so far observed in sterile media may represent something like the maximum degree of dynamic complexity which the self-organizing properties of serum components can achieve in the absence of nucleic acids (in Crile's autosynthetic cells, nucleic acids were presumably present). Nevertheless, such forms may play important roles in some disease processes or, on the other hand, may be undetected simply because they do not bother us. Fig. 1 illustrates the model.

The hypothetical scheme outlined above is not the only plausible model for neobiogenesis. We venture to suggest that a wide range of reorganizing processes involving organelles and other fragments of dying cells may occur under suitable conditions in vivo and even outside of living organisms. The fact that organelles (nuclei, mitochondria, and chloroplasts) can function for days in suitable media outside of cells lends support to this idea. Again we can envision a spectrum of sub-vital states of organization. We return to this point when we discuss experiments suggested by this model.

V. Possible Implications for Medicine

Could these reorganizing processes of molecules and cell fragments have a general importance for *in vivo* processes? One may be immediately tempted to answer that if the processes were important they would have been discovered a long time ago in medical research. But medicine is based on the Pasteur dictum that life comes from a parent very similar to itself. We have accepted the generality of the Pasteur doctrine so completely that we do not even question its validity any more. There is always the search for the infective agent.

Many agents of disease are difficult to classify phylogenetically. The customary view concerning their origin is that various species became parasitic through the forces of natural selection, and a long series of fortuitous accidents (for the parasite) was involved. The difficulty with this viewpoint has been noted by Kerkut in the case of fungi, viruses, and rickettsia (29). These organisms are difficult to fit into the evolutionary pattern and Kerkut suggests a solution is to assume that life originated on several different occasions or at different places at the same time.

The fact that L-phase, Mycoplasma, and other small forms are important in the disease process is relevant to our thesis (30). Organisms produced by *de novo* processes would be expected to be small in size, lacking a proper cell wall, and show wide variation in their genetic makeup. However, these are precisely the characteristics, especially the "cell-wall deficiency," which would favor their resistance to certain antimicrobials, host tissue factors, and ingestion by phagocytes (30). Even if some of these structures do not develop as far as living microbes, they would still be of clinical importance. The clinical

importance of the calcareous structures found by Cinati was shown when the same calcifying processes were observed around necrotic cells *in vivo* (31). The fatty deposits of atherosclerosis also appear to be related to the calcareous structures (8).

VI. Conclusions and Suggested Experiments

The developments of the past decade in microbiology and molecular biology force us to adopt a new picture of evolution. We see movements in both directions along the continuum between the non-living and the living states, as shown in Fig. 1. The strict differentiation between the non-living and the living states is no longer possible. One can say that this fact has been known for a long time, in fact, since the discovery of the viruses. But in practice the sharp distinction between the two realms remains because the self-organizing tendency of biopolymers and cell fragments was not recognized sufficiently. Prebiological investigations have been responsible for demonstrating explicitly the inherent tendency of matter and energy to move toward the living state, the so-called "biochemical predestination" (2) exhibited at virtually every level of the complexification of carbon compounds.

To some extent, the case for *de novo* origin of cells has already been shown. Assuming that biopolymers and cell fragments can organize themselves, we are led to several predictions.

1) Under suitable conditions *in vivo* we will observe fusion of cell components and aggregations of molecules to form more highly developed forms. This is partially shown by the cell fusion work and the observations that calcareous structures form *in vivo*.

2) Since many bizarre formations are found in the sera of disease cases, we can predict that filtered sera from these patients will produce such forms on incubation. These forms are now regarded merely as cell breakdown products but such an experiment may show them to be more fundamental to the disease process.

3) If cells are killed in a mild manner (resembling death under natural conditions), the subunits will reorganize themsevles to form new entities exhibiting some of the properties generally called living. The experiment of Crile gives a strong indication that this will occur, and this work should be repeated using modern methods of detecting metabolic activity. Additional plausibility is given by that fact that isolated nuclei can carry on incorporation of precursors of nucleic acid synthesis (32), and DNA synthesis using endogenous DNA as primer (33).

4) Where could this action be occurring? Inside our bodies. The sterile sera work implies this. The great chemical richness of the body fluids provides an ideal starting medium for many of the processes discussed here. In aging and disease, the body fluids contain a large number of structures generally regarded as debris. Of course, macrophages are constantly cleaning this debris, but the fact that it is visible shows that this function cannot be performed completely.

Most biologists have not given much thought to the fate of cell components following cell death. They assume that because a cell undergoes autolysis under sterile conditions, the same thing happens *in vivo*. We are willing to predict that a great many surprises, including reorganization of dead cell subunits to higher states of functioning are ahead.

5) If microorganisms do arise de novo, they would be attributed to contamination or be discounted as "artifacts." It would be interesting to supplement sterile sera with mycoplasmal DNA and other sources of genetic information and follow the development of the organized structures.

In summary, we are proposing that a new dimension be considered in biology, the back and forth movement between what is commonly considered life and death. The practical import of these considerations to medicine and biology can hardly be overestimated.

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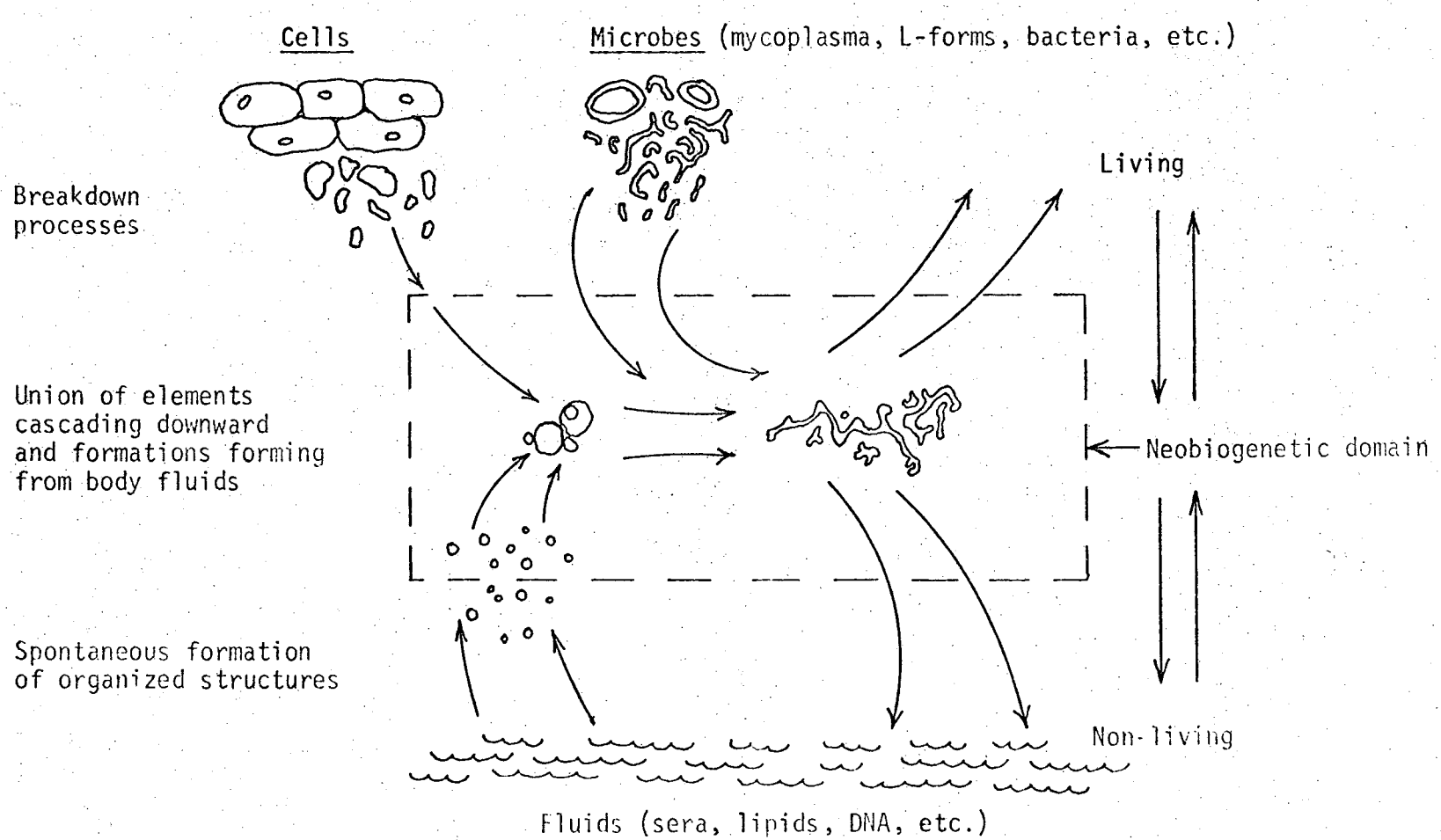
FIG. 1. MODEL OF NEOBIOGENESIS



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