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Precipitation Membranes, Osmotic Growths and Synthetic Biology

Experimental osmotic growths of now extant synthetic biology suggest that much of the morphogenetic self-organization might follow similar pathways in both inorganic and organic systems. The organic-like shapes and forms of inorganic chemicals lead to intriguing philosophical-paradigmatic implications for biochemistry, biology, general systems theory and philosophy of science.

Classes, divisions, and separations are all artificial, made not by nature but by man. All the forms and phenomena of nature are united . . .

—*Stephane Leduc*

As all distinctions, the division between life and non-life is an artificial one. Although useful for some purposes, it may narrow down alternative options of exploration.

Recent interest in spatial, periodic and wave properties of chemical systems (e.g., Liesegang patterns,⁷ Belousov-Zhabotinskii reaction,^{10,22} Benard convection cells,¹³ viscous “salt fingers” and precipitate “needles”¹⁵) reflects the resurfacing of the timeless notions of *self-organization*, *spontaneous formation*, and *membraneous*

precipitation in studying the emergence of life's forms and functions, the origins of life.

We have reproduced a number of osmotic precipitation experiments of Stephane Leduc⁶ and other representatives⁸ of the long-extant field of *synthetic biology*. These experiments confirm that the potential richness and complexity of spatial-periodic spontaneous patterns goes beyond modern wave-propagation systems and periodic precipitation patterns. It is obvious that the *osmotic growths* of Leduc were complex membranous precipitates, mimicking not only the most intricate forms and shapes of the "organic" world, but exhibiting phenomena of dynamic growth and transportation, self-maintenance, self-organization and self-renewal, on a scale previously unsuspected.

By immersing a fragment of fused inorganic salt, a crude lump of brute inanimate matter, in a simple inorganic salt solution, the osmotic growth germinates before one's eyes—putting forth structures of "bud and stem and root and branch and leaf and fruit," without any presence of organic matter. These growths are not crystallizations: they grow through intussusception, not by accretion. They exhibit phenomena resembling those of circulation, respiration, and periodic structuralization; they go through a vigorous growth, stable platitude and decay; they reproduce through budding and mimic the forms, colors, textures and even microstructures of "organic" growths to the extent of *deceiving experts*.

In 1907, the Academie des Sciences excluded Leduc's report from its *Comptes Rendus*; his experimental researches on diffusion and osmosis touched too closely on the then-discredited notion of spontaneous generation.

The book by Stephane Leduc was newly reviewed by Zeleny²⁴ in 1979. It is now possible, some 80 years later, to take a new dispassionate and scientific look at the field of synthetic biology.

HISTORICAL REMARKS

Stephane Leduc was a Professor at L'Ecole de Medicine de Nantes in 1911. His research created a heated controversy at the time, since his experiments touched on the notion of spontaneous generation in a way that reached far beyond the crudeness of "Pasteur's proof" ("The majority of scientists seem to consider that the question of spontaneous generation was definitely settled once and for all when Pasteur's experiment showed that a sterilized liquid, kept in a closed tube, remained sterile."⁶).

Leduc insisted that the study of life must begin with the study of physico-chemical phenomena which results from the contact of two different liquids: biology then is a branch of the physico-chemistry of liquids, studying electrolytic and colloidal solutions and the molecular forces brought about by osmosis, diffusion, cohesion, and even crystallization. This turns out to be a remarkably modern and

useful view: it provides for the precursory self-organization of the milieu in which modern genetic materials have to interact and function.

It was l'Abbe Nollet (1748) who first experimented with osmosis, followed by Fischer (1812), Dutrochet (1837), Vierordt (1846), Graham (1854), and others.^{6,12} Both Leduc⁶ and Pfeffer¹² agree that it was Moritz Traube of Breslau¹⁶⁻¹⁸ who first discovered (in 1866-67) the "artificial cell"—a precipitated (or osmotic) membranous growth" "...one of the most important, if not all together the most important, steps forward since the discovery of osmosis."¹²

The list of researchers who followed up on Traube's discovery is indeed large: E. Montgomery (1867), G. Quincke (1869), H. de Vries (1871), O. Butschli (1892), L. Rhumbler (1906), R. Liesegang (1907), M. Kuckuck (1907), and A. and A. Mary (1909), just to name a few.⁶

After the field of synthetic biology sank into full scientific obscurity, only the rings of Liesegang,⁷ wave-propagations of Belousov-Zhabotinskii,¹⁰ dissipative structures of Prigogine,^{13,14} and similar simple periodic phenomena continued to make their on-and-off appearances. The original richness and complexity of patterned precipitation was either lost or delegated into the realm of "curiosities."⁹

EXPERIMENTS

Typical textbook presentations of the concept of precipitation (the mixing of two solutions) and their discussion (the formation of an insoluble salt) are rather unimpressive and boring (a cloudy solution).

In contrast, *osmotic growths* precipitate and grow over a five- to thirty-minute period (sometimes days) and go from a transparent to an opaque state. Some can even be removed from the mother solution for inspection.

From the hundreds of varied and differentiated experiments, a typical experiment of "synthetic biology" might be presented as follows:

MATERIALS

CaCl₂—fused and broken into fragments

Na₃PO₄—saturated solution

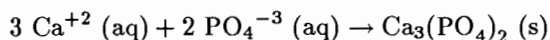
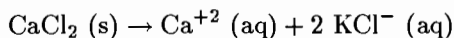
A 250-ml Beaker

PROCEDURE

Pour 200 ml of the saturated Na₃PO₄ solution into the 250-ml beaker. Drop three or four fragments of fused CaCl₂ into the solution and let them sink to the bottom.

DESCRIPTION

The precipitation is almost immediate: the precipitate is a transparent film that becomes white as time progresses and further precipitation occurs. The reactions are



describing the ion-interactions that produce the precipitation.

The description of the osmotic growth can be left to Leduc: "The membranous substance, the chloride of calcium, diffuses uniformly on all sides from the solid nucleus, and forms an osmotic membrane where it comes to contact with the solution. The spherical membrane is extended by osmotic pressure, and grows gradually larger. Since the area of a sphere increases as the square of its radius, when the cell has grown to twice its original diameter, each square centimetre of the membrane will receive by diffusion but a quarter as much of the membranous substance. Hence, after a time, the membrane will not be sufficiently nourished by the membranous substance, it will break down, and an aperture will occur through which the interior liquid oozes out..."⁶ In Figure 1, we present the diagrammatic representation of the described process. In Figure 2, the photographs capture the dynamics of another example of precipitation and osmotic growth.

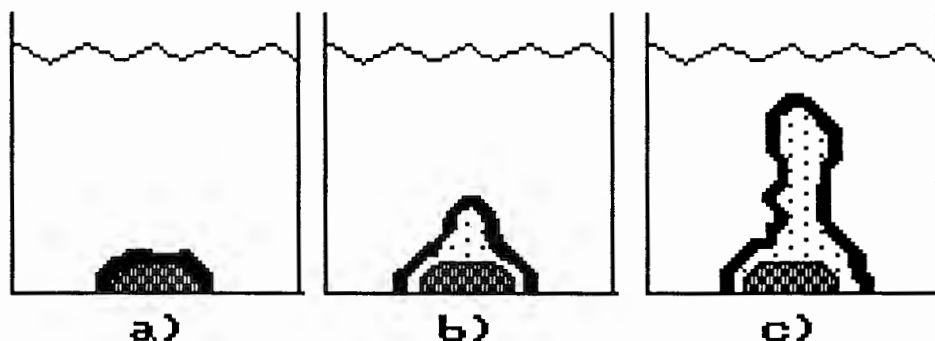


FIGURE 1 a) immediate precipitation, b) osmotic growth begins via osmosis and H_2O passes through membrane, and c) continues. Key: sodium phosphate (sat.); manganese phosphate (s); manganese chloride (s); and . . . manganese & chloride ion (aq).

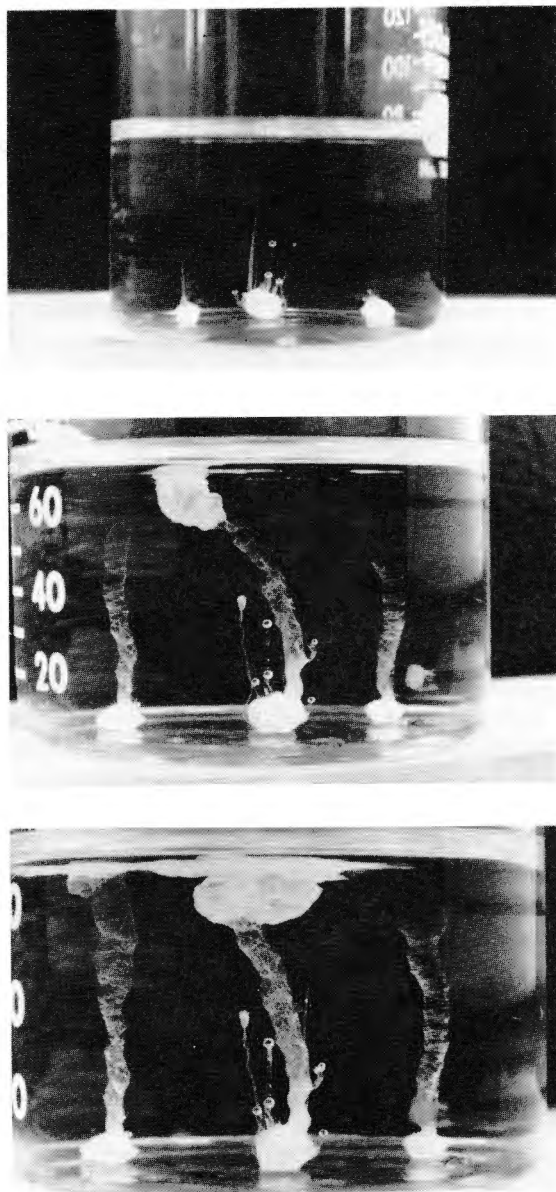


FIGURE 2 CaCl_2 (s)/ Na_3PO_4 (aq) system: from top to bottom, 10:39 a.m., 10:53 a.m. and 11:05 a.m.

Most of Leduc's osmotic growth experiments are analogous to the above $\text{CaCl}_2/\text{Na}_3\text{PO}_4$ system. We have also reproduced the following systems and have achieved results as described by Leduc:

- 1) $\text{CaCl}_2/\text{NaSiO}_4, \text{Na}_2\text{CO}_3, \text{Na}_2\text{HPO}_4$
- 2) $\text{CaCl}_2/\text{K}_2\text{CO}_3, \text{Na}_3\text{PO}_4$
- 3) $\text{CaCl}_2/\text{K}_2\text{CO}_3, \text{Na}_2\text{SO}_4, \text{Na}_3\text{PO}_4$
- 4) $\text{MnCl}_2/\text{Na}_3\text{PO}_4$
- and 5) $\text{MnCl}_2/\text{K}_2\text{CO}_3, \text{Na}_2\text{SO}_4, \text{Na}_3\text{PO}_4$

OSMOSIS

Relevant study of osmosis and membrane phenomena comes mostly from Pfeffer.¹² Traube's artificial precipitation membranes (e.g., of copper ferrocyanide) were most important for the accurate measurement of the osmotic pressure, developed later by Pfeffer (who induced the membranes to form within porcelain walls) and also used by Leduc.

Membraneous precipitates are formed at the interface of two solutions, or of a solution and a solid, both colloidal and crystalloid. Major membrane-formers used by synthetic biologists were, for example, potassium ferrocyanide and copper nitrate. Osmotic membranes behave exactly as colloids. They grow as long as an osmotic current of water, flowing in, produces a pressure in the interior which tends to distend the membrane. At the same time, they are capable of thickening. The membranes evidently cannot form (or persist) if too active a diosmotic exchange takes place between the solutes.

When the osmotically active solute *does not* pass through the membrane, then the endosmotic (not diosmotic) water flow takes place. This is due to the unequal ratio between the water molecules that collide with the unit surface of the membrane, and the water molecules that pass through it. Let us assume that a membrane has formed and is in contact with water molecules on both sides: an unequal number of water molecules hit the membrane from the surrounding water. However, if one side of the membrane comes in contact with a solute that does not diosmose, its molecules bounce off the membrane, the number of water molecules hitting from that side decreases, and a water flow moving into the salt solution must necessarily result.

OSMOTIC GROWTH: A CELLULAR-SPACE MODEL APPROACH

Varela, Maturana, and Uribe¹⁹ in their discussion of *autopoietic (self-producing) systems* define a unity as a complex system of components that is realized as a whole through its components and their mutual relations. Upon studying Stephane Leduc's descriptions and reproducing some of his experiments, one can show that osmotic growths fall into this category of autopoietic systems.

As stated above, the term autopoiesis means self-production. Self-production has the potential to mean and to be interpreted many different ways by a variety of people. Thus "autopoiesis" has been coined (not translated from greek) as a label, a definition for a clearly defined interpretation of "self-production." This phenomena of self-production can be observed, intuitively, in living systems. A cell consists of a complex set of production processes that synthesize proteins, lipids, enzymes, etc. that renew the entire macromolecular population of the cell thousands of times during its lifetime. Yet, throughout this turnover, the cell maintains its identity, cohesiveness, relative autonomy and distinctiveness. This lasting unity and wholeness, in the midst of continual turnover of constituents, is called "autopoiesis."

An autopoietic system is defined as an entity that can be distinguished from its background by an observer and is realized through a closed (circular) organization of production processes such that (1) the same organization of processes is created through the interactions of its own products (constituents) and (2) a discernable topological boundary emerges as a result of the same processes. The organization of components is maintained and remains invariant throughout the interactions and continual flux of the components: what changes is the system's structure and its parts, *not* its organization.

Guiloff²⁵ states that the *theory of autopoiesis* does essentially three things:

1. It defines, without reference to the whole, the relations that the components must satisfy in the integration of an autopoietic system.
2. It makes a clear distinction between the phenomonic domain of the components of an autopoietic system (as the domain of its states in autopoiesis) and the phenomonic domain in which the autopoietic system operates as a unity (as the domain of its relational states), showing that these two phenomonic domains do not intersect.
3. It shows that reproduction is not a definitory feature of the organization of living systems, but that it is necessary for evolution.

Varela, Maturana, and Uribe¹⁹ along with Zeleny,²³ have developed computer models of autopoiesis. In their models, the boundary (membrane/autopoietic unity), that is created (realized) and maintained through the functioning of autopoietic organization, remains essentially constant in the volume that is enclosed as time proceeds. Figure 3 summarizes the components (essential building blocks) and

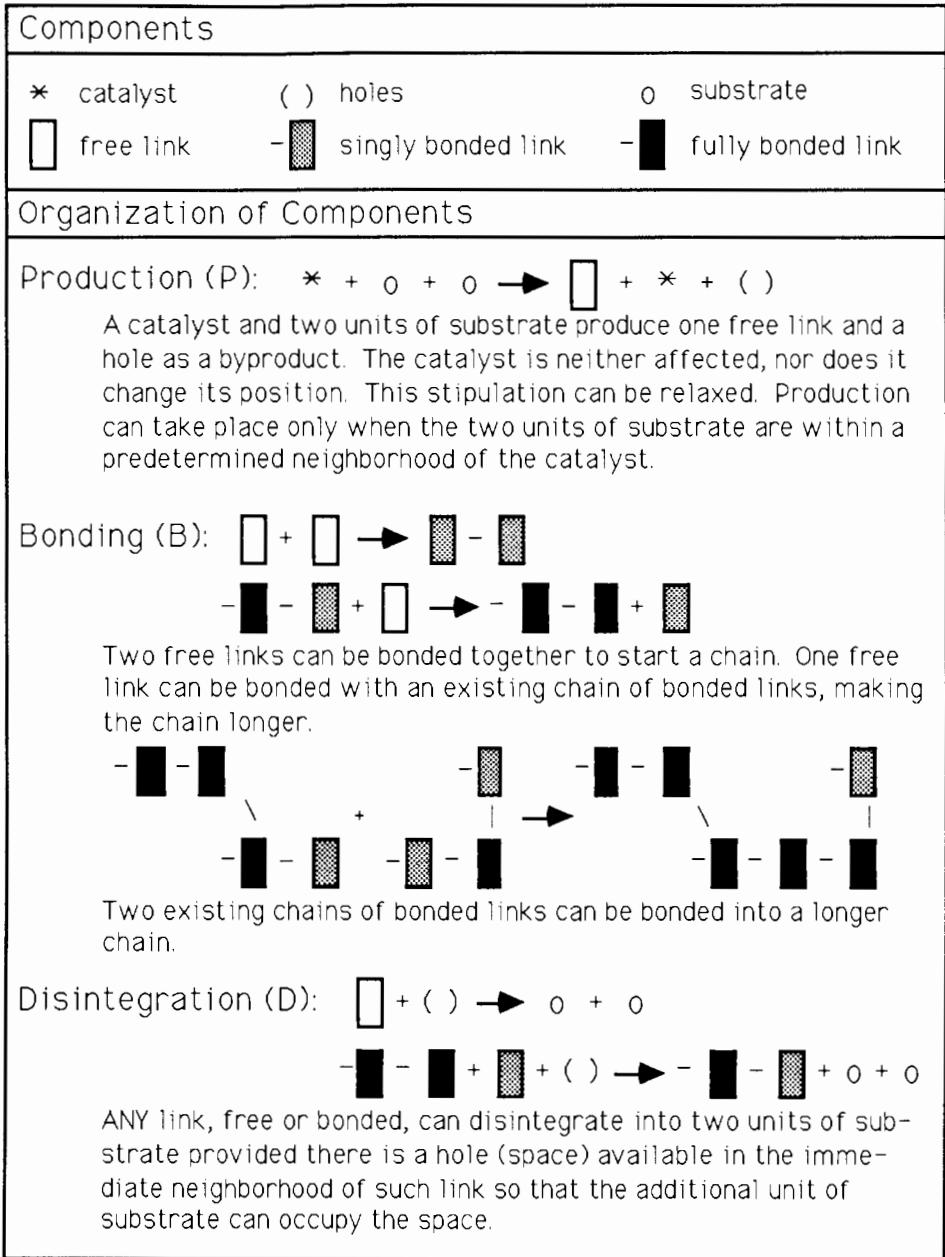


FIGURE 3 APL-Autopoiesis: a model of the cell.

organization of components in Zeleny's APL-Autopoiesis.²⁵ It is the three processes (productions)—(1) production, (2) bonding, and (3) disintegration—that produce the autopoietic unity or cell when concatenated in a circular fashion. Zeleny has found that the circular organization in itself is not sufficient to maintain the autopoietic unity. The rates of the productions must be balanced in an equilibrium state such that neither the production nor the disintegration is too vigorous. Figure 4 shows four frames from an APL-Autopoiesis computer simulation of a balanced autopoietic organization. The emergence of a topological boundary occurs in the fourth frame and the autopoietic unity is then maintained indefinitely. In experimenting with the models of autopoiesis, it is necessary to remember that this three-process model represents the minimum conditions necessary for the emergence of autopoiesis and that the phenomenon of autopoiesis has to be "tuned into" the underlying circular organization.

To model Leduc's osmotic growths, it will be necessary to modify and expand upon these models to account for the membrane production as well as for the osmotic growth processes while maintaining the autopoietic organization.

Pfeffer¹² has provided very interesting descriptions of the processes of osmotic membrane formation and osmosis along with details of the membrane structure. Pfeffer's concept that membranes are formed from structural units, which allow the paths that the solute and water molecules follow to be defined, employs the principles of localized structural unit interactions, structural unit-solute interactions, and structural unit-water interactions. At the membrane-solution interface, a solute diffusion zone and, therefore, a concentration gradient develops which influences the flow of water through the membrane. Pfeffer also used kinetic molecular theory to explain osmosis and osmotic pressure as a result of the differing number of water molecules that affect the membrane surface, on each of its sides, when unequal solute concentration occurs.

Both of these mechanisms view osmosis and osmotic pressure as complex and dynamic systems, where the processes that occur are based on the current states of the membrane, solute, and water molecules. These states and processes change in time and space according to their localized (neighborhood) interactions. Cellular-automata approaches, similar to those of Vichniac,²⁰ Goel,⁵ Gernert,⁴ and others²¹ seem promising for modeling such systems. Since cellular-automata are dynamic models in which space, time and variables are considered to be discrete, an overall outline for the osmotic growth process may be as follows:

1. *Initial Conditions.* Definition of the initial membrane, that forms by precipitation, between the solid fragment and the solution.
2. *Endosmosis.* Definition of the states of the membrane, solvent, and solute and their transition rules for passage through the membrane.
3. *Membrane Expansion.* Definition of conditions (states) of the membrane, solvent, and solute for expansion to occur.
4. *Formation of a Membrane Component.* After separation, a new unit forms; define the appropriate conditions.

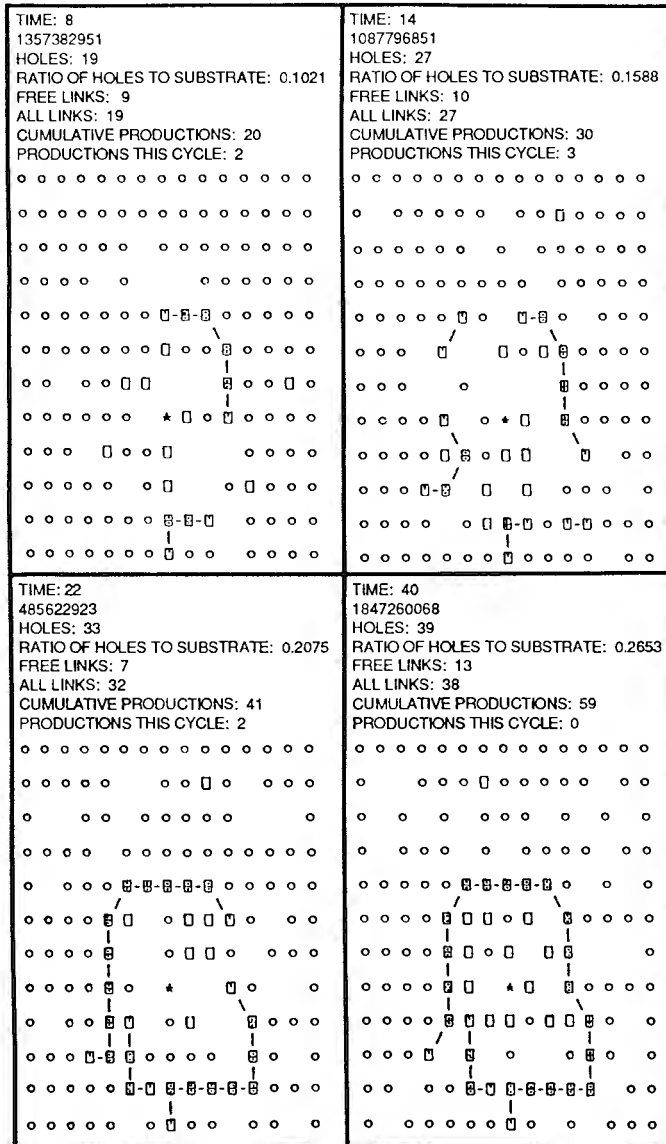


FIGURE 4 APL-Autopoiesis: computer simulation. Catalyst is represented by an asterisk. Holes are represented by a blank space. Substrate is represented by a small circle. Free link is represented by an empty square. Singly bonded link is represented by a square with a quota inside and a single dash. Fully bonded link is represented by a square with an APL division sign inside and two dashes.

5. *Repeat* steps two through five.

The above five steps provide a general outline for the process of growth. Other aspects that are to be considered to completely model the entire "lifetime" of this autopoietic system are rules (a) to direct the growth in a vertical direction, (b) to slow, thicken, and eventually stop the growth, and (c) to describe the decay of the osmotic growth structure.

ORGANIC FORMS

What is interesting about inorganic osmotic growths is their striking resemblance to living shapes, forms and behavior. These are the forms and behavior which we intuitively associate with living organisms. Leduc reports osmotic growths resembling striated stems, leaves and buds, mushrooms and fungi, shells, capsules and flowers, amoebas, fins, worms, and, of course, assorted spheres, spheroids, beads, drops, compartments and cilia. These are not more or less rigid geometrical lattices of crystals, crystalloids or liquid crystals. The shapes are round and smooth, interconnected by stems and pathways, growing, changing, reshaping and repairing themselves throughout their life span. The fact that "inorganic" matter, under the most common geophysical conditions, can give rise, spontaneously and without "coaching," to such apparently "organic" forms, is challenging: organic and "living" forms would then be "in-formed" in the system of chemicals and their interactions, rather than "informed about" through a particular structural component.

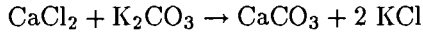
Identical forms of organic elements, cells, tubes, etc. may be produced either in organic liquid or a semi-organic liquid such as sucrate of lime, or in an absolutely inorganic liquid such as silicate of soda. The sulfates and phosphates generally produce tubes while the carbonates form cells.

The periodicity of some precipitates and their light diffraction give first insight into the nature of color in natural objects. The variety of forms and colors is bewildering, exhibiting not only periodicity and wave propagation, but also rhythmic catalysis and undulatory movements.

Osmotic growths may be viewed as models of morphogenesis. They consist of a number of cells instead of one large cell. Some of these artificial cells can actually grow out of the solution into the air, changing shape and color, and dissolving gases from the atmosphere.

Osmotic growths absorb nutriment from the medium in which they grow: comparing the morphogenic germ (small mineral fragment) with the system (osmotic growth) it "produced," we can register an increase of many hundreds times in weight. Of course, the surrounding liquid has lost an equivalent weight.

The absorbed nutrients undergo chemical transformation before they can be assimilated. For example, calcium chloride growing in a solution of potassium carbonate is transformed into calcium carbonate:



So the osmotic pressure possesses the remarkable power of organization and morphogenesis. It is a matter of surprise that this peculiar faculty has hitherto remained almost unsuspected.

All of these osmotic growths not only imitate the *forms and shapes*, but also imitate the *microstructure* and various *functions* of living systems such as nutrition, metabolism, growth, movement and evolution.

These osmotic forms have the potential to grow wherever suitable conditions exist. Combined with the discovery of rock-dwelling life forms³ and the new theories of mineral origins of life,¹ osmotic forms could have played a large role in the evolution of life. Thus, when searching for the physical embedding of life, we should also consider osmosis and osmotic growths and not only clay crystals and crystallization.²⁵

UNRESOLVED QUESTIONS

The number of unresolved questions is staggering and that is why this paper was written. Let us state only the most important:

1. How much of what is in the fossil record should be attributed to the imprints of osmotic growths and how much to clearly defined biotic events?¹¹ Precipitated osmotic membranes could have been widely distributed in nature. Can osmotic growths be operationally distinguished from organic forms, especially since their persistence and resilience is comparable to that of living matter?
2. What are the philosophical-paradigmatic implications of the capability of inorganic matter to exhibit phenomena which are strikingly similar, externally and internally, to the morphogenesis of living matter? If "organic" forms is the way matter can organize itself, what is the true role of "information programs" residing on some of the components?
3. What are the possibilities of "organic components invading inorganic forms and templates"? See Cairns-Smith¹ for an example of such. Can osmotic growths be the basis for a hypothesis on the origins of life?
4. What role, if any, have osmotic growths played in the formation of the various rocks, e.g., siliceous, calcareous, barytic, magnesian, the fibrous and nodular rocks, and atolls?

5. What are the most appropriate conceptual frameworks for developing models of spontaneous osmotic growths?
6. What are the implications of “life as organization” as opposed to “life as stuff” in searching for life processes? Should the search for organic debris take precedence before the search for autopoietically organized and self-renewing processes?
7. Why was both Leduc’s work and synthetic biology so effectively removed from scientific and non-scientific consciousness, even though the phenomena of osmotic growth still remained unexplained and challenging?

SUMMARY

The dismissal of Leduc’s work seems to have occurred due to the narrow view of biology and biological processes that his peers held in that era of science. The scientists of Leduc’s time did not have as broad a conception regarding the correct domain of biology as science does today. The advanced thinkers, the mainstream scientists of today, are willing to explore the fringes of life much further than the Academie des Sciences of Leduc’s time.

According to Leduc⁶ and also Zeleny,²⁵ there is no sharp division, no precise limit where inanimate nature ends and life (animate nature) begins. The transition is gradual. Individual attributes of life are also found outside of the living organism. Just as a living organism is made of the same substances found in the mineral world, so life is a composite of the same physical and chemical phenomena found in the rest of nature.

Leduc’s osmotic growths, like Cairns-Smith’s clay crystals,²⁵ should be considered as representatives of protobiological, primitive systems. They do not represent the actual (complete) biological processes of life, but they are similar and they do resemble the biological processes in their shape, form and behavior. It is for these reasons that they have a potential place in the origin (evolutionary tree) of life and are definitely worth investigating. Life is not a substance but an organization.

The fact that an organic body is formed of certain elements should not be of greater importance than the manner in which these elements are organized. The implications of this view are potentially far reaching.

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