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PANEL

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# Was the environment for primordial life hypersaline?

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All known life-forms consist of cells with semipermeable membranes capable of generating the energy necessary for growth and procreation by utilizing available energy from concentration gradients across the membranes. All cellular life-forms also contain the genetic information necessary for their own growth and procreation.

All forms of “life as we know it” contain DNA, RNA, and protein. Shortly after the double-helix structure of the DNA and its genetic code had been established, the “central dogma” for genetic information was formulated as follows: Genetic information is transcribed from DNA to RNA and then translated to protein, and only DNA is capable of “self-replication.” Scientific research on “generation of life” seems to have been stumped by the realization that while DNA was essential to all known forms of life, life itself was essential for the formation of DNA. This classical “chicken and the egg” impasse seems to have been a stumbling block for fruitful scientific research into how life was first created.

Fairly early the central dogma started to erode, first by the realization that RNA could serve as template for the formation of DNA, and later by the realization that in some cases, as for example in some pathogenic conditions in animals, such as scrapie in sheep and “mad cow disease,” and also kuru and Creutzfeld’s disease in humans, the infectious agent is not any known life-form, but simply a special type of protein, a “prion.” These prions, when present in living mammalian neural cells producing normal cell protein, may “subvert” the cell to produce prions instead. Prions thus seem to carry “information” for their own formation.

Even earlier, another form for non-DNA “information” had been noticed, as when cell-wall-deficient L-forms of bacteria were only able to produce cell walls after being given the “information” residing in a small performed fragment of cell wall. This “wall information” is, however, perhaps more analogous to the information inherent in nucleation crystals; it only carries growth information and seems thus, by itself, unable of Darwinian evolution. Nevertheless, the erosion of the monumental central dogma makes it attractive to search for life “as we don’t know it,” i.e., life-forms without developed DNA as its source of genetic information.

Primordial life may well have been very different from present life-forms, but the central conditions for existence of life must have been present before the emergence of even life as we don’t know it. Very naively, these conditions seem to be:

The presence of liquid water
The presence of abiologically formed “organic” molecules
The presence of semipermeable membranes
The presence of a suitable energy source
A tangible form of inheritable information allowing Darwinian evolution, and the eventual emergence of the vast diversity of life-forms, living and extinct

There are plausible phylogenetic arguments for considering Archaebacteria the most “primordial” existing life-forms. Preconceived notions as to what conditions seem beneficial to survival and procreation of the highly evolved modern Archaebacteria may not a be fruitful base for speculations about primordial life-forms. The absence of competition or cooperation with highly evolved life-forms posed less constraints as to efficiency and fitness of growth, than the constraints operative in modern ecological environments. The minimum generation time for a primordial cell would largely be determined by chemical and physical environmental conditions, not primarily by biological ecological constraints.

Knowledge about what environmental conditions existed when the first life-form was formed is obviously crucial to understand its emergence. Very simply put, there

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was water on the Earth, and a sun and a moon in the sky. The sea was marine, salt, with a very thin primordial soup of organic and inorganic molecules formed during eons of chemical evolution and accretion of extraterrestrial galactic matter. There is a general consensus that essentially no free oxygen existed in the atmosphere or in the sea. Geological activity, tidal action, and storms created lakes, pools, and puddles of salty seawater. Wind and solar irradiation caused the seawater to evaporate, often until the thin primordial marine soup became a supersaturated salty brine where chemical evolution proceeded under conditons of very diminished water activity.

As a research assistant for Prof. Helge Larsen, I fell in love with the halobacteria and started speculating that primordial life might conceivably have emerged in hypersaline environments (Dundas 1974). Hypersaline brines definitively have rather low water activity, and diminished water activity may enhance the formation of macromolecules from small abiologically formed "organic" molecules (de Duve 1995), but no investigations seem to have been carried out as to the course of chemical evolution in saturated salt brines. Amphiphilic molecules aggregate at gas–water interphases, and three-layered membrane fragments with a partly hydrophobic middle layer and two more hydrophilic surfaces are easily formed abiologically (Deamer 1997). Fragments of such three-layered membranes may easily form "microvesicles" encapsulating some of the liquid medium in which they are formed.

Microvesicles prepared from bacterial cell membranes are osmotically very sensitive, being easily disrupted by even very small decreases of the osmotic pressure of their external medium. Salt-saturated brines, however, may remain largely isohaline, because evaporation only results in further crystallization and addition of water only results in the dissolution of some of the salt crystals. Abiologically formed macromolecules such as proteins and tetrapyrroles did not carry any "genetic information," and vesicles containing hypersaturated salt brines with the concentrated primordial soup in which they were formed, were, in the absence of any "carrier of genetic information," not capable of Darwinian evolution.

Rain and tidal action could dilute the hypersaturated salt brine, but this need not significantly change the salinity and osmotic tension of the brine, because some of the crystallized salts would simply dissolve, keeping the brine saturated and largely isoosmotic. Even further evaporation need not greatly change the "salinity," as more salt would crystallize out, keeping the brine largely isoosmotic and saturated. Slight gradients of sodium and potassium ions and larger gradients of prebiotic molecules would nevertheless be established and reestablished across the membranes of any vesicles in the brine. Energy of potential biological importance might thus become available, waiting for the evolution of a system that could use it.

It would perhaps seem that solar irradiation would offer an immediate energy source for life, but even today solar irradiation is not a really primary biological energy source; it must be transduced into an energy gradient across membranes before it can yield useful biological energy. Solar irradiation is and was, however, of immediate potential harm to all life-forms. Photodestruction of biologically important molecules may be the most immediate effect of sunlight on life, both "as we know it" and "as we don't know it." Many mechanisms have primarily evolved to protect life from photodestruction. Only later did evolution lead carotenoids and other pigments to become useful "harvesters" of potentially harmful photons. It may be that even chlorophyll started out as a photoprotector molecule. Even the primitive and unique photosynthetic bacteriorhodopsin of halobacteria may have started its career by shielding primordial cells against photodestruction.

One can envisage, in the prebiotic world, an aqueous hypersaline environment enriched in dissolved potassium chloride and from which most of the sodium chloride had been lost by crystallization. Even today, the hypersaline bitterns remaining after sodium chloride crystallization in solar salt-producing facilities are examples of aqueous environments enriched as to potassium and depleted with regard to sodium ions (Lepierre 1935). Nothing seems to be known as to the concentration of soluble phosphate or as to possible prebiotic production of phospholipids in hypersaline potassium chloride-enriched brines. Based on current understanding of chemical evolution it seems reasonable to imagine the formation of vesicles with bilayer membranes, containing prebiotic macromolecules, proteins, tetrapyrroles, and perhaps even some photoprotective molecules. Any intravesicular molecule conferring even very slightly enhanced continuity of vesicle existence, and also capable of autocatalytical formation, would confer a modicum of "ecological fitness" on its vesicle. A vesicle containing abiologically formed prion-like molecules with even minimal positive effect on vesicle existence and with even minimal autocatalytical formation capability would be capable of "Darwinian evolution" and could thus be classified as a "life-form." Life-forms are essentially Turing machines in mathematical terms, or Darwin machines in biological terms (Plotkin 1994).

The idea of evolution of bioinformation in hypersaline brines may seem extremely far-fetched, but knowledge about the genetics and physiology of extremely halophilic microorganisms in saturated sodium chloride brines is developing fast, although no studies seem to have been made as to microorganisms in hypersaline, potassium chloride-enriched saline bitterns. The notion that concentrated salt solutions are alien to life is still tacitly generally accepted. The warm, hypersaline lakes on the bottom of the Red Sea have been reported as being devoid of microbial Life (Coleman 1993). The search for life-forms in these environments centered around sulfate-reducing bacteria, which were not found in brines and sediments with the highest concentrations of heavy metals, but could be isolated from from brines and sediments with slightly lower concentrations of heavy metals (Trüper 1969). More recently, bacterial populations of more than a thousand cells per milliliter have been reported as existing in the warm anoxic salt brines on the bottom of the Red Sea (Karbe 1987).

Potential bioenergy, in the form of membrane-separated chemical concentrations, and an extremely primitive bioinformational molecule may have been available in prebiotic hypersaturated brines, but any such hypotheticical form of "life as we don't know it" would remain a prisoner in its saturated nearly isoosmotic environment until the eventual advent of a cell-wall molecule carrying the information for its own growth. Only then might this primitive life-form invade the world of nonsaturated brine.

Many "primitive" Archaebacteria have been proposed for a role in the origin of life. Halobacteria have been bypassed, largely because of their assumed general aerobic nature. It seems that the placement of halobacteria in phylogenetic systems often is based solely on aerobic members of the group. Modern aerobic halobacteria would as such only have begun evolving rather late, after evolution of biological production of an oxic atmosphere. Halobacteria are also commonly considered as too osmotically fragile. Some extremely halophilic bacteria are flat, thin, and square (Oren et al. 1997) and would as such be extremely sensitive to even minimal decreases in the osmotic pressure of their natural environment. Halobacteria, nevertheless, seem not unlikely candidates for primordial life. Their versatility as to ATP generation seems unsurpassed. They are admittedly mainly aerobic, utilizing many organic molecules as energy sources. They are nevertheless able to obtain ATP from the non-oxygen-requiring degradation of arginine.

Halobacteria are also able to produce ATP under essentially anoxic conditions, utilizing the photosynthetic molecule bacteriorhodopsin, which from an evolutionary standpoint may have evolved from an initial role as a photoprotecting molecule. Halobacteria also seem to be nonconformists, being quite versatile as to their genetic physiology. The genetic information of their phages may be subjected to considerable rearrangement during reproduction (T. Torsvik, personal communication). Genetic plasticity would probably have been an advantage during primordial evolution. All Halobacteria are today able to protect themselves effectively against photodestruction in their natural environments such as the Dead Sea, solar salterns for producing marine salt, and nearly every little puddle of saturated salt brine formed during summer along the coasts of Portugal and Spain.

It seems interesting to speculate that, in the deep anoxic and aphotic layers of the Dead Sea and the Red Sea, there may exist descendants of primordial isoosmophilic "life as we don't know it." Such primitive life-forms may have lost the capacity for both photoprotection and photosynthesis. They would not be growing on products of anoxic photic chemical evolution, but on sedimenting organic remains from the teeming aerobic ecosystem in the photic, aerobic surface layers of the Dead Sea and the Red Sea. Such primitive microorganisms might not unlikely be very sensitive toward sunlight, toward minimal changes in osmotic pressure, and toward oxygen. They may also be quite dependent on their natural energy source, i.e., sedimenting products from photic and oxic surface layers. They need thus not be easy to isolate, but studies of bacterial DNA from the anoxic sediments of the Dead Sea or some hypersaline pools at the bottom of Red sea might conceivably yield information about some very early forms of "life as we know it."

It seems also possible that the hypersaline, nearly anoxic bitterns remaining after commercial marine salt production harbor "primordial" halophilic bacterial species. It is intriguing that the intracellular salt concentrations of modern halobacteria resemble the potassium-enriched, sodium-depleted bitterns remaining after the harvesting of marine salt. Bitterns, however, are rather difficult environments to study as to both their chemistry and ecology. It might be possible to recreate an anoxic, "primordial salt-saturated soup," including suitable organic and inorganic components assumed to be present. Inoculation of this prebiotic environment with brine from the nearly anoxic bittern layer of a modern salt pan could perhaps allow growth of some modern extreme halophilic Archaebacteria exhibiting some of their primordial physiological capabilities.

The title of this rather speculative essay contains a question mark. I would like to end with another question mark: What evolutionary connections may there be between the rhodopsin molecules in my eyes and the bacteriorhodopsin of my beloved halobacteria?

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