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REVIEW ARTICLE

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Crucial crises in biology: life in the deep biosphere

Summary The origin and evolution of life on Earth are the result of a series of crises that have taken place on the planet over about 4500 millions of years since it originated. Biopoiesis (origin of life), ecopoiesis (origin of ecosystems) and the first ecosystems (stromatolites and microbial mats), as well as eukaryopoiesis (origin of nucleated cells) are revised. The paper then focuses on the study of the deep biosphere, describing ecosystems never found before, which are independent of solar radiation and have changed previous assumptions about the requirements of life; even the concept of biosphere, as Vernadsky defined it, has increased its scope. Since the discovery, in 1987, of bacteria growing in the crevices of rocks at 500 m deep, in boreholes drilled near the Savanna River, Aiken, South Carolina, other bacteria have been found in the deep subsurface reaching depths of about 3 km (e.g., in the Columbia River Basalt Group, near Richland, Washington state), in an anaerobic, hot, high-pressure environment. Some kinds of microorganisms can thrive at such depths, living in many cases a geochemical existence, by using very specialized metabolisms, which depend on the local environments. The existence of organisms independent from photosynthetic production is the most outstanding, novel feature of the deep biosphere. Living beings might not need other energy and chemical sources than those which occur in the development of all planetary bodies. Life, therefore, could even be an ineluctable outcome of planetary evolution and, as a corollary, a natural continuation of the usual development of physical phenomena in the universe.

Key words Biopoiesis · Ecopoiesis · Stromatolites · Microbial mats · Deep-biosphere microbiota

Introduction

In the medical language, crisis is any sudden change, for better or worse, in an acute disease or infection; a sudden exaltation of the phenomena typical of a chronic disease; or a paroxysmal attack of pain, distress or disordered function. Life on Earth is not actually a disease, even though we humans may seem to be bent on making it a serious one. The origin and evolution of life on our planet, however, are the result of a series of crises which have made the Earth and the organisms which live on it undertake fundamental changes throughout time. I am considering here three turning points, which turned out to be crucial for the evolution of life.

1. Origin of life (biopoiesis). It is possible that life originated around 3800 million years ago. By then, the Earth, which had originated 4550 ± 20 million years ago, was ready to shelter life. It already had liquid water—an essential factor for the existence of life—, and a suitable temperature. The characteristics of the Earth's neighbor planets—Mars and Venus—could have also made the origin of life possible at that time. Nevertheless, if life did originate also on them, for some reason it became extinguished at some later moment.

2. Origin of ecosystems (ecopoiesis). The establishment of a kind of life that fed on the chemical compounds present on the Earth surface would have had a limit. With the repletion of nutrients—which would have happened in less than 300 million years, life would have become extinguished. It was the establishment of trophic chains, in which the products of some organism's metabolism became nutrients for others, that made it possible for life to persist. Over the first two billion years of evolution, bacteria were the only inhabitants of the planet; they "invented" most metabolic strategies that exist nowadays. In fact, prokaryotes are the only living beings that can be found in any kind of habitat, no matter how inhospitable it may be.

3. Origin of eukaryotic cells (eukaryopoiesis). The first living beings were prokaryotes; they lacked a nucleus differentiated from the cytoplasm, where their genetic material was dispersed [17]. Life might have persisted that way. In fact, bacteria are the only organisms that are not dependent on others for their survival; on the contrary, they are actually necessary for the survival of other living beings. But the level of

complexity reached in the evolution of bacteria would have been limited. Humans, for example, are the result of an evolutionary process that lasted for over 3500 million years, and the path leading to our species started when eukaryopoiesis took place. Symbiosis was an essential evolutionary mechanism in the origin of the eukaryotic cell.

Biopoiesis: the first living beings

Life is a phenomenon that may have occurred many times and at different places in the universe, provided some circumstances coincided. Liquid water is a prerequisite for the presence of any kind of life. When the Earth originated, its surface temperature must have been too high for the existence of liquid water. Most water presumably escaped to the outer space. The frequent collisions of comets with our planet, once the Earth had cooled, may have been responsible for the later presence of liquid water [6, 25, 26]. Some 3.9 billion years ago, the Earth must have reached a condition compatible with the existence of life. The mechanisms that led to the origin of life have not yet been elucidated, even though the study of the origin of life has made great advancements over the second half of the 20th century. Currently we know that, starting from such simple compounds as water, NH_4 and CO_2—all of them present on the Earth in the Archean period—, and through electrical discharges or ultraviolet radiation it is possible to obtain organic compounds spontaneously. A series of famous experiments carried out in 1953 by Stanley L. Miller [23], by then a twenty-three year old graduate student of Harold C. Urey (Chemistry Nobel Prize winner in 1934 for his discovery of deuterium in 1932), produced the amino acids alanine and glycine as well as other organic compounds by the action of electrical discharges on a mixture of water, CH_4, H_2 and NH_4. This breakthrough was the beginning of a new discipline, which has been known as prebiotic chemistry ever since. Another key discovery was the production of adenine ($H_5C_5N_5$) from hydrogen cyanide (HCN) by Joan Oró in 1959 [26]. Adenine is the main component of one of the universal nucleotides which make up DNA, RNA and ATP. Note that cyanide, a most poisonous substance to humans, which blocks the capacity of hemoglobin to carry oxygen, can also be the source for adenine, a compound indispensable for life. The primordial broth, abundant in energy-rich organic compounds, could have accumulated without oxidizing in the reducing early atmosphere of Earth. Random associations of those compounds may have produced early biological mechanisms such as the formation of proto-cells. Proto-cells might have consisted of one lipidic membrane that would have contained a precursor of nucleic acids (proto-RNA) with catalytic capabilities, and one only protein. Such proto-cells would have increased in complexity, incorporating DNA, a compound more suitable to transmit coded information. What has not yet been solved is the mechanism that made it possible for a group of randomly gathered compounds to acquire the characteristics that define a living being, that is to say, the establishment of an entity separated from the environment by a boundary, and being able to both maintain itself actively and autoreplicate. Such a system is known as autopoietic [21].

Nor has yet been elucidated when life originated on Earth, although we have advanced a lot in this quest over the second half of the 20th century. Until the 1950s the story of life before the Cambrian was a mystery. Most paleontologists believed that the earliest organisms on Earth had been soft-bodied single-cell animals, probably direct ancestors of the trilobites, some worms and other animals which dwelt on the bottom of the oceans and were very abundant in the early Cambrian [4]. In the 1950s, Australian geologist Martin Glaessner found a rock sequence that did not show the Cambrian/pre-Cambrian discontinuity that had been usually observed in most locations worldwide. Below the Cambrian rocks, Glaessner found well preserved impressions left in sand or mud by soft-bodied marine animals of what has been called Ediacaran fauna, because he found them in the Ediacaran Hills of the Flinders Mountains in South Australia. (Currently these organisms are considered to be either protists or lichens, and there is a tendency to call them Edicara microbiota.) A fossil collector, R. C. Sprigg had already described some of those fossils in 1947, but regarded them as being early Cambrian, because at those times it seemed impossible that there would be large fossils older than those from the Cambrian [10]. Later on other remains of Ediacaran fauna were found in England, Greenland and Siberia. In the mid 1950s, another finding showed the presence of microbial life in rocks two billion years old in the Gunflint Iron Formation, Ontario and northern Minnesota. Paleontologists Elso S. Barghoorn, of Harvard University, and Stanley A. Tyler, of the University of Wisconsin, found an abundant microscopic life in those sediments, with some forms that resembled some modern bacterial cells [36]. Later studies revealed the presence of fossil bacteria in all rocks studied older than 2000 million years. The oldest microfossils were found in Warrawoona, Western Australia, by Stanley M. Awramik, of the University of California at Santa Barbara. Awramik, along with the Precambrian Paleontology Research Group directed by J. W. Schopf, of the University of California at Los Angeles, described fossilized bacteria about 3500 million years old, very complex in form, that looked like some present-day cyanobacteria. Their complexity suggested that life should have already existed for a long time before they were laid down in those sediments. Later studies carried out on the oldest known rocks—the 3900 million-year old Isua Formation in Greenland—did not reveal the presence of cells. The possible presence of organic molecules, however, was not discarded. According to these evidences, the origin of life (biopoiesis) must have happened at some time from 3900 to 3500 million years ago.

Ecopoiesis: the first ecosystems

The first cells must have been very simple organisms: little more than membranous bags containing nucleic acids, soluble enzyme proteins and ribosomes. Such kinds of bacteria do not have many abilities to make their own cell components by themselves. So, they must have lived heterotrophically, feeding on organic compounds present in the environment to keep their structure, obtain energy and replicate. As the number of cells increased, however, organic molecules in the environment must have depleted. Early cells, which must have been similar to some extant thermophilic anaerobic bacteria, had to evolve their own mechanisms to yield the energy they needed. The first mechanisms were presumably fermentation and photosynthesis. Fermentation, which takes place in the absence of oxygen, is a process to obtain energy from the breakdown of energy-rich organic compounds often with the production of heat and waste gases and a wide variety of low-energy end-products that are released into the environment. The breakdown takes place by a series of chemical reactions which release energy.

Photosynthesis is a process to obtain energy from light, usually solar radiation. Early phototrophic bacteria must have been similar to purple sulfur bacteria and green sulfur bacteria, which are photolitoautotrophs and capture energy from solar radiation by means of their pigments (bacteriochlorophylls and carotenoids). They either used H_2 or took the hydrogen from H_2S to combine with carbon present in the atmosphere in the form of CO_2. They were primary producers; so, they only needed nutrients as biogenic building blocks, and produced many organic molecules that were released into the environment.

Molecules resulting from fermentation and photosynthesis accumulated in the environment until some cells evolved that were able to use them for their own metabolism. In this way, the first fermentative trophic chains originated, and as a consequence, the cycling of elements began. The origin of ecosystems—ecopoiesis, as Canadian geneticist Robert H. Haynes coined the process—was a crucial event for the persistence of life on Earth. Otherwise life would have disappeared about 200–300 million years after its origin, once the environment would have run out of nutrients. The early Earth atmosphere was anaerobic, and its composition must have been similar to those Venus and Mars still have nowadays. Over time, as atmospheric hydrogen was depleting from some niches, phototrophic bacteria used more and more H_2S. Photosynthesis carried out by those phototrophs was anoxigenic and they released pellets of sulfur as wastes from their metabolism.

Phototrophic bacteria were able to propagate on the Earth surface and to provide chemotrophic bacteria with large amounts of organic matter. This hypothesis is in agreement with the discovery, in rocks 3400 million-year old, of carbon-rich deposits which can be compared to those left by the big tropical forests in the Carbonifer (300 million years ago). They differ in that Paleozoic carbon-rich deposits originated from the remains of the luxuriant vegetation from the Carbonifer, whereas Archean deposits are the remains of both microbial mats and planktonic communities and they show a laminated structure.

Stromatolites and microbial mats

Microfossils found at Gunflint and Warrawoona are of the kind known as stromatolites. They are layered sedimentary structures made mostly of $CaCO_3$, although in some cases their main constituent can be flint, like in the Gunflint Iron Formation. Communities of bacteria, especially photosynthetic bacteria, lived and died atop one another. Nowadays, in very restricted parts of the world, we can still see these structures—domed, conical, columnar or cauliflower-shaped—alive, with their top layers dominated by cyanobacteria. The precursors of stromatolites are some characteristic microbial communities, called microbial mats, which consist of several populations of prokaryotes distributed in very thin layers. The layers have active growth and the thickness of the mat can range from just a few millimeters to a few centimeters. The uppermost layer consists mainly of cyanobacteria—oxygenic aerobic photosynthesizers [20]. Below, different populations of anaerobic anoxigenic photosynthesizers are usually found, mainly purple sulfur bacteria (PSB) and green sulfur bacteria (GSB). At the bottom layer, sulfur-reducing bacteria (SRB) are usually found. This is the living part of the mat. Below, there can be dormant bacteria, chalk, sand, gypsum and other debris bound together by the matrices of earlier mats. Multilayered microbial communities are also found in natural environments such as freshwater stratified lakes. The vertical distribution of environmental factors such as light and oxygen—whose concentrations decrease with depth—, and hydrogen sulfide—whose concentration, on the contrary, increases with depth—determine microorganisms distribution and abundance (Fig. 1).

Stromatolites and multilayered planktonic microbial communities can be compared to other ecosystems, such as tropical forests, which also depend on light as their primary energy source. As a consequence of light extinction with depth, such ecosystems tend to arrange in horizontal layers, the stratification being the result of evolution to optimize light utilization. In the case of tropical forests, the photosynthesizing structure extends through several meters, and the ecosystem's study is not easy due to the complex trophic relations among its members; in multilayered planktonic microbial communities, the layering of phototrophic communities spans in the range of several centimeters; and in microbial mats, those layers occur only along a few millimeters. Nevertheless, at different scales these three kinds of ecosystems are comparable, and different organisms have found similar niches in each one [14, 15].

Eukaryopoiesis: the origin of nucleated cells

By 1900–1700 million years ago, most metabolic pathways had already been established. The composition of the Earth's atmosphere differed significantly from the composition at the time when life originated. Oxygen and carbon dioxide concentrations must have been practically as they are at present. A major change in evolution took place with the appearance of eukaryotic cells, which contained a nucleus and several self-replicating organelles. The complexity of eukaryotes is difficult to explain by only simple mutation or genetic transfer. Nowadays it is widely accepted that symbiosis was the process that led to the appearance of eukaryotes. Independent prokaryotes may have entered other cells and provided them with some useful services that prevented the intruder cells from being eliminated by the sheltering cell. Both cells may have adjusted their reproduction clocks and have coevolved, becoming communities of microorganisms deeply interdependent, and eventually, single organisms. The symbiotic origin of chloroplasts was proposed by German biologist A. Schimper in 1893. Some decades later American anatomist Ivan Wallin and Russian biologist Konstantin S. Mereschovsky reached the same conclusion independently [19]. In the late 1960s, American biologist Lynn Margulis went further in the study of symbiosis as the original source of several eukaryotic structures. Margulis' symbiotic theory is based on evolutionary ideas developed by geneticists, ecologists and cytologists, and relies on interdisciplinary research carried out in fields such as molecular biology, biochemistry, micropaleontology and even atmospheric physics and chemistry [18]. From our anthropological vision, it may seem to us that the evolutionary pathway that led to humans since the Cambric explosion may have been a major step in the history of life. Nevertheless, since the origin of eukaryotes—which has been the latest major evolutionary step—, evolution has only produced many variations of the same kind of organisms, i. e. eukaryotes.

The latest crisis: life in the deep biosphere

At the end of the 20th century we face other crises in biology. Nevertheless, the current crises will not produce changes in the structure of living beings. One of them only affects a small group of humans: researchers studying evolution and phylogeny that claim to be right about the existence of either five kingdoms or three phylogenetic domains. Besides, another crisis has taken place in the fundamental concepts we had about life, and some classical paradigms in biology could be replaced by new ones. Until recently it was thought that life was dependent on light. The deep-sea bottom was considered to be a wasteland, where no living beings could develop. In 1977, however, the deep-sea vents were discovered, and we are now aware of the existence of fertile submarine oases, at several kilometers from the ocean surface. There, organisms thrive regardless of the total absence of light, high pressure and high temperature. Some researchers even suggest a hot origin of life in such ecosystem.

Until the late 1980s, it was a common belief that microorganisms in soils only inhabited the uppermost levels. Our ideas are changing: an extensive array of independent reports show that microbial life is widespread at depth in the crust of the Earth. Such subterranean life may be independent of solar energy.

This new concept of life not depending on solar radiation has changed our previous assumptions about the requirements for life on Earth. Besides, it will surely change the way humans search for extra-terrestrial life. Potentially, life may be found inside other solid bodies of the Solar system and beyond. Even the concept of biosphere will increase its scope. He who introduced the term "biosphere", Edouard Suess (1831–1914), never defined it. Mightily concerned about the rising of the Alps, he envisioned the Earth's concentric circles as background to his monograph *Der Enstehung der Alpen*, 1875. "Lithosphere" he coined for the rocks, "hydrosphere" for the ocean, lake, stream and other waters to match the term "atmosphere", already in wide use referring to the blanket of Earth's air. By logical extension, the covering of living matter he dubbed "biosphere". Vladimir I. Vernadsky (1863–1945) put the term of hard work by publishing *The Biosphere* (1926, Russian edition, 1929 French edition; for the latest translations see [30]). He defined the biosphere as "the area of the Earth's crust occupied by transformers which convert cosmic radiation into effective terrestrial energy, i. e. electric, chemical, mechanical, etc." Discoveries made recently, however, have invalidated his definition. Vernadsky research shared some traits with that of biologists of the 1990s: the quest for the boundaries of life. To that topic, he dedicated his last efforts. In 1937, eight years before his death, he wrote an article under the title "On the limits of the biosphere." Over his scientific career, Vernadsky had found life wherever he had sought it. Previously, his predecessor Alexander von Humboldt (1769–1859) had called the living beings of the planet the "all-animateness of the Earth". Christian G. Ehrenberg (1795–1876), German biologist, scientific explorer and founder of micropaleontology, had spoken about the "everywhereness" of life. About half a century after Vernadsky's death, more and more localities where strange living beings thrive have been discovered. Both archaebacteria and algae thrive in the so-called Dead Sea; the rocks of the Antarctic are replete with greenish layered communities of algae, fungi and bacteria; about a dozen kinds of bacteria have been identified in the perpetual snows of the high Himalayas (at 8399 m); the black smokers, the superboiling vents blowing out sulfur-rich waters at about 3000 meters at the bottom of the ocean harbor giant red-bodied tubeworms, huge clams and weird poutfish.

Views of our green and blue Earth from space have thrilled us more than ever as we compare our lively planet with the barren wastelands of the Moon or Mars or Venus. Pock-marked Mercury, stormy Jupiter, cockeyed Phoebe, eruptive Io and ice-ringed Saturn are object lessons today at which Humboldt, Ehrenberg, Suess and Vernadsky all would have marveled. Because our international satellite programs can imagine our planet from the barren black

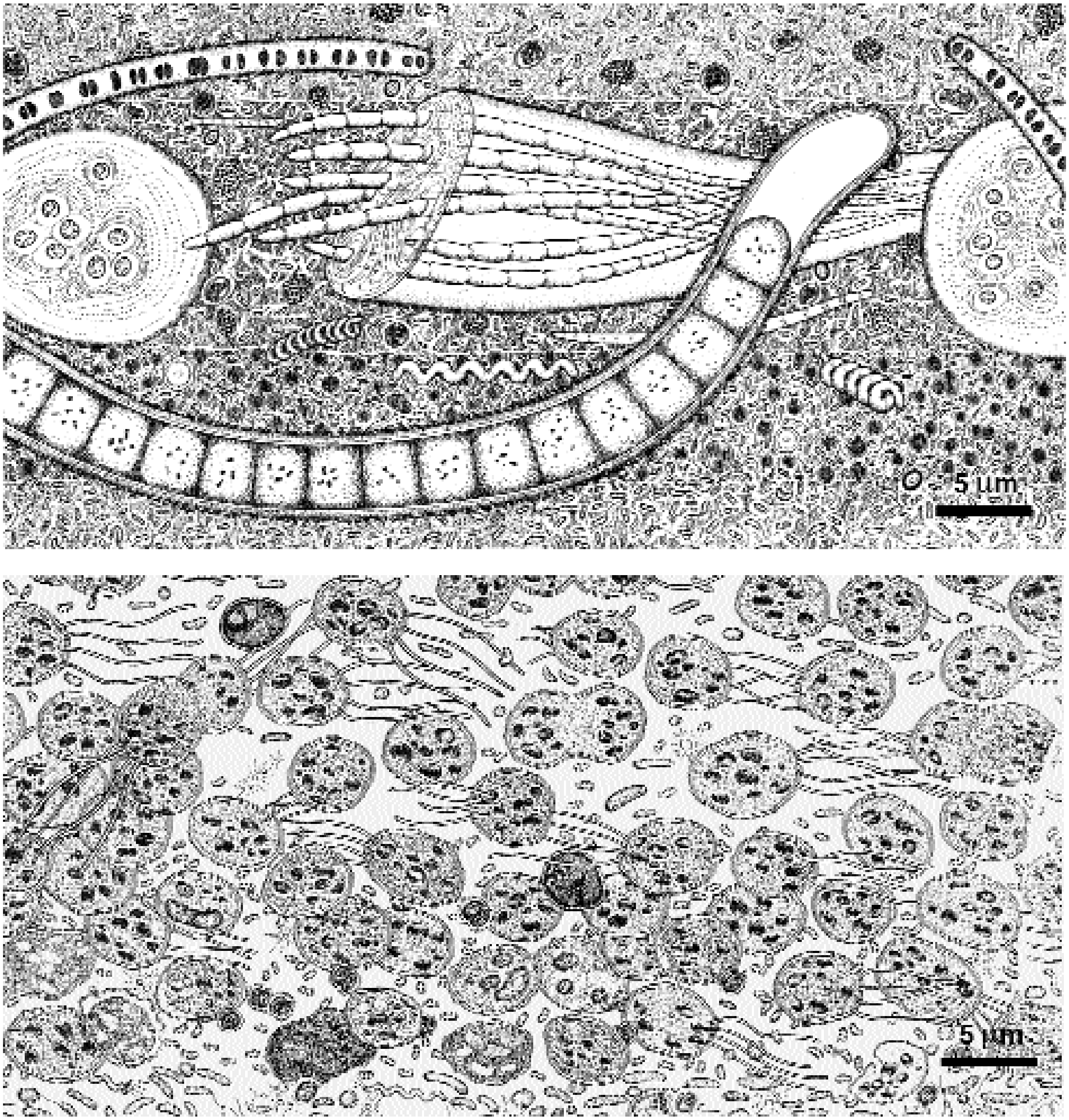

Fig. 1 Extant representatives of two of the earliest ecosystems on the young Earth, about 3500 milion years ago: Benthic (microbial mats) and planctonic (water column of a sulfated lake) multilayered microbial communities (MMC). (Top) MMC in the "algal" mats at Bidos Lagoon, near Matanzas (northern Cuba).The three conspicous cyanobacteria are: *Microcoleus chthonoplastes*, the dominant mat-building organism (seen in two transversal and one lateral view), *Johannesbaptistia* sp., the thick ensheathed individual filament, and *Oscillatoria limnetica*, the two thin straight filaments. (Bottom) MMC in the water column of Lake Cisó, near Banyoles Lake (Girona, northeastern Spain). Among other bacteria which can be seen are: *Chromatium* spp. (flagellated large ovals), and *Chlorobium* spp. (small non-flagellated rods). (Drawings by Christie Lyons, based on transmission and scanning electron micrographs.)

space, scientists have had to grow more tolerant of their own avant-garde. The years between Suess' monograph and the Martian Pathfinder (1997), serious Earthbound scientists peer review has discouraged and even punished a global outlook. Instruments of torture for scientists are, from an historical vantage point, relatively mild. Retraction of funding and rejection of manuscripts submitted for publication are the usual means by which the opinions of scientists are suppressed. Even today, after the blue marble image has become cliché, any scientist who takes on the entire Earth at once as an object of study is ignored or castigated by his highly focused peers. Vernadsky's immense corpus of contribution is still ignored, especially outside Russia. Hardly anyone in the English-reading world knows that Vernadsky's research directed the Russian space and military establishment to native sources of the radioactive elements, uranium and plutonium. The worldwide and world class attempts to understand chemical cycles of the elements (C, N, S and global geochemistry) was inspired by Vernadsky work; he considered living matter to be the greatest of all geological forces (Fig. 2).

Vernadsky and Lovelock

But meanwhile, as Vernadsky's works were ignored especially in the west, a modern scientist proceeded with his highly original study of samples of atmospheric gases diluted inside a barn on the Devon-Cornwall border. Working from a very different knowledge base and entirely independent of Vernadsky, the English atmospheric chemist James E. Lovelock described the Earth's life and its environment as "a tightly coupled physiological system." Beginning in the late 1960s when, in his search for criteria for existence of life on Mars, he already inferred life from the mixed-up composition of the atmosphere of Earth, and tried to explain his Gaia hypothesis. He used many tropes and metaphors. "The Earth is alive." "The atmosphere is the circulatory system made by and for the biosphere." "The atmosphere and the biosphere co-evolve." "Life optimizes its own environment." Lovelock's great concern for being understood overrode any deference he felt to provide his scientific colleagues with precise definitions. Lovelock, until the 1990s, when he was elected as one of the very few nonacademics to the Royal Society and was awarded the Volvo Prize and the Blue Planet Prize, was, to put it mildly, ignored.

Ecology is touted as the study of organisms in their environment. The largest unit of investigation, therefore, for ecologists is the biosphere with all of its living matter. The biota, the sum of flora, fauna and microbiota, is defined as all living matter on Earth. The biosphere then is the place where this matter resides. Ironically Vernadsky, who was a mineralogist, cartographer and crystallographer, would have never called himself "ecologist". Nor would Lovelock. However, since the main object of study of both these sages has been the biosphere itself, life in its environment, it is likely that these two are among the greatest ecologists science has ever witnessed.

Vernadsky was not able to answer the question "what are the boundaries of the biosphere? Nor can we today. The best we can do is investigate the limits to life. What are the highest and lowest temperatures that living beings survive? For how long can any organism be subjected to drying or to freezing? How much acid and how much alkali can any organism tolerate? What are the highest hydrostatic pressures and the lowest atmospheric pressures that living bodies survive? With the answers to such questions on environmental limits of life and exploration into strange localities improbable as habitats, we can begin to answer Vernadsky's query of the limits of the biosphere.

The deepest roots of life

For many years, geologists and engineers had found small bacteria-like bodies in cores they extracted from deep boreholes in rocks. The shapes and sizes matched perfectly those of bacteria observed in laboratory cultures. Whenever the geologists took these bodies in cores or electron micrographs to the attention of microbiologists, they received similar explanations for the presence of those bodies: either the bacteria were contaminants that had reached the samples during or after the core-sampling or they were fossilized bacteria, remains from the time when the rock had formed. A third possibility was always posed as remote: they might be true, original bacteria, living in the rock. Nobody believed that life were possible so deep, at such high pressures and temperatures, in such nutrient-deprived conditions.

All this has changed. Contamination during drilling at some locations has already been controlled. The aim of this control, however, was not to find the boundaries of life in the terrestrial crust, but to elucidate how microorganisms could affect polluted aquifers. For such investigation, the U.S. Department of Energy (DOE) launched its Deep Subsurface Microbiology Program. In 1987 a team of researchers sponsored by DOE drilled several boreholes near the Savannah River nuclear materials-processing facility in Aiken, South Carolina, where fissionable materials had been disposed of. For the first time since geologists and engineers had drilled the Earth's crust, the retrieved subsurface samples could be proclaimed contamination-free. Nevertheless, the culture of samples from perforations in deep rocks and sediments was conclusive: many kinds of bacteria that lived in the pores and crevices of rocks were extracted even from the deepest core, taken at 500 m below the Earth surface. Since then, many scientists sponsored by the same U.S. Program have studied other locations. The results indicate that drilling bacteria are ubiquitous. They are recovered from temperatures as high as 75°C and from locations as deep as 2800 m under the surface.

Granite and basalt are both the most common rocks in the Earth's surface and the hardest ones. Aquifers in such rocks run deep between fractures and faults. Different from aquifers near the surface, which are more or less horizontal, those at the deep subsurface may be oriented in any direction, vertically or horizontally. Hard rock fractures are partially filled with water; the remaining parts contain precipitated minerals, clay and other materials. Sampling hard rock hundreds of meters deep is a difficult task; it requires strong drilling with high fluid pressure. This technique implies the risk of contamination due to intrusion of the drilling fluid. There are studies on the potential of contamination with microorganisms coming from the surface of

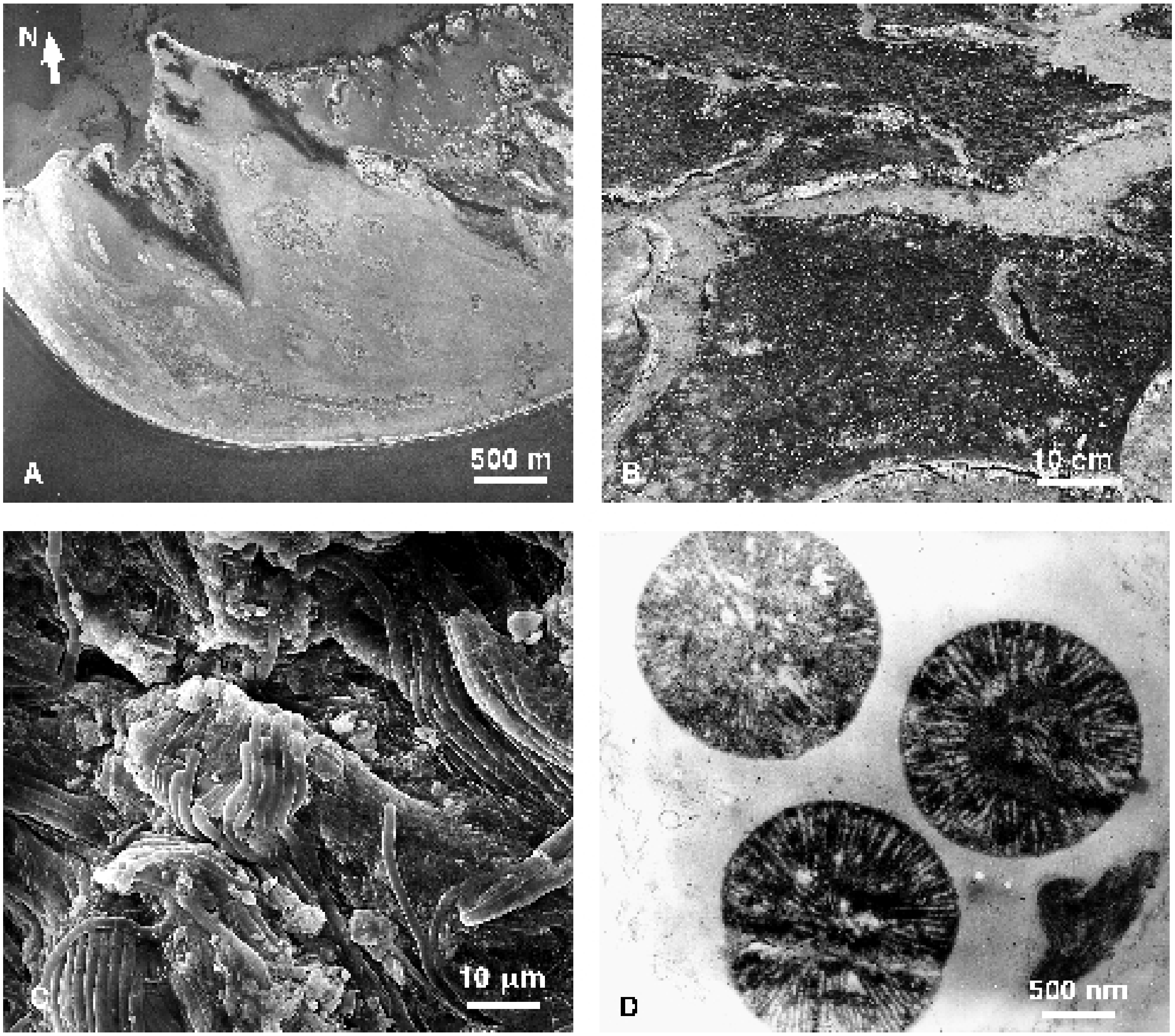

Fig. 2 From kilometers to nanometers, a microbial perspective of the physiology of the Earth. Progressive increase of the scale in the observation of microbial (almost exclusively prokaryotic) ecosystems, in this case the microbial mats thriving in Punta de la Banya, south spit of the Ebro Delta, Tarragona, northeastern Spain. (A) Air view of the microbial mats. (B) Surface of the microbial mats in A. (C) Microscopic structure of the mats in B. (D) Ensheathed bundle of *Microcoleus chthonoplastes* seen in transversal section from C; observe the radial photosynthetic lamellae and the dense cytoplasm containing carboxysomes (small black dots). (Photographs from Servei Cartogràfic de Catalunya, R. Guerrero, J. Mir, and M.-O. Soyer-Gobillard, respectively.)

boreholes during drilling. Microorganisms present in drilling equipment have never been found in samples from the deepest zones of drilled boreholes. The direct microscopic counts of bacteria from the subterranean rock habitats range from 1000 to 10 million cells per millimeter of groundwater. (Similar results could be expected in samples from surface habitats.) The number of viable cells, however, are much lower. Many microorganisms from the deep subsurface samples cannot grow in culture media in the conditions provided by the laboratory, which differ considerably from those in their natural habitats.

Life underground

Microorganisms are very abundant in all ecosystems on Earth. The water in a pond can contain a million bacteria per milliliter. One gram of agricultural soil can have more than a billion bacteria. The numbers, however, diminish rapidly with depth. For this reason, it seemed unlikely that biosphere could extend more than a few dozens of meters deep from the continental surface.

Table 1 The limits of microbial life. Known extreme conditions that allow the growth of some microorganism, provided that liquid water is present

Environmental factor	The lowest	The highest
Temperature	–12°C (psychrophilic microrganisms)	*ca.* 120°C (bacteria in deep-sea thermal vent regions; sulfate-reducing bacteria in the deep subsurface)
pH	0 (*Thiobacillus*, *Helicobacter*)	13 (*Plectonema*)
Redox potential	–450 mV (methanogenic bacteria in the deep subsurface)	+ 850 mV (iron bacteria)
Hydrostatic pressure	0 (various microorganisms)	1400 atm (barophilic marine bacteria; bacteria in the deep subsurface)
Salinity	0 (*Hyphomicrobium*)	Saturated brines (extreme halophilic bacteria)

The extension of the deep hot biosphere depends mainly on four factors: pressure, temperature, availability of nutrients and presence of water in the liquid state. Other factors such as pH and salinity are more relevant at the Earth surface (Table 1). Increasing pressure has little effect on life, even at several kilometers under the surface. Temperature increases with depth. For oceanic crust, under an average ocean-bottom temperature of 4°C, temperature increases about 15°C per kilometer. For continental crust, under an average surface temperature of 15°C, temperature rises about 25°C per kilometer. Therefore, considering a tolerance up to 110°C, microbial life could reach about 7 km under the seafloor and 4 km below continental floors. This estimate for a temperature maximum is actually conservative—in the laboratory, bacteria can be grown up to 113°C—, and we can assume that microorganisms in their natural habitats of the deep-sea vents can grow at temperatures near 120°C.

To synthesize their cellular components, microorganisms need nutrients such as C, N, P, S, several metal ions and various trace elements. They also need some kind of fuel to obtain their energy for living. Rocks and deep sediments often contain nutrients and potential energy sources, but they must be supplied in a form and at a rate usable to specialized bacteria living in the rock fractures. As to the necessary presence of liquid water, no problem; the cracks in rocks are filled with it.

Metabolism at the deep subsurface

Several kinds of metabolisms explain how different microorganisms are able not only to live sparely, but also to thrive at several kilometers in depth. All bacteria found so far in the deep subsurface are very specialized. Although they differ from the usual surface bacteria, all kinds of metabolisms found at the deepest habitats have already been described in bacteria from specialized habitats at the surface. Both chemolithotropic and heterotrophic bacteria were found at depth. “Lithotrophic” means literally “stone eater”. The term was coined in the 19th century, long before deep microorganisms were detected, and it refers to inorganic compounds rather than to carbon compounds that serve as a source of energy for these bacteria.

In the subsurface, chemolithotrophic bacteria include sulfate-reducing bacteria, those that breath SO_4 rather than oxygen. Iron reducing and methane-producing bacteria have also been detected. Not only are these organisms present in such environments, but they are metabolically active. By measuring the discrimination against the heavier isotopes (^{13}C, ^{34}S) in favor of the lighter ones (^{12}C, ^{32}S) in the sulfide and methane gas in those rocks, the biological origin of these “breathed out” gases clearly established, therefore, the presence of active metabolism.

Microbiologist T. O. Stevens and geochemist J. P. McKinley, of the Pacific Northwest National Laboratory of Richland, Washington, studied carefully the different kinds of metabolism in bacterial populations from deep crystalline rock aquifers within the Columbia River Basalt Group (CRB) [34]. Near Richland, the Columbia basalts are 3 to 5 km thick. With increasing depth, the age of the water in the aquifers between basalt flows may exceed 35,000 years. The researchers measured the population sizes of sulfate-reducing bacteria (SRB), of bacteria able to breath iron and CO_2, and of hydrogen consumers, including methanogens (MB). They also estimated the numbers of microorganisms that could grow on simple organic compounds such as sugar (heterotrophs). SRB and MB turned out to outnumber any other group of microorganisms, and their abundance ranged from 10 to 10,000 per ml in the different aquifers sampled. In fact, in nearly every sample, autotrophic microorganisms outnumbered the heterotrophic ones by several orders of magnitude. In such deep habitat, hydrogen was an abiotic energy source. Stevens and McKinley hypothesize that H_2 generates in a reaction between iron-rich minerals in basalt and ground water. For the first time hydrogen-eating bacteria have been found that do not depend on other microbes to produce hydrogen nor do they require oxygen to metabolize it. Iron trapped in rock as ferromagnesian silicates in the Columbian basalts reduces H_2O itself making H_2 available to bacteria. The discovery of free H_2 in rocks of the Earth’s crust supports their contention. This hydrogen apparently results from the weathering of iron-rich silicates at high rock:water ratios. The dissolution of ferrous silicates and the precipitation of magnetite drive the generation of hydrogen gas. The exact reactions responsible for hydrogen gas production in rocks have not yet been elucidated. The extrapolation from field observations and thermodynamics calculations suggests the following reaction:

$3\ FeO + H_2O \rightarrow H_2 + Fe_3O_4$ (magnetite)

The metabolic types and numbers of bacteria found at the deep subsurface depend on the local environment. Karsten Pedersen [28, 29], from the University of Göteborg in Sweden, detected bacteria in water flowing through fractures in granite, up 100,000 per milliliter in groundwater at 870–1240 m depth. R. John Parkes [27], from the University of Bristol, UK, found acetate, a microbial product, at two sites of the Atlantic Ocean at depths about 150 m in the sediments under the sea floor. P. McKinley and co-workers [22] have recently seen sandstone-shale interfaces in Cretaceous formations in Cerro Negro, in central New Mexico, that harbor rich bacterial populations. Presumably these bacteria ferment organic matter and reduce sulfate at least.

The difference between the different metabolic groups that use hydrogen as the source of electrons is their electron acceptor. SRB, MB and acetogenic bacteria obtain their energy from the following reactions:

Sulfate reduction: $4\ H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4\ H_2O$
Methanogenesis: $4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$
Acetogenesis: $4\ H_2 + 2\ HCO_3^- + H^+ \rightarrow CH_3COO^- + 4\ H_2O$

Microorganisms with these kinds of metabolisms have both their electron donor, hydrogen, and their electron acceptors, carbon dioxide or sulfate, as inorganic compounds. They live indeed a strictly geochemical existence. This deep hot biosphere is also novel because it is independent of photosynthetic production. Photosynthesis by cyanobacteria, algae and plants generates the molecular oxygen used by so many other organisms. The chemolithotrophic bacteria of the deep-sea vent oases use photosynthetically produced oxygen in their metabolism. The organic matter (food) needed by most of the organisms (both micro- and macro-) at the surface is also fueled by photosynthetically-derived oxygen.

Aliquit novum sub sole

All microbes found in the deep subsurface so far are directly related to known groups living at the crust surface. Given the high temperature necessary to form igneous rocks, microorganisms now living in them must have reached their current habitats coming from the surface or near the surface. They probably have generation times of months or years. The inside-rock metabolism seems to be independent of photosynthesis and therefore of solar energy. If that proves to be true, it may be among the major discoveries about life made over the 20th century. The sun has been the ultimate source of energy for all other ecosystems on Earth known until deep-subsurface microorganisms were discovered. If the only necessary condition for maintaining microbial life is liquid water, and fuel and nutrients may come from basalt, which is the most common rock, formed by chemical reactions in the developments of planets, then the number of possible places for life to exist increases astronomically. Literally, “billions and billions” of habitats are possible in the universe.

Entomologist Edward O. Wilson, from Harvard University ends his autobiography [40] with the following statement: “If I could do it all over again, and relieve my vision in the twenty-first century, I would be a microbial ecologist. Ten billion bacteria live in a grain of ordinary soil, a mere pinch held between thumb and forefinger. They represent thousand of species, almost none of which are known to science. Into that world I would go with the aid of modern microscopy and molecular analysis. I would cut my way through clonal forests sprawled across grains of sand, travel in an imagined submarine through drops of water proportionately the size of lakes, and track predators and prey in order to discover new life ways and alien food webs.”

The observation of extant life in the subsurface of Mars or other body in our solar system might be the discovery of the 21st century. Perhaps life is present in many places in the universe, prowling in solid planets surrounding many stars. Life may not need other energy and chemical sources than those which occur in the natural development of planetary bodies. Perhaps these discoveries of living beings at depth force us to realize that life is an ineluctable outcome of planetary evolution.

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