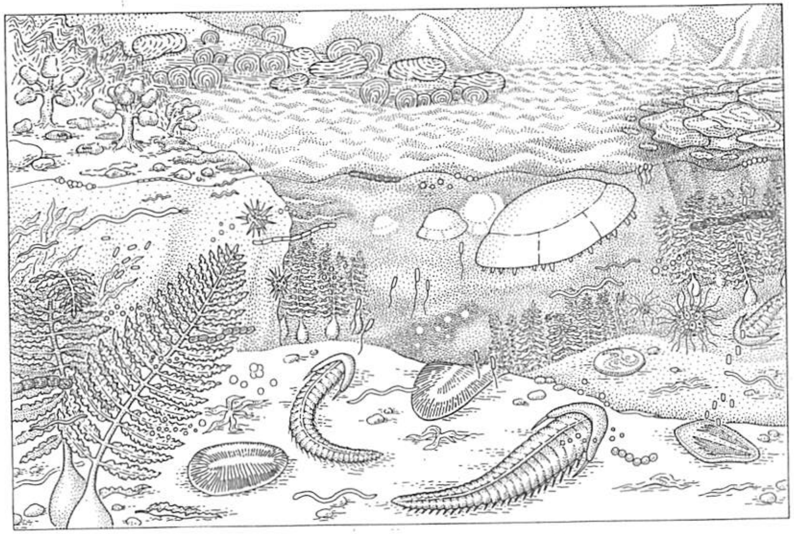
pounds through the Earth's atmosphere and water had created the fundamentals of the planetary ecosystem. Although the oxygen revolution to come was to drive these Archean anaerobes underground and under water, many bacteria living during this time have survived essentially unchanged for more than three billion years.

in MICROCOSMOS by Lynn Margulis and DORION SAGAN



0.7 billion years ago *Late Proterozoic Eon*
Soft-bodied marine animals encroach upon microbial empire.

Chapter 6 The Oxygen Holocaust

THE oxygen holocaust was a worldwide pollution crisis that occurred about 2,000 million years ago. Before this time there was almost no oxygen in the Earth's atmosphere. The Earth's original biosphere was as different from ours as that of an alien planet. But purple and green photosynthetic microbes, frantic for hydrogen, discovered the ultimate resource, water, and its use led to the ultimate toxic waste, oxygen. Our precious oxygen was originally a gaseous poison dumped into the atmosphere. The appearance of oxygen-using photosynthesis and the resulting oxygen-rich environment tested the ingenuity of microbes, especially those producing oxygen and those nonmobile microorganisms unable to escape the newly abundant and reactive gas by means of motion. The microbes that stayed around responded by inventing various intracellular devices and scavengers to detoxify—and eventually exploit—the dangerous pollutant.

The unceasing demand for hydrogen initiated the crisis. Life's need for carbon-hydrogen compounds had already almost depleted carbon dioxide from the atmosphere. (The atmospheres of Mars and Venus today are still more than 95 percent carbon dioxide; the Earth's is only 0.03 percent.) The lighter hydrogen gas kept escaping into space where it reacted with other elements, becoming ever less available. Even the Earth's hydrogen sulfide, gurgling up through volcanoes, was becoming insufficient to supply the vast communities of photosynthetic bacteria that by the late Archean dominated the soils and waters.

But the Earth was still full of an abundant hydrogen source: dihydrogen oxide, a.k.a. water. Until now, the strong bonds between the hydrogen and oxygen atoms in the water molecule (H_2O)—much stronger than those holding together the two hydrogens in hydrogen gas (H_2), hydrogen sulfide (H_2S), or organic molecules (CH_2O)—had been unbreakable by the resourceful, hydrogen-craving bacteria. Sometime after photosynthesis in the oxygen-poor atmosphere of the early Earth had been well established, however, a kind of blue-green bacteria solved the hydrogen crisis forever. These were the ancestors to modern cyanobacteria.

The cyanobacterial ancestors seem to have been mutant sulfur bacteria desperate to continue living as their store of hydrogen sulfide dwindled. These organisms were already photosynthetic, and already had proteins inside them organized into so-called electron transport chains. In some of the blue-green bacteria, mutant DNA which coded for the electron transport chains duplicated. Experts at capturing sunlight in their reaction center to generate ATP, the new DNA led to the construction of a second photosynthetic reaction center. This second reaction center, by using light-generated electron energy from the first center, absorbed light again; but this was higher-energy light, absorbed at shorter wavelengths, that could split the water molecule into its hydrogen and oxygen constituents. The hydrogen was quickly grabbed and added onto carbon dioxide from the air to make organic food chemicals, such as sugars. In an evolutionary innovation unprecedented, as far as we know, in the universe, the blue-green alchemists, using light as energy, had extracted hydrogen from one of the planet's richest resources, water itself. This single metabolic change in tiny bacteria had major implications for the future history of all life on Earth.

The new dual light-powered system not only generated more ATP but accessed an almost inexhaustible hydrogen source; with it, the first cyanobacteria were spectacularly successful. Colonizing every available spot that guaranteed sunlight, carbon dioxide, and water, they spread over the Earth's surface. Today they grow like weeds on rocks, swimming pool surfaces, drinking fountains, shower curtains, and sand flats—wherever there is water and light. When the Lascaux caves of southern France, whose walls bear paintings made by paleolithic hunters, were opened to the public in the late 1970s they were soon forced to close again. Once light and water were let in, the cyanobacteria grew and divided, threatening the dazzling 40,000 year-old cave paintings by covering their surfaces.

On the early Earth the blue-green tint crept over the minerals and muds. It glided, grew, crept, and swelled, gradually expanding along river shores and meteoritic rubble, upon volcanic debris and in puddles. Like all rapidly growing living systems, the cyanobacteria produced prodigious amounts of waste. Whereas their ancestors had taken in hydrogen sulfide (H_2S) and released sulfur (S), they took in water (H_2O), and released oxygen gas(O_2). The toxicity of uncombined oxygen is well established and the new oxygen gas produced by the photosynthetic colonies immediately threatened them most, since they were closest to the source. Oxygen—a deadly poison to all early life—bubbled up from the mats and muds, polluting the marshes, pools, and riverbeds.

Oxygen is toxic because it reacts with organic matter. It grabs electrons and produces so-called free radicals: highly reactive, short-lived chemicals that wreak havoc with the carbon, hydrogen, sulfur, and nitrogen compounds at the basis of life. Oxygen breaks down or renders useless the small metabolites—food—that otherwise become components in cellular systems. Oxygen combines with the enzymes, proteins, nucleic acids, vitamins, and lipids that are vital to cell reproduction. Oxygen also quickly reacts with atmospheric gases, including hydrogen, ammonia, carbon monoxide, and hydrogen sulfide. In a word, oxygen burns: it "oxidizes," dramatically changing soil minerals such as iron, sulfur, uranium, and manganese to oxidized forms such as hematite, pyrite, uraninite, and manganese dioxide—that is, to new, oxygen-bound compounds of these metals.

The biosphere at first could absorb the oxygen pollution. As long as there were abundant metals and gases that could react with it, oxygen did not build up in the atmosphere. Moreover, the production of oxygen probably varied with the season—more in summer when photosynthetic activity was the greatest, less in winter. Some photosynthetic microbes must have been able to alternate between oxygen-producing and oxygenless photosynthesis, depending on whether hydrogen and hydrogen sulfide were in good supply. (As in the beautiful *Oscillatoria limnetica*, oxygen production was at first optional. Discovered by Professor Yehuda Cohen of Eilat Marine Station in the hot water of Solar Lake in the Negev Desert in 1975, *O. limnetica* has a "chameleon" physiology. It can use hydrogen sulfide for photosynthesis when that compound is plentiful, in which case the cyanobacterium gives off no oxygen. But when starved for hydrogen it switches to using the hydrogens from water, and the leftover oxygen is released as waste into the air.) The amount of poisonous oxygen fluctuated with season, volcanic activity, cyanobacterial population, and many other variables.

As paleobiologist J. William Schopf has pointed out, "The paleobiological record shows . . . that the advent of oxygenic photosynthesis was the singular event that led eventually to our modern environment."[25] Although the mineral record clearly shows a sudden build-up in the amount of atmospheric oxygen, exactly when oxygen-producing photosynthesis left earth-changing quantities of oxygen in the atmosphere is hotly debated.

An intriguing sign that oxygen production existed long before most think it did is displayed in the same ancient Isua rocks that held concentrations of graphite signaling the possible remains of photosynthetic bacteria. Some beautiful banded rock formations made of alternating stripes of different types of iron oxides—oxidized hematite and less oxidized magnetite—exist there. In some places the alternating bands are only microns, rather than meters, in size. These banded iron formations (BIFs) are important to us because they supply our raw sources of mineable iron. In fact fewer than twenty BIFs, all dating from the Proterozoic Eon, make up more than 90 percent of the world's commercial iron supply. For such BIFs to occur, both large bodies of water and fluctuating amounts of oxygen are thought to have been needed. Oxygen-producing photosynthetic bacteria may have thrived on the surface of warm, volcanic pools along vents and rifts in iron-rich waters, their seasonal bursts of growth and accompanying puffs of oxygen waste producing the colorful mineral layers.

Photosynthetic bacteria may also have had collaborators in the making of banded iron. Certain iron-oxidizing or pipe-clogging bacteria can take oxygen from the environment and derive energy in nutrient-poor waters by combining it with iron. The combination of oxygen and iron leads to a chemical reaction which makes rust. These bacteria extract energy from the chemistry of that reaction and the rust gets deposited on their long and fibrous bodies. In an oxygenless world such bacteria probably proliferated both above and below the zones of oxygen production. Year after year they could have scavenged oxygen at the edges of the cyanobacterial communities, precipitating it as rust. Iron-oxidizing bacteria may have helped form the vast quantities of ancient iron ore. And the alternating bands of iron ore may be a record of their ancient relationship with cyanobacteria: hematite would have been produced in summer, when the cyanobacteria produced more oxygen, leading to rustier iron, whereas the magnetite layers probably built up in the winter, when photosynthetic oxygen production and therefore iron oxidation were at a lull.

During a part of the Proterozoic Eon from 2,200 to 1,800 million years ago, there was a great burst of banded iron formation unparalleled since. But more than 3,000 million years ago? If microbial life is indeed implicated in the making of banded iron, these glittering bands of metal ore in Labrador and Greenland represent the naissance of bacterial communities and would be the oldest evidence of bacterial oxygen-producing photosynthesis and, indeed, of life itself.

Another spectacular clue to the earliest aerobic activities of life is the accessibility of gold, the metal so prized throughout human history, that was brought up from the molten center of the earth sometime in the Archean Eon. Sediment-embedded gold is limited to several suites of rocks from Archean times, and what little there is worldwide is startlingly concentrated. The Witwatersrand mines in the Transvaal of South Africa, for example, account for some 70 percent of all the gold that has circulated throughout the history of civilization. Lesser deposits are located in northwestern Australia, Elliot Lake in northern Ontario, and southern Russia—none approaching the yield of the Transvaal.

Gold miners descending in elevator shafts thousands of feet into the Earth are effectively going back to earlier times, past layers of volcanic ash and the debris of ancient rivers to older surfaces. To seek new gold deposits, they follow the carbon leader—a distinct layer of conglomeritic rock that contains a great deal of organic carbon in it. The carbon leader, trapped between limestones and shales, contains thin seams of pyrite, gold, and often even uranium ore. The Witwatersrand carbon leader also contains microscopic filamentous and ball-like structures that are inexplicable by mineralogy alone.

D. K. Hallbauer, a South African economic geologist, first related these structures to life by interpreting them as the fossilized components of lichens. Since lichens are a complex alliance of algae and fungi of which no fossil record appears until 2,000 million years after the South African gold was deposited, no one accepted Hallbauer's specks as lichens. A more convincing possibility is that filamentous and coccoid bacteria trapped detrital flakes of gold.

Flowing from the interior of a tectonically active Archean Earth, hot magma brought tiny amounts of the heavy, molten gold from the Earth's mantle to the surface in a finely dispersed form in rocks of iron, magnesium, and silicate. Because gold moves in and out of rocks more fluidly in the absence than in the presence of oxygen, it would have been readily eroded from the rocks by rivers and streams and carried to the sea. If it encounters high concentrations of oxygen and organic carbon, however, gold comes out of solution—it "flocculates." Any colonies of photosynthesizing bacteria dwelling along the river shores could have played a role in this. By producing quantities of oxygen and carbon-rich compounds, they may have coaxed gold from the water to come out in gooey flocs and be deposited along the banks and beds of the ancient waterways.

Certain bacteria (*Chromobacterium violaceum*) today produce cyanate, a chemical used by gold mining companies in the extraction of gold from carbon-rich sediment. Perhaps the ancestors of these microbes lived in the mineral-laden Archean rivers, consolidating dissolved gold into discrete particles. The oxygen and carbon from cyanobacteria itself may have been enough to precipitate the gold from solution. In southern Africa gold from the interior of the earth was laid along a river system estimated to be five times the size of the Mississippi. Eventually the great Witwatersrand river ways, draining huge amounts of water into the ocean 2,500 million years ago, dried out. The rivers were buried under kilometers of sediments and folded. They were not found until the last century, when Dutch-derived Afrikaner settlers of South Africa were attracted by the gold speckles of some dark rocks that had outcropped in the Transvaal desert. They followed the outcrop deep into the ground, finding the gold by tracing the buried ancient river system of which the carbon leader was a part.

The clearest evidence of the lives of ancient and extensive bacterial confederacies, however, are stromatolites. Stromatolites were to the Proterozoic landscape what coral reefs are to the present ocean: rich and beautiful collectives of intermingled, interdependent organisms. These domed, conical, columnar, or cauliflower-shaped rocks, found throughout the fossil record and still in existence today, are composed of rock layers that were once microbial mats. Communities of bacteria, especially photosynthetic cyanobacteria, lived and died atop one another. When some stromatolites, such as those in the hot springs in Saratoga, New York, were first described in the late nineteenth century by geologist Charles

Walcott and others, they were termed *Cryptozoa*, Greek for "hidden animals." Some of the ancient stromatolites exceeded thirty feet in height.

Today—in very restricted parts of the world—we can see that the top layers, only a few centimeters in width, are dominated by photosynthetic blue-green bacteria. This is the living part of the mat. Everything below is composed of dormant bacteria, chalk, sand, gypsum, and other debris bound together by the matrices of earlier mats. The top layer is horizontally striped. Beneath the top layer of photosynthesizers are thriving populations of anaerobic purple photosynthesizers, which are sulfur depositers. Beneath them are dependent microbes, living on the produce or the bodily remains of the others. Living stromatolites may be found today in the Persian Gulf, western Australia, and the Bahama Islands. Breaking them open reveals with a hand lens the gelatinous growth of many different kinds of bacteria, but the actual carbonate precipitation is still done by cyanobacteria. In soft bacterial mats and hardened ones (which are by definition stromatolites) bacteria grow in multicellular layers that are as complex and differentiated, in their own way, as the tissues of animals.

The oldest known stromatolites are about 3,500 million years old, their carbon-rich layers convincing evidence that photosynthetic microbial communities—aerobic or not—were thriving by that time. Though they are scarce in Archean rocks, they skyrocketed to success in the Proterozoic Eon, dominating the landscape.

For tens of millions of years excess oxygen was absorbed by live organisms, metal compounds, reduced atmospheric gases, and minerals in rocks. It began to accumulate in the atmosphere only by fits and starts. Many local populations were killed off, and many adaptations and protective devices evolved. From blue-green cyanobacteria that produced oxygen part-time emerged grass-green bacteria that emitted it continually. Thousands of species of aerobic photosynthesizers arose adapted to rocks, hot water locales, and scums. But by about 2,000 million years ago, the available passive reactants in the world had been used up and oxygen accumulated rapidly in the air, precipitating a catastrophe of global magnitude. People are gravely worried today about an increase in atmospheric carbon dioxide from 0.032 to 0.033 percent caused by our massive burning of fossil fuels. It is supposed that the "greenhouse effect" of the additional heat trapped by extra CO_2 could melt the ice caps, raising the level of the seas and flooding our urban coastlines, resulting in mass death and destruction. But the industrial pollution of our present, Phanerozoic Eon is nothing compared to the strictly natural pollution of Archean and Proterozoic times. About 2,000 million years ago—give or take a couple of hundred million years—oxygen started rapidly increasing in our atmosphere. The Archeo-Proterozoic world saw an absolutely amazing increase in atmospheric oxygen from one part in a million to one part in five, from 0.0001 to 21 percent. This was by far the greatest pollution crisis the earth has ever endured.

Many kinds of microbes were immediately wiped out. Oxygen and light together are lethal—far more dangerous than either by itself. They are still instant killers of those anaerobes that survive in the airless nooks of the present world. When exposed to oxygen and light, the tissues of these unadapted organisms are instantly destroyed by subtle explosions. Microbial life had no defense against this cataclysm except the standard way of DNA replication and duplication, gene transfer, and mutation. From multiple deaths and an enhanced bacterial sexuality that is characteristic of bacteria exposed to toxins came a reorganization of the superorganism we call the microcosm.

The newly resistant bacteria multiplied, and quickly replaced those sensitive to oxygen on the Earth's surface as other bacteria survived beneath them in the anaerobic layers of mud and soil. From a holocaust that rivals the nuclear one we fear today came one of the most spectacular and important revolutions in the history of life.

From the earliest days of local oxygen exposure, gene duplication and transfer resulted in many protective mechanisms. The new genes were as important as survival manuals. The information they contained, so valuable to life on the newly oxygenic Earth, spread through the reorganizing microcosm. Bioluminescence and the synthesis of vitamin E are some of the innovations scientists surmise arose in response to the oxygen threat. But adaptation didn't stop there. In one of the greatest coups of all time, the cyanobacteria invented a metabolic system that *required* the very substance that had been a deadly poison.

Aerobic respiration, the breathing of oxygen, is an ingeniously efficient way of channeling and exploiting the reactivity of oxygen. It is essentially controlled combustion that breaks down organic molecules and yields carbon dioxide, water, and a great deal of energy into the bargain. Whereas fermentation typically produces two molecules of ATP from every sugar molecule broken down, the respiration of the same sugar molecule utilizing oxygen can produce as many as thirty-six. Pushed—not, probably, to its breaking point, but to a point of intense global stress—the microcosm did more than adapt: it evolved an oxygen-using dynamo that changed life and its terrestrial dwelling place forever.

Some cyanobacteria respire only in the dark—apparently because they use some of the same molecular machinery for both their respiratory and their photosynthetic electron transport chains. The shared parts can't be used simultaneously for both pathways. (Algae and plants both respire and photosynthesize because the two processes take place in different parts of the cell: photosynthesis in the chloroplasts and respiration in the mitochondria. These two organelles are tantalizing hints to the evolutionary fate of two kinds of microbes—hints to be elaborated on in the next chapter.)

Cyanobacteria now had both photosynthesis which generated oxygen and respiration which consumed it. They had found their place in the sun. Given only sunlight, a few salts always present in natural waters, and atmospheric carbon dioxide, they could make everything they needed: nucleic acids, proteins, vitamins, and the machinery for making them. If biosynthetic ability alone were considered a measure of evolutionary advancement, we humans would be far behind the cyanobacteria. Our complicated nutritional requirements leave us utterly dependent on plants and microbes to supply what we cannot make for ourselves. We are, in a very real sense, parasites of the microcosm.

Not surprisingly, with the greater quantities of energy available to them, cyanobacteria exploded into hundreds of different forms, tiny (most only a few micrometers in diameter) and large (80 micrometers or eight percent of a millimeter). They made simple spheres embedded in a gelatinous matrix of sheets of many cells, elaborately branched filaments that could release wet spores from their tips, and cells containing special oxygen-proof cysts that carried on anaerobic nitrogen fixation.

They spread into greater extremes of the environment, from cold marine waters to hot freshwater springs. New food relationships developed as other bacteria fed off cyanobacterial starch, sugar, small metabolites, and even the fixed carbon and nitrogen of their dead bodies. But most significant, cyanobacteria's continuing air pollution forced other organisms to

acquire the ability to use oxygen, too. This set off waves of speciation and the creation of elaborate forms and life cycles among them.

The stabilization of atmospheric oxygen at about 21 percent seems to be a mute consensus reached by the biota millions of years ago; indeed it is a contract still respected today. If the oxygen concentration had ever risen much higher than this, the fossil record certainly would reveal evidence of worldwide conflagration. The present high, but not too high, level of oxygen in our atmosphere gives the impression of a conscious decision to maintain balance between danger and opportunity, between risk and benefit. Even the rain forests and grasslands are extremely flammable when water levels are low. If oxygen were a few percent higher, living organisms themselves would spontaneously combust. As oxygen falls a few percent aerobic organisms start to asphyxiate. The biosphere has maintained this happy medium for hundreds of millions of years at least. While just how this works is still a mystery, we will see in our last chapter how worldwide regulatory mechanisms controlling temperature and gas composition can hypothetically arise from the normal growth properties of organisms. The leveling off and subsequent continuous modulation of the quantities of oxygen in the atmosphere was an event as welcome as the holocaust was terrible. One way of putting it is that, assuming life halted the oxygen build-up, it must have developed tremendous knowledge of antipollution engineering systems. The alternative perspective is that the cybernetic control of the Earth's surface by unintelligent organisms calls into question the supposed uniqueness of human consciousness. Microbes apparently did not plan to bring under control a pollution crisis of amazingly daunting proportions. Yet they did what no governmental agency or bureaucracy on Earth today could ever do.

Growing, mutating, and trading genes, some bacteria producing oxygen and others removing it, they maintained the oxygen balance of an entire planet.

Although it makes up only one-fifth of our atmosphere, from a chemical point of view atmospheric oxygen is extremely abundant: it should react with other chemicals to form stable compounds such as carbon dioxide and nitrate salts. As Jim Lovelock puts it, "The present level of oxygen tension is to the contemporary biosphere what the high-voltage electricity supply is to our twentieth-century way of life. Things can go on without it, but the potentialities are substantially reduced. The comparison is a close one, since it is a convenience of chemistry to express the oxidizing power of an environment in terms of its reduction-oxidation (redox) potential, measured electrically and expressed in volts."[26]

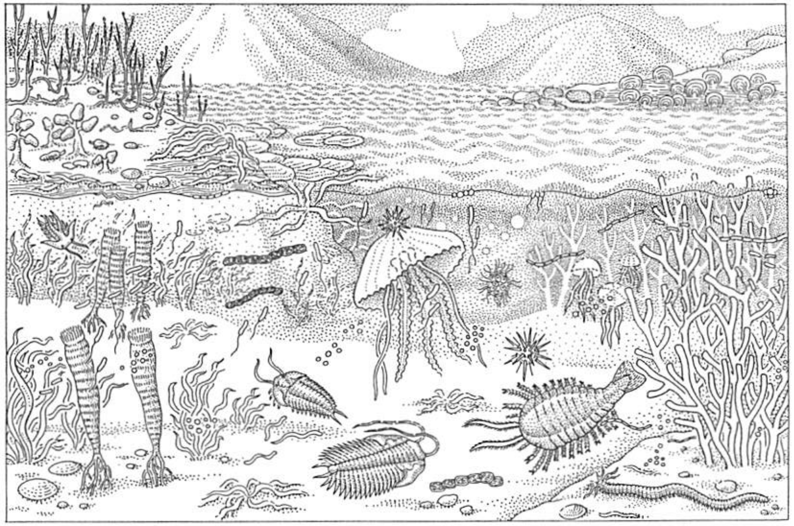
As soon as there were significant quantities of oxygen in the air an ozone shield built up. It formed in the stratosphere, floating on top of the rest of the air. This layer of three-atom oxygen molecules put a final stop to the abiotic synthesis of organic compounds by screening out the high-energy ultraviolet rays.

The production of food and oxygen from light were to make microbes the basis of a global food cycle that extends to us today; animals could never have evolved without the food of photosynthesis and the oxygen in the air. The energy dynamo created by cyanobacterial pollution was a prerequisite for a new unit of life—the nucleated cell which is the fundamental component of plant, animal, protist, and fungal life. In eukaryotes, genes are packaged in a nucleus and there is an elaborate orchestration of internal cell processes, including the presence in the area surrounding the nucleus (the cytoplasm) of mitochondria—special structures that metabolize oxygen for the rest of the cell. So different is the organization of the eukaryotic from the prokaryotic or bacterial cell that the two types represent the most fundamental separation among known life forms. Perhaps their origin in the midst of the extreme selection pressures of the oxygen catastrophe made eukaryotic cells so different. But the difference between nonnucleated bacterial cells and cells with nuclei is far greater than that between plants and animals.

Before cyanobacteria split water molecules and produced oxygen, there was no indication that the Earth's patina of life would ever be more than an inconspicuous scum lying on the ground. That it did develop and expand into gardens and jungles and cities is testimony to the power of microbial mats and seaside slime to alter each other in their local habitats. But the microbes created an even greater impact. They altered the entire surface of the Earth. The biosphere, humming with the thrill and danger of free oxygen, eventually emerged from the crisis. But the Earth was a changed place. It had become a planetary anomaly.

By the middle of the Proterozoic Eon 1,500 million years ago most of biochemical evolution had been accomplished. The Earth's modern surface and atmosphere were largely established. Microbial life permeated the air, soil, and water, cycling gases and other elements through the earth's fluids as they do today. With the exception of a few exotic compounds, such as the essential oils and hallucinogens of flowering plants and the exquisitely effective snake venoms, prokaryotic microbes can assemble and disassemble all the molecules of modern life.

Judging from the perspective of the planetwide accomplishments of early life, it is not surprising that the development of life's biochemical repertoire occurred over a full two billion years. The microbial stage lasted nearly twice as long as the rest of evolution to the present day. As Abraham Lincoln is reported to have said, "If I had eight hours to chop down a tree, I'd spend six sharpening my ax." The microcosm did just that. It set the stage for the respective evolutions of fungi, animals, and plants, all of which arose in relatively rapid succession. Just as in psychology, where the early years of infancy and childhood are known to be crucial to the development of adult personality, the early eons of life defined the contours of modern living. The Age of Bacteria transformed the earth from a cratered moonlike terrain of volcanic glassy rocks into the fertile planet in which we make our home. The alien primeval world lacking an atmosphere of oxygen was to be no more. Unlike neighboring Mars and Venus, whose atmospheres settled down to become stable chemical mixtures of carbon dioxide, the Earth had gotten energized. Delivered from the mercy of time, it became engulfed in the creative, autopoietic processes of life.



0.5 billion years ago *Early Paleozoic Era*
Animals develop hard parts from deposits of cellular waste.

Chapter 7 New Cells

WITH the invention of aerobic or oxygen-using respiration, prokaryotes had tapped into an energy source far beyond their ability to fully exploit. Unaware of the global power they were generating, the respiring bacteria flourished in their local niches all over the globe for hundreds of millions of years. But as the level of atmospheric oxygen was rising up to 21 percent, perhaps about 2,200 million years ago when it still formed only a few percent of the atmosphere, a new kind of cell formed. This was the eukaryotic cell with its key feature, the nucleus, and its important secondary characteristic, oxygen-using cell parts known as mitochondria. When eukaryotes live as single cells they are called protists. Their fossils are known as acritarchs. As Stonehill College astronomer Chet Raymo points out, the difference between the new cells and the old prokaryotes in the fossil record looks as drastic as if the Wright Brothers' Kitty Hawk flying machine had been followed a week later by the Concorde jet.[27]

The biological transition between bacteria and nucleated