An artist's rendering of what the Archean earth looked like at the time of the rise of oxygen.

The Birth of Oxygen

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We take it for granted that our atmosphere contains oxygen, but we and most other animals would die within minutes if the oxygen was removed. It is not widely appreciated that for half of the earth's history there was virtually no oxygen in the atmosphere. Oxygen appeared 2.45 billion years ago, and it has been present ever since – though not always at its present level of 21 percent.

Photosynthesis produces more than 99 percent of the oxygen in the atmosphere. Arguably, the biological invention of photosynthesis was, after the origin of life itself, the most important development in the history of our planet. About twelve times as much energy is derived from the aerobic metabolism of a molecule of glucose as the energy obtained from anaerobic metabolism. Without the invention of oxygenic photosynthesis, multicellular organisms could not have evolved. Furthermore, the presence of oxygen in the atmosphere leads to an ozone layer that protects life from the lethal effects of ionizing radiation.

The closely linked evolution of photosynthesis and the evolution of the atmosphere is perhaps the best example of the interdependence of biological and geological processes. In chronicling the rise of oxygen, I will first describe photosynthesis and its origins. Then I will turn to a discussion of the state of the earth and its atmosphere before and during the rise of oxygen. After the rise of oxygen, the atmosphere and the oceans went through some initially cataclysmic and then very slow changes. Finally, 540 million years ago – almost 2 billion years after the initial rise of oxygen – roughly the present levels of oxygen in the atmosphere and in the ocean were attained. It was only then that multicellular life began to flourish.

The story of oxygen and its effects takes place over a long period of time (see Figure 1).

One way to comprehend this vast expanse of time is to compare it to the time it took the continents to rearrange themselves completely via plate tectonics. Two hundred and twenty-five million years ago, all of the continents were together in the supercontinent Pangaea. Over 225 million years the continents separated and the Atlantic and Indian oceans were formed. This process represents about 5 percent of the earth's history and about one-tenth of the time period we chronicle here.

It is also useful to consider how much biological change can take place in 2 billion years. A heritable and selectable change – a mutation – can take place at every cellular division. The earth's oceans contain about 4×10^{24} ml of water. If we conservatively assume a steady state of 1000 cells/ml in the ocean and a division time of one week (during this period most cells are unicellular microorganisms), then in 2 billion years something like 10^{39} divisions could take place. Specific mutations in bacteria take place at a frequency of about 10^{-8}. Even more rapid changes can occur when genes are transferred between different organisms. In 2 billion years there is enormous potential for evolutionary change.

Photosynthesis

In photosynthesis, the energy of light is used to extract electrons and protons from a donor molecule H_2A, which are then used to reduce carbon dioxide:

$$2H_2A \rightarrow 4H^+ + 4\,e^- + 2A$$

$$CO_2 + 4H^+ + 4e^- \rightarrow (CH_2O) + H_2O$$

The donor molecule H_2A can be a variety of reduced compounds, including H_2S, Fe^{++}, H_2, various organic compounds, and H_2O. The use of the former group of donors probably predated the use of water in photosynthesis. The cellular machinery for oxygenic photosynthesis (in which water is used as the donor) is, in part, derived from its predecessors.

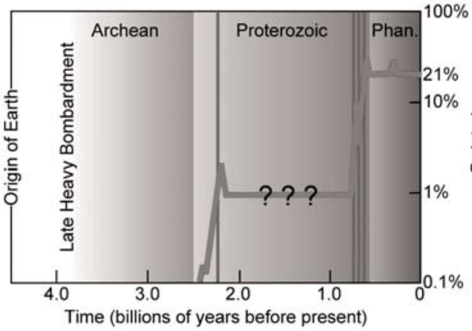

Figure 1

In oxygenic photosynthesis, the electrons from water are extracted and used to generate energy and to reduce carbon dioxide to a carbohydrate according to the equation:

$$H_2O + CO_2 \rightarrow (CH_2O) + O_2$$

Since the work of Martin Kamen and Samuel Ruben more than fifty years ago, we know the O_2 generated in photosynthesis is entirely derived from H_2O. Water is therefore dissociated in photosynthesis according to the equation:

$$2H_2O \rightarrow 4H^+ + 4e^- + O_2$$

It takes an enormous amount of energy to extract an electron from water because oxygen has a high affinity for electrons. One photon of light is required to extract each electron, making photosynthesis a four-electron process.

Oxygenic photosynthesis takes place in one class of bacteria, namely, cyanobacteria. It also takes place in a number of eukaryotic organisms, e.g., algae and plants. Photosynthesis is nearly identical in eukaryotes and cyanobacteria because photosynthetic eukaryotes came from a symbiotic event in which a primitive eukaryote captured a cyanobacterium. In discussing photosynthesis and its origin, therefore, it is appropriate to focus on cyanobacteria.

The photosynthetic machinery in cyanobacterium is located in a system of layered *thylakoid* membranes. The membranes enclose an interior space, the *lumen*. The machinery consists of pigmented proteins, many of them extending across the thylakoid membrane to the exterior space, the *stroma*. Some of the proteins and pigments in the thylakoid membrane serve as antennae to funnel light energy into the reaction center.

The reaction center consists of two complex multiprotein assemblies, termed Photosystem I and Photosystem II (PSI and PSII). At the heart of both PSI and PSII is a cofactor chlorophyll molecule.

Figure 2 depicts the major multiprotein complexes involved in photosynthesis.

We don't have sufficient space here to discuss photosynthesis in depth, so I will focus on the mechanisms of oxygen synthesis. This reaction takes place in PSII. The active site for dioxygen synthesis, called the Oxygen Evolving Center (OEC), contains four manganese atoms and one calcium atom, coordinated mainly to one core PSII protein. The water-splitting mechanism is unique; so far, at least, a related metallo-protein has not been identified. The OEC allows for the integration of a one-electron process (the excitation of cytochrome P680) with a four-electron process (the splitting of H_2O to form O_2). A beautiful experiment done fifty years ago by Pierre Joliot and Bessel Kok proved that the OEC abstracts protons and electrons from water stepwise to evolve oxygen. Alternative models ruled out by this experiment include the cooperation of four reaction centers to cleave a single molecule of H_2O, and the accumulation by one center of four oxidizing equivalents prior to oxidizing water in a single concerted step.

The OEC can now be understood more clearly because J. Barber in London obtained a 3.5A crystal structure of PSII, and K. Sauer, W. Saenger, and colleagues found a

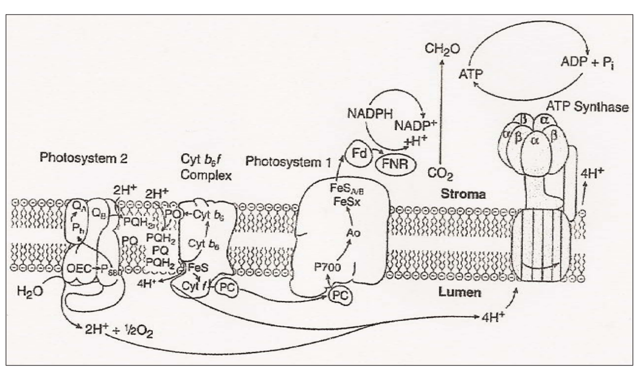

Figure 2. From Robert E. Blankenship, *Molecular Mechanisms of Photosynthesis*. Reprinted with permission from Blackwell Publishing.

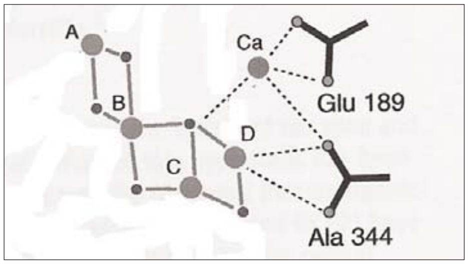

Figure 3. From Junko Yano et al., "Where Water is Oxidized to Dioxygen," *Science* 314 (November 3, 2006): 821–825. Reprinted with permission from AAAS.

higher resolution structure of the OEC manganese-oxide core by X-ray absorption spectroscopy on single crystals of PSII.

In photosynthesis the manganese-oxide cluster binds two molecules of H_2O. The energy of one quanta of light abstracts one proton and one electron. This structure then integrates four electron-transfer reactions that result in the synthesis of one molecule of dioxygen from two molecules of water. The invention of this mechanism was a unique event in evolution. When did it happen?

A Date for the Evolution of Oxygenic Photosynthesis

When did oxygenic photosynthesis evolve? One piece of data suggests that it had evolved by 2.7 billion years ago, 250 million years before the appearance of oxygen in the atmosphere.

This piece of data was discovered using powerful analytical techniques (gas chromatography and mass spectrometry) developed by Roger Summons at MIT for detecting minute traces of biological compounds (biomarkers) in ancient rocks. Rocks formed billions of years ago have gone through cycles of heating (diagenesis). The preservation of organic chemicals in ancient rocks is rare, and when they are present they are limited to hydrocarbons.

A class of hydrocarbons called stearanes was found in samples taken from black shales deposited in northwestern Australia 2.7 billion years ago. Stearanes are derived diagenetically from steroids, e.g., cholesterol, now found almost exclusively in eukaryotic cells. Steroid synthesis involves a number of steps requiring molecular oxygen. For example, in the synthesis of cholesterol starting with squalene, eleven separate steps require molecular oxygen. It seems very unlikely that all of these steps used some other oxidant and different enzymes prior to the advent of oxygen and then were somehow altered with the arrival of oxygen. Thus one could make the argument that the presence of stearanes in the Australian black shales indicates the presence of molecular oxygen in the ocean 2.7 billion years ago.

But even though the rocks from which these samples were extracted are correctly dated, it is more difficult to be sure that the biomarkers were deposited in the rocks at that date. They could have been the result of groundwater penetration from the surface or penetration of oils from younger rocks into the older rocks. Or they could have been contaminated by drilling fluid. Great precautions are now taken to avoid this type of contamination. Cores are obtained using only water as the drilling fluid. The exterior surface of the drill cores is shaved off, and the sample is taken from the interior of the core.

The biological invention of photosynthesis was, after the origin of life itself, the most important development in the history of our planet.

It is also important, insofar as it is feasible, to investigate biomarkers in yet older rocks. The possibility that oxygenic photosynthesis evolved 300 million years before the advent of oxygen in the atmosphere poses the obvious question of why it took so long for oxygen to appear in the atmosphere. To answer that question, we need to know what the earth was like before the appearance of oxygen in the ocean and what events might have triggered its rise in the atmosphere.

The Archean Earth and the Rise of Oxygen

In the Archean eon, more than 2.5 billion years ago, the major components in the atmosphere were N_2, CO_2, and perhaps CH_4, methane. The argument for methane is that, at the origin of the earth, the sun was 30 percent fainter than it is now. Without a greenhouse gas, the earth would have been frozen until 2 billion years ago. The geological record, however, shows that liquid water was present

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during the Archean eon and that the temperature was likely warmer than it is now.

Certainly carbon dioxide would have provided a greenhouse effect, but without oxygen in the atmosphere, methane, likely produced by methanogenic bacteria, could have accumulated to 1000 ppm (it is present at about 2 ppm now). The composition of the Archean ocean is less certain, but geological evidence suggests that there was much less sulphate than there is now and very little dissolved oxygen because the dissolved iron Fe^{++} was abundant. In the Archean world, organisms only lived in the ocean, and the primary producers were likely the nonoxygenic photosynthesizers (although, remember, we do not know for certain how early oxygenic photosynthesis evolved).

Geologists have known for more than fifty years that oxygen appeared in the atmosphere about 2.3 billion years ago. Preston Cloud and Dick Holland were the first to make this observation. The Huronian Supergroup in southern Canada provides a good example of what they realized early on and could see at many places around the world.

In the Matinenda formation (formed 2.45 billion years ago) are conglomerates that contain uraninite and pyrite. These conglomerates are detrital deposits, meaning that they were washed into the sea by ancient rivers. Uraninite, UO_2, is insoluble whereas the more oxidized form, UO_4, is soluble. If oxygen had been present in the atmosphere, UO_2 would have been oxidized and solubilized. In addition, pyrite (FeS_2) is rapidly converted to hematite, Fe_2O_3, in the presence of oxygen. Pyrites and uraninites are not seen in the sediments above the Matinenda formation in the Huronian, and they are generally not present anywhere in detrital deposits younger than 2.3 billion years.

When we reach the Lorraine formation (formed 2.2 billion years ago), we first encounter red beds. These are sandstone beds, deposited by rivers or windblown dust. Red crystals of hematite coat the sandstone grains. The presence of red beds is indicative of an oxidizing atmosphere. The earliest red beds were formed about 2.2 billion years ago. So oxygen must have appeared in the atmosphere after the deposition of the Matinenda formation, and before the deposition of the Lorraine formation.

A more recent result has firmly pegged the rise of oxygen at 2.45 billion years ago. In order to understand this result, we must briefly review the use of atomic isotopes in geochemistry. Four isotopes of sulfur occur naturally: ^{32}S (94.9 percent), ^{33}S (0.76 percent), ^{34}S (4.29 percent), and ^{36}S (0.02 percent). In biological processes, like SO_4 reduction to SO_2, ^{32}S is the most popular isotope. ^{34}S is used about half as much as ^{33}S. Starting with the work of James Farquhar and Mark Thiemens of the Scripps Institution of Oceanography, the isotopic abundances of the sulfur isotopes in various rocks have been measured. All modern rocks contain the same ratio of ^{33}S to ^{34}S because in modern rocks the preferential use of ^{33}S to ^{34}S in biological processes has determined this ratio. A quantity $\Delta^{33}S$ is a measure of the deviation of the abundance of ^{33}S from that ratio. In all modern rocks $\Delta^{33}S$ is zero. Figure 4 shows a recent compilation of the data.

In rocks younger than 2.45 billion years, the value of $\Delta^{33}S$ is zero; in rocks older than 2.45 billion years the value is negative if the sulfur is derived from barite ($BaSO_4$) and positive if the sulfur is derived from pyrite (FeS_2).

The variation of $\Delta^{33}S$ from zero is called *mass independent fractionation*. We can conclude that nonbiological processes were at work on sulfur in rocks older than 2.45 billion years: these processes were photochemical. Because of the presence of oxygen in the atmosphere, however, the ozone shield formed 2.45 billion years ago. Ozone absorbs ultraviolet light, active in a number of photochemical processes in the atmosphere. For sulfur, these could include reduction or oxidation of SO_2 or H_2S, leading to elemental sulfur or H_2SO_4, both of which can be incorporated into rocks. In the modern ocean all atmospheric sulfur is protected from photochemistry by the ozone layer and is subjected to *mass dependent fractionation*. A level of oxygen in the atmosphere that is 1/100 the present level would lead to an effective ozone shield.

The sulfur-isotope data determine the time for the rise of oxygen at some level. But the biomarker data suggest that oxygenic photosynthesis originated at least 300 million years earlier. What prevented oxygen from appearing in the atmosphere earlier? Though this question has been frequently asked, we do not have a universally accepted answer yet. There could have been either geological or biological reasons for the delay, or both. Perhaps the level of reductants supplied to the atmosphere and the ocean by volcanic activity decreased because of altered chemistry in the mantle. Or perhaps oxygenic photosynthesis, though it evolved earlier, had only become effective enough to alter the atmosphere 2.45 billion years ago.

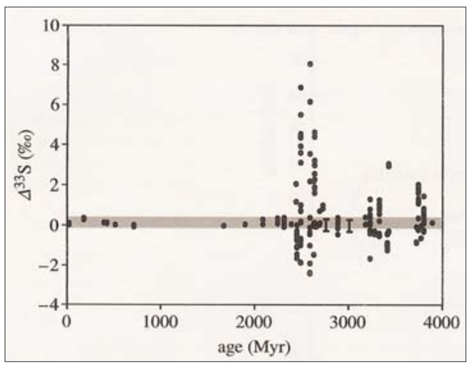

Figure 4. See J. Farquhar et al., *Science* 289 (August 4, 2000): 756; updated by S. Ohno.

Interestingly, the appearance of oxygen in the atmosphere had some relatively near-term effects on the geology of the earth but did not markedly influence the biology of the earth (at least as seen in the fossil record) for another 1.8 billion years.

The Proterozoic Earth after the Rise of Oxygen

In the Huronian Supergroup there is evidence of three separate glaciation events between the anoxygenic uraninite conglomerates (formed 2.45 billion years ago) and the oxygenic red bed deposits (formed 2.2 billion years ago). Large dropstones are seen, left behind in the sediment as the glacier recedes or as scratches in the bed rock made as the glacier moves over it. The earth evidently went through a pronounced cooling period between 2.45 and 2.2 billion years ago.

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In South Africa, in the Makganyene formation, there is evidence of another glaciation event 2.2 billion years ago. Joe Kirschvink at Caltech has shown by paleomagnetism that the Makganyene glacial event took place when the Transvaal Craton was near the equator. This means that the entire earth was glaciated, a "snowball earth" event. The most plausible cause of the cooling is that the rise of oxygen in the atmosphere destroyed the methane, and thus the greenhouse effect, that was warming the earth. As the earth cooled and ice formed, more and more solar radiation was reflected (ice reflects eight times as much radiation as water). Once ice covered the poles to the thirtieth latitude north and south, a positive-feedback loop ensured that a sheet of ice about two kilometers deep would cover the earth.

Why did the earth not remain in a frozen state? How could life have survived? Vulcanism is the most probable answer. Life was most likely confined to heated regions near vents. Eventually, carbon dioxide escaping into the atmosphere would have accumulated because it could not have dissolved in the ocean and hence been lost in weathering processes, as it is normally. After 30 to 50 million years, a sufficient level (350 times the current level) of carbon dioxide probably accumulated, enough to create a greenhouse effect that would have melted the ice. Once that level had been attained, there would have been a reverse positive-feedback loop, and the ice would have melted in a few hundred years.

In the aftermath of snowball earth, the intense greenhouse effect is hypothesized to have raised the surface temperature to 50°C – a hothouse earth. Carbon dioxide dissolved in the ocean, and a massive precipitation of $CaCO_3$ and $MgCO_3$ (dolomite) occurred. These precipitates are called cap carbonates, and they can be as much as four hundred meters thick.

The post–snowball earth ocean was rich in nutrients; cyanobacteria therefore flourished, raising the level of oxygen in the ocean and in the atmosphere. Dissolved iron precipitated as hematite, and manganese as MnO_2. South Africa thus possesses some of the richest manganese deposits in the world as a result of this event.

The Makganyene glaciation was the first snowball-earth event (there were earlier regional glaciations), but it was not the only one. Two more snowball-earth events took place between 800 million years and 600 million years ago. In the intervening billion years the earth was relatively quiet. Geologists call this period the "boring billion."

The Boring Billion

Following a proposal made by Don Canfield in 1998, consensus is building among geologists that, except for the likely spike after the Makganyene glaciation, the level of oxygen in the atmosphere remained low for more than 1 billion years and did not rise to present levels until the end of the Proterozoic eon 540 million years ago (see Figure 1).

The modest levels of oxygen in the atmosphere could have led to an ocean weakly oxygenated at the surface, and an anoxic and sulfidic (like the Black Sea today) ocean below. It is not possible here to review all of the geological data supporting this conclusion, but one line of evidence from Ariel Anbar and Tim Lyons, involving the level of molybdenum in Proterozoic black shales, strongly supports this model. In an oxic atmosphere, molybdenum is washed into the ocean by rivers as the soluble MbO_4^{2-} anion. Molybdenum is thus abundant in today's oceans. A survey of molybdenum in black shales through time reveals that molybdenum is low during the Archean, slightly elevated in the mid-Proterozoic, and abundant in the Phanaerozoic period.

The relatively anoxic ocean of the mid-Proterozoic could not have supported multicellular life and would have been a poor environment for eukaryotes. There is plenty of evidence for single-cell eukaryotes in the Proterozoic, but cyanobacteria would have dominated the shallow oceans and tidal flats. Beginning in the late Proterozoic, as oxygen levels rose, the multicellular eukaryotes make a modest appearance in the fossil record. This is the end of the boring billion.

The Rise of Multicellular Eukaryotes

Two snowball-earth events punctuated the end of the Proterozoic eon: one occurred 750 million years ago, the other 600 million years ago. These were not caused by oxidation of methane in the atmosphere but, more likely, by a fall in carbon-dioxide levels. At this time, all of the earth's land mass was near the equator, and so none of it would have been covered with ice as Antarctica is today. The entire land mass of the earth would have been available for removing CO_2 from the atmosphere by atmospheric weathering, leading to a gradual cooling of the planet. The rich aftermath of these snowball earth-events could have oxygenized the oceans and led to the initial rise of multicellular animals. We can see fossils from this period (called the Ediacaran or Vendian period) in many parts of the world. At the boundary between the Cambrian and the Precambrian eras, 542 million years ago, a mass extinction occurred. The Ediacaran animals disappeared, and the modern world followed.

The relatively anoxic ocean of the mid-Proterozoic could not have supported multicellular life.

However, we have to look at rocks deposited some 40 million years later to see the blossoming of animal life in the Cambrian period as seen in the Burgess shales. The Burgess shales record a wonderful zoo of animals that clearly had developed many of the body parts seen later in evolution as well as mind-boggling creatures that were never seen again. By the Cambrian period, oxygen was near its present level in the atmosphere and the ocean. Animal evolution was on its way.

Epilogue

The unique and powerful process of oxygenic photosynthesis nearly resulted in the extinction of all life in the Makganyene glaciation. The earth itself, with its molten core, came to the rescue in that instance. After a period of nearly 2 billion years, photosynthesis ultimately made the evolution of multicellular animal life possible, a process that continues today.

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Although it is in its infancy from a geological perspective, human intelligence may be as unique and potent a force for change on earth as photosynthesis was. Will human intelligence lead to a flowering of the earth, or will it lead to the extinction of life? It is too early to say. Geology tells us that we will have to wait 2 billion years to know.

Acknowledgments

I should say that I am not a geologist. I am a biochemist, but I have been a student of geobiology for the past five years and, as President of the Agouron Institute (see www.agi.org), I have been a patron of the field. For the past five years we have supported a course in geobiology, which has included a geology field trip led by John Grotzinger of Caltech and Andy Knoll of Harvard. I have been on all of the field trips. We have also carried out a drilling project in South Africa in which we obtained some 3000 meters of core that cover the period of about 2.5 billion years to 2.2 billion years. It was during that period that oxygen first appeared in the atmosphere. Last year we sponsored an interdisciplinary meeting on "Oxygen" in Santa Fe, New Mexico. About forty chemists, biochemists, geologists, and microbiologists discussed the problem of the origin of oxygenic photosynthesis. This report represents my attempt to synthesize the ideas that were expressed in this exciting meeting. I wish to thank my geobiology mentors, John Grotzinger and Andy Knoll, for helping me to appreciate what rocks can tell us about biology. I thank Andy Knoll, Robert Blankenship, Judith Klinman, Don Canfield (the organizers of the Oxygen meeting), and many of the participants for helping me to write this article. Thanks also to my longtime partner in science, Mel Simon, and to Joan Kobori, the Agouron Institute administrator who has made the execution of our programs possible.

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