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Evolution Of Biogeochemical Element Cycles With Emphasis On The Role Of Metal-organic Interactions In The Accumulation Of Heavy Metals In Organic-rich Sediments

John Dennis Meloche

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EVOLUTION OF BIOGEOCHEMICAL ELEMENT CYCLES
WITH EMPHASIS ON THE ROLE OF METAL-ORGANIC
INTERACTIONS IN THE ACCUMULATION OF HEAVY
METALS IN ORGANIC-RICH SEDIMENTS

by

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Submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Graduate Studies
The University of Western Ontario
London, Ontário
June, 1981

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Probable eukaryotic microfossils in ~1 billion-year-old chert of the Glénegl Formation, Victoria Island.

bar = 20 microns
ABSTRACT

Bacteria constitute one of the most interesting and least studied potential metal-accumulating agents in sediments due to their widespread occurrence, large biomass and generally high affinity for metals. Model diagenesis experiments were carried out to ascertain the relative stability of organically-complexed metals (U^{+6}, Fe^{+2}, Cu^{+2} and Zn^{+2}) as a function of Eh and pH up to 100°C in equilibrium with water. Metal-loaded cells of the ubiquitous bacterium, *Bacillus subtilis*, artificially aged in a variety of synthetic sediments under confined, anoxic conditions at 100°C for up to 200 days, gave rise to mixed assemblages of crystalline metal phosphates, metal sulphides and metal-complexed, polymeric organic residues (kerogen). The influence of Eh, pH and the nature of the complexed metal on thermal degradation of cellular matter and authigenic mineralization is illustrated. The significance of authigenic phosphate and sulphide formation as a consequence of thermal maturation of sedimentary organic matter is discussed.

The association of microfossils, complex organic residues and graphite with Precambrian sedimentary metal deposits has often been cited as evidence for the probable interaction of microorganisms in metal accumulation and mineralization. Recent biological/biochemical, geological/geochemical and micropaleontological/organic chemical data
challenge the current wisdom that the marine biomass throughout the Archean and early Proterozoic eras consisted solely of anaerobic bacteria adapted to life in an anoxic hydrosphere in equilibrium with an atmosphere devoid of free oxygen. Major evolutionary events within the lithosphere, atmosphere, hydrosphere and biosphere might be resolved ultimately in terms of thermal decay of an early planetary system. Horizontal expansion of an oxygen minimum as a consequence of temperature-modulated rates of oxygen supply and thermocline circulation within the Precambrian hydrosphere may account for the preponderance of reduced sediments. The concept of gradual oxygenation of the photic zone as a consequence of decreasing water temperature and increasing oxygen solubility is consistent with the progression of biospheric evolutionary events leading up to and including the development of the eukaryotic cell. Micropaleontological studies provide at least circumstantial evidence that oxygenation of the photic zone was locally realized as early as 3.4 By BP.
ACKNOWLEDGEMENTS

It is a pleasure to thank the many teachers and friends who in some way contributed to this thesis. I am indebted to T. J. Beveridge for the many hours that he unselfishly devoted to the preparation and study of the metal-loaded cells, for the use of his photographic facilities, and for the enlightening discussions on how to best approach the interpretation of TEM images; to R. G. Murray for the invaluable use of his laboratory and darkroom facilities, and for his helpful advise and unrelenting encouragement throughout this project; to B. Kronberg for her continued suggestions and much-needed criticisms during preparation of this thesis; and, above all, to my supervisor, W. S. Fyfe, for his inspiration and help in initiating this project, for his unfailing financial and moral support, and for his generosity with observing time whenever additional discussion was required for clarification of ideas.

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"It has taken the world 4.5 billion years to discover it is 4.5 billion years old..."

George Wald, 1979

PREFACE

The absolute age of the Earth is unknown. Based on lunar studies and analyses of meteorites, solid objects existed in the solar system 4.5 billion years ago. Estimates based upon lead isotopic ratios would place an age limit of 5.5 billion years for the Earth. The oldest known terrestrial rocks are about 3.9 billion years old. The age of the Earth is generally accepted by geologists as 4.5 billion years.

Through his studies on cooling rates, Sir Issac Newton concluded that the Earth must be much older than the six millenia envisaged in the Bible. However, in the absence of collaborative data at the time, his calculations meant very little and the chief burden of extending the age of the Earth fell upon geologists.

The Scottish geologist, James Hutton, believed that the present surface of the Earth could be accounted for by the action of every day processes, often apparently imperceptible, working steadily over long periods of time. With the publication of his 'Theory of the Earth' (1795), Hutton established the basis for research into sedimentology. To explain most features observed in ancient sedimentary rocks, he cautioned "...no powers are to be employed that are not
natural to the globe, no actions to be admitted of except those of which we know the principle, and no extraordinary events to be alleged in order to explain a common experience."

Through the careful examination of sedimentary rocks with consideration paid to natural geomorphological mechanisms, Hutton interpreted a myriad of textures and structures within stratified rock sequences. He came to the realization that the concept of geologic time encompassed a slow evolutionary process evidenced by the gradually changing succession of paleodepositional environments. Yet, while he could appreciate the magnitude of geologic time, like Newton, he could come no closer to approximating the age of the Earth. In fact, Hutton ventured even further to insist that there was no evidence to suggest that there ever was a beginning.

The concept of natural evolution has intrigued mankind for well over a thousand years. Classical Greek philosophers such as Anaximander and Heraclitus developed theories of creation and evolution. However, it was the ideas of Aristotle that proved to survive the test of time. Aristotle envisioned the emergence of life from the inanimate and the gradual metamorphosis of one life form to another. Evolution has been given a number of definitions but implicit in all of them is the concept of gradual change or development from a pre-existing entity. Applicable to all processes, the term evolution is more commonly employed synonymously
with biological evolution.

Charles Darwin occupies in biology a position comparable to that of Hutton in geology. Like Hutton, he supplied a great unifying concept in his subject. With the publication of his 'Origin of the Species' (1859), he shocked the scientific community and set the foundation for further research into the evolution of life with the proposition that evolution proceeded through natural selection "...worked by and for the good of each being..." (each organism).

Although Darwinism has undergone a number of major revisions and additions through the years, biologists have maintained evolution of life as the product of random mutation and natural selection. Historically since Darwin, scientists who have researched biological evolution adhered remarkably close to the basic principles proposed by Hutton for the interpretation of geological data. For the most part, biological evolution is viewed in terms of known principles and 'processes natural to the globe'.

Most current theories on the evolution of the lithosphere, hydrosphere and biosphere are invariably contingent upon a major evolutionary change in the composition of the atmosphere. It is commonly assumed that the primitive atmosphere was anoxygenic and reducing for almost half of the Earth's geological history. Proponents of a reducing atmosphere cite as evidence the reduced nature of the few sedimentary rocks that are preserved in Precambrian se-
quences, and have sought to gain support from biological theories for the origin of life. Recent studies are beginning to show currently popular evolutionary models to be essentially inconsistent owing to an inherent misinterpretation by geologists of environmental conditions throughout the Precambrian. It appears somewhat ironic at this point in time that such a fundamental error should be propagated by geologists whose basic approach to Precambrian rock interpretation is a contravention of Hutton's 'law of uniformitarianism'. They chose to ignore what is perhaps the most important environmental variable: the influence of microorganisms and the role of organic matter in sedimentation and diagenesis.
"What was formerly called natural history is the perennial foundation of the biological sciences. It has given rise to all of the theoretical branches and will no doubt give rise to others in the future."

William Morton Wheeler (1934)

CHAPTER 1

INTRODUCTION

1.1 Purpose

Biogeochemistry, geomicrobiology and organic geochemistry collectively represent a facet of each science dedicated towards biological input into what was once an almost exclusively inorganic area of environmental study. Bacteria constitute one of the most interesting and least studied potential metal-accumulating in sediments due to their widespread occurrence, large biomass and generally high affinity for metals. Using cell walls of the ubiquitous bacterium, Bacillus subtilis, as a model for studying the interaction of metal ions with anionic cell wall components, T. J. Beveridge and R. G. Murray recently demonstrated the ability of cell walls to fix significant quantities of over forty metals from dilute solutions. In this study, metal-loaded cells of Bacillus subtilis were used specifically to complement the model study of Beveridge and Murray, in an attempt to ascertain the rela-
tive stability of both the cellular constituents and complexed metals as a function of Eh and pH at temperatures up to 100°C in equilibrium with water.

Initially, the author's intent was to attempt to assess the potential significance of metal-organic interactions to heavy metal accumulations in Precambrian sedimentary rocks. It became apparent, however, that such an endeavor necessitated the establishment of a set of parameters characterizing Precambrian depositional environments. Diversity and physiological complexity of the microfauna, biomass productivity, pH, Eh, salinity and temperature of the depositional waters are but a few of the variables which must be defined prior to any investigation of biomineralization. Unfortunately, much of this information for the Precambrian failed to survive the rigours of diagenesis and metamorphism. Consequently, the geologist is reduced to the application of generalizations emerging from the collective study of Precambrian sedimentary rocks which vary widely in both age and depositional environment.

To date, much of the information available to the geologist for the Precambrian eras exists enmeshed in a web of contradictory and often internally-inconsistent evolutionary models for the lithosphere, atmosphere, hydrosphere and biosphere. Most currently-popular evolutionary models for the atmosphere and biosphere invariably hinge upon a major atmospheric redox transition as a
consequence of the evolution of oxygen-producing photosynthesis. The main objective of this thesis is to critically examine the high degree of uncertainty surrounding the biogeochemistry and geomicrobiology of Precambrian sedimentary rocks. Recent biological/biochemical, geological/geochemical and micropaleontological/organic chemical data challenge the accepted views that the Archean atmosphere was reducing. Arguments are assembled to show that both anoxygenic and anaerobic systems can be developed and sustained beneath an oxygen-rich atmosphere and it is difficult to exclude the possibility that the atmosphere could have been oxidizing throughout most of the Earth's history.

1.2 Method of Approach

Chapter 2 presents a comprehensive review of selected aspects of biogeochemistry, geomicrobiology and organic geochemistry essential to the accurate interpretation of organic-rich sedimentary rocks. The following chapter evaluates the currently popular evolutionary models and their supporting evidence, concluding with a generalized scheme for Precambrian global evolutionary development of biogeochemical element cycles. Chapter 4 reviews the principles of metal-organic interaction and biometal accumulation processes. The experimental section of this chapter provides new insight into one of the lesser known mechanisms and illustrates some of the interrelationships
between organic and inorganic processes during diagenesis. Chapter 5 summarizes biomineralization in the marine environment and illustrates with examples how known biogeochemical principles can be applied in an uniformitarian approach to the interpretation of enigmatic Precambrian sedimentary mineral deposits.
CHAPTER 2

BIOGEOCHEMISTRY AND THE REDUCING ENVIRONMENT

2.1 Introductory Statement

Microorganisms make up the bulk of the Terrestrial biosphere. Adapted to life in a wide range of environments, they exist in one form or another in nearly every surface environment providing there is free water below 100°C. The presence of free water is a fundamental prerequisite to all life on Earth, acting both as a nutrient reservoir and as a means by which to transport the nutrients into the cell (1).

The essence of life can be considered as a series of complex chemical reactions taking place under the appropriate physico-chemical conditions. All living organisms, by virtue of their metabolic requirements, must impart some degree of chemical modification to their environment and, in turn, are themselves sensitive to chemical variations within their environment. Nowhere is this more obvious than in the hydrosphere where microorganisms not only regulate the levels of many dissolved constituents but also mediate a large number of inorganic chemical re-
2.2 Fundamentals of Oxidation and Reduction in the Natural Milieu

The term oxidation implies a loss of electrons. Naturally occurring oxidations result in the concomitant reduction of a second chemical species through the transfer of electrons. The substance which undergoes the reduction is termed the oxidant or oxidizing agent and, conversely, the substance which is oxidized is called the reductant or reducing agent.

The redox potential (Eh) is a measure of the tendency of a substance to either receive or donate electrons. To the biogeochemist, the redox potential represents the sum total of oxidizing and reducing agents within an environment or, in other words, the capability of an environment to either accept or donate electrons. In the hydrosphere, it is often difficult to separate redox reactions from simple hydrolysis reactions (2). The redox potential is to some extent reciprocal to the hydrogen ion activity (pH) but is also influenced by other variables which allow the biogeochemist to plot domains of measured Eh-pH values for various aqueous environments.

The natural milieu is subject to an infinite number of intensive and extensive variables. The biogeochemist is faced with the constant problem of determining which set of parameters best defines the situation at hand.
Fortunately, the global study of Baas Becking and his coworkers (3) has shown that virtually all aqueous environments can be characterized by their limited range in Eh and pH. Their study further demonstrated that the Eh-pH limits of biological systems and natural environments almost coincide (Fig. 1) suggesting that there are few sterile surface environments.

Redox reactions in the surface environment are controlled to a large extent by the metabolic activity of the groups of organisms which inhabit them. The common natural oxidative reactions comprise nitrification, oxidation of reduced sulphur species (i.e. hydrogen sulphide, sulphide ion etc.), oxidation of ferrous and manganous chemical species, and the oxidation of organic compounds. Through the mediation of oxidative chemical reactions, microorganisms act to lower the redox potential of their environment as a whole both through the consumption of oxidants and through the concomitant generation of reductants.

2.3 Microbial Energetics

A necessary corollary to the study by Baas Becking et al. (3) must be that there are few, if any, surface environments unaffected by the metabolic activity of living organisms. For the most part, microorganisms act only as catalysts in reactions which, although thermodynamically favourable, would require the addition of
Figure 1

Approximate 'areas' of Eh and pH for some biogeochemically-important groups of prokaryotes and natural marine environments (after Baas Becking et al. (3)).

A •••• cyanobacteria
       •••• purple photosynthetic bacteria
       —— green photosynthetic bacteria

B •••• thiobacteria
       •••• iron bacteria
       •••• denitrifying bacteria
       •••• sulphate reducing bacteria
       —— anaerobic heterotrophic bacteria (fermentators)

C •••• open sea sediments
       —— marginal marine sediments
       •••• seawater
considerable time and energy (4). The type of chemical activity of a group of organisms is determined by the nature of the available metabolites which in turn are a function of the environmental chemistry.

All organisms are composed essentially of water and complex organic macromolecules. Organic macromolecules, by virtue of their high degree of structural order, are inherently thermodynamically unstable. It follows that living organisms, through the production and storage of organic compounds, accumulate a state of 'negative entropy' during growth. Organisms increase and maintain their high degree of internal complexity at the expense of free energy gained from their immediate surroundings, thereby acting to maximize the net entropy of their environment as a whole.

As long as an organism is alive it is the centre of complex and integrated biochemical reactions. Death ensues when the organism either loses its ability to regulate its chemical activities or can no longer compensate for changes that result in an imbalance in its activities. Metabolism is defined herein as the sum total of chemical reactions taking place within an organism "embracing the flux of materials and energy between the organism and the environment as well as the transformation of matter and energy within the organism" (5, pg. 209).

Metabolites constitute all of the inorganic and organic requirements for growth of an organism. Basically,
they are required for only one of two processes: 1) to assimilate for cell synthesis; and 2) for use in energy yielding processes (6). Generally, there is no direct relationship between metabolites required in biosynthetic reactions and those required for energy production (5). Consequently, microorganisms can be classified according to the nature of both the carbon source and the energy source.

On the basis of carbon assimilation, microorganisms are grouped into two major categories: the autotrophs and the heterotrophs. Autotrophs typically acquire the necessary carbon through the assimilation and reduction of carbon dioxide. Heterotrophs obtain the necessary carbon through assimilation of pre-existing organic molecules.

Photosynthesis is the name given to the metabolic process in which, by the aid of suitable transfer substances and enzymes (termed the Calvin reductive diphosphate pathway), hydrogen obtained from the photolysis of a reductant is made to combine with carbon dioxide to form organic compounds (carbohydrates). Photoautotrophs can be either aerobic (requiring free oxygen) or anaerobic (not requiring free oxygen). Anaerobic and aerobic photosynthetic systems resemble each other in that they employ the same reductive Calvin cycle for carbon fixation, but differ in their choice of hydrogen donors. Obligate anaerobic photosynthetic bacteria are able to use a wide range of hydrogen donors to obtain the necessary reducing
power (i.e. $\text{H}_2\text{S}$, $\text{HS}^-$, $\text{H}_2$, Corg etc.). Strict aerobic photosynthesizers such as some cyanobacteria and the eukaryotic algae are restricted to the use of water as the sole electron and hydrogen donor.

All photosynthetic reductions of carbon require a concomitant oxidation step. The electron donors are 'dissimilatively' oxidized, which is to say that they are not incorporated into the cell structure but rather released back into the external environment. Hence, anaerobic photosynthesis utilizing hydrogen sulphide results in the formation of elemental sulphur, sulphate and thiosulphate. Aerobic photosynthesis results exclusively in the generation of molecular oxygen from water.

Most bacteria are chemolithotrophic, obtaining the necessary energy for biosynthesis through the oxidation of appropriate substrates from the external environment (chemosynthesis). Through the catalysis of oxidative reactions, they are able to recover the energy stored in inorganic molecules (chemolithotrophy), organic molecules (chemoorganotrophy) or both simultaneously (mixotrophy) (5). They can be either autotrophic or heterotrophic, aerobic or anaerobic.

Respiration is the term used to denote dissimilative, energy-yielding cellular oxidations utilizing inorganic oxidants. Aerobic respiration relies entirely upon molecular oxygen as the oxidizing agent whereas anaerobic respirations can proceed using a variety of suitable oxi-
dants (ie. sulphate, nitrate, Mn and Fe oxides etc.). The respiration of organic compounds (chemoorganotrophy) often results in the near complete combustion of the organic substrate to carbon dioxide and reduced forms of the oxidant employed.

Fermentation represents the obligate anaerobic, chemooorganotrophic energy-yielding reaction in which part of the organic substrate is oxidized at the expense of part of the substrate being further reduced. It is much less energy efficient than most respiration reactions and results in the conversion of part of the energy substrate into end products at an oxidation state lower than carbon dioxide (6).

From the viewpoint of carbon assimilation, most bacteria are heterotrophic and depend upon a pre-existing organic carbon substrate. Chemoautotrophy is restricted to only a few types of bacteria which are able to fix carbon dioxide in the absence of light by the same metabolic pathway (ie. reductive Calvin cycle) found in photoautotrophs. Termed chemolithotrophic, they obtain the necessary energy for carbon reduction through the oxidation of reduced inorganic substrates which in most cases are the by-products of anaerobic respiration.

2.4 Biogeochemical Element Cycling

On the global scale, chemical elements are continuously being transformed within two interlocking cycles;
the endogenic cycle and the exogenic cycle. The endogenic cycle, powered by the Earth's internal heat flow, encompasses the element transformations associated with diagenesis, metamorphism, orogenesis and epierogenesis within the lithosphere. The exogenic cycle, extended to include all element transformations within the atmosphere, hydrosphere and biosphere, is powered by solar energy and includes the processes of weathering, erosion, transport, sedimentation and diagenesis (7).

The biosphere represents the sum total of all living organisms and freely available organic matter on the surface of the Earth. To date, at least twenty-eight elements are known or suspected to be physiologically essential to life (8). The elements carbon, oxygen, hydrogen, nitrogen, sulphur and phosphorous constitute the bulk of the basic biological metabolites required for the synthesis of new cellular material. According to Trudinger et al. (9), "the synthesis of the overall biosphere is largely a reduction of carbon dioxide, water, sulphate and nitrate to provide the major element components of living matter. Oxidized forms of these elements are regenerated by subsequent respiration, fermentation and other degradative processes" (pg. 7).

As previously discussed, metabolites are either assimilated for cell synthesis or oxidized dissimilatively as a source of energy. Although separate, these processes are related in that the bulk of the cells'
energy budget is expended in biosynthetic reactions and nutrient transport (6). Further energy must be spent if the assimilation of materials for cell synthesis requires a reduction step.

Reductive photosynthesis is considered by many as the master reaction in the biosphere, converting solar energy to bond energy and passing the stored energy through the various trophic levels in the form of complex organic molecules. The biosphere, however, has no similar extraterrestrial source for the chemical elements for life. Hence, these elements must be continuously recycled through the biosphere if it is to persist. Biogeochemical cycles, according to Odum (10), more or less represent the flux of chemical elements in the biosphere "from the environment to the organism and back to the environment". Of the six biologically most essential elements (C, N, O, H, S, P) all but phosphorous form volatile, soluble and insoluble compounds and therefore are free to circulate through the atmosphere, hydrosphere and lithosphere. Phosphorous, which forms only non-volatile compounds, is restricted to the lithosphere and hydrosphere. On this basis Odum distinguished two basic types of biogeochemical cycles: a gaseous cycle in which the atmosphere and hydrosphere serve as a main reservoir; and a sedimentary cycle in which the lithosphere acts as a reservoir.

Biogeochemical element cycles represent the complex interaction of biochemical, chemical and, in many cases,
physical processes in the surface milieu (9). Since organic matter is composed of many, if not most, of the biologically essential elements, it follows that their biogeochemical cycles must intersect within the biosphere or, more specifically, within the living cell such that "one cycle may be influenced by processes which are mechanistically unconnected to that cycle" (9, pg. 21).

It is apparent that for many chemical elements the concepts of exogenic cycling and biogeochemical cycling are synonymous. It is also apparent that by virtue of their metabolic requirements all living organisms must impart, either directly or indirectly, some degree of modification to the exogenic cycle of virtually every element. Bacteria comprise perhaps the most important group of organisms which contribute to the biogeochemical cycling process. As well as participating in the cyclic transformation of elements, they are actively involved in the formation of mineral deposits, sedimentation and rock weathering.

2.5 Carbon Cycling

Carbon, in its reduced state, is the most important structural element in the biosphere. With its small size and four valence electrons, carbon forms at least ten times as many compounds as all other elements combined. Carbon atoms form strong covalent bonds and, unlike the other elements, are able to bond to each other to form an
infinite variety of ring and chain structures. The affinity for strong covalent binding inhibits ionization and explains, in part, the low solubilities and slow reactivities observed for reduced carbon compounds.

In a real sense, life on Earth is 'carbon-based', made possible by the unique properties of this element. On the global scale, carbon is partitioned by its valency state into an oxidized pool and a reduced pool. Within its exogenic cycle, the two pools are linked through the transformation of carbon compounds within two mutually interdependent cycles: the inorganic or carbonate cycle in which carbon compounds pass through a series of chemical equilibria; and the organic cycle in which carbon compounds pass through the processes of biosynthesis and mineralization (7).

The inorganic carbon cycle encompasses the series of hydrospheric chemical transformations leading to the precipitation or dissolution of carbonate minerals. Atmospheric carbon dioxide dissolves in water to form carbonic acid which, in turn, dissociates to form bicarbonate and carbonate ions plus protons. The equilibria between the gaseous, dissolved and solid phases is largely dependent upon the aqueous saturation concentration of carbon dioxide and the hydrogen ion activity. These in turn are strongly influenced by temperature, pressure and biological activity. In the hydrosphere, microbial activity strongly influences the bicarbonate-carbonate buffer
system by the release or uptake of dissolved carbon
dioxide and/or by effecting changes in the hydrogen ion
activity.

Within the exogenic organic carbon cycle, carbon
dioxide is biologically reduced to form new organic matter
by autotrophic organisms. Upon the death of an organism,
carbon is returned to the environment as carbon dioxide
through a number of biological and non-biological oxida-
tive processes collectively referred to as mineralization
processes. Mineralization of organic matter is perhaps
the single most important role of bacteria.

The oceans represent, by far; the most important
reservoir of freely-available carbon, predominantly in
the form of bicarbonate and carbonate ions. Covering
seventy percent of the Earth's surface, the oceans are
thought to contain little more than two percent of the
living biosphere but more than eighty percent of the dead
biosphere (8). Microorganisms concentrated near the air/
water and sediment/water interfaces comprise the major
part of the modern marine biomass. In the surface waters,
where the penetration of light is a maximum, marine phyto-
plankton photosynthetic efficiency is 1500 times greater
than terrestrial plants (11). This, combined with the
short life span of microorganisms in general, is largely
responsible for the generation of such high concentrations
of metabolic wastes.

The exogenic cycles of carbon and oxygen are intim-
ately joined through the reciprocal processes of oxygenic photosynthesis and aerobic respiration. The exchange of carbon dioxide and oxygen between the hydrosphère and atmosphere is dependent upon a number of biological and chemical equilibria ultimately poised by the aqueous solubility of the gases. In the marine environment, only the highly productive surface waters are maintained at or near saturation with respect to dissolved gases through the aerating action of waves and other local disturbances.

Below the zone of maximum aeration, the exogenic cycles of carbon and oxygen are somewhat self-contained; the oxygen produced during photosynthesis being consumed in bacterial oxidations of organic carbon to carbon dioxide and water. The net effect, according to Bolin (12), is to prevent oxygen saturation and to enrich the deeper marine waters with carbonate and bicarbonate ions.

When organic matter is no longer subject to biodegradation through natural causes, it is considered removed from the biosphere and hence from the exogenic cycle. Generally, in the absence of microbial catalysis, organic compounds are slow to react at low temperatures and can persist for an indefinite period. As a consequence, the greatest bulk of the Earth's organic carbon budget is tied up as fossil organic matter in the sedimentary fraction of the lithosphere. While a considerable amount is found in massive deposits as coal and petroleum, the greatest portion of fossil organic carbon occurs in the form of finely
dispersed, complex polymeric compounds referred to as kerogen.

The amount of organic carbon that is eventually trapped in sediments depends upon a large number of factors including the rate of primary productivity, the environment of deposition, the efficiency of microbial recycling processes as well as the nature and intensity of post-depositional diagenetic alterations. Within the marine environment, nearly the entire exogenic organic carbon cycle is located within the photic zone at depths not exceeding 200-300 metres (13). Biological oxidations in the photic zone account for the recycling of 93-97% of the dead biomass back to the hydrosphere as carbon dioxide. Generally, the amount of organic carbon which is permanently trapped in the bottom sediments represents less than 1% of the primary production in the surface waters (11).

In recent marine sediments, the amount of organic matter varies from as low as a fraction of a percent in the open ocean to as high as twenty percent in restricted euxinic basins (14). The organic content of sedimentary rocks, regardless of age, appear to correlate well with the levels observed in recent sediments deposited under similar conditions (15).

2.6 Chemical Diagenesis

Many rocks, particularly those formed under conditions
of high temperature and pressure, are in chemical disequilibrium under surface conditions. Emergence, resulting in the exposure of these rocks to surface conditions, begins a series of chemical transformations leading to the formation of compounds of greater stability as the various rock components pass through a sequence of sedimentation processes including the physical and chemical weathering of rock; solubilization, transport and deposition of minerals and detritus by surface waters; and the consolidation of cementation of the deposited materials.

Diagenesis is commonly defined as the sum total of physical and chemical changes that convert unconsolidated sediment to rock after deposition. Fairbridge (16) extended the concept of diagenesis to further include all changes in sedimentary rocks, in the absence of metamorphism, up to the point of emergence. However, many of the characteristic diagenetic processes occurring below the sediment/water interface are not fundamentally different from those already taking place between the suspended particle and the surrounding water. On this basis, Howell (17) broadly defined diagenesis as the physical and chemical changes that sediments undergo during and after their accumulation.

Based upon marine sedimentological data, Fairbridge (16) divided diagenesis into three main phases as follows:

1) syn-diagenesis (the sedimentation phase)
2) anadiagenesis (the compaction-maturation phase)
3) epidiagenesis (the emergent-pre-erosion phase)

Syndiagenesis is defined herein as the physico-chemical-microbiological changes that begin the moment the sediment component is formed at the surface and continues after deposition to the point where biological activity ends. During this phase sediments are characterized by large amounts of interstitial water, as high as seventy-five percent by volume in the upper several metres (18, 19). Steady-state diagenesis in the upper part of the sediments is an acceptable assumption as compaction, leading to expulsion of interstitial water, undergoes linear change during burial.

Hydrolytic, oxidative and reductive reactions are probably the most important chemical changes associated with syndiagenesis. Microbial activity accounts for many, if not most, of the rapid fluctuations in redox potentials, ion activities and dissolved gas levels within the sediment pore waters during this phase. Normal surface waters are oxidizing and only slightly alkaline or neutral. However, confined pore waters rapidly lose their oxygen content in the presence of aerobic bacteria and become reducing as well as slightly acidic. Dapples (20) recognized two distinct stages in syndiagenesis: the initial or oxidizing stage controlled by the chemistry of the overlying waters; and the early or reducing stage controlled by the microbially-modified chemistry of the interstitial waters. As sediments accumulate and are
buried, the uppermost oxidized layers are moved into the reducing zone resulting in the reduction or "sulphidation" of the oxidized components (21). In the euxinic environment, where the Eh of the bottom waters is low enough to produce reducing conditions, the initial or oxidizing stage occurs within the water column.

The depth limit of syndiagenesis is defined generally by the lower limit of vigorous bacterial activity (16). In all sediments, both the population size and metabolic diversity of microorganisms decrease with depth. Oxygen depletion results in a logarithmic decrease in the number of aerobic bacteria below 5-10 cm (22), coinciding roughly with an inflection point in the redox gradient (termed the redoxcline) around +200 mV ± 50 mV (21). In most sediments, anaerobic bacteria display a similar population decrease below 40-60 cm. Under ideal conditions the syndiagenetic phase may extend to depths from 100-1000 metres (18) but generally is restricted to within 100 metres of the sediment/water interface.

Anadiagenesis and epidiagenesis represent the later phases of diagenesis beginning when bacterial activity ends through to the point of emergence. Anadiagenesis is the deep burial, dewatering phase where diagenetic changes occur at a much slower rate and the interstitial waters are gradually expelled from the sediment. The trapped waters during this phase become progressively more alkaline and the Eh is gradually neutralized. Epi-
diagenesis, the emergence phase associated with uplift, is marked by late stage oxidative reactions due to the reintroduction of oxygenated meteoric waters. The extent of epidiagenetic oxidation depends largely upon the accessibility of the oxygenated waters which, in turn, is a function of the degree of porosity loss during anadiagenesis. Where uplift rapidly succeeds deposition, anadiagenesis may be bypassed with the sediments going directly from the reductive syndiagenesis phase to the oxidative epidiagenesis phase. In this situation, porosity loss would be minimal, allowing for the complete oxidation of the reduced sediment constituents.

2.7 Reducing Environments

Aqueous environments characterized by negative redox potentials are effectively reducing. Naturally occurring redox potentials are dependent upon the aqueous solubilities of oxidants and reductants under a given set of physico-chemical conditions. Yet, while thermodynamics defines their equilibrium saturation concentration, biochemical and geochemical processes determine their degree of saturation (23).

At surface temperatures many of the natural oxidizing and reducing agents exist as gases whose aqueous solubilities are determined by the intermolecular attractive forces between the gas molecules and water molecules. The presence of solutes, particularly electrolytes, gen-
Aqueous solubility of some biogeochemically-important gases as a function of temperature and salinity.

A) Equilibrium saturation concentration (moles O$_2$/litre H$_2$O) in pure water ($S = 0\%$) with respect to an atmosphere of 21% oxygen, 100% relative humidity and atmospheric pressure of 760 mm Hg (reference 42). Increase in salinity (i.e. $S = 40\%$) results in an overall decrease in oxygen solubility (reference 43) referred to as the 'salting-out effect'.

B) Equilibrium saturation concentration (moles gas/litre H$_2$O) of hydrogen sulphide and carbon dioxide in pure water with respect to an atmosphere of 100% H$_2$S and 100% CO$_2$ respectively at an atmospheric pressure of 760 mm Hg and 100% relative humidity (reference 42).
erally reduces the aqueous solubility of gases. This process, referred to as the 'salting-out effect', varies with the nature and concentration of electrolytes. With any given concentration of salts, the relative decrease in aqueous solubility is nearly the same for all gases (24).

Temperature affects a large number of properties of water. Since heat is generated in the solution process at low temperatures, the solubility of gases usually decreases with increasing temperature (below 100°C). The combined effect of temperature and salinity on the aqueous solubility of oxygen is outlined in figures 2A. By 90°C the equilibrium saturation concentration of oxygen is less than 15% of the saturation concentration at 10°C and decreases to near zero by 100°C.

In the surface environment, molecular oxygen is the ultimate oxidant reacting spontaneously with reduced chemical species, particularly hydrogen sulphide. Figure 2B illustrates the relative aqueous solubilities of carbon dioxide and hydrogen sulphide as a function of temperature alone. At any given temperature below 100°C, the solubility of these biogeochemically-important gases is nearly three orders of magnitude greater than that of oxygen. Under natural conditions, the half-life of hydrogen sulphide in the presence of molecular oxygen is on the order of thirty minutes (26) although it has been suggested that they may coexist for longer periods at
lower pH values (1).

A common mistake made by geologists is to confuse the terms reducing, anoxic (anoxygenic) and anaerobic. Anoxic implies extremely low ambient oxygen levels whereas anaerobic describes the physiological nature of the organisms adapted to life in the absence of molecular oxygen. Generally, it is through the generation of reduced metabolic by-products that anaerobic bacteria act to lower the environmental redox potentials and maintain the physiological requirement of anoxia.

Within the hydrosphere, oxygen depletion leading to anoxia is essentially a product of aerobic biodegradation of organic matter produced in the photic zone. Anoxia results when the natural biological demand for oxygen exceeds its supply. While the demand is directly related to primary photosynthetic surface productivity, oxygen supply throughout the water column is dependent upon the saturation concentration, degree of saturation, and circulation of the oxygenated water masses. These in turn are governed to a large extent by water temperature, salinity and global circulation patterns.

"Reducing environments are strictly syndiagenetic, evidently produced during or soon after deposition, with \( \text{Eh} \) ranging from 0 to -400 mV and pH slightly above or below 7..." (16, pg. 55). Bacterial oxidations and reductions in the bottom waters are natural processes by which biogeochemical balance in the modern oceans is system-
atically maintained between biologically essential elements removed from the hydrosphere during photosynthesis and those returned during respiration and fermentation. Most depositional environments today are oxidizing. Primary deposition of reduced sediments is a relatively uncommon phenomena restricted to stagnant abyssal water masses and some areas of geothermal activity. However, the interstitial waters of most sediments, particularly those rich in organic detritus, are reducing only a few centimetres below the surface. Anaerobic metabolic activity within confined pore waters frequently generates redox potentials into the range -400 to -600 mV.

Primary productivity in the modern ocean varies geographically over two orders of magnitude (27), with over eighty percent of the photosynthetic carbon fixation occurring in the nutrient limited open ocean and the remainder in fertile upwelling areas and shallow seas (28). Where the water column is relatively stable below highly fertile and productive surface waters, aerobic biodegradation of phytoplankton debris depletes the oxygen supply with depth resulting in the development of a mid-water oxygen minimum. The stratification of oceans in terms of dissolved oxygen levels has long been recognized. Mid-water anoxia has been reported in the northern Pacific Ocean (23) and in the northwestern Indian Ocean (29). Modern oceans would be completely anoxic with depth if not for aeration of the bottom waters by mixing with cold oxygenated polar
Deposition of reduced organic-rich sediments in the open ocean is restricted to areas along continental margins and the flanks of mid-oceanic ridges where surface water fertility is maintained by continuous upwelling and where the oxygen minimum impinges on the sediment/water interface. Depositional environments of this nature occur along the continental margins of North America, South America, India and Pakistan (29).

In restricted stratified bodies of water, complete anoxia is the straight-forward consequence of a slow convective overturn and ineffective abyssal circulation. Stabilization of the water column below a permanent thermocline (temperature), pycnocline (density) and/or halocline (salinity) inhibits the downward diffusion of oxygen, promoting the development of strongly reducing conditions. In the Black Sea, a well marked halocline and thermocline (around 100-200 metres depth) separates less saline, well oxygenated, seasonally stratified surface waters from saline, anoxic and reducing deep-water masses (26). The interface between the two zones is marked by a strong sulphide-rich chemocline (30) where the downward diffusion of oxygen is effectively buffered by the upward diffusion of biogenic hydrogen sulphide.

In the Black Sea, as in all marine environments, sulphate reduction by bacteria of the Desulfovibrio and Desulfotomaculum types is considered the principal source
of reduced sulphur species. Obligate anaerobic photosynthetic sulphur bacteria are the primary producers of organic matter in the Black Sea, developing maxima directly below the oxygen minimum within the photic zone. Sorokin (31, 32) located an active zone of chemosynthesis corresponding with the transition from oxygen-rich to sulphide-rich waters where the redoxcline has a maximum gradient between +50 to -100 mV. Within this zone, chemolithotrophic bacteria of the *Thiobacillus* type (33) catalyze the oxidation of hydrogen sulphide by molecular oxygen.

The sedimentary products of long-term stagnation of abyssal waters are sulphide-rich sapropels; sediments which have accumulated up to twenty percent organic matter by weight (14). In the Black Sea, seasonal mass mortality of photosynthetic bacteria arising from vertical mixing of cold oxygenated surface waters was suggested by Dickman and Artuz (30) as a mechanism to explain rapid deposition of iron sulphides and organic matter characteristic of varved sapropelic sediments.

In some areas of high geothermal gradient, particularly areas of dormant volcanism, hot-spring generation has resulted in the precipitation of thin, laterally-limited deposits of primary reduced chemical sediments. Geothermal activity is responsible for the majority of modern high temperature aqueous environments. At the surface, hot-springs span the pH range from 1 to 11 and
range in Eh from reducing to oxidizing depending upon the nature of dissolved constituents and the intensity of microbial activity (1).

Insufficient data exist to date upon which to generalize about the redox conditions of hot-spring effluents. In many cases, however, where Eh data are unavailable, redox conditions can be approximated through the physiology of the microbial population and the nature of the chemical precipitates. In the Yellowstone Park geyser fields, for example, the presence of anaerobic photosynthetic and sulphide-oxidizing chemolithotrophic bacteria are evidence for dissolved hydrogen sulphide and low primary redox potentials of the thermal waters (1). Likewise, the presence of chemolithotrophic sulphur bacteria and metal sulphide precipitates associated with hot-springs along the Galapagos rift zone (34) and the East Pacific Rise (35) attests to the relatively low redox potentials of the undiluted thermal waters.

2.8 Organic Diagenesis

Organic diagenesis can be defined as the microbiological and chemical processes involved in the natural alteration, mineralization and post-humous formation of organic matter during and after its incorporation into sediment or soil. Based upon the main driving force behind the diagenetic change, organic diagenesis can be divided into two main phases:
1) Early diagenesis, dominated by the processes of chemical and microbial degradation of complex biopolymers into simple monomers, the abiotic chemical reorganization of the monomers into complex geopolymers, and the interaction of the geopolymers with minerals and metal ions.

2) Late diagenesis, during which the metal-complexed or mineral-complexed geopolymers undergo slow thermal maturation.

In reality, the concept of early organic diagenesis cannot be easily separated from the concept of early chemical diagenesis of sediments as many of the microorganisms responsible for the alteration of organic matter are also responsible for the modification of the sediment pore water chemistry. In addition, the generation of reactive organic metabolic wastes can drastically alter the activities of numerous metals and initiate the precipitation of inorganic chemical species at levels well below their predicted values.

In the marine environment, Curtis (18) recognized distinctive depth-related diagenetic zones within organic-rich sediment burial sequences. Each zone is defined by a reaction or set of reactions that may be responsible for the triggering of other reactions in response to modifications in the pore water chemistry. Zone 1, termed the oxidation zone, extends effectively from the air/water interface to a few centimetres below the sediment surface. Within this zone the bulk of the organic matter produced
within the photic zone is extensively mineralized through aerobic bacterial activity. The depth of the water column strongly influences the flux of organic matter to the bottom sediments, but generally only a small fraction of the photosynthetically-derived organic matter reaches the sediments without extensive alteration. The organic matter which is eventually buried consists primarily of naturally occurring biopolymers such as proteins, polysaccharides, lipids and pigments.

Below the sediment/water interface, aerobic bacterial oxidations of organic matter result in the establishment of anoxic conditions within the pore waters. When molecular oxygen is depleted, oxidation of organic matter proceeds utilizing the next most efficient oxidant until either all oxidants or oxidizable organic matter are consumed (36). Zone II, extending several metres below the sediment surface, is termed the sulphate reduction zone. Within this zone, oxidation of organic matter is carried out by anaerobic bacteria through the concomitant reduction of Fe and Mn oxides, nitrate and sulphate. If burial rates are slow, sulphate reduction can result in the combustion of up to 35% of the primary organic matter trapped within the sediments (37).

Within zone III, termed the fermentation zone, organic matter is further degraded through bacterial fermentative processes. The upper limit of zone III is defined by the effective depth of sulphate penetration and may extend
to a kilometer below the surface corresponding with the lower limit of syndiagenesis.

Throughout zones I-III, biopolymers are partially of fully biodegraded to smaller molecules and often down to simple monomers (i.e. polysaccharides to sugars, lipids to fatty acids, proteins to amino acids). The resultant degradation products can persist within the sediments to become unique 'chemical fossils' or they can condense or polymerize into complex anionic heteropolycondensates (i.e. humic acids, fulvic acids, humins), commonly referred to as geopolymers (22). Disproportionation, reduction and cyclization reactions associated with the formation of geopolymers begin at the sediment/water interface and continue with depth into zone IV, termed the decarboxylation zone. Within this zone, the loss of functional groups from the organic matter results in the release of carbon dioxide, ammonia, hydrogen sulphide, nitrogen, phosphate and sulphur. Extending as far as 2.5 kilometres below the surface, this zone represents the transition into the high temperature late phase of organic diagenesis.

Non-synchronized microbial degradation processes result in the formation of intermediate organic compounds which may act as metal chelates, as well as co-ordinating to surfaces of associated mineral matter. The formation of metal-organic and mineral-organic complexes appears to fix and enhance the stability of the organic matter 'in situ' through intermolecular crosslinking and the neutral-
ization of reactive sites (22).

In oxygenated environments, intensive biodegradation of carbohydrates produces soluble humic and fulvic acid compounds which behave as negatively charged species due to the ionization of their acidic carboxyl and/or hydroxyl groups (38). Neutralization of the negative charge through mineral and/or metal ion interactions leads to their precipitation as biologically-inert metal humates and fulvates.

The formation of condensed metal-complexed geopolymers is directly related to the intensity and duration of aerobic biodegradation. On the average, in sediments under oxic waters, geopolymers of the humic and fulvic acid types comprise up to 40% of the organic matter and locally up to 70% (39).

Preservation of natural organic biopolymers and monomers is favoured by conditions of acid pH (1-6) and negative Eh within the depositional waters where biodegradation proceeds predominantly through anaerobic processes. In euxinic environments, such as the Black Sea, sugars and amino acids are largely uncomplexed with metals implying that a large proportion of the organic matter is potentially biodegradable. According to Degens and Mopper (11), the high degree of organic preservation reflects a low degree of aerobic biological activity. However, Richards (40) argued that the enhanced preservation may reflect an increase in the organic sedimentation rate due
to the restricted circulation and/or higher rates of primary productivity. Alternatively, preservation of biopolymers in reducing environments might be the consequence of some physiological effect exerting a greater control on biological activity than the chemistry of the surrounding waters and the supply of metabolites. Degens and Mopper (11) suggested that, under acidic and reducing conditions, heavy metal staining of the bacterial cell wall may account for the inhibited activity of anaerobic bacteria. Preservation of cell wall fragments as metal-organic complexes within 7000-year-old sediments from the Black Sea (41) provides compelling evidence for the natural staining of bacterial walls under reducing conditions.

The synthesis of the late phase of organic diagenesis involves the slow, abiotic maturation of organic matter leading to the production of compounds of increasing thermal stability. With increasing depth of burial, organic matter is moved into zone IV, termed the liquid hydrocarbon generation zone, where elevated temperatures (50-150°C) result in the breakdown of large geopolymers into smaller, more stable compounds. The major reactions characterizing zone V, as outlined by Brooks (22), are: 1) the disproportionation and redistribution of hydrogen atoms and the cracking of carbon-carbon bonds producing a lighter paraffinic hydrocarbon with increasing volatility and increasing hydrogen content (with methane
as the ultimate end product); and

2) the loss of hydrogen producing an aromatic condensate of decreasing hydrogen content and increasing carbon content (with graphite as the ultimate end product).

Below the zone of intensive bacterial activity, the diagenetic reactions are predominantly a function of temperature and time. Below 150°C, an increase in temperature by 10°C will halve the time required for thermal maturation (22). However, the rate of chemical change with temperature generally decreases as the maturation level increases to the point where increased depth of burial will produce only minor changes in the high-carbon residues. Above 150°C, organic metamorphism (zone VI) signals the end of diagenesis with the ultimate conversion of organic matter to methane and graphite.
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"Nature which governs the whole will soon change all things thou seest, and out of their substance will make other things, and again other things from the substance of them, in order that the world will be ever new."

Marcus Aurelius (121-180 AD)

CHAPTER 3

EARLY CRUSTAL DEVELOPMENT AND THE EVOLUTION OF MODERN-BIOGEOCHEMICAL CYCLES

3.1 Introduction

The previous chapter dealt with the broad aspect of biogeochemical element cycling in the hydrosphere, emphasizing the interrelationships between living organisms and their chemical environment. The projection of biogeochemical cycling through time depends entirely upon the extrapolation of processes observed in the modern environment. This in turn requires that basic assumptions be made on the physico-chemical conditions prevailing throughout past geologic eras. Biological evolution, in the broad sense, represents ecological specialization in response to environmental pressures. The regulation and maintenance of biogeochemical cycles in the hydrosphere by microbial metabolic processes implies that biogeochemical element cycling has undergone progressive changes with
time paralleling the evolution of biological systems (1). The development of aerobic respiration, oxygenic photosynthesis and the eukaryotic cell represent milestones in the early evolution of terrestrial life forms and, as such, must have had a profound influence on the evolution of biogeochemical element cycles. It follows that any model proposed for early prokaryotic and eukaryotic evolution, if it is to be acceptable, must complement models proposed for the early evolution of the lithosphere, atmosphere and hydrosphere. Conversely, any models proposed for the latter must be consistent with the progression of evolutionary events evidenced in the biochemical makeup of extant microorganisms.

Evolutionary models rely upon the accurate interpretation of biological, geological and paleontological evidence. Stemming from the early work on atmospheric evolution, a strong school of thought has developed which views the present oxygen-rich atmosphere as a consequence of evolution of oxygenic photosynthesis under globally reducing conditions. Simply stated by Schopf (2), "the mineral record tells when the change took place (reducing to oxidizing), the fossil record reveals the organisms responsible, and the distribution of biochemical capabilities among modern organisms puts the development in its proper context" (pg. 114). Proponents of this idea have not surprisingly emphasized particular lines of evidence over others (depending upon their disciplines and
predilections) creating a somewhat disjointed debate.

A necessary corollary to the concept of a reducing atmosphere would have to be that the early hydrosphere was even more reducing owing to the aqueous solubility and stability of reductants in the absence of molecular oxygen. The existence of abundant microbial marine life throughout the Precambrian is gradually gaining popular acceptance and, in addition, there is little evidence to suggest that the organisms living during the Precambrian did not interact with their chemical environment in a manner similar to the modern marine biomass. However, the concept of global reducing conditions places severe restrictions on the physiological complexity and metabolic diversity of the organisms inhabiting the early seas. If such was the case, all aspects of biogeochemical element cycling, diagenesis, chemical sedimentation, primary biomass productivity, etc., would have had to be radically different from the processes observed in the modern environment.

The test of any good theory lies in its ability to tie up previously unrelated facts. Recent biological/biochemical, geological/geochemical and micropaleontological/organic chemical data are inconsistent with currently accepted evolutionary models which advocate a biological origin for atmospheric oxygen. Furthermore, the evidence sheds doubt on the existence of a reducing
atmosphere as a prerequisite for early prokaryotic evolution. The following sections represent a synthesis of available evolutionary data, culminating with a simple scheme which views the evolution of biogeochemical element cycles as natural phenomena in response to global cooling.

3.2 Origin of the Lithosphere, Atmosphere and Hydrosphere

The period of approximately 4.5 By to 3.8 By, termed the Hadean era by Cloud (4), represents the time of origin of the Earth’s internal structure, outgassing of much of the atmosphere, local and transient sialic crustal formation, development of the hydrosphere, and the beginnings of life. Definition of the physico-chemical conditions that prevailed on the Earth’s surface in past geologic eras relies heavily upon the interpretation of ancient depositional environments. Unfortunately, nearly the first quarter of the Earth’s geologic history is absent from the sediment record and consequently the geologist is reduced to mere speculation based upon available models for the early planetary development. The purpose of this section is not to reiterate the details of each model but rather to outline only those aspects relevant to surface conditions on a protoEarth in order to establish a firm base for discussion in the following sections.

Recent advances in lunar petrology and cosmochemistry have lent broad support to a widely accepted theory that
views planetary bodies as coalesced dust-condensates of nebular gas. This theory, in turn, has given birth to two currently popular theories of Earth origin; the homogeneous accretion theory, and the inhomogeneous accretion theory. The homogeneous accretion theory, as summarized by Shaw (5), maintains that complete nebular condensation preceded accretion of small dust planetesimals to form a relatively chemically-homogenous protoEarth. During the following accretion, a major thermal event accompanying core formation resulted in the fractionation of liquids to form protocrustal material and to degas the protoEarth of much of its volatiles. In contrast, the inhomogeneous accretion theory, according to Walker (6), holds that the protoEarth formed initially from high-temperature, volatile-poor nebular condensates which accreted and subsequently differentiated into core and mantle. Upon cooling, the protoEarth aquired a 'veneer of volatile-rich, low-temperature condensates' that now comprise the upper mantle, crust, hydrosphere and atmosphere.

While it has yet to be determined whether the Earth is currently heating or cooling, it is difficult to envision surface conditions throughout the Hadean and early Archean eras as being the same as or cooler than the present. Heat sources in the early Earth system, regardless of which accretion model is most plausible, would have included gravitational collapse, continuing radioactivity
(ie. U, Th, K, etc.), extinct radioactivity (ie. $^{26}\text{Al}$) (5), as well as meteorite impacts and 'normal tectonic activity' (6). At present, the terrestrial heat flux is four orders of magnitude lower than the incident solar heat flux. Terrestrial heat flux in the Hadean and Archean eras must have been greater by virtue of the higher $^{235}\text{U}$ abundance alone (5).

A considerable body of geophysical and geochemical data concerning early crustal evolution favour an initial, early segregation of sialic material which, upon cooling, crystallized to form an Earth-girdling protocrust (5, 7) similar in mass and composition to the present crust (8). Higher total heat production in the Earth, arising in part from the concentration of radioactive elements in the crust and upper mantle, would have been reflected in steeper geothermal gradients and hot surface temperatures. As cooling progressed, vigorous mantle convection coalesced the thin global crust into a few supercontinents (7) and/or a number of small continental-type crustal nuclei (5).

It is now generally accepted that the Earth's primordial atmosphere was a secondary feature in a sense that it was not directly inherited from the solar nebula but formed as a product of high-temperature outgassing. Primordial anoxicogenic conditions have been postulated on astronomical (9), geochemical (10, 11) and biochemical (12-14) grounds, but little is known about the actual composition of the early atmosphere. Intrinsic to any
model of atmospheric development is the temperature and oxidation state of the upper mantle where the gases originated (6). According to the homogeneous accretion theory, the rudimentary atmosphere would have been hot, hydrogen-rich and highly reducing, consisting primarily of $H_2O$, $CO_2$, $N_2$ and $H_2$ with minor amounts of $CO$, $CH_4$, $S_2$, $H_2S$ and $NH_3$ (5, 7, 8). In variance, Walker (6) argued that by inhomogeneous accretion the primeval gases would have closely resembled modern volcanic gases in overall oxidation state, consisting primarily of $H_2O$ and $CO_2$ with only trace amounts of reduced gases.

Assuming that the salient features of the protoEarth system where high geothermal gradients, high terrestrial heat flux and globally hot surface temperatures, it follows that the early hydrosphere could not have developed until the Earth's surface had cooled sufficiently to allow extensive condensation of water vapour. Under conditions of high atmospheric pressure and high water vapour content, it is conceivable that the first ocean began to form at temperatures as high as 150°C. Continued condensation accompanying decreasing surface temperatures could have increased the volume of the primitive hydrosphere to near present day levels, gradually inundating the early protocrust to a mean depth of 2-3 kilometres (5, 7). The result would have been a hot (100°C) global ocean separating the lithosphere from the atmosphere, marked only by the occasional emergent volcanic
pile. To account for the oldest Archean sedimentary rocks, this point must have been reached or even surpassed during the Hadean era, pre 3.8 By BP.

Both the Archean (approx. 3.8 to 2.6 By BP) and the Proterozoic (approx. 2.6 to 0.7 By BP) eras appear to have witnessed the process of continental plate thickening and decreasing geothermal gradients in response to decreasing heat production in the Earth. Extensive geologic data indicate that the Archean-Proterozoic boundary marks a transition in global tectonic regime from predominantly volcanogenic to stable cratonic. A comprehensive review of global tectonics is impossible here, but due to the impact of tectonic change on biological evolution and biogeochemical element cycling, some discussion cannot be avoided. Shaw (5) contended that progressive sialic volcanism, deep partial melting and greenstone belt suturing led to an increase in 'continental freeboard' favouring the development of incipient ocean basins and continental land masses characteristic of the Proterozoic era. Based upon earlier isostatic models and geophysical data, Hargraves (7) postulated a general emergence model which contended that since sialic crustal thickness is controlled by the depth of the 750°C granite-minimum-melting isotherm, continental land masses could not have breached the surface of a primitive global ocean, through isostatic balance alone, until global geothermal gradients had decreased sufficiently to
allow extensive crystallization at depth. He maintained that general crustal emergence could not have occurred until late in the Proterozoic and in all likelihood did not occur until the onset of the Cambrian. Unfortunately, neat as his model might be, general emergence as such is not collaborated by geologic data as emergence is known to have occurred locally throughout both the Archean and Proterozoic eras. However, the basic concept of emergence leading to the formation of extensive shallow-water shelf environments as a function of decreasing geothermal gradients, when coupled with the concept of concomitant decreasing global surface temperatures, holds the solution to a number of problems encountered in evolutionary studies.

3.3 Oxygen Isotopes and Paleotemperatures

The currently popular models for Earth origin imply that as the early Earth system cooled from perhaps 1200°C or higher (15) to more biologically congenial surface temperatures, life evolved from a purely physico-chemical system. The oldest 'bona fide' record of life is represented by the 3.5 By-old stromatolites of the Warrawoona Group at North Pole, Western Australia (16, 17), although the presence of carbonaceous spheroids in the 3.8 By-old metasediments of the Isua series, Greenland (18, 19) supports the view that life evolved prior to their deposition. Brock (20) concluded that tempera-
ture is one of the most important environmental factors controlling the activity and evolution of microorganisms, particularly in view of the effects temperature has on the stability of certain biochemical constituents. Modern microorganisms are unable to survive temperatures much in excess of 100°C and there is no cause to believe that organisms during the Archean were any more tolerant of high temperature. Hence, it is safe to assume that the evolution of the first organisms and incipient biogeochemical element cycles could not have taken place until hydrospheric temperatures had cooled to 100°C or lower. It is appropriate, therefore, to discuss the evidence for global surface cooling prior to discussing biological and geological evolution.

Isotope fractionation during any chemical process is most apparent in the low-temperature environment. Within the hydrosphere and biosphere, significant fractionation occurs between isotopes of carbon, oxygen, sulphur and hydrogen. The oxygen isotopic composition of natural waters varies considerably and, in general, marine waters are enriched in the heavier isotope over fresh water. Likewise, when cherts and calcium carbonates are precipitated from marine waters, they are preferentially enriched in the heavier isotope. The extent of fractionation frequently demonstrates an approach to equilibrium and, if equilibrium is achieved, the isotopic ratio is a function of temperature. As
temperature increases, the amount of the heavier isotope incorporated into the crystalline phase decreases or, in other words, the minerals become isotopically lighter or enriched in $^{16}$O. To summarize, the isotopic composition of cherts and carbonates is a function of both the initial isotopic composition and the temperature of the depositional waters.

In the past, the traditional approach to interpreting stable oxygen isotopic ratios in ancient sediments has been to assume a temperature approximately equal to that of the modern marine environment and, based upon that temperature, calculating the isotopic composition of the ancient depositional waters. Degens and Epstein (21) demonstrated a progressive decrease in the $^{18}$O values in both cherts and carbonates from the Cretaceous to the Cambrian. Schidlowski et al. (22) traced this trend back into the Archean. The decreasing isotopic ratios with increasing time has led a number of workers, such as Perry (23), to conclude that the isotopic composition of the oceans has somehow changed with time; the early oceans being enriched in the lighter oxygen isotope relative to the present.

While the deficiency in $^{18}$O in progressively older cherts and carbonates could be the result of partial reequilibration with meteoric waters and/or changes in the initial isotopic composition of sea water, in view of the popular theories for Earth origin, it is more likely
that the observed trends reflect higher ocean temperatures in the past. By reinterpreting the isotopic compositions of chert as a function of equilibration with seawater of similar present day isotopic composition, Knauth and Epstein (24) calculated the corresponding temperature at the time of crystallization. While their data showed variations by as much as 20°C within individual geologic periods, there was an overall rise in temperature from the Tertiary to the Proterozoic. Subsequently, higher temperatures averaging approximately 70°C were claimed by Knauth and Lowe (25) for the Archean Onverwacht cherts (3.4 By BP). In agreement with the latter trend, Oehler and Smith (26) reported 16O enrichments in carbonates from the 3.8 By-old Isua metasediments and tentatively suggested higher ocean temperatures. Perry et al. (27) observed similar enrichments in the cherts of the Isua series which they interpreted as close to the primary equilibrium fractionation values at the time of crystallization. Based upon this assumption, Knauth and Lowe (25) estimated water temperatures for this time at around 80°C. For reference purposes, a summary plot of temperature estimates as a function of geologic time is presented in figure 3.

It must be acknowledged that the temperatures indicated by stable isotopic ratios represent the temperatures of the chemical precipitates at the time of their original crystallization. Since chert formation is either
Figure 3. Compilation of paleotemperature estimates for depositional waters as a function of time (billion years before present) based upon oxygen isotopic ratios from sedimentary cherts and carbonates (after Knauth and Lowe (25)).
Penecontemporaneous or early diagenetic, the temperature of chert crystallization, for the most part, approximates the temperature of shallow burial. Generally, the sediment temperature in the upper few metres equals that of the overlying bottom waters. It follows that temperatures under conditions of early burial are equal to or only slightly higher than the coexisting depositional waters.

Inasmuch as the 2 By-old Gunflint cherts are isotopically heavier than the 1.3 By-old cherts from the Beck Spring Dolomite, and isotopically lighter than the 1.2 By-old Arizona cherts, it is apparent that the Gunflint cherts do not follow the general trend in which progressively younger cherts are progressively enriched in the heavier oxygen isotope (24). Alternatively, the isotopically heavier Beck Spring cherts may present the anomaly and not the Gunflint cherts. Temperature estimates for the Precambrian range from 20-33°C for the Arizona cherts, about 38°C for the Gunflint cherts, and about 52°C for the Beck Spring (Death Valley) cherts. The discrepancy between the temperatures of the Gunflint and Beck Spring cherts was explained by Knauth and Epstein in terms of past climatic fluctuations. However, of all the cherts represented in their study, only the Gunflint cherts appear to be predominantly the product of primary silica precipitation. The others apparently formed as a consequence of early diagenetic silicification.
of carbonate sediments within a few metres of the surface. This would explain why, as noted by Knauth and Epstein, the water content of the Gunflint cherts if four times greater than that of the silicified Beck Spring dolomites. It follows that, while climatic variations cannot be discounted, the higher temperature of chert crystallization of the younger Beck Spring cherts may well reflect the higher temperatures associated with preconsolidation burial which, in turn, would be defined by the depth at which silicification occurred. For this reason, in figure 3, the temperature of the Beck Spring Dolomite was omitted when the cooling trend was drawn.

3.4 Precambrian Atmospheric Evolution

The study of Precambrian atmospheric evolution presents an enigma. While it is generally accepted that the ancient atmosphere-hydrosphere system was anoxygenic and reducing well into the Proterozoic era, extensive direct and indirect evidence exists for primary oxidation of sediments and volcanics throughout the Archean and Proterozoic eras (28, 29). There is no way of knowing the exact composition of the early atmosphere and no factual evidence exists to date to support the view that it was ever strongly reducing. However, regardless of its oxidation state, it is accepted that the primeval atmosphere was initially devoid of free oxygen and that oxygen levels increased with time to the present level of roughly 21%.
The principal questions facing the evolutionary student are when and by what mechanism did the atmosphere become enriched in free oxygen. Only two principal sources of atmospheric oxygen are considered plausible: abiotic photodissociation in the upper atmosphere and biotic photosynthesis within the hydrosphere.

While there is little disagreement that photodissociation of water vapour produces free oxygen, its contribution to the early terrestrial oxygen budget is a matter of considerable debate. Through the application of various self-regulating mechanisms based upon a number of assumptions involving gravitational field effects, solar constants, water vapour mixing ratios and oxygen sinks, estimates for the photochemical production of oxygen prior to the evolution of photosynthesis range from the classical 'Urey level' of 0.001% PAL (present atmospheric level) (9, 14) to 25% PAL or higher (31).

The assumption of an early anoxicogenic, reducing atmosphere-hydrosphere system is based largely upon the models for atmosphere origin, the antiquity of anaerobic metabolism in prokaryotes, the apparent retarded evolution of eukaryotes, the mobilization of ferrous ions in the hydrosphere, and the generally reduced nature of Archean and Proterozoic sediments. While it is conceded that photodissociation of water vapour made a minor contribution to the terrestrial oxygen budget, most workers favour a biotic model that views the evolution of an oxygen-rich
atmosphere as a corollary to the evolution of oxygen-producing photosynthesis within an anoxygenic hydrosphere. The following sections assemble arguments against a reducing, anoxic atmosphere throughout the Archean and against a photosynthetic origin for oxygen, in favour of an early oxidizing atmosphere derived ultimately through abiotic photodissociation of atmospheric water vapour.

3.5 Biological Evolution: Environmental Considerations

Perhaps the greatest task facing every student of evolution today is the separation of factual data from mere conjecture which, over a period of years, has been accepted 'a priori' as fact. Traditionally, biologists have attempted to unravel the evolutionary scheme for early prokaryotic and eukaryotic development through studies on the antiquity of metabolic and biochemical pathways in extant organisms. The limitations of this approach were readily apparent, however, in that only certain groups of organisms could be compared directly resulting figuratively in a phylogenetic evolutionary tree consisting of a number of separate branches lacking any common stem or trunk. More recently, an entirely new body of evolutionary evidence has come from studies on the three-dimensional structure of a variety of proteins and nucleic acids believed to have developed early in the history of life and to have evolved somewhat systematically with time. Through the characterization of the
constituent amino acid and nucleotide sequences within these 'biochemical fossils', biologists have been able to define genetic relationships between a number of physiologically unrelated organisms.

In the past, the successful adaptation of organisms to progressively more extreme environments has stimulated consideration of the evolutionary events in teleological terms whereby the ability to adapt has been regarded as developing towards a specific goal. The proponents of teleological theories have been unable to find any natural mechanism to account for their postulated finalism and, in fact, the possibility that any such mechanism could have ever existed has been ruled out by the findings of molecular biology.

Biological evolution, in the broad sense, represents ecological specialization in response to environmental pressures whereby genetic variants, arising through random mutations, are selected largely by the process of survival. This, in turn, results in speculation and the propagation of the beneficial trait. A taxonomic species can be defined as a given stage of biological evolution at which actually or potentially interbreeding life forms become segregated by environmental, geographical, morphological, behavioral or physiological differences into two or more forms incapable of interbreeding (31). With any given environment, as a biota evolves from a single taxonomic stem over time, it would be expected
that ecological specialization would inevitably reduce the population size and area covered by an average species as the required structural habitats become more restrictive and the metabolic requirements more selective (32). Collectively, however, on a global scale, ecological specialization within a single taxonomic line should expand the area covered by the group as a whole as new environments are progressively opened to colonization.

It is safe to assume that, in a hot, primitive atmosphere-hydrosphere system, abiotic synthesis of organic compounds would continue undisturbed only until the physico-chemical conditions favouring abiotic synthesis changed or until the first life forms developed. It is generally accepted by students of evolution that life arose as a chemical response to changing physico-chemical conditions in the primitive hydrosphere. Unfortunately, with no actual knowledge of the conditions required for abiotic synthesis, or the nature of the first life forms, it is impossible to speculate as to when the former ceased and the latter began. From laboratory studies, it is certain that abiotic synthesis required low ambient oxygen levels or complete anoxia.

The location of the residence of the first life forms presents a formidable problem to the biologist and chemist alike. The presence of free water is a fundamental prerequisite to life so it is without question
that life arose within the hydrosphere. Stemming from the early work on abiotic synthesis of organic compounds, chemical models for life origin favour the evolution of the primitive organisms from a primordial 'organic soup' within a shallow-water environment (i.e. tidal pool, lagoon, shallow sea). However, shallow-water environments throughout the Hadean and Archean eras were probably relatively rare and highly transitory, primarily associated with areas of active volcanism. It would be expected that those that did exist were subject to rapid and perhaps extreme fluctuations in aqueous chemistry as well as frequent disturbances associated with tidal and storm activity. A more plausible idea would be that early life forms resided in a somewhat static environment, characterized by relatively long-term physical, chemical and thermal stability, until they evolved the ability to tolerate some degree of physiological stress. Unconsolidated deep-water sediments provide such a habitat where, as suggested by Cairns-Smith (34), the unique properties of clay minerals might have played a critical role in the accumulation and polymerization of organic compounds, the concentration of phosphate, and the catalysis of primitive biochemical reactions.

"Life as a whole is selectively adapted to growth in a common environment" (20). The study of physiological changes which made it possible for microorganisms to adapt to various environments provides some of the clea-
est insight into early prokaryotic evolution. As previously discussed, the global study of Baas Becking et al. (35) demonstrated that virtually all groups of extant microorganisms can be characterized in terms of the limited range in Eh and pH of their environment. Figure 4 represents a composite of the approximate 'areas' of Eh and pH for some biogeochemically important groups of prokaryotes together with a number of common aqueous marine environments.

Taking all of the biological and biochemical evolutionary evidence together, it is hard to escape the conclusion that early life forms arose and developed to a large degree under general conditions of anoxia and low redox potentials. The earliest bacteria to leave modern descendants were probably anaerobic fermentative heterotrophs and/or chemolithotrophic methanogens. The fact that methanogens have such a limited environmental distribution (stippled area, fig. 4) suggests that there was some physico-chemical limitation which was impossible to overcome through further evolutionary change. Assuming that life arose within a common environment, it may be suggested that the distribution of methanogens reflects the conditions of that environment in terms of Eh and pH.

To date, no samples of 'bona fide' Precambrian seawater have been found and, consequently, the actual composition of the early seawater is unknown. The gen-
Figure 4. Compilation of approximate 'areas' of Eh and pH for some biogeochemically-important groups of prokaryotes and natural marine environments (refer to figure 1) showing the limited distribution of primitive methanogenic bacteria (stippled area). The shaded area depicts the range of Eh and pH common to all groups of organisms and natural marine environments. Arrows indicate the direction of ecological diversification in terms of Eh and pH as a function of progressive prokaryotic evolution (data from Baas Becking et al. (35)).
eral consensus, however, is that the composition of seawater has remained relatively constant throughout geologic time (36, 37). Our knowledge of the early seas is based largely upon our understanding of the chemical reactions involved in the deposition and diagenesis of chemical sediments found within Precambrian sedimentary sequences and upon inferences drawn from postulated chemical interactions in an early Earth system. Of particular interest is the computer simulation study by Lafon and Mackenzie (38). Their calculations of steady-state chemical interaction in a primeval hydrosphere yielded a seawater composition close to that of the modern oceans, with a pH of 7.7, and supports the view that seawater pH has remained at or slightly below the present value of 8.3 throughout much of the Precambrian.

From figure 4, it is apparent that modern open ocean sediments, marginal marine sediments and deep-marine waters provide a common environment conducive to all groups of anaerobic bacteria represented in the study (shaded area). Assuming that the interpretation of the antiquity of fermentation and methanogenesis is correct, and that the composition of seawater has remained relatively constant through time, the distribution of prokaryotes in figure 4 supports evolutionary evidence for ecological adaptation from conditions of relatively low Eh and neutral to slightly alkaline pH to conditions of high Eh and strongly acidic to basic pH. More important,
however, the area covered by each group reflects the degree of ecological expansion associated with progressive evolutionary physiological change within each group as a whole.

3.6 Biological Evolution: Energy Considerations

The living biosphere represents a complex energy cycle whereby incident solar radiation is converted to chemical bond energy and passed through the various trophic levels in the form of complex organic molecules to be ultimately released as heat or stored in sediments as potential fossil fuels. The biosphere itself is comprised primarily of water and low-entropy macromolecules such as proteins, nucleic acids, polysaccharides and lipids. These in turn consist primarily of the elements C, O, N, H, P and S. The elements C, N, and S share the common property of having an eight electron difference between the most oxidized and most reduced species. However, there are significant differences in the free energies obtained from the oxidation of the most reduced species of each. This phenomenon results in a marked decrease in 'reducing potential' through the series $\text{H}_2$, $\text{CH}_4$, $\text{H}_2\text{S}$, $\text{NH}_3$ to $\text{H}_2\text{O}$, and increase in 'oxidizing potential' through the series $\text{CO}_2$, $\text{SO}_4^{-}$, $\text{NO}_3^{-}$ to $\text{O}_2$ (39). It is from this unique feature that stems much of the physiological complexity and metabolic diversity characterizing the modern biosphere.
Living organisms share a number of common characteristics which, collectively, come close to a definition of life. All possess both the requirement and the capability to extract free energy from their environment and to expend this energy efficiently in the assimilation of essential elements, the synthesis of low-entropy molecules, and the excretion of non-essential elements, while maintaining both internal and external homeostasis. More importantly, however, all possess the capability to pass on these attributes to successive generations and, with time, to modify them to accommodate environmental stress.

For the most part, there are only two sources of energy available to an organism: incident solar energy and chemical bond energy. Likewise, there are only two principal carbon sources: organic compounds and carbon dioxide. While this at first appears to be an understatement, it is fundamental, particularly to the concept of parallel evolution, to the understanding of why and to a lesser extent, how prokaryotes and eukaryotes evolved with time, for it is the nature and efficiency of utilization of both the energy and carbon substrate which, in a broad sense, defines microbial metabolism.

Microorganisms, in general, act only to catalyze redox reactions which, although thermodynamically favourable, are kinetically slow. The amount of cellular matter that can be synthesized (hence the degree of metabolic and structural sophistication of an organism)
is directly related to the energy yield of the redox reaction which, in turn, is a direct function of the difference in standard redox potentials of the primary electron donors and acceptors (39). In other words, more widely separated reactants (i.e. $C_{org}/O_2$) release more energy per mole than reactants with similar redox potentials (i.e. $C_{org}/C_{org}$). The redox potential ($E_h$) of an environment is determined by the relative concentration and standard redox potential of its chemical constituents. It follows that organisms maintaining homeostasis with their external environment are restricted in their choice of primary electron donors and acceptors. From the viewpoint of competition and survival, it is only advantageous for an organism to utilize the highest energy-yielding couple made available.

Efficient energy transfer and storage is a prerequisite to a successful metabolic pathway. Adenosine triphosphate (ATP), synthesized through either substrate-level or electron-transport (oxidative) phosphorylations of adenosine diphosphate (ADP), acts as the principal energy-coupling and storage unit in nearly all organisms. Not only does this provide compelling evidence for the antiquity of ATP-based metabolism, but it enables early microbial evolution to be reduced, in part, to the evolution of metabolic pathways (i.e. fermentation, respiration, photosynthesis, etc.) or, more basically, to the evolution of chemical energy transfer systems.
Substrate-level phosphorylations of ADP typify the low energy-yielding redox reactions carried out by fermentative organisms whereby part of the organic substrate is oxidized at the expense of part of the substrate being further reduced. The energy yield of fermentation is therefore defined by the difference in redox potentials between the electron-donating and accepting couplets. Although generally relatively energy-inefficient, fermentative organisms (particularly bacteria) are highly diversified owing to their versatility in utilizing a wide range of organic compounds.

Electron transport (oxidative) phosphorylations of ADP typify both respiration and photosynthesis. The former involves the generation of reduced pyridine nucleotides through the tricarboxylic acid (TCA or Krebs) cycle at the expense of oxidation of low molecular-weight molecules. From the reduced electron carriers (NADH), the electrons pass through a flavoprotein and cytochrome chain to produce ATP.

The decomposition of low-entropy macromolecules by living organisms generally requires two separate steps; digestion and respiration. Digestion involves the cleavage of macromolecules into their smaller constituent molecules usually through hydrolytic reactions catalyzed by enzymes termed hydrolases. This, in turn, is followed by oxidation (respiration) of the smaller molecules and the extraction of chemical bond energy.
In nature, the microorganisms which are responsible for the initial hydrolytic attack on complex carbon compounds are principally those which oxidize its low molecular-weight derivatives. Their diversity and complexity lie both in the range of hydrolases they possess and in the range of oxidants they can utilize rather than in the actual chemical energy transfer system itself.

Studies have shown that, in the upper sediment column in marine sediments overlain by oxygenated waters, decomposition of photosynthetically-derived organic matter proceeds through a number of depth-related trophic levels (40, 41). Oxidants are consumed with depth by chemotrophic bacteria in order of decreasing energy yield per mole of organic carbon through the series \( \text{O}_2 \), \( \text{NO}_3^- \), Fe oxides, \( \text{SO}_4^{2-} \) to \( \text{CO}_2 \). The utilization of available oxidants, in turn, reflects the physiology of the bacterial population comprising each successive trophic level, from obligate aerobic, strict aerobic and facultative anaerobic, strict anaerobic through to obligate anaerobic.

Metabolic activity in organic-rich marine sediments is used to define depth-related zones of early diagenesis: oxidation, sulphate reduction, and fermentation (methanogenesis) zones (42). The lower limits of the oxidation and sulphate reduction zones are determined largely by the maximum depth of penetration of \( \text{O}_2 (\text{NO}_3^-) \) and \( \text{SO}_4^{2-} \) respectively which, in turn, is controlled by their relative
rates of supply and consumption. Decreasing Eh with depth, characteristic of early diagenesis, is the direct product of the generation of reductants (through the series \( \text{NH}_3 \) to \( \text{H}_2 \)) concomitant with the oxidation of sedimentary organic matter.

Assuming that life originated in an environment typified by low redox potentials, and that the antiquity of fermentation and methanogenesis is essentially correct, the distribution of heterotrophic bacteria in modern open ocean sediments supports the accepted view of ecological adaptation from conditions of low Eh to high Eh during early prokaryotic evolution. More importantly, however, each successive trophic level from fermentation upwards reflects the progressively higher degree of physiological complexity made possible by the availability of higher energy-yielding oxidants associated with the gradual oxidation of the hydrosphere over three billion years ago.

3.7 Molecular-Based Phylogenetic Classifications:

A Cautionary Note

Over the past two decades, comparative analysis of constituent amino acids and nucleotides in ferredoxins, c-type cytochromes and ribosomal RNAs have been used to explore early prokaryotic and eukaryotic development. Molecular-based phylogenetic classifications are essential to the understanding of evolutionary events in
microbial physiology in that they more clearly define which groups of organisms are best compared on the biochemical and structural levels. Unfortunately, they are not without their limitations for the interpretation of variability within sequence data is frequently biased by earlier biochemical evolutionary evidence which itself is open to reinterpretation.

Ferredoxins and c-type cytochromes are small iron-containing proteins which participate as electron-transporting agents in anaerobic and aerobic, phototrophic and chemotrophic oxidative phosphorylations of adenosine diphosphate. The application of both ferredoxins and cytochromes to evolutionary studies relies upon the basic assumption that through mutations in the bases of DNA of organisms the sequencing of the amino acids in its proteins have through time undergone change at a fairly fixed rate. It follows that the greater the difference between like-proteins from two different organisms, the earlier the time of their divergence from a common ancestor. Both types of proteins are found in a broad spectrum of organisms, favouring their use in evolutionary studies, but are not exactly functionally homologous in all organisms in that cytochromes and ferredoxins of the photosynthetic/faculative respiratory centres participate to some degree differently from those of obligate respiratory centres. This is most apparent in eukaryotic organisms where the individual centres are
confined within separate membrane-bound organelles. Since both obligate anaerobic photosynthesis and respiration have aerobic analogues, it would not be unexpected that like proteins of each energy centre underwent structural change with time separately from each other even within a single taxonomic line. Hence the tentative suggestion is that prokaryotic classifications constructed from amino acid sequence data be viewed with discretion for only in eukaryotes does direct comparison appear warranted.

Ribosomal RNA's, in contrast to proteins, exhibit constancy of function, are universally distributed and, more importantly, are independent of developmental changes in the chemical energy transfer system of organisms. Recently, Fox et al. (43) proposed a general outline of prokaryotic phylogeny based upon nucleotide sequences in 16S rRNA in which they defined three distinct kingdoms emerging from a common ancestral life form. The first kingdom, containing methanogenic, halophilic and thermoacidophilic bacteria, is referred to as archaebacteria (ancient bacteria). The second and best characterized kingdom containing the Gram-negative and Gram-positive bacteria, is collectively referred to as the eubacteria (true bacteria). The last kingdom, herein termed the protoeukaryota, is defined solely upon the unique character of the eukaryota cytoplasmic 18S rRNA.
The tripartite division of extant organisms proposed by Fox and his colleagues is incompatible with conventional phylogenies and accepted models for early biological evolution in that it advocates an ancient, separate lineage for eukaryotic host cells. The assumptions, conclusions and implications of their model and other molecular-based phylogenies, as they apply to the evolution of the hydrosphere and biosphere, are discussed in the following sections in conjunction with biochemical, biological and paleontological evolutionary evidence. It is impossible to adequately review 'evolution' as a whole. However, since all indications are that sulphur played a critical role in the development of early chemical energy transfer systems (hence metabolism and life), the review is confined largely to the evolution of the biogeochemical sulphur and oxygen cycles.

3.8 Fermentation, Anaerobic Respiration and Photosynthesis: Evolution of the Organic Carbon/Sulphur Cycles

The progress of comparative biochemistry has revealed that the more selective and chemically simpler the carbon source, the more numerous are the enzymes and more complex are the metabolic pathways possessed by an organism (31). Consequently, it is customary to imagine the first primitive organisms as anaerobic fermentative heterotrophs which depended for their existence on free energy
from whatever abiotic organic substrate was available (2, 31, 33, 34). Indirect evidence supporting this hypothesis lies both in the antiquity and low redox potential of ferredoxins (close to that of hydrogen gas) (44). Ferredoxins are active both in substrate-level and oxidative phosphorylations whereas cytochromes are confined to oxidative phosphorylations and are absent in fermentative bacteria. Substrate-level phosphorylations, in general, result in the generation of a variety of reduced organic end-products which cannot be remetabolized by fermentation. It follows that, regardless of the residence of the first organisms, the gradual depletion of an abiotic foodstock would have proven sufficient environmental pressure to encourage alternative energy and carbon sources; the decomposition products of fermentation and, ultimately, photoassimilation of carbon dioxide.

Anaerobic photosynthesis, involving the photolysis of inorganic and organic hydrogen donors in the reduction of carbon dioxide, offers a much higher energy yield than fermentation and frees the organism from a substrate existence. Photosynthetic bacteria, therefore, are generally considered to have evolved early from the ancestral fermentators in response to dwindling foodstocks. Anaerobic photophosphorylations are exclusively cyclic, mediated through ferredoxins, cytochromes, quinones and some form of bacterial chlorophyll.
the reductive assimilation of carbon dioxide is similar to energy-yielding redox reactions, it is not coupled to ATP synthesis and hence constitutes an energy drain to the organism (39).

At present, four groups of photosynthetic bacteria are known: green sulphur, green filamentous, purple sulphur, and purple non-sulphur. All are Gram-negative, employ the same reductive pentose phosphate (Calvin) cycle for photoassimilation of carbon dioxide, and are capable of utilizing hydrogen sulphide to obtain the necessary reducing power, emitting sulphate, thiosulphate and sulphur as by-products. Both the purple non-sulphur and green filamentous bacteria are capable of facultative aerobic respiration in the dark. Species of the purple sulphur and green sulphur bacteria (i.e. Chromatium and Chlorobium respectively) are capable of facultative dark fermentation. None of the photosynthetic bacteria appear to respire anaerobically.

It has yet to be substantiated which group of the photosynthetic bacteria is oldest. The sulphur bacteria may have evolved first but equally likely is the prior emergence of the purple non-sulphur bacteria utilizing the end products of methanogenesis and fermentation. Certainly on energy constraints, the photolysis of hydrogen and methane should be less thermodynamically costly than hydrogen sulphide, requiring a lower initial energy input. However, for the purpose of discussion in the
following sections, the photosynthetic bacteria are largely treated as a single group in light of their ability to utilize reduced sulphur species.

It is tempting to envision a primitive, anaerobic organic carbon cycle based solely upon fermentation and photosynthesis. On ecological grounds, however, this concept would be difficult to resolve. While the location of residence of the first phototrophs is unknown, environmental factors favouring an open ocean habitat would have included the possibility for maximum occupation of the photic zone together with wide current-borne dispersion into areas of replenished nutrient supply (45). If the early fermentative bacteria were not originally bottom dwellers, the transient nature of the early phototrophs would have promoted a benthic habitat where the accumulation of particulate and absorbed organic matter would have guaranteed a relatively concentrated food supply. As in the present, primary photosynthetic surface productivity in the past must have depended upon the efficient recycling of biolimiting nutrients (i.e., phosphorous). In the absence of large-scale chemical weathering, the tentative suggestion is that fermentation alone would have proven inadequate to sustain a 'quasi' steady-state organic carbon cycle for any appreciable length of time.

Like photosynthesis, respiratory oxidations of organic matter have the potential to extract higher energy
yields than fermentation but, unlike photosynthesis, do not free the organism from a pre-existing organic substrate. Sulphate reducing bacteria (hence anaerobic respiration) provide the necessary link in an integrated, somewhat self-contained, organic carbon/sulphur cycle. Surprisingly little is known about the energy transfer system in sulphate reducing bacteria, although it is known to involve cytochromes, ferredoxins, quinones and flavins (46) and, as such, appears to be functionally and chemically homologous to the aerobic tricarboxylic acid cycle. Sulphate reducing bacteria are capable of oxidizing only a limited range of simple organic compounds which are predominantly the end products of fermentation (31, 39, 47, 48). Sulphate reduction, in turn, generates as by-products the basic metabolites required by photosynthetic bacteria.

The development of anaerobic respiration is critical to the understanding of early atmospheric-hydrospheric evolution. To date, no absolute genetic link has been substantiated between sulphate reducing and photosynthetic bacteria. Most proponents of an anoxic and reducing early Earth scenario envision the sulphate reducers as having emerged late in response to sulphate-releasing photosynthesis (2, 31, 39, 44, 46, 49, 50). To suggest otherwise is to advocate an alternative source for sulphate, thiosulphate and/or sulphur: abiotic oxidation under an oxygenated atmosphere.
Phylogenetic evolutionary trees constructed from amino acid and nucleotide sequence data serve to demonstrate the high degree of uncertainty associated with the postulated late emergence of sulphate reducing bacteria. Dickerson (49) assigned sulphate reducers an early separate lineage on the basis of marked dissimilarities in sequence as well as function of the Desulfovibrio cytochrom C₅₅₃ compared with cytochromes of the photosynthetic bacteria. Yet, by convention, he promoted their late emergence as a response to sulphate-releasing photosynthesis. The ferredoxin-based evolutionary tree of Schwartz and Dayhoff (50) also depicts a lineage for sulphate reducers separate from the photosynthetic bacteria and somehow related to the late emergence of the aerobic Gram-positive Bacillus bacterium. They admit, however, that the 'divergence of Desulfovibrio and Bacillus is unclear', arbitrary, and interpreted largely upon the functional similarities of both ferredoxins in respiration.

Eubacteria are subdivided on the basis of structural and functional properties of the cell wall into Gram-negative and Gram-positive bacteria. The existence of obligate fermentative bacteria in each group provides strong support for their early divergence and separate, somewhat parallel evolution as envisioned by Fox and his co-workers (43). Most common bacteria, including the sulphate reducing and photosynthetic bacteria, are Gram-
negative and, as such, are probably distantly related. A large number of photosynthetic bacteria are either facultative fermentators or aerobic respirers. Hence, it is not surprising that in the evolutionary scheme proposed by Fox et al. phototrophic lines of descent are intermixed with chemoheterotrophic organisms. Of relevance to this section, however, is the fact that Gram-negative obligate fermentative bacteria are poorly represented in their investigation resulting in the forced correlation of sulphate reducers to anaerobic photosynthetic and aerobic heterotrophic bacteria. In spite of this, as in the latter protein investigations, Desulfovibrio demonstrated a marked early divergence from the ancestral photosynthetic purple photosynthetic bacteria, and defined a lineage distinct from both anaerobic photosynthetic and aerobic heterotrophic bacteria. Again, apparently by convention, Fox et al. have depicted the sulphate reducers as having emerged after the evolution of photosynthesis.

On the premise that it is easier to lose a biochemical system through evolution than to develop the myriad of complex molecules required by the new one, the transformation from respiration to photosynthesis, or vice versa, must have required strong environmental motivation; either the depletion of essential metabolites and/or the potential for higher energy yields. The generally accepted late emergence of sulphate reduction is
difficult to envision in terms of basic bacterial biochemistry, energetics and ecology. First, it requires that fermentative bacteria take a giant step from simple substrate-level phosphorylations to cyclic-oxidative phosphorylations involving the creation of a cytochrome chain and photosensitive pigments (chlorophyll). It also requires that the early 'planktonic' phototrophs develop an alternate chemical energy transfer system (tricarboxylic acid cycle) and abandon an efficient energy source and inexhaustible carbon source to form a syntrophic relationship with benthic fermentative organisms. More importantly, however, it requires that the photosynthetic bacteria accommodate a significant decrease in energy input. Common oxidations of fermentation end products using sulphate as a terminal electron acceptor yield at best no more energy than fermentation itself (39), a fact reflected in the limited diversity of sulphate reducing bacteria.

It would appear more plausible that anaerobic respiration or, more precisely, cytochrome-based, low energy-yielding oxidative phosphorylations evolved in response to the necessity to extract free energy from the metabolic wastes of fermentation. The need for alternate energy sources due to dwindling foodstocks would have outweighed the fact that the energy yield was equal to or somewhat lower than its predecessor. It is even conceivable that the initial respiratory chain was
employed only facultatively to subsidize fermentation. Anaerobic respiration, however, could only have provided a short-term advantage for it was still dependent upon a pre-existing organic substrate. Hence, the same environmental pressure invoked to explain the transition from fermentation to photosynthesis can be used to explain the transition from fermentation through respiration to photosynthesis. Indirect physiological and geochemical evidence supporting this hypothesis is presented in later section.

For an organism, the maintenance of internal and external homeostasis requires a continuous expenditure of energy. All phototrophic organisms must therefore possess either a facultative fermentative or respiratory system which permits energy extraction during the regular dark periods when the photosystem is inoperative. For most photosynthetic organisms the dark respirations are largely endogenous, relying upon the oxidation of organic compounds synthesized and stored within the cell during the light period. A number of facultatively heterotrophic photobacteria, however, are able to assimilate and utilize simple organic compounds such as sugars. In the purple non-sulphur bacterium *Rhodopseudomonas sphaeroides* high oxygen tensions inhibit the synthesis of bacterial chlorophyll and induces the synthesis of a cytochrome oxidase to replace the photoreaction centre. The fact that these bacteria can readily switch over to
aerobic heterotrophy by such a simple procedure prompted Prince and Dutton (51) to suggest that the similarities between photophosphorylations and oxidative energy conservation may extend down to the molecular level and reflect a common ancestor.

The origin of photosynthesis continues to puzzle biologists. Smith and Raven (52) presented an interesting hypothesis that phototrophy evolved first as an energy conservation mechanism to decrease the rate of energy expenditure on hydrogen ion transport within the cell. Further sophistication of the mechanism provided the ability to utilize solar energy in the generation of electrons for carbon dioxide reduction. If the archaeobacteria diverged early from an ancestral life form, and developed separately with time from the eubacteria (43), the existence of an aerobic respiratory system in the Halobacteria represents a remarkable example of parallel evolution. Halobacteria possess a primitive photosystem whose sole purpose is to conserve energy in the maintenance of chemiosmotic equilibrium between the cell and its environment. This led Štoeckensius (53) to suggest that not only does respiration predate phototrophy in the archaeobacteria but that the primitive photosystem of halobacteria predates photosystem II in cyanobacteria. Considering the sulphate reducing bacteria, forced to exist upon an energy yield lower than or equal to their predecessors, the adoption of light-prompted ATP synthe-
sis as an energy conservation mechanism would certainly have proven advantageous. In addition, with the necessary cytochromes, quinones and light-sensitive pigments developed, the transition from respiration to photosynthesis should have proven simpler than the direct changeover from fermentation.

3.9 Oxygenic Photosynthesis: Evolution of Cyanobacteria, Aerobic Chemolithotrophs and Eukaryotes

Relating oxygenic photosynthesis, banded iron formations and upwelling of ferrous iron-rich waters, Cloud (4, 13), with support from Schidlowski et al. (22), Margulis (54) and numerous others, championed the currently popular model which views the oxygen-rich atmosphere as a direct consequence of the evolution of oxygenic photosynthesis in cyanobacteria. Biotic models for atmospheric oxygen origin carry with them a 'number of assumptions, corollaries and consequences' as discussed in part by Towe (55). Apart from the most obvious assumption that the early atmosphere was in fact reducing throughout most of the Archean, the major assumptions in such a model are that for some unknown reason oxygenic photosynthesis evolved initially in an anaerobic cyano-bacterium adapted to an anoxic, reducing environment and that somehow the lethal effects of intracellular oxygen were effectively mediated by extracellular ferrous ions. It follows therefore that while the iron-dependent cyano-
bacteria were evolving oxygenic photosynthesis, not only must they have carried out anaerobic dark reactions but the entire biological system must have remained anoxic and anaerobic with biologically-essential elements being recycled by fermentative and anaerobic respiratory processes alone. A necessary corollary to this must be that all aerobic heterotrophs, chemolithotrophs and eukaryotic cell forms emerged after the evolution of oxygenic photosynthesis in cyanobacteria.

Cyanobacteria (blue-green algae) constitute the most diverse and widely distributed group of photoautotrophic prokaryotes. They are readily distinguished from the photosynthetic bacteria by the nature of their photosensitive pigment (chlorophyll a) and by their capacity to perform oxygenic photosynthesis. Stanier and Cohen-Bazire (56) presented a comprehensive review of cyanobacterial metabolism. The following discussion represents a brief summary of cyanobacterial physiology, based largely upon their review, highlighting those aspects considered by most as having direct relevance to the study of early prokaryotic evolution.

Cyanobacteria, like all photoautotrophs, utilize solar radiation to provide the necessary energy for reductive assimilation of carbon dioxide. Oxygenic photosynthesis consists of two basic photochemical pathways which normally operate in series: photosystem I and photosystem II. The former is functionally homologous to the
anaerobic bacterial reductive pentose phosphate cycle. The sole purpose of photosystem II is to supply electrons to photosystem I via the photolysis of the water molecule, emitting molecular oxygen as a by-product.

Like all photoautotrophs, cyanobacteria possess both the requirement and capacity to extract free energy during the regular dark periods. Oxygen-linked respiration is the only known mechanism for dark ATP synthesis. For the most part, the tricarboxylic acid cycle is inoperative in cyanobacteria due to their inability to synthesize the requisite dehydrogenase enzymes. Consequently, as with the facultatively aerobic photosynthetic bacteria, cyanobacterial respirations are carried out via the oxidative pentose phosphate cycle. While endogenous respiration is universal in cyanobacteria, some are able to assimilate a limited range of simple sugars. However, chemoheterotrophic varieties, at best, grow only poorly in the dark.

Cyanobacteria are evidently aerobic organisms whose physiological attributes link them to an anaerobic ancestor. The best known and most elaborated upon attribute is their facultative photosystem. A number of cyanobacteria possess the capacity to switch over to photosystem I, generating electrons through the photo-oxidation of sulphide to sulphur. This 'physiological potentiality', however, can only be expressed in light and then only in the presence of dissolved sulphide. Two
factors appear to be operative in the changeover from oxygenic to anoxygenic photosynthesis; the immediate inhibition of photosystem II by sulphide and the subsequent induction of a sulphide oxidizing enzyme system.

A second line of evidence supporting an anaerobic ancestry lies in their ability to utilize molecular nitrogen. While most bacteria can readily use $\text{NH}_3$, nitrogen fixation requires specific enzymes termed nitrogenases. These enzymes are oxygen-labile, becoming rapidly and irreversibly denatured in the presence of small quantities of molecular oxygen. Consequently, while many cyanobacteria possess the latent capacity to synthesize nitrogenases, its expression is repressed in the presence of oxygen. Heterocysts are highly specialized cyanobacterial cells, harbouring nitrogenases, whose thick outer envelope is impermeable to oxygen and resistant to autolytic enzymatic attack. The ability to form heterocysts, therefore, is invariably associated with the advantageous physiological capacity to fix nitrogen in the presence of free oxygen.

Among the autotrophic prokaryotes are non-pigmented chemolithotrophic bacteria (i.e. *Thiobacillus*, *Nitrosomonas*) and cyanobacteria (i.e. *Beggiatoa*, *Thiethrix*, *Thioploca*) which possess the capacity to reduce carbon dioxide in the absence of light using ATP generated by the oxidation of reduced inorganic substrates. Not every species of each can be considered as strictly autotrophic
since some can use organic compounds as both an energy and carbon source. However, in light of the recent discovery of an entire ecosystem supported solely by chemolithotrophic sulphur bacteria, some discussion of chemoautotrophy appears warranted. The typical chemolithotroph, as described by Fenchel and Blackburn (39), is obligately autotrophic and aerobic, generating ATP by oxidative phosphorylations with molecular oxygen as the terminal electron acceptor. Like the photoautotrophs, it uses the reductive pentose phosphate cycle for carbon dioxide assimilation. However, unlike the phototrophs, the reduction stage must be carried out by reverse electron flow expending energy (ATP) generated by the normal flow of electrons to oxygen.

Exposure of obligate anaerobic bacteria to molecular oxygen can cause cellular damage and metabolic malfunction in a variety of ways. As well as raising the intra- and extracellular Eh, oxygen can interact directly with oxygen-labile compounds or be metabolized into a number of highly toxic intermediaries (i.e. superoxides, peroxides and hydroxyl free radicals). The lethal effect of oxygen in aerobic organisms are prevented by oxygen-mediating enzymes (i.e. catalases, peroxidases and superoxide dismutases) (45).

Microorganisms such as fungi, algae and protozoa represent simple eukaryotic cell forms, having two or more genetic systems each of which is contained in a
separate membrane-bound organelle (i.e. nucleus, chloroplast and/or mitochondria). Biologists customarily view the prokaryotic-eukaryotic division as a 'fundamental phylogenetic dichotomy' (43). In the past, the simple presence or absence of a nucleus was the principal point of distinction between eukaryotes and prokaryotes. This, however, has proven to be only the most obvious of a number of functional differences. Nearly all eukaryotes are aerobic or facultatively anaerobic which implies that molecular oxygen is a physiological requirement. A number of eukaryotic metabolic and biochemical pathways (i.e. respiration, sterol synthesis) represent aerobic reactions coupled to anaerobic reactions which are thought to represent the vestiges of earlier prokaryotic biochemical systems (2, 53). Other processes such as mitosis (mitotic cell division) appear to be fully oxygen dependent and have no analogues in the prokaryotic kingdom. This has led to the universally accepted conclusion that eukaryotic cells evolved at a point where environmental oxygen contents were stable and relatively high (54).

Dodson (57) presented a masterly review of the problems encountered to date in the study of eukaryotic evolution. To summarize, biologists are divided basically between the popular endosymbiotic theory and a number of continuous developmental theories. The endosymbiotic theory presumes that a large, anaerobic or facultatively aerobic, heterotrophic prokaryote engulfed and subse-
quently formed a symbiotic relationship with a smaller, aerobic prokaryote, either a cyanobacterium (chloroplast) and/or a heterotrophic bacterium (mitochondria). In variance, the developmental theories (as there are several) maintain that the various organelles of eukaryotes arose through continuous modification of an aerobic prokaryotic or protoeukaryotic system. Dodson concluded that while there is still insufficient evidence to decide which theory is most satisfactory, in view of the fact that no intermediaries exist to support the developmental concepts, isolated biochemical evidence collectively appear to support the endosymbiotic theory.

For the purpose of discussion within this chapter, the mechanism for eukaryotic origin is not of prime concern. However, the divergent theories carry necessary corollaries important to the placing of eukaryotic evolution in a proper geologic time reference. Implicit in endosymbiosis is the prior emergence of aerobic heterotrophic and photosynthetic prokaryotes. In contrast, the only limiting factor in the appearance of eukaryotes by the continuous developmental theories is an adequate supply of oxygen and, as such, the early eukaryote could have emerged at the same time or earlier than cyanobacteria. It follows that if all life forms were once anaerobic, the ancestral eukaryote by the developmental theories was also anaerobic prior to the segregation and compartmentalization of its metabolic systems.
Obligate anaerobiosis is a rarity within the eukaryotic kingdom. However, a variety of oxygen-indifferent, obligately fermentative fungi have been reported from anoxic sediments and waters (46). This would imply that while they are not strict anaerobes in a sense that molecular oxygen is lethal, they have no biochemical requirements for free oxygen and, as such, function anaerobically. Taking this further, it would mean that not only must these eukaryotes have dispensed with those materials whose fabrication requires the fixation of free oxygen, or developed an alternate anaerobic biosynthetic pathway to achieve the same result, but more importantly these eukaryotes must have abandoned a high energy-yielding metabolic pathway (respiration) to exploit a lower energy-yielding, oxygen-independent fermentative pathway. By the current wisdom, this is considered to represent a remarkable example of retrogressive evolution (58).

Oxygenic photosynthesis could not have arisen spontaneously from anaerobic photosynthesis in the one miraculous leap envisioned by Cloud and numerous others. Evolutionists, such as Schopf (59, 60), while supporting a late redox transition during the Proterozoic, admit difficulty in conceiving how extracellular iron could possibly mediate auto-oxidative toxicity when the oxygen itself is generated intracellularly. Oxygen-mediating enzymes must have evolved prior to the evolution of
photosystem II. Yet, if cyanobacteria possessed such enzymes before it could produce oxygen then some motivating force must have been behind their initial development. Cyanobacteria were not blessed with the foresight to realize that they would require these enzymes in order to photolyse the water molecule. The solution must lie in the fact that each physiological adaptation that collectively led to the generation of molecular oxygen evolved for some other purpose other than oxygen generation.

In view of the fact that cyanobacteria have an anaerobic ancestry, their ability to carry out facultative dark respirations represents a later stage in their evolution. However, no evidence exists to support the view presented by Margulis (54) that dark aerobic respirations coincide with an atmospheric redox transition initiated by cyanobacterial oxygenic photosynthesis. The existence of facultative aerobic respiration in anaerobic photosynthetic bacteria alone is evidence enough to suggest that only a source of molecular oxygen and not necessarily oxygenic photosynthesis is required to account for the transition from dark fermentation to dark respiration in both prokaryotes and eukaryotes.

The photosynthetic apparatus in cyanobacteria is similar in functional, structural and molecular respects to that contained in the eukaryotic chloroplast, but is only truly homologous with that of the red algal (rhodo-
phytan) chloroplast specifically. The general similarity between red algal chloroplasts and cyanobacteria has lent support to the endosymbiotic theory for eukaryote origin (54). However, to be essentially correct, this theory requires either that all other types of chloroplasts evolved from the red algal chloroplast during the early development of eukaryotes or that the ancestral cyanobacterial entities to the other types of chloroplasts are now extinct. Stanier and Cohen-Bazire (56) strongly favoured the latter explanation, suggesting that "the present diversity of algal pigment systems is the expression of a very ancient divergence" (pg. 267).

While no genetic link exists between cyanobacteria and aerobic obligate heterotrophic bacteria, molecular cataloguing serves to demonstrate the inconsistencies inherent in a biotic model for atmospheric oxygen. Phylogenetic evolutionary schemes derived from sequence data depict the aerobic heterotrophs as emerging independently from several lines of anaerobic photosynthetic bacteria and, more importantly, earlier than the cyanobacteria (43, 49, 50). It is generally assumed that since eukaryotes are predominantly aerobic while cyanobacteria are facultatively anaerobic, cyanobacteria emerged prior to the eukaryote regardless of its origin. Yet, Fox et al. (43) noted that the sequence data for extant cyanobacteria do not demonstrate enough diversity to warrant such a conclusion. Only after combining chloroplast data
with cyanobacterial data (employing the endosymbiotic theory) could they develop an ancient lineage for cyanobacteria compatible with that of the other photosynthetic bacteria. If, on the other hand, eukaryotes are not the product of a fortuitous relationship but rather a result of progressive development of an early prokaryote cell form, the antiquity of chloroplasts, mitochondria and the 'protoeukaryote' host cell could simply reflect the age of the organism and the successive biochemical modifications in the chemical energy transfer system from fermentation through to oxygenic photosynthesis. If such was the case, the present diversity of algal pigment systems would be an expression of a very ancient divergence, as suggested by Stanier and Cohen-Bazire, but among the ancestral protoeukaryotes not the cyanobacteria.

The paradox of cyanobacteria as the originators of an oxidizing atmosphere was neatly resolved on biochemical grounds by Towe (55). Emphasizing the universal requirement of oxygen-mediating enzymes in aerobic organisms, the inhibition of photosystem II by dissolved sulphide (hence low redox potentials), the effective quantum efficiency of photosystem I, and the large initial energy expenditure required to dissociate the water molecule, Towe resolved that oxygenic photosynthesis could only have arisen as a direct response to diminishing reduced energy substrates (hydrogen donors) in a cyanobacterium already capable of facultative aerobic dark respiration.
Paradoxically, however, while promoting photodissociation as an early source for atmospheric oxygen, Towe concluded that the forced evolution of oxygenic photosynthesis in cyanobacteria coincided closely in time with the hypothesized early Proterozoic atmospheric redox transition. In complete disregard to existing paleontological evidence, he made the startling proposal that stromatolites and 'algal' microfossils older than about two billion years represent the probable remnants of anaerobic photosynthetic bacteria.

3.10 Precambrian Micropaleontology: Uses and Abuses

Searching for knowledge of biospheric evolution in the geologic record is an arduous task. With the probability for extensive recrystallization and deformation increasing with the age of sedimentary rocks, evolutionary evidence becomes progressively rarer and generally more obscure with time. In a recent paper, Cloud and Morrison (61) attempted to differentiate between three broad categories of evolutionary evidence: permissive, presumptive and compelling. To be compelling, evidence must unambiguously demonstrate biogenicity and have accurate geochronological control. They concluded that the oldest structurally preserved microfossils for which there is conclusive evidence are approximately 2.3 billion years old and that, at best, evolutionary evidence for Archean life falls into the presumptive class. For-
tunately, others have not been so easily discouraged and compelling evidence for widespread microbial life has since been extended to 3.5 billion years or older.

In 1914, Walcott (62) first proposed an algal origin for finely-laminated structures (stromatolites) in PreCambrian sedimentary rocks. Shortly thereafter, in 1925, Gruner (63) proclaimed the discovery of filamentous microfossils in cherts of the PreCambrian Mesabi Range. Yet, not until the late 1950's, following the publication of a short note by Tyler and Barghoorn (64) on microfossils from cherts of the Gunflint banded iron formation (BIF), did world interest focus on the implications of PreCambrian microfossils to biospheric evolution. Since then the mass of literature related to PreCambrian microfossils and their evolutionary ramifications has increased to the point where computer compilation is essential simply to keep track of fossil names and references.

During the past two decades, the study of early biospheric evolution has led to encompass a broad spectrum of interrelated studies. Among such topics, however, is one area of particular importance that has received relatively little consideration, namely the application of microfossils to paleoecological studies. Out of the literally hundreds of fossiliferous localities, few are of the exact same age, paleogeographical distribution and depositional environment. From this viewpoint,
each locality represents a unique and isolated occurrence. Similarities within the microfauna from various localities arise, to a large extent, from similarities in water temperature, chemistry, depth, light intensity, etc.

Biological and geological evolutionary models, as discussed earlier, invariably hinge upon a major early Proterozoic atmospheric redox transition. It appears somewhat of a paradox that many such models, particularly those directly or indirectly concerning eukaryotic development, call upon supporting paleontological evidence whose very interpretation rests upon the validity of the model it supports. In light of the difficulties encountered with environmental and age differences, Schopf (59, 60, 65) and numerous others have confined themselves to the application of generalizations when attempting to work with Precambrian microfossils. Unfortunately, as it will be demonstrated, such an esoteric approach has not lent itself to the revision of earlier ideas but rather to the propagation of many misconceptions inherent in current evolutionary models.

Precambrian paleontological studies fall into two broad areas; the study of microfossils and related biogenic structures and the chemical analysis of sedimentary rocks. While the latter is commonly considered to include stable isotopic studies and the characterization of organic compounds from kerogen extracts, inorganic chemical analyses and organic carbon contents, if inter-
predicted in view of known syndiagenetic, microbially-mediated processes, can provide useful, albeit less direct, information on the level of metabolic sophistication within a microbial community. Population size distributions together with size, shape and the degree of morphological complexity of individual microfossils are about the limit of information that can be extracted directly from a microfossil assemblage. These must be integrated with detailed sedimentological, geochemical and organic chemical studies in order to gain a somewhat clearer picture of the evolutionary action-reaction phenomena.

The sheer mass of morphological and chemical data related to Precambrian paleontology are almost overwhelming and must be culled and sifted by any student attempting to study early 'prokaryotic and eukaryotic development. It is impossible to present a complete literature review on the Precambrian fossil record. The following discussions are restricted largely to a few well known publications and a few well documented microfossil occurrences. It must be acknowledged, however, that the ideas generated herein stem from the review of a substantially larger proportion of the published literature and are applicable to most, if not all, Precambrian microfossil studies.

Precambrian microfossils, in general, are the product of rapid entombment of microbial cells in marine
sediments within a shallow- or deep-water, low energy depositional environment. Fossiliferous rocks exhibit a low degree of recrystallization and metamorphic alteration (generally less than lower Greenschist facies) and a low degree of epidiagenetic or post-metamorphic oxidation by meteoric waters. On the basis of the mode of preservation, Precambrian microfossil assemblages can be categorized according to the nature of the host rock into three broad groups: fossils in cherts; fossils in shales; and stromatolites in carbonates (65). This categorization, in turn, can be extended to relate the relative degree of cellular preservation to physico-chemical conditions of the depositional environment.

Foregoing discussions (chapter 2) dealt largely with the subjects of degradation and preservation of organic matter in terms of hydrospheric biogeochemical carbon cycling. To summarize, the preservation of organic matter is contingent upon inhibited biodegradation which, in turn, is a consequence of the aqueous chemistry of the depositional environment. It has long been known that the mere existence of 'soft-bodied' fossils must reflect an unusual set of syn- and post-depositional conditions. Twenhofel (66), in 1942, noted "it seems a paradox that the more congenial the conditions of the sea bottom for bottom dwelling forms and more numerous the colonization by organisms, the less likelyhood there is of many fossils which finally attained entombment. In
other words, an abundant bottom population under conditions of slow deposition produces...more or less complete elimination of nutrient matter" (pg. 105). A necessary corollary to this must be that environmental conditions which are optimal to the preservation of cellular matter must be less than congenial to habitation by all forms of life. While Twenhofel's concern was primarily with metazoans, his observations are even more applicable to the preservation of microorganisms where their relative susceptibility to hydrolytic enzymatic attack results more often than not in the complete loss of cell morphology.

The best Precambrian microfossil assemblages are found in relatively uncommon black or grey, primary and secondary cherts characteristic of warm, possibly hypersaline, shallow-water depositional environments. The assemblages are commonly comprised of well preserved, three-dimensional microfossils of diverse planktonic and benthic forms. They are frequently stromatolitic, providing valuable information on the ecologic organization and growth habits within the microbial community. The preservation excellence results from the rapid permineralization of non-biodegraded cells by chemically precipitated silica or calcium carbonate which was subsequently silicified before extensive diagenetic recrystallization could destroy cell morphology. The silica matrix, in turn, provided long-term resistance to metamorphic alter-
ation and impermeability to oxidative meteoric waters.

The second category of microfossils is represented by organic-walled nanofossils from relatively common, silicified, kerogen-rich shales which, for the most part, are considered characteristic of offshore, deeper water conditions. Albeit considered to represent chiefly planktonic forms, organic-walled nanofossils are referred to generally as, acritarchs in view of the difficulty in assigning them to any known algal group. The conditions surrounding acritarch preservation have yet to be fully elucidated. Although they are commonly compressed and often poorly preserved, the mere fact that they survived at all attests to an unique depositional setting. Biodegradation-resistant cell walls are generally considered more characteristic of eukaryotes than prokaryotes. Heavy-metal staining of the cell walls, low ambient oxygen levels and early diagenetic permineralization may have played a significant role in the pre- and post-depositional inhibition of bacterial enzymatic attack. Anomalous concentrations of Cu, Ni and Fe associated with the cell walls of nanofossils were described by Pflug (67) from silicified shales of the 3.2 billion year old Fig Tree series, South Africa. The metal enrichments were interpreted as presilification sulphide coatings originating as the product of metal ion interaction with cellular sulphide compounds. If such was the case, the coatings provide support for con-
ditions of low oxygen levels and possibly low redox potentials within the depositional environment.

Stromatolites, as defined by Walter (68), represent "organosedimentary structures produced by some combination of sediment trapping, binding or precipitation as a result of growth and metabolic activity of microorganisms" (pg. 563). It follows, therefore, that where environmental conditions were not conducive to the early silicification of sediments, evidence for Precambrian microbial activity can be found in carbonate stromatolites. Although commonly enriched in kerogen, carbonate stromatolites are usually void of microfossils due, in part, to biodégradative activity but probably primarily as a result of rapid, late-diagenetic and metamorphic recrystallization of the carbonate matrix. Apart from being an excellent paleoenvironmental indicator, stromatolites alone provide little useful information relevant to global evolutionary studies.

The quest for the oldest eukaryotic cell and the earliest evidence for life continues to dominate Precambrian paleontological studies. The time of the first appearance of eukaryotes is of particular biological significance due to the eukaryotic capacity for sexual reproduction. The advent of sexual reproduction introduced the potential for increased ecological diversification and, more importantly, may have initiated accelerated evolutionary rates leading to the early development of
thallophyta, protozoa and metazoa. In addition, according to the endosymbiotic theory, the first appearance of eukaryotes provides compelling evidence for an oxygenated environment.

Evolutionary rates vary widely and any effort to extrapolate an average rate of molecular evolution from advanced eukaryotes to primitive prokaryotes must be compatible with the fossil record. Swartz and Dayhoff (50) attempted to correlate the diversification of cyanobacteria with the hypothetical atmospheric redox transition (2.0 By BP) and the emergence of photosynthetic bacteria with 3.1 By-old stromatolites by extrapolating the average rates of cytochrome, ferredoxin and 5s rRNA evolution. The purpose of this section is not to dispute the accepted relative constancy of molecular change, but rather to demonstrate the advantages of geochronological dating over molecular evolutionary rates when used in conjunction with paleontological evidence. The fossil record supports the existence of cyanobacteria by 3.5 By BP while circumstantial evidence suggests that eukaryotes may have appeared by 3.4 ByBP.

Little agreement exists as to when the first eukaryotic cell appeared. Ironically, the arguments rest not entirely upon the appearance of the microfossils (for many exhibit characteristics remarkably similar to modern unicellular eukaryotes) but rather upon the credibility of the observations and whether or not there was suffici-
ent oxygen around to support eukaryotic métabolisms. Interpretations of eukaryotic microfossils have been largely based upon one or all of the following: large cell diameter; the presence of internal dark spots thought to represent nucleii or other membrane-bound organelles; tetrad cell arrangements, triplet scars and putative mitotic cell divisions considered as evidence for sexual reproduction; and the isolation of steranes from kerogen extracts considered to be indicative of eukaryoticsterol or steroid synthesis.

The best described (and generally accepted) Pro-cambrian eukaryotic microfossil assemblages are preserved in silicified algal mats within the 800–900 My-old Bitter-Springs Formations of Western Australia and in the silicified stromatolites within the 1.3–1.4 By-old Beck Spring Dolomite of eastern California. To date, nine of the fifty described species from the Bitter Springs locality have assigned to the eukaryota (59, 60, 69-71). In contrast, five of the seven described species from the older Beck Spring Dolomite have been tentatively ascribed eukaryotes (72, 73).

Long 'ribbonlike megafossils' from the 1.3 By-old Belt Supergroup of Montana were described as multicellular eukaryotic algae by Walter et al. (74) on the grounds of size and complexity. Oehler et al. (75) tentatively ascribed an eukaryotic origin to tetragonal cell clusters from the 1.5 By-old Amelia Dolomite of northern Australia.
Barghoorn and Tyler (76), Licari and Cloud (77), Barghoorn (78), Edhorm (79), Darby (80), Tappan (81), Kazmierczak (82, 83), and Knoll et al. (84) have all noted similarities between spheroidal microfossils of the 2.0 By-old Gunflint BIF and modern eukaryotic algae. A fungal presence within the 1.8-1.9 By-old Belcher Island stromatolitic cherts was described by Hoffman and Jackson (85) along with a variety of large and small spheroidal 'spot cells' (some greater than 20 microns in diameter) but all eukaryotic interpretations were abandoned in subsequent papers (86-88) largely on the basis of a lack of unquestionable eukaryotic microfossils from rocks older than 1.2 By BP.

Knoll and Barghoorn (89) contested the credibility of fossilized cell organelles and hence the interpretation of eukaryotic cells in rocks older than the metazoan-bearing Ediacaran fauna of south Australia (680-700 My BP). Francis, Margulis and Barghoorn (90), through detailed experimental studies on permineralization and degradation of unicellular cyanobacteria, generated all of the morphological criteria used to identify eukaryotic cells in the fossil record. This led them to conclude that "there is no compelling evidence (which can be deduced from cytological studies including those 'organelles' of Precambrian microfossils) for the presence of eukaryotic organisms in the fossil record prior to the late Precambrian Ediacaran fauna of south Australia"
(pg. 97). Ironically, they discredited the very criteria by which unicellular eukaryotes of even the Ediacaran and younger rocks could be identified. Knoll, Barghoorn and Awramik (84), in a discussion on eukaryotic evolution, suggested that although the relatively large size of eukaryotes may have been inherited from prokaryotes, there is no evidence to show that eukaryotes were always large. Paradoxically, while contesting the reliability of criteria used to identify fossil eukaryotes, these same authors found 'strong evidence' to suggest a planktonic eukaryotic origin for certain spheroidal microfossils from the two billion year old Gunflint sediments.

A comparative study of population size distributions within major Proterozoic silicified, shallow-water marine microfaunas was undertaken by Schopf (59, 60) and Schopf and Oehler (91) with the objective of establishing the first global appearance of eukaryotes. They rejected the contentions that eukaryotic cells could not be conclusively traced throughout the Proterozoic but discounted the numerous suggestions for eukaryotic cells in the microfossil assemblages from the Gunflint cherts. On the basis of change in the relative abundance of large spheroidal unicells in Proterozoic cherts, they concluded that eukaryotes may have evolved by 1.4 By BP and were abundant by 850 My BP.

On the premise that free oxygen was a consequence of cyanobacterial evolutionary development about two
billion years ago, Licari and Cloud (92) attempted to bracket the origin of eukaryotes between 1.3-1.6 By BP, consistent with the timing proposed by Schopf and Oehler (91). In a subsequent summary paper on early biospheric evolution, Cloud (4) presented one of the more articulate and imaginative reviews of eukaryotic evolutionary evidence, expressing most of the prejudices and predilections inherent in an applied Berkner-Marshall atmospheric evolutionary model. Some of the proposed eukaryotic forms from the Gunflint BIF were discredited by Cloud as "simple fuzzy images of a probable budding bacteria", while the fungal presence in the Belcher Island cherts was discounted on the basis "that to interpret it as a fungus is to hypothesize the existence of fungi when (we know) O₂ was probably still barely 1% PAL or less". Employing such arguments, one is able to contest even the most compelling evidence for eukaryotic organisms regardless of age.

To date, more than 500 organic compounds have been characterized from various sedimentary sequences not including the individual constituents of crude oils. The contribution of organic geochemistry to biospheric evolutionary studies has been reviewed in depth by McKirdy (93). Isoprenoid alkanes, notably norpristane, pristane and phytane, and metal-chelated porphyrins in appreciable amounts are considered likely to represent degradation products of algal, cyanobacterial or bacterial
chlorophylls. Together with low stable carbon isotopic ratios, they provide compelling evidence of a photosynthetic biomass but collectively proffer little or no information on the nature of the photoautotrophs. Similarly, highly aromatic kerogens have been attributed, in part, to the breakdown of algal and fungal spore exines and/or the polymerization of algal polyunsaturated and cyanobacterial unsaturated fatty acids. Of all the organic compounds isolated from kerogen extracts, steranes, derived from the breakdown of sterols and steroids, provide the only unambiguous evidence for the past presence of eukaryotes.

McKirdy pointed out that so far no critical examination or systematic assessment has been undertaken on the degree of metamorphic alteration within fossiliferous and/or kerogenous Precambrian sediments. In fact, little is known about the stability of various organic chemical fossils, particularly steranes, as a function of temperature, pressure and time. Steranes have yet to be reported from the sediments of both the Bitter Springs Formation and the Beck Spring Dolomite in spite of the compelling morphological evidence for eukaryotic microfossils. The oldest biogenic steranes, to date, were extracted from the 2.7 By-old Soudan Shales (94, 95). While the authenticity of younger steranes has never been questioned, even in the absence of supporting morphological evidence, the Soudan steranes were contested by Hoering
(96), Margulis (97) and Schopf (59) in view of the fact that their antiquity contradicted current models for eukaryotic development. Schopf contended that organic chemical fossils could only be accepted as contemporaneous with lithification of the sediments if consistent with macro- and microfossil evidence; a fact that in view of the findings of Francis et al. (90) can never be satisfactorily substantiated.

To reiterate, life as a whole is selectively adapted to life in a common environment and, more than likely, life probably arose in such an environment. Advantages of an early oceanic, planktonic habitat for photoauto- trophs would have included the wide geographic area, the potential for current-borne dispersion, the availability of essential resources (i.e., light, CO₂, nutrients, etc.) and, for eukaryotes, better oxygenation of the surface waters (45). It is safe to assume that, for the most part, shallow-water environments were relatively un- common throughout much of the Archean and those that did exist were characterized by conditions less than conducive to habitation relative to the open ocean. Competition between various groups of organisms and within individual groups promotes ecological diversification and specialization. It follows, therefore, that the ability to colonize a shallow-water, 'extreme' environment would have represented an ecological advantage. Studies have shown that cyanobacteria are among the first
to colonize extreme environments (20). Compared with eukaryotic algae, they are more efficient at obtaining CO₂ and phosphate from low concentrations (39), have lower oxygen requirements (2, 20) and higher temperature tolerances (20). Walter (98, 99) was among the first to draw a parallel between the 2.0 By-old Gunflint cherts and recent 'stromatolitic' siliceous deposits from the Yellowstone hot-springs on the basis of geochemical and microfaunal similarities. In a recent paleoecological study of the Gillen Member of the Bitter Springs Formation, Oehler abandoned her support of the approach which Schopf has pursued in the quest for eukaryote antiquity. Oehler et al. (100) aptly demonstrated that microfaunas of Precambrian shallow-water hypersaline environments, like their modern counterparts, are dominated by prokaryotes, have low species diversity and can never be "used for inferring the evolutionary status of the contemporary biosphere or for considerations of evolutionary trends such as age-related changes in algal diversity and size distributions" (pg. 390).

To summarize the current state of eukaryotes in Precambrian paleontological studies; where preservation is optimal, the afforded evolutionary information is minimal. Steranes, if to be considered synchronous with deposition, must be supported by morphological evidence. Yet, size, shape and internal detail no longer are conclusive criteria for eukaryotic interpretations. Cloud (4)
cautioned that "where we seek to extend our knowledge of biospheric evolution in time and space...not to dilute the credibility of well established data" (pg. 355). However, in a discipline such as this where credibility is purely subjective, one can only rely upon the integrity and assess the knowledgibility of fellow students. With the concept of a late, major atmospheric redox transformation so entrenched in the current literature, and in light of the controversies surrounding eukaryotic origin and paleontological identification, it would appear that the universally accepted earliest age for eukaryotes will never exceed much beyond 2.0 By BP. Only through the disparagement of existing evolutionary models can the age ever be extended beyond the current limits.

Stromatolites are the least controversial evidence for early life. The oldest documented stromatolites are preserved within silicified, shallow-water carbonate sequences within the 3.5 By-old Warrawona Group of the Pilbara Block of Western Australia (16, 17, 101). Acid maceration of kerogenous stromatolites from the North Pole sediments of the Warrawona Group revealed a variety of hollow, spheroidal structures interpreted by Dunlop et al. (16) as nanofossils displaying a population size distribution similar to mixed microbial assemblages of recent ecological counterparts. Statistical analysis using size probability plot techniques ruled out an abiotic origin for the spheroids and, more importantly,
showed that the diameter of the nannofossils (within the total population and separately) lay on a nearly straight, steeply inclined curve paralleling trends for the extant cyanobacteria, *Gleocapsa*, and, more significantly, the extant eukaryotic alga, *Chlorella*. The high degree of morphological variation and range in size led Dunlop and his colleagues to propose the existence of a considerable diversity in the microbial population of early Archean shallow-water environments, and to suggest that the physiological complexity of early Archean life may have been more advanced than previously thought. No microfossils were reported in later studies by Lowe (101) and Walter et al. (17) on similar stromatolites although consideration of stromatolitic microstructure in the latter study restricted the microbial 'architects' to less than twenty microns in diameter, consistent with the earlier nannofossil data. Walter et al., however, contended that while these particular stromatolites provide evidence for an early benthic microbiota "adapted to life in an adverse, fluctuating environment", stromatolites alone do not warrant a cyanobacterial interpretation as modern examples of anaerobic bacterial stromatolites are found in hot-springs. Granted, while this may be the case, the evidence for primary oxidation of iron in hematitic cherts underlying the stromatolites and the presence of sulphates in the host sediments strongly support an oxygenated environment and hence does not
preclude a cyanobacterial origin nor the existence of eukaryotes.

Compared to stromatolites, spheroidal microfossils present a much more difficult problem of interpretation. Spheroidal nannofossils from argillaceous cherts of the Onverwacht Group, Barberton-Mountain Land, South Africa, constitute the oldest known occurrence of probable planktonic nannofossils (3.4 By BP). The isolation of porphyrins (102), pristane and phytane (103) together with low stable carbon isotopic ratios (104) from highly condensed, aromatic kerogens of the Onverwacht cherts lend support to a biogenic origin for the microfossils. The size distributions of several hundred microfossils from three chert horizons were shown by Engel et al. (105) and Nagy and Nagy (106) to display a high degree of variability, ranging from less than 10 microns to greater than 70 microns in diameter, consistent with a mixed population of microorganisms. In a subsequent study, Muir and Grant (107) described several separate size distributions within smaller, single and paired unicells and filaments from yet a fourth horizon in the Onverwacht Group. On the basis of size distribution and range in morphology they suggested a cyanobacterial interpretation for all of the microfossils from the Onverwacht.

On the subject of cell size and eukaryotic origin, Timofeev (108) indicated that Soviet planktonic acri-
tarchs displayed a slight decrease in size with increas-
ing age throughout the Proterozoic. It was this trend for deep-water acritarchs plus their own confirmation and extension of it to shallow-water environments that led Schopf (109) and Cloud (4) to question not only the alleged algal affinities of the large Onverwacht microfossils but also their biogenicity. Figure 5 shows a plot of the 'population' size distributions of the Onverwacht microfossils compared with size distributions of shallow- and deep-water Proterozoic microfaunas compiled by Schopf (2). While the remarkable similarity between the Archean Onverwacht nannofossils and modern planktonic algae does not confirm an eukaryote origin, it provides at least circumstantial evidence that prokaryotes or protoeukaryotes were attempting to increase their size early in the history of the Earth.

To conclude this section, it is imperative to discuss recent studies on the paragenesis of the Vaal Reef carbon seams within the 2.37-2.48 By-old Witwatersrand conglomerates in South Africa. These deposits are unique in that not only do they represent the oldest verified Precambrian fresh-water deposits of organic carbon, but they host the oldest recorded non-marine eukaryotic microbiota. Geological observations indicate the Witwatersrand carbon seams (thucolite) formed in a composite environment where a cool wind swept desert interfaced with a braided river system (110). Organic chemical analysis has shown that the thucolites contain amino acids
Comparison of size distributions of 3.4 By-old Onverwacht spheroidal microfossils, Proterozoic shallow- and deep-water spheroidal microfossils and modern unicellular cyanobacteria and green algae (after Schopf (2), Engel et al. (105) and Nagy and Nagy (106)).

A - Upper Onverwacht
B - Lower Onverwacht
and carbohydrates (111), porphyrins, pristane and phytane (112) which collectively support a proposed photoautotrophic origin for the biochemical precursors (93). Stable carbon isotopic ratios for the thucolites (96), when compared to isotopic ratios of recent biological systems, fall into the range of land plants (113). From a critical examination of the external appearance and internal structure of the thucolite seams, Hallbauer and his colleagues (113-115) concluded that "the carbon was the relatively undamaged remnants of Precambrian mass vegetation of comparatively large plants remaining in site" (113, pg. 479). Three morphological 'plant' forms were described in detail from SEM studies. The largest and biologically most-significant form, named Thuchomycetes lichenoides, was described as aggregates or mats of vertical columns 0.5-5.0 mm high and 0.2 mm in diameter, encrusted with U, Th, Si, Ti and Au, and exhibiting many of the characteristics of modern lichens. The second form, named Witwateromycetes conidiophorus, appeared as loose hyphae of a 'saprophytic nature' encrusted in Pb and Zn. The remaining form consisted of organic spheroids and was left unnamed on the basis that it may represent vegetative diaspores of the columnar Thuchomycetes.

The existence of advanced land thallophytes around 2.4 By BP violates all current concepts of Precambrian biospheric evolution. Moreover, they call for a complete revision of atmospheric evolutionary models and a critical
reassessement of the evidence upon which they are based. Yet, it cannot be stressed too emphatically that in spite of this major disparity, few micropaleontologists have attempted to refute the well documented studies of Hallbauer and his colleagues. One might even hazard to conclude that it was safer to ignore their work than to refute it. The only critics of the alleged thallophyte interpretation for the Precambrian thucolites are inadequate and, perhaps, even unscientific. With a figurative hand wave, Cloud (6) attempted to discredit the validity of Hallbauer's findings with an unpublished acclamation of experimental duplication of 'similar' filamentous structures by heating mixtures of carbon with lead and zinc powders and filings. Ironically, even if such was the case, no one has yet to dismiss the more significant columnar forms. In his summary paper, Cloud concluded that, as with the origin of life, we will probably never know the answers to how eukaryotes arose. Unfortunately, a corollary to this must be that we can never really know how old eukaryotes are.

3.11 Precambrian Biomass Productivity

During photosynthesis, stable carbon isotopes undergo kinetic and metabolic fractionation whereby the lighter carbon isotope ($^{12}$C) becomes preferentially enriched in the organic fraction. Within the hydrosphere, fractionation occurs between photoautotrophs and dis-
solved carbon dioxide. Once fixed within the sediments in the form of kerogen and calcium carbonate, carbon isotopic ratios appear to remain relatively constant throughout diagenesis and low-grade metamorphism (21).

In an atmosphere-hydrosphere system of relatively constant carbon content and isotopic composition, and with a continuous high degree of biological fractionation, similar stable carbon isotopic ratios in rocks of all ages would indicate a constant separation of carbon between the inorganic and organic reservoirs and, hence, a relatively unchanging photosynthetic global biomass. Holland (116) first suggested that the constancy of carbon isotopic ratios in carbonates since the Precambrian indicated a similar constancy in the burial of organic carbon. Broecker (117) used the relative constancy of carbon isotopic ratios in marine carbonates to argue for a maximum buildup of the organic carbon reservoir (biosphere) prior to the Phanerozoic. Schidlowski et al. (22) and Junge et al. (118) interpreted the constancy of carbon isotopic ratios as implying a corresponding constancy in the proportions of organic and inorganic carbon in the global sedimentary reservoir. They concluded that the ratio of 1:4 between organic and inorganic carbon (typical of recent sediments) was established by 3.3 By BP and, hence, the buildup of the global biosphere must have started much earlier, possibly pre-3.8 ByBP.

In the absence of steady-state chemical balance
whereby CO₂ fixed by photosynthesis in the surface waters is returned by bacterial oxidations throughout the water column, the underlying sediments become a more or less permanent reservoir for reduced carbon. At present, the flux of organic matter to sediments in oxygenated environments is solely a function of sedimentation rates as fluctuations in the primary surface productivity are systematically compensated for by aerobic biodegradation. However, since oxygen demand relates aerobic biodegradation to surface productivity, the flux of organic matter to sediments under anoxic waters increases proportionately with primary surface productivity. Productivity is highest today in areas of prograding terrigenous coastlines and over submarine ridges where nutrients are replenished by strong upwelling ocean currents. Where the water column is relatively stable, high surface productivity can lead to the development of mid-water anoxia, inhibition of aerobic biodegradation and high organic carbon contents in sediments. Consequently, with knowledge of the accumulation rates and organic carbon contents of sediments, it becomes possible to estimate past organic sedimentation rates and, in turn, to assess the relative degrees of oxygenation and convective over-turn within paleodepositional environments.

In the modern ocean, organic carbon contents of sediments deposited under well oxygenated waters range generally from less than 0.5% to a maximum of around 4%,
regardless of the surface productivity. Organic carbon in sediments deposited under conditions of anoxia (i.e. in the Black Sea) are commonly much higher than those of their oxygenated counterparts, frequently reaching as high as 20% by weight. Available geochemical data indicate that organic carbon contents are comparable for mudstones (argillites and shales) from similar depositional environments for all ages. Archean and Proterozoic shales sampled to date average 0.7% and 1.6% organic carbon respectively (120) and, as such, are not substantially different from the Paleozoic average of 0.5% (121). From sedimentological, geochemical and micropaleontological considerations, Reiner et al. (122) attempted to assess biomass productivity in the surface waters at the time of deposition of the 3.4 By-old Barberton Mountain Land sequences, based upon sedimentation rates and organic carbon contents of the Fig Tree argillites. Making the basic assumption that organic carbon was recycled in the past with the same relative efficiency as today, they concluded that the flux of organic carbon into the Fig Tree sediments was comparable in magnitude to that in contemporary depositional analogues. In view of the similarity in organic carbon contents of mudstones of all ages, they concluded that primary photosynthetic surface productivity was continuously high throughout the Precambrian, since the early Archean, in agreement with the estimates for the buildup
of the organic carbon reservoir derived from stable carbon isotopic ratios.

The basic tenet for a biotic model for atmospheric oxygen evolution, that ferrous iron acted as a general environmental oxygen sink for photosynthetically-derived oxygen, is inconsistent with geochemical data for Precambrian sediments. In areas of proposed upwelling of ferrous-rich waters, where surface biomass productivity would be expected to have been maximized, under conditions of general anoxia, biogenic oxygen reacting with the ferrous ions could not be utilized in aerobic biodegradative processes. Hence, biogeochemical element cycles would have been anaerobic. Anaerobic biodegradation generates strong potential reductants (i.e. $H_2$, $H_2S$) which would invariably react with and reduce simple ferric species. It follows, therefore, that the extensive deposition of iron oxide sediments (BIF), as a consequence of cyanobacterial photosynthesis, would have required a minimum of aerobic biodegradation or biological oxygen consumption. In view of the relative inefficiency of anaerobic degradative processes, a necessary corollary to this must be that the deposition of biogenic iron oxides required a concomitant high flux of organic carbon to the sediments. The chert facies of iron formations frequently host paleontological evidence of a diverse cyanobacterial population. Yet, for the most part, organic carbon is inversely correlated with iron oxides.
in banded iron formations regardless of the facies, but especially in the most oxidized hematitic facies (119). The paucity of organic carbon in iron oxide sediments provides strong circumstantial evidence for aerobic biodegradation. In the modern marine environment, oxygen and carbon cycles are somewhat self-contained with at least eighty percent of the oxygen consumption through aerobic biodegradation occurring in the photic zone. Concomitant iron oxidations, particularly in sufficient quantity to form an economic iron deposit, must have required an external oxygen source. In a review of geological and geochemical evidence for Precambrian atmospheric oxygen, Dimroth and Kimberley (29) concluded that the petrographic similarity of the Isua Complex iron formations and associated sediments to younger iron formations suggests that 3.8 By BP offers a probable minimal age for the present oxygenated atmosphere.

3.12 Atmospheric, Hydrospheric and Biospheric Evolution:
Temperature Considerations

Primary deposition of iron oxide sediments (BIF) represent evidence consistent with, but not necessarily indicative of, the proposed aerobic physiology of the earliest Archean microfossils. In the contemporary hydrosphere, aerobic biodegradation of organic matter constitutes the ultimate oxygen buffer, recycling up to 97% of the photosynthetic biomass at the expense of
photosynthetically-derived oxygen. Fluctuations in the relative rates of primary surface productivity are systematically met by changes in the rates of aerobic biodegradation and, hence, oxygen consumption. Negative feedback mechanisms presently controlling the rates of biogenic oxygen production and consumption in the marine environment were probably just as effective throughout the Archean and Proterozoic eras. This alone would have placed severe restrictions on the relative contribution of photosynthesis to atmospheric oxygen levels (117). An alternative to the biotic models is that atmospheric oxygen was solely derived through abiotic photodissociation of water vapour.

What proportion of atmospheric oxygen existed in the Precambrian atmosphere depends largely upon the relative rates of abiotic production and consumption during the Hadean era. Brinkmann (30) estimated that, under current conditions, photodissociation of water vapour alone could have produced one-quarter or more of the present oxygen level. Van Valen (123) contested Brinkmann's calculations, maintaining that he should have emphasized oxidation of reduced volcanic gases over rock weathering as the prime oxygen consumptive process. Holland (124), on the other hand, supported Brinkmann's assumption, showing weathering of sulphide-rich rocks to be more important.

In support of a biotic model for oxygen evolution,
Margulis, Walker and Rambler (125) attempted to discount both the Berkner-Marshall and Brinkmann estimates for photochemical oxygen production as being 'certainly too high' on the premise that these workers erred both in neglecting oxygen consumption in reactions with reduced volcanic gases and by assuming that every photolysis reaction was followed by hydrogen escape. They argued that under past and present conditions hydrogen escape is controlled by the rate at which hydrogen compounds, primarily water vapour, are transported upwards from the lower atmosphere. Since the abundance of water vapour in the stratosphere is controlled by condensation rates at the tropopause, the photochemical production of oxygen would depend more on the temperature of the tropopause than the actual rates of photodissociation. They concluded, therefore, that, unless the primitive tropopause was warmer than the present, photochemical oxygen production in the primeval atmosphere could have been no greater than today.

In a concurrent, contradictory publication, Wàlker (6) stressed that, in fact, there is no conclusive evidence to suggest that exhalative gases forming the primordial secondary atmosphere were ever any more reducing than modern volcanic gases. In an early earth scenario, regardless of which accretionary model is applied, hot global surface temperatures were highly probable, with an atmosphere consisting predominantly of water vapour and
lesser amounts of carbon dioxide. The properties of a steam atmosphere were reviewed by Walker with emphasis on two important features relevant to photochemical oxygen production. First, since temperature in a convecting steam atmosphere would decrease very slowly with height, the absence of a tropopause or cold trap would have resulted in high water vapour contents throughout the entire atmosphere. At the outer extremities, photochemical reactions would have resulted in large scale conversion of water vapour to molecular oxygen. The second feature, concerning the duration of a steam atmosphere, relates surface temperatures and the rate of water vapour condensation leading to the formation of the hydrosphere. Condensation rates are determined by the upward energy flux and, hence, the Earth's albedo together with the flux of solar energy. Walker noted that most studies related to condensation rates in a primeval atmosphere have considered only the solar flux, neglecting the other heat sources available. Taking the opposite approach, he calculated that accretional energy alone could have maintained a steam atmosphere for one million years. Adding to this the other sources of heat liberation (radioactivity, tectonic activity, meteorite impacts, etc.) together with the solar flux, it would be considerably difficult to estimate the lifetime of a vapour atmosphere, particularly without information on the initial water vapour content. From geochemical and geological con-
siderations, however, it is safe to assume that condensation was fully realized by at least 3.8 By BP.

Studies by Hunt (126) and Walker (127) suggested that a mass of water vapour equal to that of the contemporary hydrosphere could be converted to molecular oxygen through photochemical reactions in a primitive steam atmosphere in about 35 million years. Since the actual water vapour mass, temperature and duration of the Hadean steam atmosphere will never be elucidated, and since the modern hydrosphere represents only the remnants of a condensed steam atmosphere after loss of water vapour through photolysis, it is also difficult to speculate on atmospheric oxygen levels in the past. One point, however, seems to emerge: photochemical oxygen production was probably substantially high in the early history of the Earth and, given the 700 million year time span of the Hadean era, photochemical reactions could have converted several times the current oceanic mass of water vapour to molecular oxygen before condensation of the hydrosphere was fully realized.

It has long been argued that abiotic models for oxygen evolution fail to account for the enormous amounts of oxygen transferred from the free reservoir to the bound reservoir. Van Valen (123), however, noted that since the relative rates of oxygen production and consumption were about equal throughout the Phanerozoic, the existence of continuous inorganic oxygen sinks through-
out the Precambrian presents a formidable problem. He concluded that a strong regulator is required to explain the initial rise in oxygen levels but remains to be found.

Temperature affects a wide variety of properties of the hydrosphere but of particular significance is the influence of temperature on the aqueous solubility of oxygen. For the purpose of discussion, it is assumed that the early atmospheric oxygen levels never exceeded 21%. Figure 6 represents a plot of the aqueous equilibrium saturation concentration for oxygen (from figure 2A) at the various temperatures estimated from the stable oxygen isotopic composition of marine cherts and carbonates (from figure 3). It must be acknowledged that if the sediments reflect a somewhat higher temperature than the overlying waters due to the effects of pre-consolidation burial, lower surface temperatures would effect a shift in the curve to the left. This is significant in that oxygen supply to the hydrosphere, regardless of whether it is abiotic or biological, occurs primarily in the upper zone of surface mixing.

To reiterate, early crustal evolutionary models of Shaw (5) and Hargraves (7) envisage the formation of a global ocean, 2-3 kilometers deep, marked only by the occasional emergent volcanic pile. High atmospheric vapour pressures may have initiated condensation of the steam atmosphere at surface temperatures as high as 150°C, with the current oceanic volume realized by the time
Figure 6. Equilibrium saturation concentration of oxygen in pure water (moles $O_2$/litre $H_2O$) as a function of increasing paleotemperatures and geologic time (billion years before present) with respect to an atmosphere of 21% oxygen, 100% relative humidity and an atmospheric pressure of 760 mm Hg (compiled from figures 2 and 3).
temperatures had cooled to around 100°C. The time required for complete condensation would have depended upon the Earth's past albedo which, in turn, would have been influenced by the initial water vapour content of the atmosphere. From figure 2A, it is apparent that the aqueous solubility of oxygen would be negligible at temperature near the condensation point, even under an atmosphere of pure oxygen. Hence, regardless of the duration of a steam atmosphere, once a thin film of hot water had enveloped the protocrust, an effective oxygen barrier would have existed until condensation was complete and the global ocean cooled sufficiently to allow gradual oxygenation. Apart from the possible oxidation of reduced volcanic gases, inorganic oxygen sinks would have become available only after the loss of a steam atmosphere and, hence, after maximum photochemical oxygen production. In other words, contrary to popular belief, Precambrian atmospheric evolution might in fact have witnessed progressively decreasing oxygen levels reflecting the gradual transfer of oxygen from the free reservoir to the bound reservoir as a function of hydrospheric cooling.

Stratification of oceans in terms of oxygen and temperature has long been recognized. In fact, if not for basal mixing with colder, oxygenated polar waters, modern oceans would become entirely anoxic at depth. Certain researchers have speculated that during Pre-
Cambrian times the majority of the seas were permanently stratified into a deep, non-mixing, anoxic layer, termed the monimolimnion, and an overlying oxygenated mixing layer, termed the mixolimnion, similar in many respects to the conditions found today in the Black Sea (128). Weyl (129) first postulated the existence of a thermocline and pycnocline in Precambrian oceans. He proposed that cyanobacterial adaptation above the pycnocline led to the accumulation of oxygen in the surface waters, setting the stage for the evolution of eukaryotes. An alternative to a density stratified early ocean hypothesis might be found in the temperature dependency of oxygen solubility. Horizontal expansion of an oxygen minimum layer on a global scale, as a consequence of temperature modulated rates of oxygen supply and thermocline circulation, would explain many of the observed biological and geological phenomena which characterize the Precambrian. This hypothesis does not require the complete removal of oxygen from seawater through reactions with dissolved reductants, nor does it necessitate complete anoxia in every depositional environment. The vertical position of the oxygen minimum layer would be defined by the relative rates of the upward diffusion of reductants and the downward diffusion of oxygen which, in turn, would be governed by the temperature of the surface waters. At the extreme, when the temperature of the incipient ocean was close to the condensation point
of water vapour, the oxygen minimum layer would effectively coincide with the air/water interface. Gradual oxygenation of the hydrosphere concomitant with decreasing global temperatures would have witnessed the descent of oxygen minimum layer permitting both oxidative weathering and the deposition of primary oxide sediments in shallow-water environments, and the evolution of aerobic metabolism in marine microorganisms.

Physiological adaptations permitting ecological specialization in related taxonomic groups that can be explained in terms of their physico-chemical environment can provide valuable insight into the nature of the selection pressures acting upon the population at the time of speciation. The variable response to, and metabolic requirements for, molecular oxygen in prokaryotes and, to a lesser extent, in eukaryotes are consistent with the current views that early Archean and pre-Archean life forms evolved their characteristic metabolic systems in response to rising and fluctuating environmental redox potentials. ATP synthesis through aerobic chemotrophic and phototrophic biochemical pathways represent the later enzymatic modification of anaerobic electron-transfer systems. Aerobic respiration and photosynthesis provide potentially higher energy yields which, in turn, enabled organisms to increase their physiological complexity. Gradual oxygenation of the hydrosphere appears to have been the driving force behind the evolution of oxidative
energy conservation (respiration) and oxygenic photosynthesis.

If, in fact, there is any direct evolutionary significance in the response of various microorganisms to molecular oxygen, the implications are unclear. A major error of Berkner and Marshall (14) was to emphasize the significance of the Pasteur Point, the partial pressure of oxygen at which respiration is commonly believed to become more efficient than fermentation. Although never actually assigned any units, the Pasteur Point was defined by Berkner and Marshall at about a hundredth of the present atmospheric oxygen concentration (1% PAL). Derived from the 'Pasteur effect', the Berkner-Marshall concept of the Pasteur Point arose from the fundamental work of Louis Pasteur on alcoholic fermentative yeast. Faculative eukaryotes, such as yeasts, possess the metabolic capacity to function either in the presence or absence of oxygen. Glycolysis refers to the facultative anaerobic fermentative pathway in eukaryotes. While there is no 'a priori' reasons why glycolysis should not function normally while respiration is taking place, there is an unexpected inhibition of glycolysis termed the Pasteur effect. This phenomenon is poorly understood, complicated further by what is termed the 'Crabtree effect' whereby, under certain conditions, glycolysis continues and may be even stimulated when oxygen is introduced to the system. The Pasteur effect is general,
with the switch over from glycolysis to respiration occurring over a wide range of ambient oxygen levels for different organisms. Moreover, the oxygen threshold discovered by Pasteur for a specific group of yeasts was probably defined for a fixed medium over a very limited temperature range. Regardless of the selection pressures motivating the evolution of aerobic respiration after glycolysis (or fermentation), it can be safely stated that the levels of oxygen in the biological system need never have been directly related to the Precambrian atmospheric oxygen concentrations. The fact that facultatively anaerobic eukaryotes thrive in anoxicogenic niches today is evidence enough to suggest that Berkner and Marshall overstepped the limits for scientific deduction in applying poorly understood biological phenomena to atmospheric evolutionary studies.

Comparative biochemistry of prokaryotes and eukaryotes provides compelling evidence that the latter group underwent rapid physiological diversification only after environmental oxygen levels were significantly high and stable. In the area of metabolism, cyanobacteria occupy an intermediate position, optimally adapted to an aerobic lifestyle but retaining the physiological capacity to function anaerobically. Physiological experiments on oxygen inhibition of photosynthesis, respiration and nitrogen fixation in the heterocystous cyanobacterium, *Anabaena flos-aquae*, suggest that this organism is optim-
ally adapted to an ambient oxygen concentration of 10% or roughly 50% PAL (figure 7). Yet, if this response to oxygen has any evolutionary significance, as proposed by Schopf (2), it is inconsistent with the basic tenet for a cyanobacterial origin for atmospheric oxygen. In fact, this cyanobacterium is optimally adapted to an ambient oxygen level fifty times greater than that required to effect the switchover from glycolysis to respiration in eukaryotes (Pasteur Point).

In a series of detailed experiments, Brock (20) demonstrated that the addition of inorganic and organic reductants to the aqueous media augmented oxygenic photosynthesis in the cyanobacterium *Phormidium*. While high concentrations proved inhibitory, low concentrations stimulated photosynthesis leading Brock to conclude that the enhancement was more likely the effect of decreased extracellular redox potentials rather than specific effects of the reductants on photosynthesis. Redox potentials in oxic environments are invariably contingent upon the ambient oxygen concentration. It follows that, if Brock's conclusions are correct, the data presented by Schopf (2) need not necessarily reflect oxygen inhibition in cyanobacteria but rather the optimal adaptation of the metabolic systems to lower environmental Eh.

Precambrian life arose and evolved within the hydrosphere and, as such, was never in direct contact with the atmosphere for prolonged periods. Precambrian bio-
Figure 7. Oxygen inhibition of metabolic functions in the heterocystous cyanobacterium *Anabaena flos-aquae* (after Schopf (2)).
spheric evolutionary models are essentially incorrect not only in the inherent assumption that life evolved in waters saturated with oxygen at any given atmospheric oxygen concentration but, more importantly, that the aqueous equilibrium saturation concentrations were similar to those observed in the modern oceans. Aqueous equilibrium saturation concentrations for oxygen decrease with increasing temperature and, to a lesser degree, salinity. Moreover, even in the cooler modern ocean where oxygen solubility is high, saturation is maintained only within the upper zone of physical mixing where oxygen supply exceeds biological demand.

Thermophilic microorganisms have been reported from the sea floor at a depth of 1310 metres where the temperature is less than 10°C. One explanation of these data envisions a warm primitive ocean inhabited by thermophilic organisms which persisted to the present era (31). Currently popular models of early hydrospheric development imply that as the global ocean (or oceans) cooled from temperatures in excess of 100°C to the present average of about 15°C, life evolved from one or more primitive anaerobic heterotrophic bacterial lines to that which characterizes the complex modern biosphere. Temperature appears to be the single most important environmental factor influencing biospheric evolution, regulating the metabolic activity of the global biomass by governing the nature and supply of the gaseous metabolites.
The influence of temperature on biospheric evolution can be studied both through the upper temperature limits for growth of different taxonomic groups and through the effects of temperature within individual taxonomic groups. It has long been recognized that the upper thermal limit for growth decreases with increase in physiological complexity. Multicellular plants and animals, in general, do not grow at temperatures above 50°C whereas unicellular protozoa, fungi and algae can tolerate temperatures up to 60°C. Cyanobacteria and photosynthetic bacteria cannot function normally above temperatures of 70-73°C. Only the non-pigmented, simple chemolithotrophic and heterotrophic bacteria can thrive at temperatures in excess of 90°C (20). In other words, while 50°C is the upper temperature limit for metazoa, 60°C marks the 'fundamental phylogenetic dichotomy' between prokaryotes and eukaryotes.

The occurrence of oxygen-mediating enzymes in obligate anaerobic bacteria (46, 130), which are clearly not later adaptations, is difficult to explain in terms of evolutionary development under global reducing conditions. However, it is consistent with the concept of an early oxidizing atmosphere whereby vertical mixing of oxygenated surface waters resulted in a periodic purge of the anaerobic system with free oxygen. In contrast with the seasonal oxygen-induced mass mortality of anaerobes in the Black Sea, the effect of vertical mixing in the early
Precambrian seas was probably moderated by the initial low solubility of oxygen and the limited depth of penetration of the surface waters. An early benthic habitat for non-pigmented anaerobic bacteria, as previously discussed, is possibly evidenced in the physiological response of sulphate reducing bacteria to pressure. Maximum growth temperatures for sulphate reducers were reported by Zobell (131) to increase with an increase in pressure. He also demonstrated that the rate of sulphate reduction in a strain of Desulfovibrio (accustomed to atmospheric pressure) underwent a four-fold increase at pressures up to 1 kb. Similar effects of pressure were noted by Trudinger et al. (132) for the genus Desulfitomaculum.

While no single factor determines the upper temperature limit for growth within any taxonomic group, the fact that photosynthetic organisms are restricted to temperatures below 75°C led Brock (20) to conclude that their upper temperature limit for growth was probably defined by the thermal sensitivity of the photosystem. He further suggested that the absence of photosynthesizers above 70-73°C might reflect the organism's inability to develop a photosystem that is both functional and stable at high temperatures. Regardless of the reasons behind the physiological limitations of photoautotrophs, from a paleontological viewpoint, it follows that photosynthetic bacteria and cyanobacteria probably did not
appear until that point in time when the temperature of the surface waters cooled to below 75°C.

Recent studies by Walter et al. (133) and Brock (2) demonstrated that organisms tend to colonize the surface of a hot-spring only where the waters are less than approximately 80°C. The upper temperature limit for growth of microbial mats is about 70°C. However columnar forms (stromatolites) do not occur above 60°C, the temperature around which microbial mats attain their maximum thickness. The oldest evidence of photosynthetic activity for which there are temperature data is from the 3.4 By-old fossiliferous Onverwacht argillaceous cherts. Maximum temperatures of the depositional waters estimated from stable oxygen isotopic ratios were around 70°C (25). The oldest columnar stromatolites, to date, occur in the 3.5 By-old North Pole sediments. Stable oxygen isotopic ratios for barites from the North Pole sediments fall into the range of low isotopic values for barites from the top of the Onverwacht Group (134), possibly indicating similar water temperatures at the time of crystallization. While paleotemperature data do not confirm a cyanobacterial origin for the stromatolites and microfossils, nevertheless, they do not preclude the possibility. Moreover, since the silicification of the Onverwacht and North Pole sediments appears to have been early diagenetic in origin, it is highly likely that the overlying waters were somewhat cooler (possibly by as much as 10°C).
On the premise that physiological diversification and speciation within a single taxonomic group paralleled decreasing global surface temperatures as a function of time, an alternative explanation for the thermal limitations of photosynthesis could be simply that sufficient selection pressure did not exist for a long enough period of time to force photoautotrophs to extend the temperature limit of their photosystem beyond that which characterized the photic zone at the time of their emergence. The relationship between species diversity and temperature within the cyanobacteria was illustrated by Brock (2) whereby, as temperature increased, the population structure became progressively simpler with a marked decrease in the number of species above 60°C. From an evolutionary viewpoint, Brock remarked that the dramatic decrease in species diversity above 60°C is difficult to explain. However, since cyanobacterial metabolism is inhibited by low redox potentials (low ambient oxygen levels below 50% PAL), one explanation may reside in the coincidence that the equilibrium saturation concentration of oxygen (at 21% atmospheric concentration) at 60°C is roughly half the saturation concentration at 15°C (from figure 2A). Consequently, while anaerobic photosynthesis in both cyanobacteria and bacteria might have developed when the mean ocean surface temperatures were 70-73°C, the tentative suggestion is that oxygenic photosystems were not perfected until
temperatures of the photic zone cooled to around 60°C. From figure 6, it is apparent that cyanobacteria may have evolved their characteristic aerobic metabolisms at least by 3.0 By BP. If the surface waters were slightly cooler and/or the atmospheric oxygen concentrations slightly higher in the early Precambrian than depicted, the net result would make the age for cyanobacterial oxygenic photosynthesis earlier, possibly pre-3.5 By BP, which would still be consistent with geological and paleontological observations.

Eukaryotes cannot grow at temperatures much in excess of 60°C. Brock (20) contended that their incapacity to grow at higher temperatures may reside in the inability to form organellar membranes that are both thermostable and functional. Hence, as with the cyanobacteria, the upper thermal limit for growth might reflect, in part, an oxygen threshold defined by the equilibrium saturation concentration, as well as insufficient motivation to develop a thermostable metabolism.

In evaluating paleontological data, the aspects of habitat suitability and ecological competition among various groups of organisms must be considered. The Gunflint cherts host the oldest, well-documented occurrence of structurally preserved microfossils. Awramik and Barghoorn (135) indicated that at least thirty taxonomic entities have been described from the Gunflint. Of
the thirty, they accepted sixteen as being of undoubted
biological affinities and taxonomic assignment. In view
of their earlier work on permineralization and cellular
degradation, they concluded that the Gunflint microbiota
was entirely prokaryotic. Stable oxygen isotopic ratios
for the Gunflint cherts reflect a temperature around
38°C (24). Brock (20) noted that today eukaryotic algae
are uncommon at temperatures above 40°C largely as a
consequence of their inability to compete with cyano-
bacteria for essential nutrients. Hence, from an evolu-
tionary viewpoint, the presence of cyanobacteria, column-
ar stromatolites, primary iron oxidation and low water
temperatures in the Gunflint depositional environment are
consistent with, but not necessarily indicative of, the
proposed eukaryotic existence 2.0 billion years ago
(refer to section 3.10).

3.13 Precambrian Sedimentology and Crustal Emergence:
Implications for Biospheric Evolution

Biological and biochemical studies tell much about
early Precambrian biospheric evolution but only the sedi-
ments of that age can provide essential clues to the
nature of the physico-chemical motivations behind global
evolutionary development. The Precambrian mass of sedi-
mentary rocks, spanning some three billion years, is
approximately equal to the mass of Phanerozoic sediments
deposited over the last 600 million years (38). The major
part of the Precambrian sedimentary record is preserved in younger Proterozoic intracratonic basins or basins marginal to Archean shield areas (93). Archean sedimentary rocks, for the most part, are characterized by deep-water turbidite deposition in dominantly volcanogenic terrains. Archean shallow-water sediments are relatively uncommon and generally characterized by siliceous banded iron formations. The apparent scarcity of Archean carbonates and evaporites together with the relatively abrupt appearance of extensive early Proterozoic carbonate and red bed sequences constitutes one of the major enigmas of the Precambrian sedimentary record and presents a two-fold problem to the sedimentologist. First, it is necessary to explain the apparently weighted percentage distribution of chemical and terrigenous sediments throughout the Precambrian with the sudden appearance of extensive carbonate sequences in the Proterozoic. Secondly, it is necessary to explain observed elemental trends in the sedimentary carbonates as a function of age.

There have been a number of explanations proposed for the weighted distribution of Precambrian sediments, one of the more popular involving the continuous recycling of sedimentary rocks through erosion, metamorphism and crustal plate thickening (38). While this adequately explains the preponderence of Phanerozoic sediments, it fails to account for the low percentage of carbonates and evaporites in relatively unmetamorphosed
Archean and Proterozoic terrains where there is no evidence for differential cycling of sediments. Yet, although carbonates are volumetrically minor components of Archean sedimentary sequences, they have been documented together with evidence for evaporite formation in every major shield area, regardless of age. To date, the oldest record of evaporite formation is found associated with the oldest stromatolites in silicified, shallow-water carbonates of the 3.5 By-old North Pole sedimentary sequence in Australia (16, 17, 134).

Sidereenko (136) first noted the fundamental similarities between sedimentary processes of the Precambrian and those of the Phanerozoic. Dimroth and Kimberley (29), employing a uniformitarian approach to Precambrian sedimentary rock interpretation, emphasized that no evidence exists to support earlier evolutionary models proposing "orders-of-magnitude changes in average atmospheric or hydrospheric abundances of chemically reactive species". Variations in sediments and elemental distributions with time can be adequately explained by variations in depositional environment without the need to invoke unique or unusual global chemistries. In a subsequent paper, Kimberley (137) indicated that chronological variations in the relative abundance among major environmental types of iron formations supports an Archean to Phanerozoic tectonic-magmatic evolution from abundant shallow-water volcanic platforms through predominantly
non-volcanic platforms or continental shelves to abundant inland seas. Broadly speaking, the conclusions of Kimberley, and Dimroth and Kimberley are consistent with the aforementioned early crustal development model proposed by Shaw (5).

On the premise that Archean and Proterozoic seas were chemically homologous to the present, Cameron and Baumann (138) considered the crucial factor controlling Archean carbonate deposition to be the absence of 'stable shelf on miogeosynclinal environments'. While small amounts of carbonate sediments accumulated in local areas of tectonic stability, carbonate sedimentation was largely confined to abyssal regions to be obscured by dilution with terrigenous materials or perhaps to be re-dissolved below the carbonate compensation depth. If such was the case, a substantial proportion of the calcium from Archean seas was probably reabsorbed and recycled through subductive processes.

In spite of the general similarities between Precambrian and Phanerozoic carbonate sedimentation, one cannot neglect observed chemical trends and changes in the relative proportion of siliceous and carbonate sediments with age. Chilingar (139) and Laasko (140) noted a progressive decrease in the Ca/Mg ratio of carbonates with increasing age from North America. Vinogradov et al. (141) confirmed the trend for carbonate rocks from the Russian Platform. Two classes of explanations have
been offered to account for the increase in magnesium with age. The first includes those which call for progressive dolomitization of primary calcium carbonate as a function of time. Recent studies have shown that virtually all sedimentary dolomites are the product of magnesium metasomatism of calcium carbonate, regardless of whether they are penecontemporaneous, diagenetic or epi-
genetic in origin. There is no question that post-
depositional episodes of dolomitization must have left an overprint on the sedimentary carbonate record. Yet, progressive dolomitization as a function of time fails to account for the magnesium deficient limestones of the Proterozoic in Rhodesia, Karelia and Ontario, and for the magnesium deficient limestones within the Archean mixed assemblage of limestones, dolomitic cherts, sil-
aceous dolomites and dolomitic limestones of the Swaziland Sequence, South Africa.

The second class of explanations includes those which call for higher temperatures and magnesium concent-
trations (relative to calcium) and lower pH and bicarbon-
ate levels in the Precambrian seas which collectively favour penecontemporaneous dolomite formation. In view of the negative temperature coefficient of carbon dioxide solubility, higher temperatures would invariably affect pH, bicarbonate levels and carbonate solubility. There is moderately good correlation between stable oxygen isotopic ratios, Ca/Mg ratios and age of carbonate
rocks (38) supporting the view that temperature played a
critical role in the dolomitization process, Laasko
(140) indicated that the highest fluctuations in the Ca/
Mg ratios coincided with major orogenic events and, hence,
continental denudation. Lebedev (142) cited variations
in the relative proportions of Ca and Mg derived from
continents as a function of the character of weathering.
It is not inconceivable that, in the absence of large
stable land masses, the relative proportions of Ca and
Mg derived from the weathering of dominantly volcanic
terrains was significantly different from the present.
This 'isochronal' approach to the interpretation of Ca/
Mg ratios does not require that all primary Archean carbonates be dolomitic for both the temperature and initial
Ca/Mg ratio of depositional waters are subject to environ-
mental variation, particularly those characterized by ineffective mixing with the open ocean. In other words,
conditions favouring penecontemporaneous dolomitization
were simply more prevalent in the past than the present.

In general, the chert or silica content of marine
carbonates increase with age following the trend for
magnesium. However, an average silica content, particu-
larly for the Archean, cannot be quantified due to the
willingness of some to include the chert-carbonate
facies of banded iron formations which are demonstrably
volcanogenic in origin. Chemical coprecipitation of
silica and carbonate was advocated by Holland (37) and
Cloud (13). To explain primary chert deposition, they invoked an ocean saturated with respect to silica. Yet, as noted by Bridgewater and Fyfe (143), had the oceans been at or close to saturation with silica, there would have been a pronounced change in the chemistry of undersaturated Archean spilitic basalts. This has not been observed.

In a study on Phanerozoic chert-carbonate association, Chilingar (144) observed that the chert content of limestone decreased with an increase in Mg content. He credited the inverse correlation of chert and Mg to concomitant variations in temperature and pH of the depositional waters favouring dolomitization over silica precipitation. It follows that since a primary or penecontemporaneous origin for dolomite can be rationalized for the early Precambrian, a secondary, early diagenetic origin for the silicification appears to be the more likely possibility.

Particular importance of the extensive carbonate and red bed sequences to early biospheric evolution lies in the fact that broadly speaking their appearance coincides both chronologically and geographically with the habitation of stable shelf and platform environments by a diverse population of photosynthetic planktonic organisms. Early crustal development models imply that decreasing geothermal gradients favoured continental emergence concurrent with decreasing surface temperatures as
a consequence of progressive global thermal decay. Implications of continental emergence for evolution of planktonic communities were considered by Chamberlain and Marland (145). In particular, they emphasized that without large scale land masses to aid in the upwelling of nutrient-rich waters, average global primary productivity probably would have closely corresponded to the low productivity of the modern open ocean. With continental emergence and the gradual stabilization of shelf and platform environments, shoaling of nutrient-rich waters promoted the migration of open ocean planktonic algae into shallow water habitats with a concomitant increase in biomass productivity and species diversity. Decreasing surface-water temperatures and large-scale convective overturn coinciding with the establishment of widespread shallow-water environments would, in turn, have accelerated oxygenation of the hydrosphere, particularly in the proximity of the stable land masses, as evidenced by the sudden appearance of continental red bed sediments.

3.14 Concluding Remarks

To summarize, biological and biochemical studies indicate an early origin for the integrated anaerobic carbon-sulphur cycle supported by anaerobic photosynthesis. Gradual oxygenation of the hydrosphere resulted in the depletion of reduced metabolites and the forced evolution of oxygen-mediating enzymes and aerobic meta-
bolic systems. Paleontological and geochemical studies confirm that the transition to a self-contained, integrated carbon-sulphur-oxygen cycle was locally established at least by 3.4-3.5 by BP. At this point in time, physico-chemical conditions in the open ocean environment appear to have been able to support eukaryotic (or proto-eukaryotic) organisms. Decreasing global surface temperatures effecting an increase in the aqueous solubility of oxygen led to an increase in the physiological complexity and degree of speciation within a variety of taxonomic groups, particularly algae and cyanobacteria. The opening up of stable shelf environments concurrent with decreasing global temperature promoted the gradual takeover of exotic shallow-water cyanobacterial habitats by eukaryotic algae.

In his review on early biospheric evolution, Cloud (4) posed the question "what is the biogeochemical consequence of biospheric evolution?" (pg. 353). However, it is evident that if we are ever to unravel the mysteries of evolutionary change, regardless of whether it is biological, physical or chemical in nature, we must attempt to define the motivation behind the change. In other words, to understand the evolution of life, we must first ask - what are the biospheric consequences of biogeochemical evolution?

"There is nothing permanent except change."

Heraclitus (530-470 B.C.)
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CHAPTER 4

DIAGENESIS OF BACTERIALLY-COMPLEXED METALS: THE FORMATION OF METAL-PHOSPHATES, METAL-SULPHIDES AND METAL-ORGANIC CONDENSATES IN SEDIMENTS

4.1 Introduction

Substantial concentrations of soluble organic compounds and insoluble metal-complexed organic residues (kerogen) are associated with a broad spectrum of economic stratiform ore deposits of early Precambrian and later ages (1). Similarly, substantial enrichments of a wide variety of trace metals are associated with the organic fraction of many, if not most, sediments and sedimentary rocks so far studied (2-8). However, in spite of a decade of intensive multidisciplinary research focussed on the correlation between the organic carbon and trace metal content in sediments, little is known about the actual role of living organisms and their derivatives as direct metal-accumulating agents in sediments, largely because of the difficulty in isolating any specific fraction for study. Even less is known about the stability of organically-complexed metals in sediments.
as a function of increasing pressure, temperature and time. Excluding the metal-porphyrin complexes, the actual molecular form in which the metals occur in organic-rich sediments and sedimentary rocks, in most cases, is unknown (9). In this section, artificial diagenesis experiments using metal-enriched bacterial cells are described. Probable interrelationships between inorganic and organic metal-complexes in contact with confined pore waters during anadiagenesis, or more specifically, during the decarboxylation stage of organic diagenesis are described.

It has long been recognized that microorganisms possess the ability for intra- and extracellular metal accumulation, often against enormous concentration gradients, to satisfy specific physiological requirements (10-15) and/or to isolate and sequester potentially-toxic metals (16-22). Enrichment factors as high as $10^6$ have been reported for microbial accumulation of metals from seawater (23). However, the limitations of this direct bioaccumulation mechanism in nature are poorly defined and little quantitative data exist on the specific binding processes and binding capacities of aquatic microorganisms relative to other common organic constituents of natural waters. Furthermore, taking into consideration the number of variables involved in metal-organic complexing, the applicability in nature of much of the currently-available data is questionable owing to the simplicity of
the systems studied.

All microorganisms require for growth a number of trace metals which must be drawn in contact with, and across, the cell wall into the cytoplasm. The microbial cell wall represents a highly organized assemblage of complex organic polymers essential for both nutrient uptake and the protection of the organism from physical damage and osmotic disruption. The walls of eukaryotic cells derive their tensile strength largely from cellulose and other related complex polysaccharides (or from chitin in fungi), whereas prokaryotic cells rely upon mucopolysaccharides - complex polymers composed essentially of amino acids, amino sugars, their derivatives (i.e. muramic acid) and highly phosphorylated polymers (i.e. teichoic acid). Both muramic acid and amino sugars are absent from eukaryotic walls (24).

More important than the structural components of cell walls are their functional group content (carboxyl, hydroxyl, phosphate, etc.) for it is the nature, concentration and degree of dissociation of free functional groups that determines the reactivity of the wall towards metal ions. Cell walls, as envisioned by James (25), act as a series of merging ionic shells, the outermost layer being an ionic atmosphere held in place by ionic groups within the wall. The attractive interaction of metal ions with reactive cell walls, in general, may range from weak forces (physical adsorption) leaving the ions easily
replaceable, to strong forces (chemisorption or specific adsorption) indistinguishable from chemical bonds (9).

Metal accumulation by microorganisms generally comprises two distinct phases, the first representing a rapid, but limited, non-specific, often easily reversible, energy- and temperature-independent, pH-dependent binding of metal ions to the cell wall surface. The second phase involves a slower, selective, commonly irreversible, metabolism- and/or temperature-dependent translocation of the metal ions into and across the cell wall, followed by intracellular accommodation. Both phases of metal accumulation have been described for essentially non-toxic and potentially-toxic metals in fungi (26-29), algae (30-32) and bacteria (33-35).

Increasing concern over heavy-metal toxicity in nature has led to increased research activity in order to define the relationships between metal binding, accommodation, tolerance and toxicity. As yet metal tolerance in microorganisms is poorly understood and few generalities can be addressed herein. Marine microorganisms have been reported to be less susceptible to heavy metals than fresh water varieties (18, 19), and anaerobic growth appears to be more sensitive to metals than aerobic growth (36, 37). Some authors have suggested that metal tolerance may not necessarily be a deciding factor in microbial activity in nature if the rate of inorganic precipitation of the metals exceeded their rate
of supply. Temple and Le Roux (38), for example, empha-
sized that natural copper toxicity to sulphate-reducing
bacteria would occur only if the rate of supply of copper
ions exceeded the rate of biological hydrogen-sulphide
production, owing to the insoluble nature of copper sul-
phides. However, a single heavy metal (such as copper)
is rarely dominant in natural waters and the few studies
concerned with multi-metal systems (39, 40) appear to
support the opinion of Wong and Beaver (41) that natural
heavy-metal mixtures would, more commonly than not, re-
sult in an overall decrease in the degree of metal toler-
ance in aquatic microorganisms. The maximum tolerable
metal concentration for any particular organism is, in
many cases, unrelated to the actual concentration of the
metal in the environment due to a number of synergistic
and antagonistic effects by chemical variables (Eh, pH),
other metal ions, and other organic and inorganic chelat-
ing agents.

Natural bioaccumulation of potentially-toxic heavy
metals by a mixed assemblage of microorganisms was de-
scribed by Thomas (42) for a scum developed in a settling
pond enriched in Zn, Pb and Cd (37). Generally speaking,
in such a situation it is safe to assume that as long as
the organisms are viable, they are able to actively iso-
late or assimilate the non-essential metals. Heavy metals
can prove toxic to microorganisms by binding with, and
deactivating, functional groups in the cell wall con-
cerned with nutrient uptake and by entering into the cytoplasm and interfering with normal metabolic activity (43, 44). It follows, therefore, that inherent in any concept of metal tolerance is an energy-dependent detoxification mechanism by which an organism can safely sequester or expel toxic metals. It also follows that the absence, taxation and deactivation of such a mechanism, in the presence of heavy metals, should result in the modification; inhibition or cessation of growth.

Generally, under ideal conditions, heavy-metal accumulation during the metabolism-dependent second phase of intracellular accommodation far exceeds the concentrations of metals taken up during the initial phase of surface binding in microorganisms (26-32). However, high intracellular metal concentrations are contingent upon the organism's ability to evade toxicity and hence upon its survival. In addition, to be geologically important, the resultant metal-organic complexes must be sufficiently stable to survive the death and partial decay of the organism (9). Of biogeochemical significance is the fact that the energy- and temperature-independent initial phase persists beyond the death of an organism and, in many cases, is enhanced by post-mortem changes in the structure and chemistry of the cell as a whole. Goldberg and Arrhenius (45) first reported that organic matter arising from the death of aquatic microorganisms retained the ability to accumulate metals through ion adsorption and
exchange reactions. This was substantiated by the work of Ferguson and Bubela (46) from which they concluded that sorption of Cu, Zn, and Pb onto dead, non-decomposed particulate algal matter provided a feasible mechanism for the incorporation and accumulation of these metals in sediments. Similar observations of post-mortem cellular metal-uptake were made by Engle and Owen (47) for four groups of bacteria. They reported that, for a number of metals studied, uptake values for dead cells were as much as fifty percent greater than for living cells.

In nature, the concepts of primary biomass productivity, carbon cycling, organic matter preservation, organic diagenesis, sediment diagenesis, and biogeochemical element cycling and accumulation are inseparable. The depositional environment, nature of organic compounds, microbiological and chemical alterations, and pore water chemistry all play important roles in the fixation and diagenesis of organically-complexed metals in sediments. From the point of view of organic diagenesis, the positive correlation between organic carbon content and trace metal contents in sediments is neither an unusual nor unexpected phenomenon in that it reflects the degree of resistance of inert metal-organic complexes to further biodegradation and, to some extent, chemical and thermal degradation. Also, as suggested by Degens and Mopper (48), the enhanced preservation of organic matter in some sediments may reflect the deactivation of anaerobic degradation
processes through natural heavy-metal staining of bacterial cell walls under conditions of low pH and high metal ion concentrations associated with post-deposition-al pore-water anoxia.

Studies into the efficiency of biomass recycling in the open oceans have shown that the flux of particulate organic matter to the bottom sediments represents less than ten percent of the primary photosynthetic surface productivity (48). Aerobic and anaerobic bacterial respirations, at and below the sediment/water interface, accomplish the further combustion of organic matter to the extent that the quantity of organic carbon permanently trapped in sediments represents generally less than one percent of the surface productivity (48). During the bacterial oxidations nearly a third of the organic carbon is assimilated by bacteria to synthesize new cellular material which in turn can become the substrates for bacterial enzymatic attack upon death of the bacteria (49). Adding to this the poorly-understood contribution of organic carbon by non-photosynthetic, chemoautotrophic bacteria (ie. the sulphur oxidizers), it becomes readily apparent that the living bacterial population can, and probably does, represent a significant fraction of the total organic carbon content in sediments.

The ability of organic compounds to scavenge trace metals from surface waters is well known (50-54). In addition, studies have shown that the presence of organic
chelators in natural waters imparts a profound modifying influence on the solubility, mobilization and fixation of trace metals (55, 56). However, below the sediment/water interface, the nature of the metal-organic interactions is, in most cases, uncertain. In order to understand the role of living and non-living organic matter in sediments it is imperative to have clear knowledge of the nature of metal-organic, mineral-organic and metal-mineral interactions, the stability of the resultant metal complexes, and the adsorption-desorption behavior of both the organic ligands and metal ions. To date, most of the available quantitative data concerning metal-organic interactions relate to the metal-complexing ability of the heterogeneous class of humic substances. The adsorption-desorption behavior of any particular metal is, directly or indirectly, a function of the relative binding strengths of competing anionic ligands. Irving and Williams (57) published a stability order for complexing of divalent metals which applied independent of the ligand type (Pb>Cu>Ni>Co>Zn>Cd>Fe>Mn>Mg). Perrin (58) pointed out, however, that in any system the nature and concentration of metal complexes will be determined by pH, $pK_a$ values of the complexing ligands, total concentration of each metal ion and complexing ligand, and the stability constants of all possible metal complexes. Stabilities in turn are affected by temperature, and conditional stability constants can vary with ionic strength (59). Consequently, the
Irving-Williams stability sequence cannot be expected to hold true for natural multi-ligand systems typical of organic materials: a fact that is borne out by the divergent stability sequences published for divalent-metal uptake by soil humus, aqua humus, humic acids and fulvic acids (60-64).

The importance of humic matter in sediments has been speculated upon by a number of authors (65-67), but it is the masterly review of Jackson et al. (59) that best lends perspective to the complexities encountered in the study of natural metal-organic interactions. Humic matter in general can be divided into three components: base-soluble humic acid, acid- and base-soluble fulvic acid and insoluble humins. Humic and fulvic acids, as described by Jackson et al., are complex, high but variable molecular-weight, weak acid polyelectrolytes of poorly defined structure and containing essentially carboxyl, phenolic and alcoholic hydroxyl, methoxyl, carbonyl and quinone functional groups. They differ from each other in that the fulvic acids are generally of a lower molecular-weight and contain considerably more acidic functional groups, particularly carboxyl, than the humic acids.

Inert metal-complexed humic matter is considered to represent the end products of intense, non-synchronized bacterial oxidations of organic compounds in oxic environments (48). Aqueous humic matter tends to be highly aliphatic, less condensed, poorer in phenolic hydroxyl groups
but richer in carboxyl and carbonyl groups than soil humus (59). Of the functional groups present in humic matter, it is generally agreed that the easily-ionizable groups (particularly carboxyl and phenolic hydroxyl) control the reactivity of the material towards metal ions (59). The affinity of certain metals for specific organic ligands has been reviewed by Saxby (9) and Jernelov and Martin (68). In general, small, complex, highly electropositive metal ions having both large positive charge or oxidation state and low polarizability (i.e. divalent Ca, Mg, Mn, UO$_2^-$, trivalent Fe, Co) preferentially form strong electrovalent bonds with carboxyl, hydroxyl, phosphate and amino functional groups. Large metal ions characterized by low electropositivity, high polarizability, small positive charge and low oxidation numbers (i.e. divalent Cu, Fe, Pb, Zn, Hg, Ag, Co, Ni) preferentially form covalent or covalent-like bonds with sulphur ligands. The high affinity of the chalcophile elements for sulphur ligand binding would, in part, explain the observed discrepancies between the Irving-Williams stability series and published stability data. It would also explain the observations of Baker (69), where the percolation of hydrogen-sulphide through artificial seawater caused a marked decrease in the stability of copper humates and fulvates (copper salts of the organic acids). Similarly, Pauli (70) found that in the presence of hydrogen-sulphide more than sixty percent of
all metal humates studied (Cu, Zn, Ni, Pb, Cd) were transformed to metal sulphides.

Metal-organic (71-74) and mineral-organic (75-78) interactions with the ionogenic functional groups of bacterial cell walls have been adequately demonstrated. The preservation of 'fossilized' bacterial cell wall fragments as metal-organic complexes in the 7000-year-old sediments of the Black Sea (79) provides tangible evidence for the natural uptake and retention of heavy metals by bacterial walls in anoxic environments. Using cell walls of the ubiquitous, gram-positive bacterium, *Bacillus subtilis*, as a model for studying the interaction of metal ions with anionic wall components, Beveridge (80) and Beveridge and Murray (81, 82) chose forty different metals to demonstrate the ability of cell walls to fix substantial quantities of metal ions from relatively dilute solutions. By chemically modifying reactive sites in the cell wall, they also showed that the interaction of metal ions with the wall of *B. subtilis*, like some humic compounds, resulted for the most part from the ionization of free carboxyl groups interspersed throughout the network of partially-crosslinked and interwoven linear, homo- and heteropolymers constituting the wall fabric. For a number of metals studied, they observed that the binding process appeared to be at least two steps: firstly, the interaction between metal ions and carboxyl groups within the cell wall, followed secondly by an inorganic precipi-
tation leading to the accumulation of larger quantities of the metals.

The preservation of bacterial cells after a population becomes static depends largely upon post-mortem changes in the permeability of the cell wall and whether or not the bacteria possess autolysins (self-dissolving enzymes). If such enzymes are present, and they are not inhibited by heavy metal interactions, the cells will rapidly lose their morphology and disappear (49). On the other hand, if they are absent or ineffective, the cells can persist for an indefinite period subject to biodegradation by other bacteria or chemical attack.

In sediments and sedimentary rocks, the isolation of bacterial cells from the non-descript condensed humic matter is a difficult task owing to their lack of definitive external morphology and generally poorer degree of preservation (relative to higher organisms). To date, few studies have documented the presence of 'bona fide' fossil bacteria in either consolidated or unconsolidated sediments. It can be argued (and rightly so) that the unique preservation of bacterial wall fragments in the Black Sea sapropels is but a fortuitous event reflecting a rare set of physico-chemical diagenetic conditions. Alternatively, it is conceivable that the apparent paucity of fossil bacteria in the literature may reflect the high degree of resolution required, but seldom attained, in the visual study of organic-rich sediments.
Bacteria are among the most interesting and least studied potential organic metal-accumulating agents in sediments due to their widespread occurrence and generally high affinity for metals. In this work, metal-enriched cells of Bacillus subtilis were used specifically to complement the model study of Beveridge and Murray, in an attempt to ascertain the relative stability of the cellular constituents and complexed metals as a function of Eh, pH and time at elevated temperature and pressure. In addition, the fact that metal-binding in the walls of B. subtilis is dominated by carboxyl ligand interactions should enable predictions to be made on the relative stability of other natural metal-carboxyl ligand complexes such as fulvates and aqueous humus.

The metals U, Cu, Zn, and Fe used in this study were chosen for a variety of reasons. Firstly, they have high affinity for cell wall binding as evidenced in their uptake values (80, 81). Secondly, they demonstrate differences in relative mobility under conditions of low Eh and pH. Thirdly, they respond differently towards hydrogen-sulphide (considered by the author as perhaps the most important inorganic chelating agent of chalcophile elements in the depositional environment). Lastly, and most importantly, they are prevalent in organic-rich sediments, sedimentary rocks and stratiform ore deposits.
4.2 Materials and Methods

4.2.1 Preparation of Bacterial Cells

Metal-loaded bacterial cells were prepared for this study by Dr. T. J. Beveridge at the Department of Microbiology and Immunology, U.W.O., following his methodology outlined in (80). Whole cells of Bacillus subtilis Margurg (U.W.O. coll. no. 1032) were incubated in dilute (5 mM) solutions of FeCl₂ · 6H₂O, CuCl₂ · 2H₂O, ZnCl₂, and UO₂(C₃H₃O₂)₂ (0.5 mg dry wt. cells/ml. metal sol.) at 22°C for 10 minutes, filtered, washed in deionized, distilled water and freeze-dried under vacuum. For each metal the location and relative efficiency of cellular uptake was checked and recorded by transmission electron microscopy (unstained thin section) using the sorbed metals as the sole electron scattering agent. The relative efficiency of uptake onto the cell walls of whole cells appeared consistent with the uptake values for B. subtilis wall fragments listed below.

<table>
<thead>
<tr>
<th>Metal</th>
<th>ug/mg (dry wt.)</th>
<th>Wt.% (dry) wall</th>
<th>approx. Wt.% (dry) whole cell*</th>
</tr>
</thead>
<tbody>
<tr>
<td>U⁶⁺</td>
<td>1300</td>
<td>130</td>
<td>26</td>
</tr>
<tr>
<td>Fe²⁺</td>
<td>200</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>Cu²⁺</td>
<td>190</td>
<td>19</td>
<td>3.8</td>
</tr>
<tr>
<td>Zn²⁺</td>
<td>45.8</td>
<td>4.6</td>
<td>0.9</td>
</tr>
</tbody>
</table>

*assuming that the cell wall represents 20% of the dry weight of the whole cell (T. Beveridge, pers. comm.)
4.2.2 Preparation of Synthetic Sediment

Spec pure calcium carbonate, crystalline quartz and montmorillonite clay (bentonite) were chosen as components for the synthetic sediments used in the diagenesis experiments. Each mineral was ground in an agate mortar and sieved to less than 200 mesh. Elemental sulphur and crystalline magnetite (1 mm grains) were used as redox buffers to remove dissolved oxygen and to ensure adequately low Eh values throughout the experimental runs. All minerals were checked by powder X-ray diffractometry.

4.2.3 Experimental Procedure

Diagenesis experiments were carried out by mixing approximately 25 mg dried bacterial cells, 50 mg of either elemental sulphur or magnetite, 250 mg of prepared synthetic sediment and 0.5 ml of pure, deionized type II water (Milli-R/Q Water Purifier, pH-7.0) in a pyrex tube (1 mm wall thickness, 6 mm bore, 1-1.5 ml capacity). When combinations of minerals were used in the sediment preparation, they were mixed on a 1:1 basis to the required amount. After sealing by flame, the tubes were heated at 100°C ± 2°C for periods of 1, 10, 100 and 200 days.

A complete list of the metal-cell-sediment combinations used is listed in Table 1. Controls for the experiments consisted of:
List of the metal-sediment-redox buffer combinations, and the controls used in the artificial aging of metal-loaded cellsoof *Bacillus subtilis* for 1, 10, 100* and 200 days** at 100°C.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Sediment Mixture</th>
<th>Redox Buffering Agent</th>
<th>Time (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1  10  100  200</td>
</tr>
<tr>
<td>Uranium</td>
<td>SiO₂</td>
<td>magnetite</td>
<td>x  x  x</td>
</tr>
<tr>
<td></td>
<td>SiO₂</td>
<td>sulphur</td>
<td>x  x  x  x</td>
</tr>
<tr>
<td></td>
<td>SiO₂-CaCO₃</td>
<td>magnetite</td>
<td>x  x  x</td>
</tr>
<tr>
<td></td>
<td>SiO₂-CaCO₃</td>
<td>sulphur</td>
<td>x  x</td>
</tr>
<tr>
<td></td>
<td>absent</td>
<td>magnetite</td>
<td>x  x  x</td>
</tr>
<tr>
<td></td>
<td>absent</td>
<td>sulphur</td>
<td>x  x  x  x</td>
</tr>
<tr>
<td>Copper</td>
<td>CaCO₃</td>
<td>magnetite</td>
<td>x  x  x</td>
</tr>
<tr>
<td></td>
<td>CaCO₃-bentonite</td>
<td>magnetite</td>
<td>x  x  x</td>
</tr>
<tr>
<td></td>
<td>SiO₂</td>
<td>magnetite</td>
<td>x  x  x  x</td>
</tr>
<tr>
<td></td>
<td>SiO₂</td>
<td>sulphur</td>
<td>x  x  x  x</td>
</tr>
<tr>
<td></td>
<td>SiO₂-CaCO₃</td>
<td>magnetite</td>
<td>x  x  x</td>
</tr>
<tr>
<td></td>
<td>SiO₂-CaCO₃</td>
<td>sulphur</td>
<td>x  x</td>
</tr>
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</table>

continued ....
Table 1 (cont'd.)

<table>
<thead>
<tr>
<th>Zinc</th>
<th>Bentonite</th>
<th>Magnetite</th>
<th>X</th>
<th>X</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiO₂-CaCO₃-bentonite</td>
<td>Magnetite</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>SiO₂-CaCO₃-bentonite</td>
<td>Sulphur</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Absent</td>
<td>Magnetite</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Absent</td>
<td>Sulphur</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Iron</td>
<td>Absent</td>
<td>Magnetite</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Absent</td>
<td>Sulphur</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Control</td>
<td>SiO₂</td>
<td>Magnetite</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Cells</td>
<td>SiO₂-CaCO₃</td>
<td>Magnetite</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>SiO₂-CaCO₃</td>
<td>Sulphur</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>SiO₂-bentonite</td>
<td>Magnetite</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>SiO₂-bentonite</td>
<td>Sulphur</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Absent</td>
<td>Absent</td>
<td>Magnetite</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Absent</td>
<td>Absent</td>
<td>Sulphur</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

**Not all combinations were carried through to 200 days.**
* Some samples from the 1, 10 and 100 day runs were lost due to breakage of the pyrex tube during heating.
1) runs employing unreacted control cells and various sediment mixtures,

2) runs employing the same control cells without the addition of synthetic sediment,

3) runs employing metal-loaded cells without the addition of synthetic sediment,

4) duplicate runs employing the alternate redox buffering agent, and

5) runs consisting solely of the redox buffering agents without the addition of either cells or sediment.

4.2.4 Electron Microscopy

On cooling, the pyrex tubes were opened, releasing the gaseous products, and a portion of the cell-sediment mixture removed by disposable pipette for analysis.

For transmission electron microscopy analysis, the cell-sediment mixture was enrobéd in Noble agar (Difco), dehydrated through an ethanol-propylene oxide series, embedded in Epon 812 and sectioned on a Reichert model OMU2 ultramicrotome to a thickness of approximately 60 nm. Both fixed (with glutaraldehyde) and unfixed specimens were used. Sections were collected on carbon-Formvar-coated 200-mesh copper grids, examined with a Philips EM300 transmission electron microscope operated under standard conditions at 60 kV, and the results recorded on film. No stains were employed, relying upon the complexed metals for the electron scatter. Selected speci-
men's were examined in a Philips EM400 transmission electron microscope fitted with a goniometer stage, field emission gun, STEM attachment and a 30 sq. mm. Kevex Si(Li) energy dispersive X-ray spot-mode analysis with resolution down to 20 nm (82). The instrument was operated at an accelerating voltage of 80 kV. Count time was 200 seconds with a 20 degree specimen-to-detector angle.

For SEM-EDS analysis, the cell-sediment mixtures were washed in 5 ml deionized, distilled water, centrifuged, and dehydrated through an ethanol-propylene oxide series. Unfixed, unstained whole mounts of selected samples were made by depositing the cell-sediment mixture from suspension in propylene oxide onto carbon-Formvar-coated 200-mesh nylon grids and blotting them dry. Specimens were examined with a JEOL JEM 100 cx type transmission electron microscope adapted with a goniometer stage, EM-ASID-4D high resolution scanning attachment and an EM-NDS dispersive X-ray spectrometer. The instrument was operated with an accelerating voltage of 100 kV. Count time was 200 seconds with a 40 degree specimen-to-detector angle.

Energy dispersive X-ray spectra obtained from both whole mounts and thin sections include the characteristic peaks due to elements with atomic numbers greater than 10 present in the experimental products. Where organic matter was analyzed, an associated continuum was also present arising from the organic matrix. Some spectra also
contain an instrumental copper peak (at around 8.05 keV) due to the copper specimen grid. Since cells of B. subtilis are generally low in chlorine (T. Beveridge, pers. comm.), the relatively large chlorine peaks (at around 2.69 keV) are considered to be directly or indirectly due to contamination from the Epon 812 matrix. Likewise, the presence of a prominent silicon peak (at around 1.75-1.8 keV) in every X-ray spectrum, regardless of the instrumentation used or material analyzed, strongly suggest contamination probably from the glassware.

The products of experimentation are all extremely fine-grained (less than 5 microns) and consequently their identification relies upon interpretation of TEM images supported by qualitative X-ray spot analysis. Thin sections of the crystalline products, for the most part, were too electron-dense to provide accurate electron diffraction images. However, in most cases, the crystal symmetry and chemical analysis provided sufficient information for tentative identification.

Both X-ray diffraction analysis using an 11 cm Debye-Sherrer camera and standard SEM analysis were attempted. However, the results proved inconclusive due to the high matrix to cell ratio and the extremely fine nature of the authigenic constituents.

4.2.5 pH Analysis

The pH of the water was checked for 19 selected
### Table II

pH analyses for selected metal-sediment-redox buffer combinations artificially aged for 100 and 200 days at 100°C.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Sediment Mixture</th>
<th>Redox Buffer</th>
<th>Time (Days)</th>
<th>Initial pH</th>
<th>Final pH</th>
<th>Δ pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uranium</td>
<td>SiO₂</td>
<td>sulphur</td>
<td>200</td>
<td>7.0</td>
<td>4.8</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>absent</td>
<td>magnetite</td>
<td>200</td>
<td>7.0</td>
<td>5.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Copper</td>
<td>SiO₂</td>
<td>sulphur</td>
<td>200</td>
<td>7.0</td>
<td>4.5</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>SiO₂</td>
<td>magnetite</td>
<td>200</td>
<td>7.0</td>
<td>5.3</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>SiO₂</td>
<td>magnetite</td>
<td>100</td>
<td>7.0</td>
<td>5.0</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>SiO₂-CaCO₃</td>
<td>sulphur</td>
<td>200</td>
<td>9.0</td>
<td>6.9</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>SiO₂-CaCO₃</td>
<td>sulphur</td>
<td>100</td>
<td>9.0</td>
<td>6.8</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>SiO₂-CaCO₃</td>
<td>magnetite</td>
<td>200</td>
<td>9.0</td>
<td>6.8</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>SiO₂-CaCO₃</td>
<td>magnetite</td>
<td>100</td>
<td>9.0</td>
<td>6.8</td>
<td>2.2</td>
</tr>
<tr>
<td>Zinc</td>
<td>absent</td>
<td>sulphur</td>
<td>200</td>
<td>7.0</td>
<td>4.8</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>absent</td>
<td>magnetite</td>
<td>200</td>
<td>7.0</td>
<td>5.0</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td>Iron</td>
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continued .....
<table>
<thead>
<tr>
<th>Control Cells</th>
<th>$\text{SiO}_2$</th>
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<th>7.0</th>
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<th>2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{SiO}_2$</td>
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<td>100</td>
<td>7.0</td>
<td>5.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>$\text{SiO}_2$--CaCO$_3$</td>
<td>sulphur</td>
<td>200</td>
<td>9.0</td>
<td>6.9</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>$\text{SiO}_2$--CaCO$_3$</td>
<td>sulphur</td>
<td>100</td>
<td>9.0</td>
<td>6.8</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>$\text{SiO}_2$--CaCO$_3$</td>
<td>magnetite</td>
<td>200</td>
<td>9.0</td>
<td>6.8</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>$\text{SiO}_2$--CaCO$_3$</td>
<td>magnetite</td>
<td>100</td>
<td>9.0</td>
<td>6.8</td>
<td>2.2</td>
<td></td>
</tr>
</tbody>
</table>
samples covering a wide range of metal-sediment-redox buffer combinations heated for 100 and 200 days (Table II). All pH analyses were carried out in a nitrogen atmosphere to prevent oxidation of dissolved constituents. On opening the tubes, a quantity of the water was taken up in a disposable pipette and blotted onto three different manufacturer-brand indicator papers having overlapping pH ranges graduated into 0.3 pH units which enabled the pH to be approximated within ± 0.2 pH units.

4.3 Results

4.3.1 Organic Diagenesis

The following plates illustrate the results of simulated diagenesis experiments under confined, essentially reducing conditions at 100°C using metal-loaded cells of Bacillus subtilis. What is not shown is that the fluid phase in all reaction vessels containing bacterial cells took on a fair to strong yellow colouration, after heating for 10 days, which was unrelated to the presence or nature of test metals, sediment or redox buffering agent. No attempt was made to characterize the colour source due to the small fluid volume (less than 0.5 ml). In natural surface waters, yellow colourations are commonly due to the presence of dissolved, complex carboxylic acids, phenols and amines (84). While it is questionable whether a direct comparison can be drawn between the
two situations, it appears probable that the discolouration of the test fluids is a consequence of the synthesis of soluble organic compounds during thermal degradation of the bacterial cells.

Non-synchronous thermal degradation of uranium-loaded cells yielded an assemblage of crystalline phosphate interspersed throughout a matrix of insoluble, electron-dense organic residues. The reactions involved in the thermal degradation of the cellular constituents have not been fully elucidated, but a somewhat predictable sequence of events leading to the polymerization of uranium-complexed organic residues is here summarized:

1) mineralization and loss of cell walls; release and migration of uranium into the cytoplasm; and incipient extracellular crystallization of phosphate (Plates 2A, 3A, 5A, 7A, 9B).

2) mineralization, condensation and incipient polymerization of cytoplasmic constituents; intra- and extracellular phosphate crystallization (Plates 2B, 3A,B, 9A,C,D, 10A, 11A).

3) continued mineralization, condensation and polymerization of cytoplasmic constituents resulting in the loss of basic cell morphology; phosphate recrystallization and growth; and incipient organic-phosphate interaction (Plates 3D, 5B,C, 7B,C, 10C,D, 11B).

4) intense polymerization of uranium-complexed organic residues; phosphate aggregation, recrystallization and
growth; and pronounced organic-phosphate interactions (Plates 6A, 8A,C, 11C,D).

The early migration of uranium from the cell wall into the cytoplasm was confirmed by X-ray spot-mode microanalysis (Plate 4D). Uranium migration accounts for the generally higher electron density of the test cells (Plates 2A, 5A, 7A) relative to unreacted uranium-loaded test cells (Plate 1A). Highly electron-dense polymeric organic residues (Plate 11C,D), together with a prominent uranium peak in the EDAX spectrum for 200-day-old organic residues (Plate 6Eh) provides support for the synthesis of insoluble metal-organic complexes. Diffuse, electron-dense coatings on crystalline phosphate (Plates 5D, 7D, 8B, 10C,B, 11B) are interpreted as adsorption of organic compounds onto the phosphate.

Apart from the mineralization and loss of the cell walls, cellular degradation appeared less pronounced in runs employing Fe-, Cu- and Zn-loaded cells. Degradation was most apparent by the formation of phosphate microcrysts (Plate 12), large spheroidal structures interpreted as organic colloids (Plates 13, 14, 18A), and, less commonly, incipient polymerization of cytoplasmic constituents (Plate 20B). Tests carried out employing unreacted control cells yielded similar results. After aging for 100 days, loss of the cell wall and incipient crystallization of what is tentatively identified as a phosphate mineral occurred (Plate 1B).
4.3.2 Phosphate Mineralization

Thermal degradation of uranium-loaded cells resulted in the crystallization of a phosphate mineral in all experimental runs independent of the nature of the sediment or redox buffering agent. After one day, the phosphate appeared as extremely fine (less than 60 nm length) acicular to tabular microcrysts associated with the walls (Plates 3A, 5A, 9B) and/or cytoplasm (Plates 3A, 9A,C, 11A) of the bacterial cells. Continued aging of the cells for 10, 100 and 200 days resulted in the recrystallization and growth of the fine microcrysts producing larger (greater than 1 micron length) coarse, granular aggregates (Plate 6C), platy microcrysts (Plate 10E), tabular microcrysts (Plates 5D, 6B, 7D), tapered bundles (?) of fine phosphate needles (Plate 8B) and coarse (greater than 10 microns) acicular aggregates, not unlike the 'colloform' habit, containing individual phosphate needles up to 4 microns in length (Plate 8C).

With the exception of chlorine, EDAX spectra for 10-day-old microcrysts (Plate 4B) and 200-day-old microcrysts (Plate 6Ea) are essentially identical, showing the microcrysts to be composed predominantly of uranium, potassium, and phosphorous. Insufficient information is available to accurately name the phosphate mineral or minerals. However, the platy habit (Plate 10E) and perfect micaceous, basal (001?) cleavage (Plates 5D, 7D, 11D) are consistent with the hydrated phosphate, meta-ankoleite
(K₂(UO₂)(PO₄)₂·6H₂O).

Artificial aging of ferrous-loaded cells in the presence of elemental sulphur resulted in the synthesis of iron phosphate as isolated hexagonal plates (Plate 12B) and large (greater than 2 microns) electron-dense aggregates composed of a mixture of fine phosphate microcrysts and free sulphur (Plate 12A,C). EDAX analysis of the hexagonal plates (Plate 12D) showed them to be composed essentially of iron and phosphorous. The minor amounts of potassium, chlorine and sulphur evident in the spectrum may indicate an organic coating on the crystalline phosphate. There are several ferrous phosphate minerals, such as ludlamite (Fe₃(PO₄)₂·4H₂O) and vivianite (Fe₃(PO₄)₂·8H₂O), which are considered to form under natural biogeochemical influence. However, the platy habit and hexagonal symmetry of the iron phosphate in this study appears more consistent with the hydrated ferric phosphate, cacoxenite (Fe₉(PO₄)₄(OH)₁₅·18H₂O) (85).

Electron-dense granular aggregates and fine to coarse, acicular, tabular and platy microcrysts were observed in a number of runs employing copper-loaded cells (Plates 15A, 16B, 21B, 22, 23) and control cells (Plate 1B). The crystal forms and close association with bacterial cells resembles the previously described U-K-phosphate assemblages, suggesting minor phosphate mineralization in these runs. However, as they were not analyzed,
they cannot be elaborated upon.

4.3.3 Sulphide Mineralization

Metal-sulphides were synthesized in all experimental runs where copper- and zinc-loaded bacterial cells were aged in the presence of elemental sulphur without calcium carbonate in the system. After aging for 10 days, copper sulphide formed a mixed assemblage of frambooids, crystalline aggregates and discrete euhedral microcrysts (Plate 13A). The sulphide euhedra appeared most frequently associated with electron-transparent organic (?) colloids (650 nm to 2 microns diameter) in the form of equidimensional; tabular, hexagonal microcrysts (250-750 nm diameter) occurring singly, joined at crystal faces (Plate 13B), as dense internal crystal aggregates (Plate 13D), or all three together (Plate 13C). In one instance, concentric layering of copper sulphide within the colloid resulted in the development of hexagonal symmetry and a polygonal outline to the colloid (Plate 14). Continued aging of the copper-loaded cells for 200 days produced a dramatic increase in crystal size and marked changes in crystal form. At 200 days, the copper sulphides occurred as frambooidal aggregates (Plate 15A), 'books' of stacked hexagonal or pseudohexagonal plates (Plate 15B), cubic crystal aggregates (Plate 15C), hexagonal crystal aggregates (Plate 16A,B), and 'quasi-spheroidal' crystal aggregates (Plate 16D). One large polygonal crystal
aggregate appears to have formed by epitaxial growth infilling the space between precursor hexagonal microcrysts (Plate 17A,B). EDAX microanalysis (Plate 16Da) indicates that the copper sulphides, for the most part, are pure phases with the exception of the cubic form which contains a small amount of iron (Plate 16Db). Covellite (CuS) is the likely hexagonal copper sulphide although hexagonal chalcocite (Cu$_2$S) cannot be ruled out as its minimum temperature of formation is documented at 103°C (86). The tapered edges of the stacked sulphide plates are reminiscent of the pseudohexagonal rhombic prism form typical of the lower temperature orthorhombic chalcocite polymorph (87). The cubic form and trace amounts of iron are consistent with the mineral digenite ((Cu,Fe)$_9$S$_5$) which is stable at temperatures above 83°C (86).

Well crystallized zinc sulphide, from the 10-day-old runs, formed dense, framboïd-like crystal aggregates (Plate 18B), isolated, short-prismatic to platy hexagonal microcrysts (70 nm to 1 micron) (Plate 18C,D), large hexagonal crystal aggregates (greater than 1 micron) (Plate 18D) and discrete hexagonal microcrysts (less than 200 nm) enrobed in electron-transparent colloidal organic (?) matter (Plate 18A). The bulk of the zinc sulphide, however, formed as electron-opaque granules and granular spherules (less than 500 nm diameter) intimately associated with the bacterial cells (Plate 19A,B). SEM images of the whole mounts (Plate 20D) indicated that each
spherule was attached to a single bacterium. Aging of the bacterial cells up to 200 days produced a slight increase in the size of the spherules and, perhaps, a slight tendency towards a polygonal outline (Plate 20A,B). Comparison of TEM images for the 10, 100 and 200 day runs indicates that the relative increase in spherule size with time is accompanied by a decrease in the number of isolated sulphide granules, suggesting growth by accretion. This, in turn, would imply that the hollow nature of the spherules is the consequence of accretion onto an electron-transparent colloid. There is at least circumstantial evidence to suggest that separated cell wall fragments were involved in the accretion and/or nucleation of zinc sulphide granules (Plates 19B, 20C). EDAX analysis (Plate 20E) confirmed that the granular and euhedral zinc sulphides are relatively pure phases. While the exact mineralogy of the granular form is undetermined, the coexisting euhedral form is tentatively identified as hexagonal wurtzite (ZnS).

4.3.4 Conditions of Product Formation

The pH and ΔpH values of 19 selected test runs are shown in Table II along with the corresponding metal, sediment, redox buffer and initial pH values. The final pH values of the fluid phase varied from 6.8–6.9 and 4.5–5.3 depending upon the presence or absence of calcium carbonate in the system. The ΔpH values were fairly con-
sistent for all runs, ranging from 1.7-2.5 pH units, regard-
less of the nature of constituents or initial pH. No attempt
was made to measure redox potentials. However, in
runs employing a sulphur redox buffer, the strong
smell of hydrogen sulphide upon opening the tubes attested
to relatively low final Eh values.

Clumping of the bacterial cells (Plate 21A) resulted
in non-synchronous cellular degradation and precipitation
of authigenic mineral phases (Plate 21B), suggesting that
diffusion played a critical role in the early stages of
diagenesis. After one day, crystallization was evident
along the perimeter of the cell aggregates (Plate 22A).
By 100 days, uniformity in cellular degradation and
crystallization was achieved throughout the cell aggre-
gates (Plate 22B). In view of the clumping effect, some
of the apparent discrepancies within individual runs and
between various 1 and 10 day runs may reflect more the
relative location of the section within a cell clump than
actual differences in degradation and crystallization
rates. After 100 days, the effects of clumping appear
negligible.

Organic degradation and phosphate formation, in
general, were most pronounced in runs using uranium-
loaded cells. Within these runs, cells appeared to de-
grade most rapidly at higher pH values (6.8-6.9) with the
organic residues polymerizing most intensely in the
presence of hydrogen sulphide. Phosphate formation cor-
relates well with cellular decomposition although aggregation and recrystallization of the U-K-phosphate was most intense at lower pH values (i.e. in the absence of CaCO$_3$). The relative paucity of phosphate mineralization in the copper and zinc runs suggests that highly electro-positive cations are a prerequisite for phosphate formation and/or that uranium catalyzes the breakdown of cellular constituents promoting the release and subsequent precipitation of organic phosphate. All copper and zinc sulphides formed at lower pH values (4.5-5.3). No sulphides were observed in similar runs containing calcium carbonate, possibly indicating some pH control on sulphide mineralization and/or the hydrolysis of sulphur.

4.4 Discussion

Laboratory studies concerned with petroleum generation have defined a large number of chemical reactions, as summarized by Brooks (88), leading to the slow, abiotic maturation of sedimentary organic matter to kerogen at temperatures generally less than 150°C. At lower temperatures (less than 50°C), the major reactions include: chemical hydrolysis, the breaking of C-C bonds, redistribution of hydrogen atoms, and cyclization yielding predominantly secondary carbon structures; and the loss of functional groups from the precursor biochemical molecules releasing carbon dioxide, hydrogen, ammonia, nitrogen, methane, hydrogen sulphide, sulphur and phosphate into
the sediment pore waters. If kerogen maturation processes occur in a permeable sediment, the latter soluble and gaseous products would be expected to migrate. The initial alkalinity of many sediments range from 8.0-9.8 due to open equilibrium of the interstitial waters with calcium carbonate. Decarboxylation reactions, both abiotic and biological, lower the pH of the pore waters through the liberation of carbon dioxide and hydrogen ions (89). With increasing temperature associated with burial, sufficient energy becomes available for free radical cracking reactions yielding volatile, hydrogen-rich paraffinic hydrocarbons and insoluble, hydrogen-poor, carbon-rich aromatic condensates (kerogen).

Many, if not most, of the chemical reactions involved in the abiotic maturation of sedimentary organic matter are rate-dependent as well as temperature-dependent. In short-term (100 hr.) laboratory experiments, Saxby (90) synthesized insoluble carbonaceous residues (kerogen) together with soluble and gaseous products from metal-cystine complexes at temperatures up to 400°C. Data from similar diagenetic experiments, according to Brooks (88), appear to support the physical law that reaction rates are halved by an increase in temperature of 10°C.

Owing to the inherent simplicity of short-term laboratory studies, their relevance to natural sediment diagenesis is always open to some question. The present
study is relevant to late-stage diagenetic alteration of non-biodegraded, metal-complexed organic matter in wet, essentially reduced, impermeable sediments. The formation of highly polymerized, insoluble carbonaceous residues together with the overall decrease in pH of the test fluids are consistent with the anticipated kerogen maturation processes outlined by Brooks up to, and possibly including, free radical cracking reactions.

Considerable attention has been given in recent years to the post-depositional, diagenetic mineralization of phosphate leading to the formation of marine phosphorites. In a discussion on bedded phosphates, Youssef (91) noted the frequent association of phosphate with organic matter, metal sulphides and black shales. On the basis of the common close correlation between phosphate and organic matter, the direct, purely-inorganic precipitation of phosphate has been questioned (85, 92, 93). Romankevich and Baturin (94) described a parallel trend between the precipitation of phosphate and the loss of organic matter during lithification. Proponents of phosphate bioaccumulation models held that burial of organic matter followed by phosphate release during later diagenesis provides a feasible mechanism for the incorporation of phosphorous in fine-grained sedimentary rocks (85, 91, 95). Trudinger (96) suggested that, to be geologically significant, large scale incorporation of organic phosphate in sediments would require shallow-water
conditions, allowing for the rapid deposition of essentially non-decomposed organic matter, and efficient post-depositional phosphate release and mineralization during diagenesis. Bioaccumulation models have gained considerable support from the recent discoveries of shallow-water stromatolitic phosphorites from the Precambrian of India (92, 97-99) and China (100) and the Cambrian of Australia (101).

The biogeochemistry and, in particular, the aqueous geochemistry of sedimentary phosphate formation was reviewed by McConnell (85) and Tooms et al. (102). Carbonate fluorapatite and carbonate hydroxyapatite comprise more than 99% of the phosphate minerals in marine sediments. The question of apatite formation during diagenesis is unresolved. According to McConnell, it must depend on complex energy relationships to explain the selective incorporation of fluorine over chlorine. Like carbonate minerals, phosphate formation is commonly considered a pH phenomenon (103) controlled by the partial pressure of carbon dioxide, with apatite being the dominant phase in the pH range 7.1-7.8 (99). However, McConnell was quick to note that the geological importance of organic matter in the natural transformation from organic phosphate to crystalline phosphate minerals has not been adequately evaluated except for very simple systems not involving other minerals (i.e. silicates and carbonates). While low redox potentials favour natural
phosphate mineralization, reduced forms of phosphate have yet to be identified in marine sediments (85). It is likely that this natural association reflects more the close correlation of phosphate minerals with reduced organic matter than a requisite condition for phosphate crystallization.

Phosphate in non-decomposed cellular organic matter is contained largely in organic phosphate esters, phospholipids and nucleic acids (12). In the aquatic habitat, algae and cyanobacteria have been shown to be effective phosphate accumulators (104, 105). The ash of some marine algae can reach as high as 7% P$_2$O$_5$ (3% P) (106). Like higher order cells, the chemical composition of bacteria vary directly with that of their environment. In the present study, cells of Bacillus subtilis, grown in the presence of phosphate, contain substantial amounts of phosphorous (up to 6% dry wt. (107)) most of which occurs as phosphodiester groups in the cell wall or associated with nucleic acids in the cytoplasm (T. Beveridge, pers. comm.). The crystallization of fine phosphate microcrysts early in the experimental runs resulted from the direct release of phosphate concomitant with the mineralization and loss of cell walls. Progressive organic degradation resulted in the efficient release of cellular phosphate promoting the growth of large microcrysts and crystalline aggregates. In the present experiments, phosphate precipitation occurred at pH values never exceeding 7 and
was most pronounced in the mildly acidic pH range. The apparent absence of calcium in the phosphates precipitated in the presence of calcium carbonate indicates either that low pH values inhibit apatite formation or that the kinetics of apatite formation were too slow to have been realized in a short-term study. If apatite is inhibited by low pH, the availability of suitable metal ions may be a critical factor in the precipitation of less complex metal phosphate compounds.

In modern marine sediments (108) and fine-grained sedimentary rocks (109) uranium frequently demonstrates a marked positive correlation with phosphorous. Tooms et al. (102) recorded an average uranium content of 190 ppm in marine phosphorites. Yet, while phosphorous enrichments in sediments can be explained by the accumulation of organic matter, uranium enrichments are more frequently attributed to the post-depositional sequential adsorption-reduction of soluble uranium species from seawater. Until recently, the mobilization and transport of uranium in the hydrosphere was considered to be primarily by inorganic processes involving oxyionic species: free uranyl ions and complex bis- or tri-carbonate anions. However, it has been adequately demonstrated that uranium is readily complexed by free anionic ligands of humic substances and living cells.

The role of organic matter in the hydrogenic cycle of uranium was the subject of a review by Baturin (108)
in which he noted that high syngenetic concentrations of uranium with organic matter are favoured by higher than normal concentrations in the bottom waters. He emphasized that uranium did not appear to be concentrated by organic matter in suspension but rather by organic matter at the sediment/water interface. His observations are consistent with the current concepts for the formation of reactive humic matter through aerobic biodegradative processes in the oxidizing upper few centimeters of marine sediments. In a study of authigenic uranium precipitation in sediments near Vancouver Island, Kolodny and Kaplan (110) concluded that half of the uranium in the sediments was in the form of organic complexes in contact with uranium-enriched pore waters.

The stability of organically-complexed uranium throughout diagenesis appears to be affected to a large extent by the formation of authigenic phosphate minerals. In a summary report on the geochemistry of black shales, Vine and Tourtelot (2) indicated that uranium enrichments in phosphatic black shales were primarily associated with the phosphate phase. In phosphate-deficient shales, uranium was retained by the organic fraction. A similar association between organic matter, metal sulphides and uranium was described for the Proterozoic uraniferous conglomerates of the Elliot Lake and Witwatersrand deposits. Through a comprehensive molecular and elemental study of the Witwatersrand thucolites, Zumberge et al.
(111) concluded that syngenetic accumulation of uranium within decomposing microbial mats played a significant role in the genesis of the deposits. Important to this study, however, is the fact that the organic matter in these deposits retained the complexed uranium well into low grade metamorphism. In the present experiments, the formation of uranium-complexed organic residues indicates that a fraction of the uranium initially sorbed onto non-decomposed cells remained fixed by the organic fraction throughout diagenesis in spite of phosphate mineralization and the complex transformation from biochemical macromolecules to kerogen.

To date, the question of uranium in natural sedimentary apatites is unresolved. Both hexavalent and tetravalent uranium occur in apatites substituting either for calcium or phosphorous. Hence, there is no clear redox control on its incorporation. Occasionally, uranium also seems to be associated with organic substances trapped in the phosphate phase (85). Taylor (112) proposed that the incorporation of uranium in sedimentary apatites may reflect the lower solubilities of uranyl phosphates relative to uranyl carbonates in sea water. In the present experiments, the crystallization of U-K-phosphate was most intense at low pH values. It is tentatively suggested that these phosphates might represent an intermediary stage in the transformation from organic phosphate to uranium-bearing apatite as the
pH of the pore waters are progressively neutralized.

In reducing environments, polysulphide ions serve as good redox and acidity buffers. In addition, the incorporation of polysulphide chains in organic matter stabilizes the organic matter and enhances its complexing properties (113). Both elemental sulphur and the sulphide ion are strong bases with hydrolysis reactions leading to the formation of hydrogen sulphide and bisulphide ions. In laboratory experiments, Chambers et al. (114) generated significant quantities of hydrogen sulphide in aqueous solutions containing organic matter at 220 °C. In view of their study, hydrogen sulphide generation in the present experiments is attributed to the hydrolysis of elemental sulphur concomitant with the thermal degradation of the bacterial cells.

At surface temperatures, the stability of natural bivalent metal-organic complexes is determined to some extent by pH and the nature and concentration of competing sulphur ligands. The influence of pH on metal-binding by organic ligands has claimed the interest of many workers. Guy et al. (115) indicated that copper in aqueous solutions containing particulate matter was distributed evenly as adsorbed and organically-complexed species at pH 6 but at lower pH values (3.8-6.0) copper was primarily in an uncomplexed form. Similarly, the sorption of bivalent metals (Cu, Zn, Pb) onto particulate algal matter was shown by Ferguson and Bubela (46) to be strongly
suppressed by a decrease in pH. Mierle and Stokes (116) found that copper binding onto the walls of living algae was completely inhibited below pH 4. Data presented by Baker (69) and Gardner (117) on the stability of metal-organic complexes in aqueous sulphide solutions indicated that competing reduced sulphur species, in many cases, actively sequester bivalent metal ions from anionic organic ligands. Pauli (70) found that, in the presence of hydrogen sulphide, sixty percent of previously-prepared metal-humates (Cu, Zn, Ni, Pb, Cd) were converted to metal sulphides.

The importance of organic chemical processes in the partitioning of bivalent metals and the formation of metal sulphides in sediments is poorly understood; further complicated by the fact that, in general, the low temperature relationships of sulphide minerals are not as well defined as those at higher temperatures. Nissenbaum and Swaine (65) examined the role of humic matter in metal sulphide formation in recent marine sediments. In terms of metal partitioning, they concluded that Cu, Zn and Mo were primarily associated with humic acids whereas Fe, Ni and Co were associated with metal sulphides. In contrast, in a similar study on organic-rich lake sediments, Timperly and Allan (118) found that Cu preferentially formed sulphide minerals while Fe and Zn were retained by the organic fraction.

The biogenic versus abiotic formation of sulphide
minerals in sediments has been the focus of numerous laboratory experiments. Ultimately, the debate revolves around the origin for the reduced sulphur species or, more precisely, the reduction of sulphate in sea water. Chambers et al. (114) concluded that the kinetics of abiotic sulphate reduction, even at temperatures up to 220°C, are too slow to account for the dissolved sulphide concentrations in natural sediments. Consequently, it is generally accepted that organic chemical processes are involved, directly or indirectly, in the formation of reduced sulphur species in most low-temperature diagenetic environments.

Baas Becking and Moore (119), Rickard (120) and Hallberg (121) are among a large number of investigators who have demonstrated the 'biogenic' formation of a broad spectrum of important sedimentary sulphide minerals using sulphate reducing bacteria. Yet, through the abiotic synthesis of many of the same minerals, a number of workers have argued against the necessity of bacterial activity to explain sedimentary sulphide genesis (122-124). In both the biological and abiotic studies, little attention has been given to a biological source for the bivalent metal ions. The occurrence of metal sulphides other than the iron sulphides appears to be relatively rare in recent, unconsolidated sediments suggesting that mixed metal sulphide assemblages commonly found in fine-grained sedimentary rocks are predominantly of a late-
stage diagenetic origin. The question arises as to whether sedimentary organic matter can supply sufficient metal ions to account for diagenetic sulphide mineralization. Ferguson and Bubela (46) concluded that non-specific adsorption of bivalent metals by particulate algal matter provided a feasible mechanism for trace metal incorporation in sediments. Likewise, undifferentiated sedimentary humic matter has been shown to bind and fix substantial concentrations of bivalent metals and, in the case of black shales, to retain them throughout diagenesis. Saxby (90) demonstrated that thermal degradation of metal-cystine complexes produced a number of sulphide minerals including covellite and sphalerite. However, like most biogenic sulphide studies, his experiments were concerned more with cystine as a source for reduced sulphur than organic matter as a source for metal ions. The present experiments demonstrate the fate of organically-complexed metals in the presence of excess dissolved sulphide during progressive organic maturation. Desorption of metals, augmented by decarboxylation reactions and a reduction in pH, led to the precipitation of wurzite, digenite, covellite and/or chalcocite in a mixed assemblage of forms including spherules, framboids, framboidal aggregates, multicrystalline aggregates and polyhedra, and isolated euhedra. In view of the limited reactions involved in the release of metal ions, this study provides compelling evidence to suggest that thermal
maturation of carboxyl-rich, metal-complexed humic matter should yield similar products if sufficient reduced sulphur is available.

Framboidal texture is one of the more interesting and least understood sulphide textures characteristic of fine-grained, organic-rich sediments. Since framboids have also been recognized in a number of stratiform base-metal sulphide deposits (i.e. Rammelsberg, Mount Isa, Kupferschiefer), the genesis of sulphide framboids is important to the discussion of sedimentary sulphides in general. Framboids are isolated, metal sulphide spherules, ranging in size from 1 to 100 microns, composed essentially of discrete, somewhat equidimensional, euhedral microcrysts which impart a raspberry appearance to the structure. The definition of framboid can be further limited to include only those sulphide spherules having an arbitrary maximum ratio of microcrystal size to spherule diameter of 1:10 (125). The constituent microcrysts can be separate and/or joined at crystal faces.

Framboid genesis has long claimed the attention of sedimentologists. Schneiderhohn (126) first proposed an organic origin for framboids by suggesting that they represented the pyritized remains of bacterial aggregates. Subsequent findings of Ramdohr (127) and Love and his colleagues (128-131) propagated the concept of biogenic framboid development. Their studies showed that natural framboids frequently had an organic 'skin' or
matrix and, in some cases, the removal of mineral matter revealed organic structures which were interpreted as fossilized bacteria. As with the genesis of sedimentary sulphides in general, the biogenic versus abiotic origin for framboids has been extensively debated. The purely abiotic formation of framboids has been confirmed in a number of low-temperature aqueous sulphide solutions (122, 124, 132) and explains the observations of framboids in a variety of igneous rocks. Nevertheless, while abiotic synthesis remains a distinct possibility in sediments, the intimate association of organic matter with some framboids must inevitably cast some doubt on a purely inorganic origin for all framboids.

All framboids, regardless of the host rock, share a common basic feature: spheroidicity. In a theoretical discussion on framboid development, Rickard (125) concluded that the spherulicar nature could only be explained through the pseudomorphism of a pre-existing body: immiscible organic globules, spherulicar organic coacervates, gaseous vacuoles or possibly unicellular microorganisms. He further held that the formation and stability of the constituent microcrysts were genetically unrelated to spherulicar shape (and hence unrelated to the material being replaced). He neglected to discuss any influence that the organic matter may have in the concentration or partitioning of metals. Pauli (62), on the other hand, recognized the importance of organic
matter in defining both the shape and composition of framboids. He interpreted the formation of framboids as metal-humic colloid growth around framboid nuclei with subsequent conversion to metal sulphides. In contrast to both Rickard and Pauli, Farrand (124) argued against the inherited spheri
cular nature of framboids. According to him, framboids are the direct result of aggregation of discrete sulphide particles in free suspension. Organic matter, while not a prerequisite in his model, can have a profound effect on the precipitation and aggregation of the sulphide microcrysts. Miscible organic compounds can alter the aqueous metal sulphide solubilities and promote their precipitation over a wider range of concentrations than for pure water. Hydrophobic organic compounds, in turn, can increase surface tension on the microcrysts promoting aggregation while protecting the framboids from corrosion and infilling.

In most sediments and base-metal deposits, framboidal texture is almost exclusive to pyrite although the conditions that promote its development should also favour the formation of other metal sulphide framboids (ie. Cu, Zn). Farrand (124) suggested that their relative paucity in recent sediments might reflect a different response to the conditions of progressive diagenesis. Preservation of framboids, according to him, demands that they be effectively isolated from the aqueous media before they further react, corrode or coalesce. Hydro-
phobic organic matter, on the short-term, provides a good seal. However, the effectiveness of an organic coating is contingent upon its thermal and chemical stability throughout diagenesis. If framboids are not sealed off completely from the reacting media (sediment pore water) or if the seal is destroyed, continued sulphide precipitation may occur as overgrowths or by replacement of the original sulphide phase.

To assess the relevance of framboids to natural metal sulphide formation in sediments, it is important to understand the relationship between framboids and sulphide polyhedra. Love and his colleagues (130, 131) suggested that growth of euhehedral pyrite occurs through the homogenization of first generation framboidal pyrite followed by the precipitation of second and, in some cases, third generation overgrowths in optical continuity. Ostwald and England (133) used trace arsenic contents in a mixed assemblage of pyrite forms to demonstrate a genetic relationship between euhehdra and framboids. They confirmed that whole framboids pass through a more compact, intermediate form prior to their conversion to sulphide euhehdra. Rickard (125) suggested that continuous epitaxial parallel growth, infilling the spaces between a number of dendritic constituent microcrysts of a framboid, could form 'quasi-spheroidal' or irregular shaped crystal aggregates.

In the present study, the incipient formation of
framboidal copper and zinc sulphides in 10-day-old runs supports, in part, each of the proposed mechanisms for framboid development. Amorphous, electron-transparent organic (?) spherules with dense crystalline cores (Plates 13B,C,D, 14, 18A) are reminiscent of organic colloid growth around a pre-existing nucleus as suggested by Pauli (62). The concentric layering of sulphides in some of these structures (Plates 13B,C, 14) is consistent with Rickard's (125) model for inheritance of spheroidicity. While it could not be determined whether the sulphides were precipitated by or from the organic colloids, or whether they simply accreted onto the colloids from free suspension, as the organic colloids were observed only in those runs in which sulphides were precipitated and then only in contact with the sulphide phase, it appears that some genetic relationship exists between the two phases. On the other hand, the formation of isolated microcrysts which are unassociated with organic matter (Plate 18C,D) can be used to argue for inorganic precipitation in free suspension "from metal ions and sulphide ions in true solution", as proposed by Farrand (124). The granular zinc sulphide spherules (Plates 19, 20), while not true framboids by strict definition, certainly appear to have formed by accretion of finely-dispersed sulphide granules possibly onto a pre-existing, electron-transparent colloid.

Continued aging of the copper sulphides for 200 days
resulted in the near complete conversion of framboideal sulphide to a mixed assemblage of euhedral, single and multicrystalline forms. The growth of polygonal crystal aggregates (Plate 17) might be explained through the precipitation of second stage overgrowths onto homogenized first stage hexagonal 'framboids' (Plate 14). However, regardless of the mechanism involved, this study indicates that, while copper and zinc sulphides readily form framboideal textures under diagenetic conditions, the frambooids are rapidly recrystallized or completely replaced by coarser crystalline forms in which the framboideal nature is perhaps more deductive than actually apparent. The results of this study support Farrand's (124) explanation for the relative scarcity of framboideal copper and zinc sulphides in sedimentary rocks. Diagenetic recrystallization, for the most part, destroys any evidence of primary frambooids.

It is without question that iron sulphides represent the most common sedimentary sulphide minerals regardless of age or depositional environment. In the laboratory, numerous variétés of iron sulphide have been synthesized in both biogenic and abiotic systems under a range of chemical conditions. Hence, this discussion would not be complete without an attempt at explaining why iron phosphate and not an iron sulphide was synthesized in the presence of excess dissolved sulphide. To reiterate, bivalent Cu, Zn and Fe readily form covalent-like bonds
with sulphide ions whereas trivalent Fe and U (both hexa- and tetravalent) preferentially form strong electrovalent bonds with carboxyl and phosphate ligands. In this study, degradation of ferrous-loaded bacterial cells led to the precipitation of iron phosphate. The symmetry of the crystal form together with the fact that phosphate sequestered iron in the presence of dissolved sulphide suggests that the mineral is a ferric phosphate. This would necessarily imply the oxidation of ferrous ions to ferric ions under essentially reducing conditions.

In sulphide marine sediments, the oxidation of aqueous polysulphides was shown by Berner (134) to be the principal controlling reaction:

\[ \text{polysulphide} \rightleftharpoons S^0 + 2e^- \]

In aqueous solutions the nature and abundance of the polysulphides are largely determined by pH in the following manner:

\[ H_2S \rightleftharpoons H^+ + HS^- \rightleftharpoons 2H^+ + S^{2-} \]

The sulphide ion can be oxidized to elemental sulphur by ferric ions:

\[ 2Fe^{3+} + S^{2-} \rightarrow S^0 + 2Fe^{2+} \]

with the resulting ferrous ions reacting to form ferrous sulphides:

\[ Fe^{2+} + S^{2-} \rightarrow FeS \]
The conversion of ferrous sulphides to pyrite, according to Berner, depends upon the ability of elemental sulphur to act as an oxidant in the reverse disproportionation reaction:

$$\text{FeS} + S^0 \rightarrow \text{FeS}_2$$

Lyons and Fitzgerald (135), to explain the cycling of phosphate in shallow waters and sediments, described the inorganic redox reaction involving ferrous ions and ferric phosphate:

$$\text{Fe}^{2+} + \text{PO}_4^{-3} \rightleftharpoons \text{FePO}_4 + e^-$$

In the systems employed in this study, redox reactions are controlled to a large degree by the reduction and hydrolysis of elemental sulphur to sulphide ions:

$$S^0 + 2e^- \rightleftharpoons S^{-2}$$

The tentative suggestion is that ferric phosphate was produced in the presence of excess reduced sulphur during the reduction of elemental sulphur following the general reaction:

$$S^0 + 2\text{Fe}^{2+} + 2\text{PO}_4^{-3} \rightleftharpoons 2\text{FePO}_4 + S^{-2}$$

This reaction is supported by the intimate association of iron phosphate with elemental sulphur (Plate 12C,E).
4.5 Concluding Remarks

In this chapter, an attempt has been made to demonstrate the complex interrelationships between metal-organic interactions in the surface waters and the formation of authigenic minerals during sediment diagenesis. In view of available information, one conclusion seems to emerge: that straightforward inorganic processes alone fail to account for the nature, concentration and distribution of trace metals and authigenic mineral phases in sediments and sedimentary rocks. This study provides compelling evidence for the direct involvement of organic matter in the formation of late-stage diagenetic phosphate and sulphide minerals. For the most part, these minerals were formed early in the experimental runs and, in the case of the uranium phosphate, aging did little to alter the basic composition. Since the dominant processes leading to the formation of phosphates and sulphides in this study are governed primarily by low temperature reactions, it is likely that the abiotic formation of similar phases in nature can proceed at temperatures well below 100°C if given sufficient time.
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"Isotope fractionation, mobilization and immobilization, oxidation and reduction, mineralization and storage in biological material, transfer, volatilization, catalysis and equilibrium of systems — all these are ruled mainly by biological processes in the microscale and most frequently by microorganisms."

W. E. Krumbein, 1978

CHAPTER 5

DISCUSSION AND CONCLUSIONS

Biogeochemical element cycles reflect the complex interaction of biological, biochemical, geological and geochemical processes that affect, directly or indirectly, the mobilization, transport and fixation of every element in the surface milieu. Much attention has been accorded in recent years to the biogeochemical principles underlying exogenic element cycling and mineral genesis in the modern oceans. Mánheim et al. (1) outlined four simultaneous requirements for marine phosphorite formation which are: sediments rich in organic matter; low ambient oxygen concentrations; slow rates of terrigenous sedimentation; and low but not negligible concentrations of calcium carbonate. Similarly, environmental factors, favouring the incorporation of hydrogenic uranium in re-
cent marine sediments, as outlined by Baturin (2), include: high phosphate and organic carbon contents in sediments; low Eh and pH and slow circulation of the bottom waters; and slow rates of terrigenous sedimentation. In separate investigations, Tin Mo et al. (3) and Pluman (4) proposed that the low redox potentials favouring uranium incorporation, in many cases, were the consequence of a buildup of biogenic hydrogen sulphide arising from ineffective circulation of the bottom waters. Factors conducive to the generation of biogenic hydrogen sulphide, as indicated by Trudinger et al. (5), include: an adequate supply of photosynthetically-derived organic matter; anoxic and reducing conditions; slightly acidic to mildly alkaline pH; and a complex biological ecosystem to degrade the organic matter. In brief, the formation of biogenic uranium, sulphide and phosphate accumulations are contingent upon a high flux of photosynthetically-derived organic matter to the bottom sediments. This, in turn, is dependent upon inhibited aerobic biodegradation as a consequence of anoxia augmented by ineffective turnover of the water column.

Bacterial enzymatic attack of primary biochemical macromolecules ultimately leads to the synthesis of humic substances which constitute the most reactive class of metal-chelating agents in the hydrosphere. Numerous studies have demonstrated that the ability of organic ligands to bind and fix trace metals from dilute solu-
tions far outweighs that of common anionic species. The formation of stable metal-organic complexes, in turn, inhibits further degradation via cyclization reactions and neutralization of reactive functional groups. Preservation of organic matter leading to significant concentrations of sedimentary organic carbon is favoured by: high surface productivity; bottom water anoxia; and high concentrations of heavy metal ions acting concomitantly to promote metal-organic interactions and to inhibit bacterial metabolic activity.

It is generally accepted that syngeneric accumulation of uranium by sedimentary organic matter and phosphate played an important role in the genesis of some uranium ores via the sequential adsorption-reduction of the uranyl ion from sea water. Baturin (2) indicated that hydroospheric uranium appeared to be most effectively concentrated by organic matter at the sediment/water interface (where organic detritus is biodegraded to humic matter) below waters already enriched in uranium. In an earlier paper, Baturin (6) concluded that "the final outcome of the uranium concentration process...generally is the same in basins with a hydrogen sulphide type of environment (Black and Baltic Seas, productive ocean shelves) and those with normal aeration" (pg. 192). This would suggest that, on the short-term, sorption of the uranyl ion onto sedimentary organic matter is more important than the reduction of oxanionic species in the
uranium concentration process. Subsequent reduction, following burial, ensures the 'in situ' fixation of uranium with organic matter throughout diagenesis.

The association of organic carbon (kerogen) and microfossils with Precambrian sedimentary mineral deposits has often been cited as evidence for the possible interaction of organisms in metal accumulation and mineral formation. The assessment of biological influence in the genesis of mineral deposits in the past necessitates extrapolation of known biogeochemical principles which, in turn, requires that basic assumptions be made on the level of biomass complexity together with physico-chemical conditions characterizing the paleodepositional environment. Definition of physical, chemical and biological processes which were active throughout past geologic eras, particularly the Archean, relies heavily upon the validity of currently popular evolutionary models. Numerous articles have been written on the possible roles of organic matter and microorganisms in ore formation employing an uniformitarian approach. Yet, no systematic study was ever made to warrant the projection of modern biogeochemical processes into the Precambrian. In fact, the misconception of global reducing conditions during the Archean and early Proterozoic eras jeopardizes such an approach.

The main objective to this thesis is to present a working philosophy of research into Precambrian biogeo-
chemistry and geomicrobiology. By way of summarizing some of the conclusions reached in the preceding sections, an attempt has been made to demonstrate that biogeochemical processes within the Precambrian hydrosphere were much the same as the present. Major evolutionary events within the lithosphere, atmosphere, hydrosphere and biosphere can be resolved ultimately in terms of thermal decay of an early planetary system characterized by a secondary water vapour atmosphere. Oxygenation of the atmosphere probably occurred early in the Earth's history as a consequence of photodissociation of water vapour. Temperature-induced anoxia characterized the hydrosphere throughout much of the Archean and early Proterozoic. Life emerged and evolved to a large degree within a hot, anoxic and reducing hydrosphere. Gradual oxygenation of the Precambrian hydrosphere as a consequence of decreasing water temperatures forced the evolution of aerobic metabolic systems and the transition from an integrated anaerobic biogeochemical carbon-sulphur cycle to the integrated carbon-sulphur-oxygen cycle that characterizes the modern hydrosphere. Paleontological, organic chemical and geochemical evidence supports the view that this transition was realized by 3.5 By BP but may have occurred prior to the deposition of the 3.8 By-old Isua iron formations.

Potentially, if not practically at the moment, the integration of geochemical, paleontological and sedi-
mentological data can prove invaluable as paleoenvironmental indicators in the genesis of sedimentary mineral deposits. To reiterate, most of the arguments for global anoxia in the past are based largely upon negative evidence; simply the absence of widespread oxidation. Yet, if the concept of a gradual oxygenation of the hydrosphere as a consequence of thermal decay is to be acceptable, evidence should exist for anoxia within those paleodepositional environments where anoxia is not observed in modern counterparts.

With the exception of an isolated Cambrian occurrence, stromatolitic phosphorites are unique to the Precambrian. No adequate explanation for their apparent disappearance from the geologic record of younger rocks has yet been found. Southgate (7) noted that stromatolitic phosphorites appear to have formed in shallow-water environments (lagoons or intratidal pools) where fluctuations in both water levels and temperatures may have created the "stressed-environment which created the appropriate physicochemical conditions" for phosphate formation. To reiterate, phosphate formation, while regulated by pH, is ultimately controlled by the flux of non-biodegraded, phosphate-rich cellular matter to the bottom sediments. This, in turn, requires that the biological demand for oxygen far exceeds its rate of supply inhibiting aerobic biodegradation. In an environment where water circulation is severely restricted, and
mixing with the open ocean is discontinuous (i.e. lagoons and intratidal pools), oxygen supply is fully dependent upon its solubility as a function of temperature and salinity. Hence, in the scenario envisaged by Southgate for the formation of stromatolitic phosphorites, generally higher Precambrian surface temperatures compounded by diurnal temperature increases and perhaps hypersalinity might have ensured requisite low oxygen solubilities leading to extended periods of shallow-water anoxia. Cyanobacteria, by virtue of their facultative photosystem and tolerance of extreme conditions, could in turn have guaranteed high photosynthetic productivity as long as nutrients were periodically replenished. With global cooling effecting a general increase in oxygen solubility, conditions favouring shallow-water phosphorite formation would have become progressively rarer, explaining the paucity of Phanerozoic stromatolitic phosphorites.

Sedimentary gold and uranium deposits of the Lower Proterozoic Witwatersrand formations, South Africa (2.2-2.6 by BP) provide an excellent example where known biogeochemical principles can be applied in an unformatarian approach to the interpretation of enigmatic Precambrian metal-mineral associations. The bulk of the gold and uranium in the western Witwatersrand goldfields is contained within thin layers of carbonaceous material, termed thucolite, normally situated at the top of typical fluviatile fining-upward sequences characterized by oligo-
mitic quartz-pebble conglomerates hosting detrital gold, uraninite and pyrite. Individual thucolite seams are patchy, seldom exceeding a few square metres in area and a couple of centimetres in thickness. The origin of thucolite has been subject to considerable debate. Most geologists currently favour primitive microbial mats as precursors to the organic carbon.

Hallbauer and Van Warmelo (8) presented evidence to argue for the assimilation of gold and uranium during growth of the 'lichens' along with concentration of detrital gold and uraninite between the fungal columns. Oxidation of the carbonaceous residues followed by SEM examination revealed that the internal and external structure of Thuchomyces lichenoides was effectively preserved as encrustations of U, Th, Si, Ti and Au (9). Subsequent studies by Zumberge et al. (10) on the Vaal Reef thucolites supported the dual authigenic-detrital origin for the metals. In variance, however, they maintained the primary mineralization as a consequence of syndiagenetic biodegradative processes effecting reduction of the uranyl ion and 'in situ' crystallization of colloidal gold or gold-organic complexes within the decomposing mats. Yet, while the probability of post mortem accumulation cannot be ruled out, this process alone fails to account for the remarkable degree of preservation of internal 'plant' structures which, by current knowledge, should have been destroyed by bacterial enzym-
atic attack.

Numerous studies have demonstrated that metal uptake by microorganisms generally comprises two distinct phases: the non-specific, energy-independent sorption of metal ions onto the cell wall, followed by the selective, metabolism-dependent translocation across the cell wall into the cytoplasm. By virtue of the anionic nature of most reactive functional sites in the cell wall, indiscriminate metal binding is unavoidable. Studies by Beveridge and Murray (11) indicated that free carboxyl ligands of the cell wall effectively concentrate both gold and uranium from solution to substantial quantities. While the mechanisms have yet to be elucidated, their study further showed that the cell wall of Gram-positive bacteria (specifically B. subtilis) possessed the capacity for energy-independent redox reactions such as the reduction of auric chloride to elemental gold.

All organisms require for growth a number of polyvalent metal ions. Microorganisms vary widely both in their metal requirements and in the mechanism by which they sequester potentially toxic metals. Metal toxicity occurs when the metals interfere with the normal function of the cell wall or when they enter into the cell and disrupt normal physiological activity. Algae have been shown to sequester copper intracellularly as cuprous-organic compounds while some fungi precipitate unwanted copper in the form of intracellular copper sulphides.
Unicellular microfossils from the Proterozoic Balbirini Dolomite were reported by Oehler and Oehler (12) to contain fine-grained sulphides inside their cells or cell walls. Similarly, spheroidal microfossils from the Archean Fig Tree Series were described by Pflug (13) as containing significant enrichments of copper and nickel associated with the cell walls. He interpreted the metal enrichments as indicating the organism's capacity to precipitate metal salts from solution. While this may be the case, a more plausible explanation would appear to be the organism's inability to prevent indiscriminate metal binding to the cell wall and, in the case of the Proterozoic microfossils, perhaps an early mechanism for evading toxicity through the precipitation of intracellular sulphides. This would imply that cell walls of Precambrian microorganisms were functionally and possibly structurally homologous to the walls of extant organisms; a view supported by the proposed antiquity of Gram-negative, Gram-positive and protoeukaryotic organisms in the phylogenetic scheme of Fox et al. (14).

Generally speaking, many fungi possess a remarkably high degree of tolerance towards potentially-toxic metals relative to various other groups of microorganisms. Studies by Zajic and Chiu (15), for example, demonstrated that growth of *Penicillium* sp. was uninhibited by uranium on the short-term over the range 100-1000 ppm, with only moderate long-term growth retardation occurring at 1000
ppm. They argued that not only was uranium non-toxic but it probably served a physiological function as it was actively concentrated intracellularly. Regardless of why uranium was concentrated, it is noteworthy that this fungus actively extracted 70% of the uranium from a 100 ppm solution to a maximum of 17% of the organism's dry weight.

In view of the observations of Zajic and Chiu, the proposal for active assimilation of gold and uranium during growth of the Witwatersrand microbial mats is consistent with the proposed lichen interpretation for the mats. It is conceivable that the process of active assimilation of uranium played a significant role in the inhibition of biodegradation; effectively preserving primary 'plant' structures through a natural uranium 'pickling' process.

In a recent review on the biogeochemistry of uranium, Taylor (16) concluded that "there is no way in which biology can have entered into the process of transport and precipitation of uranium wholly under highly reducing conditions" (pg. 499). Yet, detrital pyrite from the Witwatersrand sediments is commonly cited as direct evidence for the proposed reducing atmosphere throughout the early Precambrian. Under an oxygenated atmosphere, pyrite should not be an important detrital component of fluviatile sediments. Hence, the detrital pyrite of the Witwatersrand appears to be inconsistent with biogenic
models for syngenetic uranium accumulation.

The oxidation of pyrite is largely a function of aerobic bacterial catalysis which, in turn, is sensitive to ambient oxygen levels. The involvement of biological agencies in the accelerated oxidation of pyrite has been unequivocally demonstrated by numerous investigators and is adequately reviewed by Ralph (17). The organisms most commonly associated with pyrite degradation are the sulphur oxidizing bacteria, *Thiobacillus* spp., and the iron oxidizing bacteria, *Methylococcus* spp. and *Thiobacillus ferroxidans*. The catalytic ability of these bacteria far outweighs that of any other natural oxidizing agent. *Thiobacillus ferroxidans*, while mediating the oxidation of pyrite, is capable of catalyzing ferrous iron oxidation at rates $10^5$ to $10^6$ greater than abiotic rates.

Physical factors can severely limit the rates of bacterial oxidation of pyrite by restricting access of dissolved nutrients and gases (ie. CO$_2$, O$_2$). If sediment deposition is rapid, and reducing conditions established soon after deposition, extensive oxidation of pyrite is unlikely to occur. However, in the Witwatersrand conglomerates, the high degree of rounding of the detrital grains does not suggest rapid transport and deposition from a near source. Hence, an alternative explanation must be sought to account for the survival of pyrite.

There are a number of environmental factors which
come into play to inhibit growth of pyrite oxidizing bacteria (i.e. nutrient supply, temperature, pH, Eh, etc.) but of particular significance to the Witwatersrand sediments are the possible implications of high uranium concentrations in the surface (river) waters. The tolerance and toxicity of the uranyl ion in *Thiobacillus ferroxidans* was investigated by Tuovinen and Kelly (18). They found that the uranyl ion depressed growth of these bacteria on ferrous iron at low uranium concentrations between 60-95 ppm. While uranium resistant cultures could be developed with mutant strains, toxicity at higher levels could not be avoided. Uranium toxicity of *Thiobacillus ferroxidans* resulted from the blocking of reactive sites of the cell wall and/or by interference with normal physiological functions within the cell. High uranium concentrations in the transporting and depositional waters therefore might explain the apparent retardation of bacterial pyrite oxidation in the Witwatersrand sediments prior to the establishment of post-depositional anóxia. High dissolved uranium levels would be consistent with the concept of an oxidizing atmosphere throughout the Archean, oxidative weathering and leaching of the source rocks (particularly in conjunction with microbial activity), active assimilation of uranium by primitive fungal mats, and inhibited bacterial enzymatic attack on the uranium-loaded cellular matter.

In conclusion, it must be acknowledged that the con-
cept of evolution remains only a theory. While enough
evidence has accumulated to compel natural scientists to
accept the concept of evolution, it can never be cate-
gorically stated. Evolutionary models provide a consis-
tent explanation to a vast number of questions that can-
not be answered satisfactorily in any other way. The
physiological responsibility, so to speak, of the living
biosphere is to maintain a fine state of homeostasis with
the chemical milieu. It follows that any gradual change
within the chemical environment must be met by a gradual
metamorphosis within the biosphere as a whole. For this
reason, it is impossible to separate the concepts of-
liothospheric, atmospheric, hydrospheric and biospheric
evolution. This thesis views the evolution of biogeo-
chemical element cycles as a single entity with tempera-
ture change as a common denominator linking major evolu-
tionary events throughout the Precambrian. Like the
myriad of earlier models, the evolutionary theme pre-
presented herein may be considered incorrect at a later date,
but for the moment it provides a simple plausible explan-
ation to the enigmatic coincidence of a number of appar-
ently unrelated evolutionary events.

"Men's curiosity searches past and future
and clings to that dimension
but to apprehend;
the point of intersection of the timeless,
with time, is an occupation for the saint"
T. S. Elliot

from the Dry Salvages "Four Quartets"
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PHOTOGRAPHIC PLATES
Plate 1

Unreacted and reacted control cells of *Bacillus subtilis*.

A. **TEM image of a typical uranium-loaded cell of B. subtilis** showing the homogeneous appearance of the cytoplasm (cp) and the high electron-density of the cell wall (cw) indicating the cite of uranium uptake (bar = 500 nm).

B. **TEM image of unreacted cells of B. subtilis artificially aged at 100°C for 100 days with a magnetite redox buffer.** Note the incipient crystallization of the cell walls (small arrows) and the formation of large platy aggregates of a 'probable' phosphate mineral (large arrow) (bar = 500 nm).
Plate 2

Thermal degradation of uranium-loaded cells of *B. subtilis* artificially aged with a magnetite redox buffer for 1 and 10 days at 100°C.

A. TEM image of a cell of *B. subtilis* after 1 day. Note separation and loss of the cell wall and high electron-density of the cytoplasm (bar = 200 nm).

B. TEM image of a cell of *B. subtilis* after 10 days. Note complete loss of the cell wall and intracellular crystallization of fine, acicular and tabular phosphate microcrysts (bar = 200 nm).

C. TEM image of cells of *B. subtilis* after 10 days. Note extracellular crystallization of fine, tabular to platy phosphate microcrysts (bar = 200 nm).
Plate 3

Thermal degradation of uranium-loaded cells of *B. subtilis* artificially aged in SiO₂ with a magnetite redox buffer for 1, 10 and 100 days at 100°C.

A. TEM image of cells of *B. subtilis* after 1 day. Note separation, incipient crystallization and partial loss of cell walls (arrow) and incipient crystallization of cytoplasmic constituents (bar = 200 nm).

B. TEM image of cells of *B. subtilis* after 10 days. Note intense intracellular phosphate crystallization (bar = 200 nm).

C. TEM image of a cell of *B. subtilis* after 10 days. Note intracellular crystallization of fine and coarse, tabular to platy phosphate microcrysts. Relics of the cell wall are preserved as aligned platy phosphate microcrysts (arrow) (bar = 200 nm).

D. TEM image of cells of *B. subtilis* after 100 days. Note loss of basic cell morphology and intense crystallization of coarse, tabular to platy phosphate microcrysts displaying hexagonal symmetry (bar = 200 nm).
Microanalysis of degradation products of a uranium-loaded cell of *B. subtilis* artificially aged in SiO₂ with a magnetite redox buffer for 10 days at 100°C (bar = 200 nm).

A. EDAX spectrum for Epon 812 matrix. Note trace amounts of chlorine and silicon.

B. EDAX spectrum for a single phosphate microcryst associated with the relict cell wall.

C. EDAX spectrum for the cytoplasmic constituents undergoing incipient crystallization (solid profile) compared with the spectrum for the extracellular phosphate microcrysts (dotted profile).
Plate 5

Thermal degradation of uranium-loaded cells of *B. subtilis* artificially aged with a sulphur redox buffer for 1 and 100 days at 100°C.

A. TEM image of cells of *B. subtilis* after 1 day. Note loss of cell walls and incipient extracellular crystallization of tabular to platy phosphate microcrysts (bar = 200 nm).

B. TEM image of cells of *B. subtilis* after 100 days. Note general loss of basic cell morphology, intense crystallization of phosphate microcrysts (black areas) and incipient polymerization of organic cellular constituents (fine, electron-dense fibrils) (bar = 500 nm).

C. TEM image of a cell of *B. subtilis* after 100 days. Close-up of a single cell showing polymerization of cellular constituents along the perimeter of the cell and extracellular phosphate crystallization (bar = 200 nm).

D. TEM image of a single phosphate microcryst synthesized in the 100-day run. Note the perfect micaceous, basal (001?) cleavage (bar = 200 nm).
Plate 6

Thermal degradation of uranium-loaded cells of B. subtilis artificially aged with a sulphur redox buffer for 200 days at 100°C.

A. TEM image of cellular degradation products at low magnification. Note the complete loss of cell morphology and the homogeneous distribution of phosphate microcrysts and organic condensates (bar = 1 micron).

B. TEM image of a large, tabular phosphate microcryst associated with condensed organic residues (bar = 200 nm).

C. TEM image of coarse, granular phosphate aggregates (bar = 200 nm).

D. TEM image of a typical tabular phosphate microcryst (a) surrounded by uranium-complexed, polymerized organic condensates (b). Note the perfect basal cleavage in the phosphate crystal (bar = 200 nm).

E. EDAX spectra for the authigenic phosphate microcryst (a) and the condensed organic matrix (b) shown in D.
Plate 7

Thermal degradation of uranium-loaded cells of B. subtilis artificially aged in SiO₂ with a sulphur redox buffer for 1 and 100 days at 100°C.

A. TEM image of a cell of B. subtilis after 1 day. Note increased electron-density of the cytoplasm and partial breakdown of the cell wall (bar = 200 nm).

B. TEM image of a cell of B. subtilis after 100 days. Note intense polymerization of uranium-complexed cellular constituents and extracellular crystallization of coarse phosphate microcrysts (bar = 200 nm).

C. TEM image of cellular degradation products at low magnification. Note the complete loss of cell morphology (bar = 500 nm).

D. TEM image of euhedral phosphate microcrysts and uranium-complexed organic residues at higher magnification. Note the diffuse, electron-dense coatings on the crystalline phosphate and the perfect, basal (001?) cleavage in the largest microcryst (bar = 200 nm).
Plate 8

Thermal degradation of uranium-loaded cells of *B. subtilis* artificially aged in SiO$_2$ with a sulphur redox buffer for 200 days at 100°C.

A. TEM image of cellular degradation products at low magnification. Note growth and recrystallization of phosphate microcrystals and pronounced condensation of organic residues (bar = 1 micron).

B. TEM image of tapered 'bundles' of fine phosphate needles with diffuse, electron-dense organic matter possibly coating the entire aggregate (bar = 200 nm).

C. Coarse crystalline aggregates comprised of individual phosphate needles (bar = 1 micron).
Plate 9

Thermal degradation of uranium-loaded cells of \textit{B. subtilis} artificially aged in SiO\textsubscript{2} and CaCO\textsubscript{3} with a magnetite redox buffer for 1 day at 100°C.

A. TEM image of a cell of \textit{B. subtilis} showing complete loss of the cell wall and incipient crystallization of the cytoplasmic constituents as fine, acicular phosphate microcrysts (bar = 200 nm).

B. TEM image of a cell of \textit{B. subtilis} showing crystallization of the cell wall as tabular to platy phosphate microcrysts. Note incipient polymerization of organic residues (arrow) (bar = 200 nm).

C. TEM image of a cell of \textit{B. subtilis} showing intense, intracellular phosphate crystallization and incipient polymerization (arrow) (bar = 100 nm).

D. TEM image - magnification of a portion of the cell shown in 'B'. Note the alignment of platy phosphate microcrysts along the cell surface and the polymerization of uranium-complexed organic residues (bar = 100 nm).
Plate 10

Thermal degradation of uranium-loaded cells of B. subtilis artificially aged in SiO₂ and CaCO₃ with a magnétite reox buffer for 10 and 100 days at 100°C.

A. TEM image of a cell of B. subtilis after 10 days. Note loss of the cell wall and intense, intracellular crystallization of fine, acicular phosphate microcrysts (bar = 200 nm).

B. TEM image of a cell of B. subtilis after 10 days showing crystallization of the cell wall and cytoplasmic shrinkage (bar = 200 nm).

C. TEM image of a cell of B. subtilis after 100 days beginning to lose the characteristic cell outline. Note the crystallization of platy phosphate microcrysts with a diffuse (speckled) electron-dense coating (bar = 200 nm).

D. TEM image of a cell of B. subtilis after 100 days showing intracellular crystallization of coarse, crystalline phosphate aggregates which appear to be coated by diffuse, electron-dense organic residues (bar = 200 nm).

E. TEM image of a larger, single platy phosphate microcryst (twinned) produced after 100 days. Note the hexagonal symmetry and the diffuse, electron-dense organic coating (speckled) (bar = 200 nm).
Plate 11

Thermal degradation of uranium-loaded cells of *B. subtilis* artificially aged in SiO$_2$ and CaCO$_3$ with a sulphur redox buffer for 1, 10 and 100 days at 100°C.

A. TEM image of a cell of *B. subtilis* after 1 day. Note loss of cell wall and incipient crystallization of the cytoplasmic constituents (bar = 200 nm).

B. TEM image of a cell of *B. subtilis* after 10 days. Note extracellular crystallization of fine to coarse phosphate aggregates coated with electron-dense organic residues (bar = 200 nm).

C. TEM image of uranium-complexed, polymeric organic residues and crystalline phosphate synthesized after 100 days (bar = 200 nm).

D. TEM image of uranium-complexed organic residues and phosphate microcrystals retaining the original outline of the bacterial cell after 100 days. Note basal (001?) cleavage in the largest phosphate microcryst (arrow) (bar = 200 nm).
Plate 12

Thermal degradation of ferrous-loaded cells of \textit{B. subtilis} artificially aged with a sulphur redox buffer for 10 days at 100\degree C.

A. TEM image of a portion of a coarse, electron-dense aggregate composed of platy phosphate microcrystals and elemental sulphur (bar = 500 nm).

B. TEM image of a large, isolated phosphate microcryst displaying hexagonal symmetry and a platy habit (bar = 500 nm).

C. TEM image of intermixed phosphate and sulphur from the coarse electron-dense aggregates (bar = 500 nm).

D. EDAX spectrum for the isolated phosphate microcryst (B) showing it to be an iron phosphate mineral.

E. EDAX spectrum for the crystalline aggregate described in 'C' showing it to be composed predominantly of elemental sulphur with lesser amounts of an iron phosphate mineral.
Authigenic copper sulphides formed from the thermal degradation of copper-loaded cells of *B. subtilis* artificially aged in SiO₂ with a sulphur redox buffer for 10 days at 100°C.

A. TEM image of a mixed assemblage of copper sulphide euhedra, framboïds and crystalline aggregates at low magnification (bar = 1 micron).

B. TEM image of copper sulphide microcrysts associated with electron-transparent, colloidal organic(?) matter, interpreted as incipient framboïd development (bar = 500 nm).

C. TEM image of copper sulphide microcrysts associated with electron-transparent colloidal organic(?) matter, appearing both as discrete euhedra and dense internal crystal aggregates (bar = 500 nm).

D. TEM image of a dense copper sulphide crystal aggregate enrobed in electron-transparent organic(?) matter (bar = 500 nm).
Plate 14

TEM image of a copper sulphide framboid formed from the thermal degradation of copper-loaded cells of *B. subtilis* artificially aged in SiO$_2$ with a sulphur redox buffer for 10 days at 100°C. Note the concentric layering of hexagonal copper sulphide microcrysts and electron-transparent organic(?) matter imparting hexagonal symmetry and a polygonal outline to the overall structure (bar = 500 nm).
Plate 15

 Authigenic copper sulphides formed from the thermal degradation of copper-loaded cells of *B. subtilis* artificially aged in SiO$_2$ with a sulphur redox buffer for 200 days at 100°C.

A. TEM image of a frambooidal aggregate of copper sulphide (large arrow) together with a large, electron-dense granular aggregate and platy microcryst (small arrow) of a probable phosphate mineral (bar = 1 micron).

B. TEM image of a 'book' of stacked, hexagonal or pseudo-hexagonal copper sulphide microcrysts. The tapered edges of the stacked sulphide plates are reminiscent of the pseudohexagonal rhombic prism form of the orthorhombic chalcocite polymorph (bar = 500 nm).

C. TEM image of a cubic crystal aggregate of the copper sulphide, digenite ((Cu,Fe)$_9$S$_5$) (bar = 500 nm).
Authigenic copper sulphides formed from the thermal degradation of copper-loaded cells of *B. subtilis* artificially aged in SiO₂ with a sulphur redox buffer for 200 days at 100°C.

A. TEM image of a large hexagonal crystal aggregate of copper sulphide (either covellite (CuS) or hexagonal chalcocite (Cu₂S)) \( \text{bar} = 500 \text{ nm} \).

B. TEM image of hexagonal crystal aggregates of copper sulphide associated with large, electron-dense, granular aggregates of a probable phosphate mineral \( \text{bar} = 500 \text{ nm} \).

C. TEM image of a 'quasi-spheroidal' crystal aggregate of copper sulphide \( \text{bar} = 500 \text{ nm} \).

D. a) EDAX spectrum for the cubic crystal aggregate of digentite shown in plate 15C. Note the trace amount of iron which is characteristic of digentite.

b) EDAX spectrum typical of the hexagonal copper sulphide phase illustrated in A and B.
Plate 17

TEM image of a large hexagonal crystal aggregate of copper sulphide formed from the thermal degradation of copper-loaded cells of *B. subtilis* in SiO₂ with a sulphur redox buffer after 200 days at 100°C (bar = 1 micron).

A. TEM photomicrograph developed normally showing typical, high electron-density characteristic of a crystalline phase of copper sulphide.

B. The same photomicrograph underdeveloped showing a high degree of variation in the electron-density of the large crystal aggregate. Growth of the crystal aggregate appears to have occurred by epitaxial growth on at least three hexagonal microcrysts in crystallographic continuity.
Authigenic zinc sulphide formed from the thermal degradation of zinc-loaded cells of \textit{B. subtilis} artificially aged with a sulphur redox buffer for 10 days at 100°C.

A. TEM image of discrete hexagonal microcrysts and crystal aggregates of \textit{wurtzite} (ZnS) enrobed in electron-transparent organic(?) matter (bar = 500 nm).

B. TEM image of a dense, framboïd-like crystal aggregate of \textit{wurtzite} (bar = 500 nm).

C. TEM image of an assemblage of isolated, short-prismatic to platy hexagonal microcrysts of \textit{wurtzite} (bar = 1 micron).

D. TEM image of a large hexagonal crystal aggregate of \textit{wurtzite} which appears to have formed by epitaxial growth of a number of smaller hexagonal microcrysts (bar = 1 micron).
Plate 19

Thermal degradation of zinc-loaded cells of *B. subtilis* artificially aged with a sulphur redox buffer for 10 and 100 days at 100°C.

A. TEM image of cells of *B. subtilis* after 10 days. Note the intracellular formation of electron-opaque granules of ZnS and the development of granular spherules (bar = 200 nm).

B. TEM image of a possible cell wall relict consisting of electron-opaque, granular zinc sulphide (top of photo) from a 10 day-old run (bar = 200 nm).

C. TEM image of cells of *B. subtilis* after 100 days. Note the loss of intracellular zinc sulphide granules and the slight increase in size of the granular zinc sulphide spherules (bar = 200 nm).

D. TEM image of a possible cell wall relict consisting of granular zinc sulphide (left of photo) from a 100 day-old run (bar = 200 nm).
Thermal degradation of zinc-loaded cells of *B. subtilis* artificially aged with a sulphur redox buffer for 200 days at 100°C.

A. TEM image of cells of *B. subtilis* showing near complete loss of electron-opaque sulphide granules and a marked increase in the size of the granular zinc sulphide spherules (bar = 200 nm).

B. TEM image of cells of *B. subtilis* showing incipient polymerization of the cytoplasmic constituents (arrow) (bar = 200 nm).

C. TEM image of a possible cell wall relict consisting of granular zinc sulphide (bar = 200 nm).

D. SEM image of cells of *B. subtilis* from a whole mount showing zinc sulphide spherule to be attached to a single bacterium cell (arrow indicates location of EDAX analysis shown in E) (bar = 200 nm).

E. EDAX spectrum for the zinc sulphide spherule shown in D. EDAX spectra for the granular sulphide spherules were identical to the spectra for the hexagonal sulphide microcrystals (Plate 19). Hence, only one spectrum is illustrated to show that the minerals are a pure zinc sulphide phase.
Plate 21

The effects of 'clumping' of bacterial cells on authigenic mineralization and cellular degradation.

A. SEM image of copper-loaded cells of *B. subtilis* artificially aged in SiO₂ with a sulphur redox buffer for 100 days at 100°C (whole mount) showing that cells remained clumped throughout the entire experimental run (bar = 1 micron).

B. Composite TEM image of a section through a clump of copper-loaded cells of *B. subtilis* artificially aged in SiO₂ and CaCO₃ with a magnetite redox buffer for 10 days at 100°C. Cellular degradation and authigenic mineralization (undetermined composition) are most intense at the perimeter of the clump (top of the photo) decreasing progressively towards the centre of the clump (bottom of photo) where the cells appear relatively unaltered (bar = 1 micron).
The effects of 'clumping' of bacterial cells on authigenic mineralization and cellular degradation.

A. TEM image of a section through a clump of copper-loaded cells of *B. subtilis* artificially aged in CaCO$_3$ and bentonite with a magnetite redox buffer for 1 day at 100°C. Note incipient crystallization along the perimeter of the cell clump (arrows) and the relatively 'fresh' appearance of the bacterial cells (unstained) (bar = 1 micron).

B. TEM image of a section through a clump of copper-loaded cells of *B. subtilis* artificially aged in CaCO$_3$ and bentonite with a magnetite redox buffer for 100 days at 100°C. Note intense cellular degradation, loss of basic cell morphology and precipitation of platy microcrysts of a probable phosphate. By 100 days, cellular degradation and authigenic mineralization are homogeneous with respect to location within a cell aggregate (bar = 1 micron).
Plate 23

Thermal degradation of copper-labeled cells of *B. subtilis* artificially aged in CaCO\(_3\) and bentonite with a magnetite redox buffer for 1, 10 and 100 days at 100°C.

A. TEM image of a cell of *B. subtilis* after 1 day showing incipient crystallization of a probable phosphate mineral (arrow) along the perimeter of the cell (cell wall?) (bar = 200 nm).

B. TEM image of a cell of *B. subtilis* after 10 days showing loss of cell wall and intense intracellular crystallization of a probable phosphate mineral (bar = 200 nm).

C. TEM image of a normal section of cells of *B. subtilis* after 100 days showing intense cellular degradation, authigenic mineralization (phosphate?) and mineral-organic interaction (bar = 200 nm).

D. TEM image of an ultrathin section of cells of *B. subtilis* after 100 days showing intense intracellular condensation of organic matter. Large electron-dense patches represent microcrysts of a probable phosphate mineral coated in condensed organic residues (bar = 200 nm).