Lecture 1 - *Fundamentals of Microbial Physiology*

Background Reading:
- Microbiology - Review cytoplasm, cell membrane, periplasm, outer membrane
- Biochemistry - Review glycolysis, the TCA cycle, and the electron transport chain
- Chemistry - Review the periodic table, oxidation/reduction, electron shells

*How do microbes live?*
- What do they eat and what don’t they eat?
- Why do they eat it and how do they choose?
- What is left over, and who’s interested in that?
- How do they fit into the big picture?

- Physiology is at the Center of Microbiology
- The Three Basic “Food Groups”
- Rethinking Metabolism: Beyond the Mitochondrion
- Oxidation States and Oxidation/Reduction Reactions
- The Players: CHONPS

**Physiology is at the Center of Microbiology**

*Microbiology is the study of how microorganisms live*
- What do they eat? What don’t they eat? How do they choose?
- Where do they live? How are they constrained from living elsewhere?
- With which organisms do they compete? Upon which organisms do they rely?

*Microbiology is the study of molecular processes*
- Microbes select and eat certain molecules like we select and eat carrots instead of staplers.
- Microbes sense molecules in the environment like we sense cars on the street, tables in a room, and food on our plates.
- Microbes recognize molecules for attachment like we find beds for sleeping and chairs for sitting.

*Biochemistry is the heart of physiology*
- The structures of biomolecules describe the environment in which a microbe is living
- The metabolic pathways describe the microbe’s interactions with the environment
- Food webs and nutrient cycling describe interactions among microorganisms
- Therefore, understanding how different microorganisms make a living is tantamount to understanding the differences in their biochemistry.

*Microbial physiology only makes sense in the context of its environment*
- Most microbes perform only a small portion of the physiology we learn in Biochemistry
- Some microbes excrete their food “half-eaten,” performing only parts of biochemical pathways which we have defines in a relatively arbitrary way
- Other microbes make a living eating these “leftovers,” thereby “completing” a biochemical pathway
- Some microbes excrete substances as by-products of normal physiological processes
- Other microbes may recycle these byproducts for other purposes, entirely
Different microbes may use the same substances for different purposes (see below)
Some microbes perform biochemical processes that are necessary for others around them to live
Both biotic and abiotic interactions help us define a microbes way of life

The Three Basic “Food Groups”

Molecules are Food
- Humans eat a variety of very complex foods (burgers and fries) to obtain essential nutrients
- Some of these nutrients are simple (like iron) and some are complex (like methionine), but all are embedded in a complex mixture of substances
- Microbes typically eat these molecules directly
- To understand what a microbe eats, and why it eats it, we must understand its food

What Human Food is
- From a physiological perspective, human food is broken into three basic food groups:
  - Grains and starches (carbohydrates) provide carbon chains and energy
  - Meat and fish (protein) provides amino acids and nitrogen
  - Fruits and vegetables often provide vitamins and trace elements
- A balanced diet provides all of the nutrients required for health maintenance and growth
- From an ecological perspective, humans do not compete with other organisms for food; in this way, human physiology differs from the complex tradeoffs we see in microbial approaches to eating

The Hidden Human food
- There is one important component of human food that is almost never mentioned
- All energy is derived from burning food, that is, respiration to molecular oxygen
- Oxygen is the “hidden” food since we are constantly consuming it
- Yet without oxygen, we cannot use the other foods; oxygen provides the electron sink that allows us to “burn” to food (move electrons)
- Moving electrons from carbohydrates, fats, and proteins to oxygen allows us to store energy for later use

What Microbial Food is
- We must consider microbial food on a molecular scale
- Microbes need to eat different molecules for the same reasons humans do
- Microbes need sources of carbon, nitrogen, phosphorus, sulphur and other nutrients to grow
- Microbes need substances from which they can make energy (ATP) to drive their metabolism
- Microbes may also need an electron sink to utilize some energy sources

The Three Microbial Food Groups
- A microbe eats a molecule to get one or more of the three essential food groups:
  - It may want the nucleus, since it needs carbon, nitrogen, and sulphur nuclei to make cells
  - It may want the electrons, since moving electrons makes energy
  - It may want the empty electron shells, since it needs a place into which it can move the electrons
- The significance of the electrons is discussed below

Rethinking Metabolism : Beyond the Mitochondrion
Metabolism from the mitochondrial perspective
- Introductory biochemistry (metabolism) is taught from a mitochondrial (human) perspective
- This is specialized metabolism
  - Sugars are considered primary energy sources; fats and proteins get cursory attention
  - Glycolysis serves to convert glucose into acetyl-CoA
  - The TCA cycle oxidizes acetyl-CoA to CO₂, producing reduced NADH
  - The respiratory chain serves to oxidize NADH, reduce O₂ to H₂O and pump protons
  - The ATPase allow protons to return and make ATP

Microbial metabolism is more varied
- In this course, some of what we will learn will be:
  - Very few organisms use sugars as an energy source
  - Glycolysis is not really used to make acetyl-CoA; it actually evolved to make glucose
  - The TCA cycle is rarely a cycle; often only a subset of reactions occur
    - When it is a cycle, it often runs “backwards,” reducing and incorporating CO₂
  - Respiratory chains can oxidize more substances than just NADH
  - Respiratory chains rarely reduce O₂; often other substances accept electrons
  - The ATPase often serves to pump protons out of the cell, consuming ATP (hence its name)
  - Some organisms move electrons about (to make energy) without using a terminal electron acceptor

Each organism in its place
- We will consider different kinds of organisms and discuss how each makes a living
- We will learn that different kinds of organisms serve different roles in global nutrient cycling
- We will learn that the actions of some organisms create opportunities for other organisms to exist
- We will learn that some organisms must cooperate for either of them to survive
- As a result, you will learn to appreciate how microorganisms – in all their diversity – mediate the continued propagation of our biosphere.

Oxidation States and Oxidation/Reduction Reactions

Oxidation states of Atoms
- An atom with the same number of electrons as protons has zero net change
- This state is denoted an oxidation state of zero (0)
- If an atom has more electrons than protons, it has a negative oxidation state (e.g., oxygen -2)
- If an atom has fewer electrons than protons, it has a positive oxidation state (e.g. hydrogen +1)
- If an atom loses electrons, its oxidation state increases: this process is called oxidation
- If an atom gains electrons, its oxidation state decreases: this process is called reduction
- Consult your handout for a discussion of oxidation states

Oxidation/Reduction
- Chemical reaction wherein atoms change oxidation states are called Oxidation/Reduction reactions
- Oxidation/Reduction reactions are called “Redox” reactions for short
- In biological systems, these reactions effectively move electrons from one substance to another
- Yet all electrons are not created equal: electrons in some contexts have more energy than others
- If an electron moves from a context where it has much energy to a context where it has less energy, the remaining energy can be harnessed to perform other work
Fundamentally, this is how life works: moving electrons around and harnessing the energy

**Electronegativity and half-reactions**
- All electrons are not created equal; they can differ in their energetic potential
- The process of moving them about can differ, depending on the identities of the donors and recipients
- These values are summarized in Table 1
- Each “half-reaction” involves electrons, a recipient, and a product
- Two half-reactions are combined to complete a redox reaction:

<table>
<thead>
<tr>
<th>Step</th>
<th>Reaction</th>
<th>Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>a substance is oxidized: $\text{Substrate} \rightarrow \text{Product} + 2e^- + 2H^+$</td>
<td>-320 mV</td>
</tr>
<tr>
<td>2</td>
<td>NAD$^+$ is reduced: $\text{NAD}^+ + 2e^- + 2H^- \rightarrow \text{NADH} + H^+$</td>
<td>-320 mV</td>
</tr>
<tr>
<td>3</td>
<td>electrons are donated: $\text{Cytochrome b}<em>{556} \text{oxidized} + 2e^- + 2H^+ \rightarrow \text{Cytochrome b}</em>{556} \text{reduced}$</td>
<td>+46 mV</td>
</tr>
<tr>
<td>4</td>
<td>electrons are received: $\text{Cytochrome b}<em>{556} \text{reduced} \rightarrow \text{Cytochrome b}</em>{556} \text{oxidized} + 2e^- + 2H^+$</td>
<td>+46 mV</td>
</tr>
<tr>
<td>5</td>
<td>electrons are donated: $\frac{1}{2} \text{O}_2 + 2e^- + 2H^+ \rightarrow \text{H}_2\text{O}$</td>
<td>+815 mV</td>
</tr>
</tbody>
</table>

**SUM**
- $\text{Substrate} + \frac{1}{2} \text{O}_2 \rightarrow \text{Product} + \text{H}_2\text{O}$

**Following the electrons**
- In steps 1 & 2, electrons are taken from a substrate and NAD$^+$ is reduced
- In steps 3 & 4, electrons are transferred to a cytochrome, regenerating oxidized NAD$^+$
- In steps 5 & 6, electrons are transferred to oxygen, regenerating the oxidized cytochrome
- Consider a dehydrogenase that strips very low energy from the substrate ($E_{m,7} = -480$ mV)
- In each case, the electrons moved “down” the table until ending up on oxygen
- Electrons travel readily “down” compounds in Table 2 (e.g. from NAD$^+$ to cytochrome b to oxygen)
- In this process, the electrons can lose energy
- This energy can be harnessed to perform work, like pumping protons out of the cell
- This gradient (or proton motive force) can be used to drive ATP synthesis
- The ΔG “available” for work can be calculated as shown below

**The Thermodynamics**
- This should all be a review of the Chemistry and is for your edification; although you won’t be asked to solve equations on exams, familiarity with the thermodynamics makes the Biology easier to understand.
- The ins-and-outs of ΔΨ will be covered in Lecture 6.
- A proton gradient ($\Delta \mu_{\text{H}^+}$) can be expressed in terms of the steady-state charge separation ($\Delta \Psi$) and the chemical gradient resulting from asymmetric proton distribution ($\Delta p\text{H}$): $\Delta \mu_{\text{H}^+} = -F\Delta \Psi + 2.3RT\Delta p\text{H}$; where $F$ is the Faraday constant, $R$ is the gas constant, and $T$ is the absolute temperature
- The proton motive force allows easier comparison with redox potential changes: $\Delta p = -(\Delta \mu_{\text{H}^+})/F$
- The “standard” Gibbs free energy for a reaction (at 1 M or 1 atm solutes) is: $\Delta G^0 = -2.3RT\log_{10}K$
- The actual Gibbs free energy can be expressed as $\Delta G = -2.3RT\log_{10}K/\Gamma$, where $\Gamma$ is the observed ratio of products to reactants
- Therefore, $\Delta G = \Delta G^0 + 2.3RT\log_{10}\Gamma$, which is very handy
- Most redox couples are expressed in terms of their midpoint potentials at pH 7.0 ($E_{m,7}$), which is calculated from the standard redox potential $E^0$ (1 M concentration of all reactants, including protons, so at pH 0)
- The actual redox potential ($E_{h,7}$) can be calculated as: $E_{h,7} = E_{m,7} + (2.3RT/nF)\log_{10}([\text{ox}]/[\text{red}])$
- The free energy change is calculated as $\Delta G = -nF\Delta E_h$, where $n$ is the number of electrons
- So, for a $\Delta E_h$ of 1000 mV, the $\Delta G$ would be -96.5 kJ/mol per electron
TABLE 1: STANDARD HALF-REACTIONS AT PH 7.0

<table>
<thead>
<tr>
<th>Couple</th>
<th>E_{m,7} (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrodoxin_{oxidized} + 2e^− → Ferrodoxin_{reduced}</td>
<td>-432</td>
</tr>
<tr>
<td>CO_2 + 2e^− + 2H^+ → HCOOH (formate)</td>
<td>-432</td>
</tr>
<tr>
<td>HS_2O_3 (thiosulfate) + 2e^− + 2H^+ → H_2S (sulfide) + HSO_3^- (sulfite)</td>
<td>-412</td>
</tr>
<tr>
<td>2H^+ + 2e^− → H_2</td>
<td>-410</td>
</tr>
<tr>
<td>NAD^+ + 2e^- + 2H^+ → NADH + H^+</td>
<td>-320</td>
</tr>
<tr>
<td>(4Fe-4S)<em>{oxidized} + 2e^- → (4Fe-4S)</em>{reduced} in mitochondrial enzymes</td>
<td>-305</td>
</tr>
<tr>
<td>Lipoic acid + 2e^- + 2H^+ → Dihydrolipoic acid (e.g., in pyruvate dehydrogenase)</td>
<td>-290</td>
</tr>
<tr>
<td>S^0 + 2e^- + 2H^+ → H_2S</td>
<td>-270</td>
</tr>
<tr>
<td>FAD + 2e^- + 2H^+ → FADH_2</td>
<td>-220</td>
</tr>
<tr>
<td>H_3C-CHO (acetylaldehyde) + 2e^- + 2H^+ → H_3C-CH_2OH (ethanol)</td>
<td>-197</td>
</tr>
<tr>
<td>FMN + 2e^- + 2H^+ → FMNH_2</td>
<td>-190</td>
</tr>
<tr>
<td>Dihydroxy-acetone phosphate + 2e^- + 2H^+ → Glycero1-3-phosphate</td>
<td>-190</td>
</tr>
<tr>
<td>H_2C-CO-COOH (pyruvate) + 2e^- + 2H^+ → H_2C-CH_2OH-COOH (lactate)</td>
<td>-185</td>
</tr>
<tr>
<td>HOOC-CH_2-CO-COOH (oxaloacetate) + 2e^- + 2H^+ → HOOC-CH_2-CHOH-COOH (malate)</td>
<td>-170</td>
</tr>
<tr>
<td>Menaquinone_{oxidized} + 2e^- + 2H^+ → Menaquinol (reduced)</td>
<td>-74</td>
</tr>
<tr>
<td>Cytochrome b_{558oxidized} + 2e^- + 2H^+ → Cytochrome b_{558reduced}</td>
<td>+60</td>
</tr>
<tr>
<td>HOOC-CH=CH-COOH (fumarate) + 2e^- + 2H^+ → HOOC-CH_2-CH_2-COOH (succinate)</td>
<td>+33</td>
</tr>
<tr>
<td>Dimethylmenaquinone_{oxidized} + 2e^- + 2H^+ → Dimethylmenaquinol (reduced)</td>
<td>+36</td>
</tr>
<tr>
<td>Cytochrome b_{562oxidized} + 2e^- + 2H^+ → Cytochrome b_{562reduced}</td>
<td>+46</td>
</tr>
<tr>
<td>Ubiquinone_{oxidized} + 2e^- + 2H^+ → Ubiquinol (reduced)</td>
<td>+100</td>
</tr>
<tr>
<td>Cytochrome b_{562oxidized} + 2e^- + 2H^+ → Cytochrome b_{562reduced}</td>
<td>+125</td>
</tr>
<tr>
<td>(CH_3)_2NO (TMAO) + 2e^- → (CH_3)_3N (TMA)</td>
<td>+130</td>
</tr>
<tr>
<td>(CH_3)_2SO (DMSO) + 2e^- → (CH_3)_2S (DMS)</td>
<td>+160</td>
</tr>
<tr>
<td>S_4O_6^{2-} (tetrathionate) + 2e^- + 2H^+ → 2 HS_2O_3^- (thiosulfate)</td>
<td>+170</td>
</tr>
<tr>
<td>Cytochrome d_{oxidized} + 2e^- + 2H^+ → Cytochrome d_{reduced}</td>
<td>+260</td>
</tr>
<tr>
<td>Cytochrome c_{oxidized} + 2e^- + 2H^+ → Cytochrome c_{reduced}</td>
<td>+270</td>
</tr>
<tr>
<td>Cytochrome a_{oxidized} + 2e^- + 2H^+ → Cytochrome a_{reduced}</td>
<td>+290</td>
</tr>
<tr>
<td>Cytochrome c_{555oxidized} + 2e^- + 2H^+ → Cytochrome c_{555reduced}</td>
<td>+355</td>
</tr>
<tr>
<td>NO_3^- (nitrate) + 8e^- + 9H^+ → NH_3 (ammonia) + 3H_2O</td>
<td>+360</td>
</tr>
<tr>
<td>Cytochrome a_{3oxidized} + 2e^- + 2H^+ → Cytochrome a_{3reduced}</td>
<td>+385</td>
</tr>
<tr>
<td>NOO^- (nitrite) + 2e^- → NO_3^- (nitrate)</td>
<td>+421</td>
</tr>
<tr>
<td>2Fe^{3+} + 2e^- → 2Fe^{3+}</td>
<td>+771</td>
</tr>
<tr>
<td>1/2 O_2 + 2e^- + 2H^+ → H_2O</td>
<td>+815</td>
</tr>
</tbody>
</table>

The Players : CHONPS

The 12 majors
- For known microorganisms, there are only 12 elements that are needed in concentrations greater than 0.1 mM (see Table 2) for them to grow and reproduce
- Of these, only the first five or six are imported in complex forms; all others are available as simple salts
- CHON are required for 97% of all dry biomass; N is about 10% of all cell mass

TABLE 2: THE TWELVE MAJOR ELEMENTS IN MICROBIOLOGY

<table>
<thead>
<tr>
<th>Atom</th>
<th>Natural Source</th>
<th>Function in Metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>Organic compounds, CH_4, CO_2</td>
<td>DNA, protein, lipid, carbohydrate, other</td>
</tr>
</tbody>
</table>
The majors
- O, H, N, S, P, K, Mg, Ca, Fe, Na, Cl, and others are required for basic metabolism.
- These elements are essential for all organisms.
- Others are important for specific functions in some organisms.

The minors
- In addition to the big 12, other elements are required for more specific processes.
- Some of these are essential; e.g., Zn^{2+} is a constituent of both DNA and RNA polymerases.
- Others play important roles in enzymes in all organisms.
- Still others are very important only to a few organisms for specialized jobs (like Ni for F_{430}).

**TABLE 3. SOME MINOR ELEMENTS IN MICROBIOLOGY**

<table>
<thead>
<tr>
<th>Atom</th>
<th>Natural Source</th>
<th>Function in Metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>Zn^{2+}</td>
<td>Ligand in alcohol dehydrogenase, alkaline phosphatase, aldolase, Polymerase</td>
</tr>
<tr>
<td>Mn</td>
<td>Mn^{2+}</td>
<td>Ligand in SOD, photosystem II, PEP carboxykinase</td>
</tr>
<tr>
<td>Mo</td>
<td>MoO_4^{2-}</td>
<td>Molybdopterins, Molybdenum cofactors; Ligand for Nitrate reductase, formate dehydrogenase, nitrogenase</td>
</tr>
<tr>
<td>Se</td>
<td>SeO_3^{2-}</td>
<td>Ligand in reductases, present in selenocysteine</td>
</tr>
<tr>
<td>Co</td>
<td>Co^{2+}</td>
<td>Coordinated ion in coenzyme B_{120}; ligand in aminopeptidases</td>
</tr>
<tr>
<td>Cu</td>
<td>Cu^{2+}</td>
<td>Ligand in cytochrome oxidase, nitrite reductase, SOD</td>
</tr>
<tr>
<td>Ni</td>
<td>Ni^{2+}</td>
<td>Factor F_{430}, ligand in urease, hydrogenase</td>
</tr>
<tr>
<td>W</td>
<td>WO_4^{2-}</td>
<td>Ligand in some formate dehydrogenases</td>
</tr>
</tbody>
</table>

The Main Players
- In this course, we will consider how microbes get their food and their energy.
- In some cases, the same substance do not serve as food and energy sources (like potato chips do).
- We will examine how microbes get C, N, and S from their foods.
- We will examine how microbes get electrons from substances and move them around to make ATP.
- We will examine how microbes find substances to accept these electrons if oxygen is not an option.
- We will examine how some organisms make ATP without moving electrons about (mostly).

Part II - History of the World (from the microbe perspective)

Background Reading:
Evolution: Major kingdoms of life

**How does microbial diversity reflect the history of earth?**
- What was the early atmosphere like?
- What major processes evolved first?
– What evidence for microbiological processes are evident in the fossil record?
– How do extant groups of organisms reflect the earth’s evolution?

- History : Man’s perspective
- The Fossil Record
- The Evolution of Abiotic systems
- The Evolution of Biotic systems

**History : Man’s Perspective**

**History of Man : a little perspective**
– Human recorded history goes back a few 1000’s of years
– *Homo sapiens* diverged from the primate lineage ~3 MYr ago
– Mammals radiated ~100 MYr ago
– Eukaryotes evolved ~2400 MYr ago
– Life evolved ~3900 Myr ago
– The earth was formed about 4650 Myr ago

**Man’s view of history : a lack of perspective**
– Geological history is frequently examined with respect to tectonic plates, movement of continents, creation of mountains, *etc.* These factors do not impact (for the most part) the evolution or diversification of microorganisms.
– Perception of biotic history is heavily influenced by the fossil record, which is biased toward multicellular organisms that can fossilize.
– By the time organisms were fossilized at high frequency (had shells, bones and such), all major lineages extant of microorganisms had *long* been established.
– Typical summaries of the major events in earth’s history focus on large animals, especially vertebrates, and changes in climate.
– However, microbial diversification is correlated to far more fundamental changes in the environment. That is, the history of the world from a microbial perspective is vastly different, operates on a molecular scale, and extends for a much longer period of time.
– The history of microbes can be tied to the earth’s history by examination of their metabolic capabilities, the distribution of these capabilities among lineages of decent and the composition of microbial genomes; these analyses from the focus of this course.
– Often, evidence of microbial activity are found as “chemical fossils,” or chemical signatures that cannot be explained in any way other than invoking biological origins.

**What is a microbial perspective?**
– What are sources of carbon and energy available for growth?
– What are sources nitrogen, sulfur and other important nutrients that may be limiting?
– What are available electron acceptors?
– Does the atmosphere confer a reducing environment or an oxidizing environment?
– What are the regimes of temperature, salinity and other abiotic factors?

**Timetable for Earth’s history**
– For perspective, consider the history of the world as a standard 365-day calendar
– Here, the history of large eukaryotes is small compared to the time microorganisms have persisted
### The Fossil Record

**The Precambrian**
- Includes approximately about 90% of geologic time on this planet
- From 4.6 billion years ago to the beginning of the Cambrian Period (about 570 MYa)
- Includes 3 Eons:
  1. Hadean 4.6 - 3.9 GYa - no record
  2. Archean 3.9-2.5 GYa
  3. Proterozoic 2.5-0.57 GYa

**The Precambrian is not well known or completely understood.**
- Many rocks have been eroded or metamorphosed
- Most are deeply buried beneath younger rocks
- Fossils seldom found
- Most information is from cratons - large portions of continents which have not been deformed since Precambrian or Early Paleozoic time.
- Where exposed, cratons are called **Precambrian Shields**. An example is the Canadian Shield, containing mostly igneous and metamorphic rocks; few sedimentary rocks. Scrapped by glaciers.

**The Hadeon Eon**
A time of major changes and Earth formation; no rock record.
− Origin of the Earth and solar system
− Differentiation of the Earth to form crust, mantle and core
  − Cold accretion model, heating from impacts and radioactivity lead to molten Earth and gravitational differentiation
  − Hot accretion model, material accretes sequentially beginning with iron (core), similar to Bowen's Reaction Series crystallization
− Origin of the atmosphere
  Volcanic outgassing (or degassing)
  H₂O, H₂, HCl, CO, CO₂, N₂, some H₂S
  Little or no free oxygen (O₂); would lead to rapid oxidation of iron minerals
− Condensation of water vapor
  rain; runoff leads to lakes, rivers, oceans
  originally freshwater (rain); may have been acidic from sulfurous gases
  slow accumulation of salts due to weathering
− Origin of continental crust
  most of the early crust was mafic
  continental crust developed secondarily
  several models proposed involving partial melting and weathering
− Continental crust was probably present prior to 4 billion years ago.
− Oldest dated Earth rocks are 3.96 by old (Canada)

**Evidence for a lack of free O₂ in the Earth's early atmosphere**
− Urananite and pyrite are readily oxidized today, but are found unoxidized in Precambrian sediments
− There are no early PC iron oxides (no red beds)
− Banded iron formations appear in stratigraphic record in PC 1.8 - 2.5 GYa
− Evidence from Precambrian soils shows O₂ was only about 2% of modern levels
− Chemical building blocks of life could not have formed in the presence of O₂, including most amino acids and purines
− Deeply rooting Bacteria and Archaea have an anaerobic metabolism

**Macroscopic Fossils of Archean Life**
− Stromatolites (cyanobacteria or BGA - blue-green algae)
  in carbonate sediment. Oldest are 3.4 - 3.5 GYa old; more in rocks 2.8 - 3 GYa old; more abundant in Proterozoic rocks
− Algal filament fossils sometimes found in 3.5 GYa rocks at North Pole, Western Australia
− Spheroidal bacterial structures (like Fig Tree Group, South Africa); 3.0 - 3.1 GYa

**Character of Archean Rocks**
− Granulites - high grade metamorphic rocks (gneiss and anorthosite)
− Greenstone belts - volcanic and sedimentary rocks commonly metamorphosed
  − Chlorite produces green color
  − Sedimentary rocks altered to metasedimentary rocks
  − Metagraywackes, slates, schists, metaconglomerates, diamicrites
− Some relict sedimentary structures
− Banded Iron Formations red chert (jasper) and unoxidized iron-rich sedimentary rocks
Precambrian Fossil Record of Prokaryotes
- Onverwacht Series, South Africa, 3.2 - 3.7 GYa
- North Pole, W. Australia, 3.5 GYa, including Algal filaments, among the oldest cells known
- Fig Tree Group, 3.0 GYa; Spheroidal bacterial structures; may show cell division
- Gunflint Fm, Ontario, Canada 2.0 GYa; Bacteria and stromatolites with algal filaments

Stromatolites built by Cyanobacteria-Based Communities
- Oldest are 3.4 - 3.5 GYa
- Also in rocks 2.8 - 3 GYa
- Bulawayan Gp, S. Rhodesia; 3.1 - 2.7 GYa
- Pongola System, N. Natal Province, S. Africa; 3.1 GYa
- Stromatolites become quite common about 2.25 GYa
- May have been more resistant to UV radiation because of sediment covering.
- Presence of stromatolites and blue-green algae led to buildup of oxygen in atmosphere.

Precambrian Fossil Record of Eukaryotes
- First fossil cells with organelles, 1.8 - 1.2 GYa
- Beck Spring Dolomite, California; 1.3 GYa; Oldest convincing eukaryotes; branched filaments
- Bitter Springs Fm, chert, Australia; 0.8 - 0.9 GYa; Impressive eukaryote fossils

Metazoans (multicellular)
- Trace fossils (or Ichnofossils)
  - Oldest are about 0.7 GYa (700 my)
  - Trace fossils are relatively uncommon until 570 MYa
  - Trace fossils increase in diversity through time
  - Examples of Precambrian trace fossils:
    - Brookella, Grand Canyon; 1 GYa; Jellyfish-like; organic?
    - Questionable trace fossils even older. In Medicine Peak Qtzt, Wyoming; 2.0 - 2.5 GYa; Tube-like structures (found 1976, 1983) are older than oldest eukaryote cells
- Oldest Metazoan Body Fossils = Ediacara fauna
  - Originally discovered in Pound Qtzt, Ediacara Hills, S. Australia; later found worldwide (including Piedmont area of NC) at low paleolatitudes.
  - 0.59 - 0.7 GYa (590 - 700 MYa)
  - Impressions and molds of animals (associated with trace fossils)
- Oldest diversified and relatively abundant marine fauna known. No skeletons.
  - All soft-bodied, jellyfish-like animals. 26 species, 18 genera, 4 or more phyla.
  - 67% Cnidaria, 25% Annelids (worms), and 5% Arthropods
**Case Study : Evidence for Sulfate reduction in the Hadean**

**North Pole, Australia**
- An old craton (Pilbara) in Northwestern Australia
- Dated to 3.515 - 3.458 GYa by U-Pb isotope ratios

**Barite : BaSO₄**
- Found with carbonate in chert beds that were sedimentary
- Crystal interface angles indicate barite was originally gypsum (CaSO₄)
- Baritization occurs when Ba²⁺ is substituted for Ca²⁺; 30% excess sulfate is required
- Interleaving the barite is pyrite (FeS), which is deposited anaerobically
- These sulfide crystals are aligned with the face of the original gypsum crystals, indicating that the sulfide was deposited at the time of baritization. Therefore, the residual sulfate in the barite is likely original
- Also, sulfide-containing fluid inclusions are interspersed within the barite

**Sulphide Depletion**
- The microscopic sulfide deposits along the gypsum face are $^{34}$S depleted relative to sulfur deposits not so aligned
- The depletion is -7.4 ‰ to -21.1‰, with a mean of -11.6‰ when calculated relative to the coembedded sulfates
- Similar fractionation is obtained when calculated relative to the bedded barite
- This depletion is consistent with a biological signature due to fractionization of the sulfate reducing machinery of prokaryotes

**Other possibilities are not likely**
- Hydrolysis of SO₂ to sulfate and sulfide occurs in oxidizing magmic fluids at temperatures below 400 C, potentially producing a 15-20‰ disparity
  - However, the rocks of the Pilbara craton are mafic (Mg²⁺ and Fe²⁺), resulting from a reducing magmic outflow (therefore, unlikely to lead to SO₂ hydrolysis)
- Inorganic reduction of sulfate to sulfide can occur by ferrous minerals at neutral pH and temperatures greater than 200 C, potentially producing disparities from 10-27‰
  - However, gypsum is only stable below 60 C, and the alignment of the sulfide crystals along the face of the barite indicates that deposition occurred at low temperature

**The Evolution of Abiotic Systems**

**Anaerobic environments are not unusual**
- Contrary to first instinct, anaerobic environments are not unusual or unexpected
- The diversification of most extant lineages of organisms occurred under anaerobic conditions
- This becomes clear, and is important for many processes we will discuss, as we review the history of the earth from a microbiological perspective

**Primordial earth**
- Our solar system formed by coalescence of gases; gravitational attraction caused masses to accumulate, and conservation of angular momentum resulted in systems of rotating bodies
The earth was formed about 4.6 billion years ago; using British conventions, we will call this 4600 million Before Present, or 4600 MYr BP.

The sun shone at 50 - 60 % of its current intensity; the average temperature of the earth’s surface exceeding the melting point of iron (1600 °C).

Since the most abundant element in the universe is hydrogen, the primordial atmosphere was highly reduced, containing primarily H₂, CH₄, NH₃, H₂O, and H₂S.

Energy input into the Earth’s environment was large, and came from many sources:
- Ionizing radiation (no ozone layer; 4.6 x 10⁹ kcal/yr)
- ⁴⁰K decay in the earth’s crust (1.2 x 10⁹ kcal/yr)
- Vulcanism (0.04 x 10⁹ kcal/yr)
- Lightning (0.05 x 10⁹ kcal/yr)
- Meteor impact (0.05 x 10⁹ kcal/yr; atmospheric entry causes ionizing radiation; that’s why NASA loses contact with spacecraft upon re-entry, an insulating layer of ionizing radiation is formed around the ship)

Warm Little Pond

As far back as Darwin (1871), scientists have speculated that biomolecules may be formed by the action of these energy inputs and the constituents of the primordial atmosphere, as evidenced in Darwin’s famous letter (espousing the “warm little pond”):

My dear Hooker

I return the pamphlets, which I have been very glad to read. – It will be a curious discovery if Mr. Lowe’s observation that boiling does not kill certain molds is proved true; but then how on earth is the absence of all living things in Pasteur’s experiments to be accounted for? – I am always delighted to see a word in favour of Pangenesis, which some day, I believe, will have a resurrection. Mr. Dyer’s paper strikes me as a very able Spencerian production.

It is often said that all the conditions for the first production of a living organism are now present, which could ever be present. But if (and oh what a big if) we could conceive in some warm little pond with all sorts of ammonia and phosphoric salts, - light, heat, electricity &c present, that a protein compound was chemically formed, ready to undergo still more complex changes, at the present day such matter would be instantly devoured, or absorbed, which would not have been the case before living creatures were formed.

Henrietta makes hardly any progress, and God knows when she will be well.
I enjoyed much the visit of you four gentlemen, i.e., after the Saturday night, when I thought I was quite done for.

Yours affect,
C. Darwin.

The point that he is making is that even if some form of life were to evolve at a later time (when extant life forms already existed), they would be eaten, and would not be detected. He supports the argument that life likely originated only once. Delving into this issue raised many philosophical and religious issues which troubled him, and postponed his publication of The Origin of Species for decades.

Prebiotic syntheses

Stanley Miller (in Urey’s lab at the University of Chicago), among others, tested this hypothesis.

He placed a mixture of H₂, CH₄, NH₃, and H₂O in a closed system and applied energy to the system.

After a week, he analyzed the products, which included:
- Urea and hydrogen cyanide
- Formate & Formaldehyde
- Acetate, propionate, lactate
− Glycine, alanine, aspartate, glutamate, succinate
− Adenine, porphyrins
− Threonine, serine, proline, valine, isoleucine, leucine, tyrosine, phenylalanine
− Sarcosine, β-alanine, methyl-alanine, alloleucine
− Similar products have been recovered using electrical discharge, \textit{uv} irradiation, heat, and radioactive decay as energy sources, representing all major forms of energetic input into the primordial atmosphere
− These results show that complex biomolecules can be generated under primordial conditions

**Catalysis by metals**

− Syntheses of complex molecules are accelerated when heavy metals are included in reaction mixtures in tiny amounts (many are likely there in trace quantities anyway)
− For example, synthesis of ribose is greatly accelerated by lead ions at mildly basic pH’s
− This is because the pKa of lead is 7.7, where it exists as the HO-Pb-O\textsuperscript{-} ion [at pH 7 it is Pb(OH)\textsubscript{2}]
− Other ions are ineffective in these conditions since their pKa’s are too high to be effective at “physiological” pH’s (around 7)

**Ion pKa**

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<tr>
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<td>Cd</td>
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<td>Pb</td>
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**Transition to Prebiotic earth**

− Rocks have been recovered (especially in Isua, Greenland and North Pole, Australia) that date to 3800 - 3900 MYr BP; these are among the oldest rocks found to date
− These rocks show several key things:
  − Temperature had already dropped to between the boiling and freezing temperatures of water
  − The atmosphere was still reducing, but included CO\textsubscript{2}, CO, and N\textsubscript{2}
  − Most of the hydrogen, helium, and neon was lost, which was expected for a planet this size
− Miller/Urey experiments show that the stability of sugars in their experiments required an atmosphere with CO\textsubscript{2} and CO
− These data imply that the change in the atmospheric composition paralleled a change in the types of biomolecules produced by chemical processes

**The origin of life**

− Rocks dating to 3700 MYr BP show signs of biotic influences:
  − Stromatolites are biotically-induced layers of organic and inorganic materials
  − Some microfossils are found in these rocks, but may not be as old as the rocks
  − \textsuperscript{13}C/\textsuperscript{12}C ratios indicate biotic methyl- assimilation
  − Molecular/Chemical fossils show accumulation of biotically-produced chiral molecules
− Rocks dating to 2800 MYr BP show convincing microfossils
− Molecular analyses estimate the divergence of the lineages of all extant life occurred ~3100 MYr BP

**The Evolution of Biotic Systems**

**What was the first life form? What is life?**

− We can define a living organism as a physical entity that can replicate and is subject to heritable mutation
− A free-living organism encompasses all catalytic activities required for replication
− We can define quite rigorously the most recent common ancestor of all known life (MRCA):
− DNA genome, likely circular
− Transcription of DNA into RNA
− Translation of RNA with ribosomes, tRNA
− Most of the universal genetic code likely in tact
− Basic intermediate metabolism for carbon-bearing molecules
− Energy generation using ion gradients
− Membrane enclosed cell
− But this organisms was likely very much advanced from the first organism:
  − The two-polymer system (one for heritable material, one for catalysis) is a very complicated state, likely derived from a simpler system
  − RNA is a good candidate as first genome, where one polymer could serve both as heritable material and as catalyst
  − Many believe that the involvement of catalytic RNA at virtually all steps in the creation and deployment of proteins is a remnant of this state
− Therefore we can divide prokaryotic evolution into three stages
  − A. Events that predate the first prior to the formation of the first free-living organism
  − B. Events that predate the MRCA
  − C. Events that follow the formation of the MRCA

**Simplification of genetics**
− The two polymer system of today’s organisms (DNA for information and protein for catalysis) almost certainly derived from a one-polymer system
− RNA-only metabolism is certainly feasible. Some organisms do use RNA as their sole information-bearing molecule
− RNA-based catalysis is easy to achieve in the lab; every function examined to date can be performed by an RNA catalyst
− RNA catalysts remain in some of the most critical portions of biology:
  − The recent X-ray structure of the ribosome shows that it is an RNA catalyst; proteins are merely structural counter-ions
  − RNAs P is a ribozyme as well
  − The signal recognition particle (SRP) allow for protein export is an RNA particle
  − Intron splicing is RNA-mediated
  − tRNAs direct protein synthesis
  − tmRNA directs translational rescue from broken transcripts
  − snoRNAs and the like direct rRNA modification (e.g., pseudouridine insertion)
− This simplification does not completely alleviate the need for complex physiology

**Simplification of metabolism**
− Just as we can identify complex molecular biology that is derived from simpler molecular biology, we can also identify complex metabolism that is derived from simpler metabolism
− Double-photosystem, oxygenic photosynthesis is derived from single-photosystem, nonoxygenic photosynthesis
− Early food sources were likely small organic acids, readily generated in prebiotic environments
− Glycolysis almost certainly evolved as a biosynthetic pathway to make ribose for nucleic acids
− Six-carbon intermediates became important structural saccharides
− The two arms of the Kreb’s cycle arose independently for biosynthetic purposes
A continuous Kreb’s cycle like evolved to incorporate CO₂, turning in the reductive direction. Turning the Kreb’s cycle in the oxidative direction required respiration to O₂.

**What must be in operation**
- While the first organisms were likely heterotrophs, what was the ultimate energy input? Photosynthesis must have evolved early; ¹³C/¹²C biases 3.5 Gyr ago support this idea.
- Nutrient cycling must have been established for carbon early.
- Nutrient cycling must have evolved for nitrogen after the atmosphere oxidized and free NH₃ was no longer available; the strict anaerobic requirements for N₂ fixation support this idea.

**Occurrence of molecular oxygen in the atmosphere**
- The prebiotic and early biotic atmospheres were reducing environments; no molecular oxygen was present.
- Early autotrophs likely used H₂S as an electron donor in photosynthesis (later lectures); note that the midpoint potential of H₂S oxidation is much lower than that of water.
- Oxidation states of iron-bearing rock indicate that very little O₂ existed prior to 2000 MYr BP.
- However, by 2500 MYr BP, the H₂S concentration in the atmosphere likely dropped sufficiently low that organisms developed a way to extract electrons from water during photosynthesis.
- These organisms would evolve molecular oxygen.
- Gunflint shale from the north shore of Lake Superior show microfossils of *Anabaena*-like organisms (see later lecture) that indicate the presence of high quantities of O₂ in the atmosphere; this was 2000 MYr BP.
- Iron-bearing rock shows O₂ present in the atmosphere at 1% its current level (0.2%) at that time.
- CO₂ was a large portion of the atmosphere, as indicated by the ratio of dolomite to limestone.

**Atmospheric changes in nitrogen**
- As the redox state of the early atmosphere increased, less NH₃ was available; by 3000 MYr BP, very little existed.
- N₂ was present in the atmosphere as an available nitrogen source, and nitrogen fixation likely evolved under these conditions. Note that this was an anaerobic environment at that time.
- This system may have been employed to extract nitrogen from HCN and other compounds as well.
- N₂ gradually rose to its current level over the next 3000 MYr.
- Since both Bacteria and Archaea fix nitrogen using common proteins, it is likely that nitrogen fixation was invented more than 3000 MYr ago.

**Atmospheric changes in oxygen**
- The evolution of cyanobacteria ~2800 MYr BP allowed the production of O₂.
- Oxygen levels reach 0.2% by 2000 MYr BP.
- The eukaryotic lineage likely diverged from the Archaea between 1800 MYr and 2500 MYr BP.
  - Fossils of bacteria are 10 µM in size.
  - Large fossils (20 - 60 µM) occur by 1800 MYr BP.
  - Molecular evidence dates the divergence at 2100-2500 MYr BP.
- Oxygen reached 1% in the atmosphere by 1000 MYr BP; at this time, the mitochondrial endosymbiont was introgressed into the eukaryotic lineage, likely providing oxygen protection.
- Metazoans appear at 670 MYr BP; at this time, oxygen was at least 1.5%, or 7% its current level.
- Chloroplast endosymbionts were introgressed (likely multiple times) around this time.
- Oxygen levels increased dramatically over the next 250 MYr, rising to 21% in the atmosphere.
− The amount of CO₂ also decreased, as more carbon was fixed by the burgeoning plant population

**The current atmosphere is dramatically different from early atmospheres**
− Currently high oxygen levels have existed for only a short period of time
− Carbon dioxide levels are much lower than they have been, due to CO₂ fixation
− Dinitrogen levels are much higher than they have been

**Current temperature and salinity of the oceans are different than in the Archeazoa**
− More than 1.3x10²² grams of halite are evident in crust deposits; 2 to 3 times as much is found in brine below these deposits
− If this salt were suspended in existing ocean volumes, the seas would be 2 times saltier than present conditions
− Measurement of δ-¹⁸O values show convincingly that the temperature of the earth’s oceans was ~50-70 C during the Archeazoa; this is significantly higher than the 8-13 C temperatures of today’s oceans
− As a result, oxygen is far less soluble in warm, salty water. Current oxygen solubility is 5-6 ml/L, but the solubility then would be < 2 ml/L.
− Therefore, even with a moderate oxygen tension in the atmosphere, the open oceans would be nearly anaerobic.

**For Lecture 2 - Microbial fermentations**
⇒ Biochemistry : Review glycolysis and simple lactate fermentation