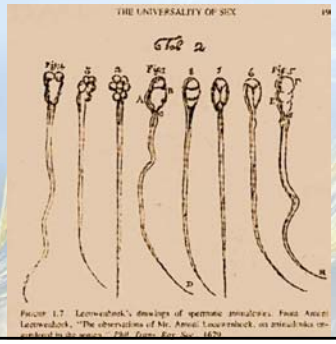
Mechanistic Ideas of Life: The Cell Theory and Basic Metabolism

Robert Hooke

- Examined thin slices of cork and discovered:
  "Yet it was not unlike a Honey-comb in these particulars...these pores, or cells, ... consisted of a great many little Boxes.... Nor is this kind of texture peculiar to Cork onely; for upon examination with my *Microscope*, I have found that the pith of an Elder, or almost any other Tree, the inner pulp or pith of ... several other Vegetables ... have much such a kind of *Schematisme*, as I have lately shown [in] that of Cork."
- Hooke called them “cellulae” (Latin word for “little rooms”).
- *Cells defined by their walls*

Antony van Leeuwenhoek



Jan Swammerdam: (1637-1680)

Describes the appearance of blood under microscope:

> "If we begin the dissection in the upper part of the abdomen, and cautiously split the skin there, blood immediately escapes from that place. The blood, when received into a glass tube and examined with a very good microscope, is observed to consist of transparent globules (globulis), in no way differing from cow's milk, a fact that was discovered a few years ago in human blood also; for this is seen to consist of slightly reddish globules, floating in a clear fluid."

Marie François Xavier Bichat: doing without microscopes

Rejected the value of observing with microscopes, but nonetheless made very astute observations:

- Two different sets of organs
  - Those under volitional control and serving locomotion
  - Those serving vital processes: digestion, assimilation, etc.
- Organs comprised of tissues, and Bichat identified different tissue types: nervous, vascular, connective, fibrous, cellular (connective)
  - Different tissues exhibit different pathologies

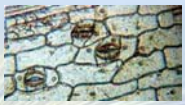
Limitations on early microscopes

- Spherical aberration: failure of light rays to fall all in one plane when focused through a lens
- Chromatic aberration: dispersive action of lenses in breaking white light into primary colors

- William Hyde Wollaston (1812): two plano-convex lenses, placed a prescribed distance apart—counters spherical aberration
- John Hershel (1821): aplanatic combination of lenses
- Joseph Jackson Lister (1824-1830): combined lenses of crown glass with others of flint glass, so adjusted that the refractive errors of each were corrected or compensated for by the other

Robert Brown

- In 1827, utilizing a very simple microscope, observed active molecules (Brownian motion)
- In 1831, observed an opaque spot in plant (Orchid) cells which he named the *nucleus* (Latin for kernel)



Matthias Schleiden

- Nucleus the most important structure in the cell—the unit from which the rest was formed
- Named the nucleus “cytoblast”
- Construed the nucleus as the defining mark of cells
  - What makes differently appearing entities all cells

Theodor Schwann

- Problem: extreme variability in animal cells
- Focused on the similarities of some animal cells to plant cells
- From Schleiden, came to focus on nucleus, which he found in embryonic tissues
- Strategy: show that despite the variability, animal cells all arose in the same manner and were all cells.
  - Cell theory: Cells the building blocks of all organisms
  - “there is one universal principle of development for the elementary parts, of organisms, however different, and this principle is the formation of cells”

Schwann's theory of cell formation

- Cells formed in the "cytoblastema": a structureless substance which sometimes is extracellular (in animals) and sometimes intracellular (in plants).
- Nucleolus appears first
- Granules coalesce around it, creating the nucleus, which then grows.
- Yet another layering of granules generates the cytoplasm

- Powerful analogy: crystal formation
  - Renders the process mechanical

Schwann's theory of the cell

- Presents this as more speculative than the claim that all organisms are made of cells, characterized by how they are formed.
- Cell as the basic unit of life:
  - "The cells, therefore, not only attract materials from out of the cytoblastema, but they must have the faculty of producing chemical changes in its constituent particles. Besides which, all the parts of the cell itself may be chemically altered during the process of its vegetation. The unknown cause of all these phenomena, which we comprise under the term metabolic phenomena of the cells, we will denominate the *metabolic power*."

Cell Division

- While Schleiden and Schwann were presenting a view of cell formation on analogy with crystals, other investigators observed what they described as cell division

- Rudoph Virchow: "Omnis cellula e cellula"

- Undermined theories of spontaneous generation that were still current

- Not until development of stains in the 1860-1870s was it possible to acquire evidence for a mechanism of cell division

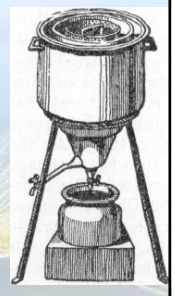
From cells to their fluids

- Hugo von Mohl: plant cells contain “an opaque, viscus fluid, having granules intermingled in it”
  - Recalled earlier observations of the movement of cell contents
  - Nucleus lies within the fluid, not bound to the cell wall
  - Named the fluid: protoplasm
- Dujardin (1835): sarcode: "I propose to give this name to what other observers have called a living jelly - this glutinous, transparent substance, insoluble in water, contracting into globular masses, attaching itself to dissecting needles and allowing itself to be drawn out like mucus; lastly, occurring in all the lower animals interposed between the other elements of structure."

Focus on protoplasm

- Cohn: “But all these properties are possessed also by protoplasm, that substance of the plant cell which must be regarded as the chief site of almost all vital activity, but especially of all manifestations of movement inside the cell.... Hence it follows with all the certainty that can generally be attached to an empirical inference in this province, that the protoplasm of the botanists and the contractile substance and sarcode of the zoologist, if not identical, must then indeed be in a high degree similar formations.”
- Max Schultz (1860): cell “a small mass of protoplasm endowed with the attributes of life.”

From Lavoisier to Physiological Chemistry



- The Chemical Revolution
  - Rejected phlogiston chemistry: Stahl, Priestley
  - Oxygen, hydrogen, carbon, nitrogen basic elements
- Lavoisier and LaPlace: respiration is slow combustion
  - Measured heat generation by melting of ice in a calorimeter
- Fermentation an ordinary chemical process (yeast had not yet been observed)
  - $C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH$

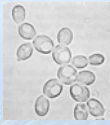
Jöns Jacob Berzelius (1779-1848)

- discovered of the elements calcium, barium, strontium, magnesium, lithium and cerium
- Established that blood contains iron (1806)
- Established that muscle tissue contains the same acid as sour milk (lactic acid; 1806-1808)
- Discovered the chemical formulae for water, ammonia, and various oxides of nitrogen, sulfur and carbon
- Gave protein its name
- Discovered pyruvic acid and aconitic acid
- Introduced the concept and term 'isomerism'
- **Advanced the concept of catalysis (1835), including organic catalysis**

Digestion of Food

- Some process of making food soluble
  - Prout (1824): gastric juice contains hydrochloric acid
  - Cagniard la Tour and Schwann independently: active enzyme in gastric juice is a catalyst: pepsin
    - Made active by hydrochloric acid
- Other digestive juices, associated with the pancreas, etc., also contain a pepsin-like substance

Schwann on the importance of yeast



- Yeast are living organisms and fermentation only occurs in the presence of yeast
- Schwann saw himself as a good mechanist
  - Proposed that fermentation due to the special combination of matter in living cells
  - But in attributing fermentation to *living* yeast cells he was viewed by some as making a concession to vitalists

The Chemists' Response

- In the 1820s Friedrich Wöhler had succeed in synthesizing urea. Wrote to Berzelius
  "I must tell you that I can prepare urea without requiring a kidney of an animal, either man or dog."
- Liebig and Wöhler—satirized the claim that fermentation requires a living cell:
  Yeast must be creatures, with champagne bottles for a bladder: "In a word, these infusoria gobble sugar, and discharge ethyl alcohol from the intestine and carbon dioxide from the urinary organs." *Annalen der Pharmacie* (a journal they edited)

Pasteur: no fermentation without life

"In what does for me the chemical process of sugar decomposition consist, and what is its intrinsic cause? I confess that I am completely in the dark about it. Can we say that the yeast nourishes itself on the sugar, only to give it off again as an excrement in the form of alcohol and carbon dioxide? Or must we say that the yeast in its development produces a substance of the nature of peptase which acts on the sugar and disappears as soon as it has exhausted itself, since we find no substance of this kind in the fermentation liquids ? I have no answer to the substance of these hypotheses. I neither accept them nor do I reject them, and I shall always try not to go beyond the facts" (1860, p. 360).

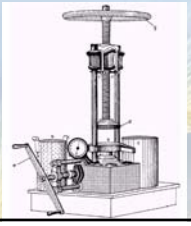
Buchner (1897): yeast really aren't necessary

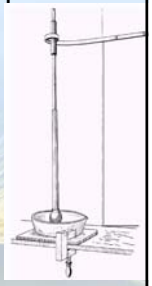
- Was preparing extracts from yeast and added sugar as a preservative
- Noted the bubbles of gas appearing—fermentation was occurring
- Living yeast cells not required for fermentation

Creating cell free extracts that perform fermentation

- Not easy to break yeast cells. Buchner added a mixture of quartz sand and diatomite to a mortar and pestle to perform the task.

Hydraulic press for producing yeast juice





Zymase

- Buchner attributed fermentation not to living yeast cells but to zymase:
  - "The active agent in the expressed yeast juice appears rather to be a chemical substance, an enzyme, which I have called 'zymase.' From now on one can experiment with this just as with other chemicals" (Nobel Lecture, 1907).

From zymase to fermentation pathway

- Overall reaction: from $C_6H_{12}O_6$ to C_2H_5OH
- Clearly not a single reaction
- Possible three-carbon intermediates: lactic acid, methylglyoxal, glyceraldehyde, dihydroxyacetone, and pyruvic acid.
- A real intermediate will
  - be found in fermenting cells
  - metabolize as rapidly as sugar itself
- Pyruvic acid ($C_3H_4O_3$) satisfied both constraints

An early proposal: Neuberg (1913)

1. $C_6H_{12}O_6 \Rightarrow 2C_3H_4O_2 + 2H_2O$
   [Hexose] [Methylglyoxal + Water]

2a. $2C_3H_4O_2 + 2H_2O \Rightarrow C_3H_8O_3 + C_3H_4O_3$
   [Methylglyoxal + Water] [Glycerol + Pyruvic acid]

2b $C_3H_4O_2 + C_2H_4O + 2H_2O \Rightarrow C_3H_4O_3 + C_2H_5OH$
   [Methylglyoxal + Aldehyde + Water] [Pyruvic acid + Alcohol]

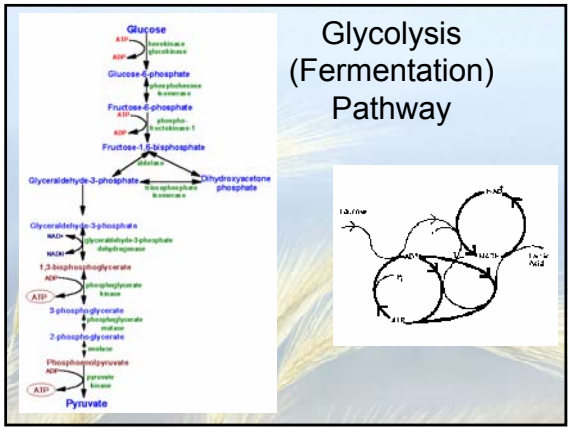
3 $C_3H_4O_3 \Rightarrow C_2H_4O + CO_2$
   [Pyruvic acid] [Aldehyde + Carbon Dioxide]

Two problems with Neuberg's proposal

- Methyglyoxal does not appear in fermentation
- In order to keep cell free fermentation going, phosphates must be added (discovered by Harden and Young in 1903)
  - Neuberg's proposal has no role for phosophate

Reconceptualizing fermentation

- What is the point of fermentation?
  - From our point of view, to make alcohol
- Discovery of adenosine triphosphate (ATP) around 1930
  - Seemed to be an intermediary between glycolysis (fermentation) in muscles and muscle activity
- Perhaps phosphates play a central role in fermentation generally
  - Creation of a (high-energy) phosphate bond that captures the energy released in fermentation
- Fermentation a means of extracting and storing energy needed for cell life



Glycolysis (Fermentation) Pathway