Mechanistic Ideas of Life: Basic Metabolism

Phlogiston and Its Demise

- Why do substances burn?
- Why do metals rust?
- Why do animals breath?
- At the beginning of the 18th century Georg Ernst Stahl developed and popularized the idea that phlogiston was a colorless, weightless, tasteless, and odorless element released in all of these processes
  - Once it is released, calx, a basic element, is left behind
  - Air could only absorb phlogiston if it was dephlogisticated
  - When air is completely phlogisticated (*fixed*), nothing burned, rusted, or lived
- Joseph Priestley isolated what we call *oxygen* but viewed it as fully dephlogisticated air

From Lavoisier to Physiological Chemistry

- The Chemical Revolution
  - Reversed the elements and compounds of phlogiston chemistry
  - 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)
  - $C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH$

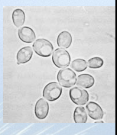
Jöns Jacob Berzelius (1779-1848)

- 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
    - Ingredient in the original Pepto-bismol, developed to treat "cholera infantum"
- Other digestive juices, associated with the pancreas, etc., also contain a pepsin-like substance

Schwann on the importance of yeast

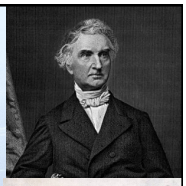


- Yeast are living organisms and fermentation only occurs in the presence of yeast
- Schwann saw himself as a good mechanist
  - Proposed that fermentation due to the special combination of matter in living cells
  - But in attributing fermentation to *living* yeast cells he was viewed by some as making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

Liebig's Grand Program



- A prodigy
  - Before he was 21 Liebig earned his doctorate, studied in Paris with the great chemists of the era, and was appointed Professor at the University of Giessen (1824)
    - Created the first large chemical laboratory devoted to analysis of organic substances
  - Founded a major chemistry journal, *Annalen der Chemie*
  - Founded Liebig's Meat Extract Company to improve nutrition

Liebig's Program of Analysis

- Invented the *kaliapparat* as a device for analyzing the composition of organic compounds using a potassium hydroxide solution to removed carbon dioxide
- Recognized the role of nitrogen in plant nuitrition and created a nitrogen based chemical fertilizer
- Proposed that chemical reactions in animals could equally be carried out in the laboratory:
  - "...the production of all organic substances no longer belongs just to the organism. It must be viewed as not only probable but as certain that we shall produce them in our laboratories. Sugar, salicin [aspirin], and morphine will be artificially produced." (Liebig and Woehler, 1838)

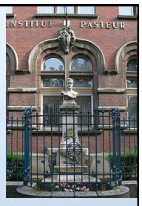
Liebig's Distinction between Plant and Animal Process

- Plants carry out synthetic reactions: create more complex substances out of simpler ones
- Since plants already do this, there is no need for animals to do so
  - They rather break down complex substances: catabolism
- What do animals do with their food?
  - Use proteins to build their bodies
    - Proteins in their bodies are broken down to perform muscle work
  - Use carbohydrates and fat to produce heat via reactions of carbon and hydrogen to produce CO_2 and H_2O
    - Carbohydrate converted to fat when insufficient oxygen available for burning it

Liebig's "Vitalistic" Empiricism

- Liebig repeatedly refers to vital forces in his account of the reactions in living organisms
  - Yet, in other respects he seems like a committed materialist/mechanist, explaining life chemically
- Two traditions in mechanism
  - Descartes—focus on parts and their detectable properties
  - Newton—identify forces even when the parts and their properties cannot be seen
    - Quantify relations between observable entities
- Liebig in the tradition of Newton: write equations to describe transformations and so characterize forces without further explaining them

Louis Pasteur



- Widespread investigation of chemical processes in living organisms
  - Explained chirality: many substances derived from living organisms exhibit optical polarity whereas apparently the same substances made in laboratory do not
    - Two forms of the substance mixed in laboratory
  - Explained spoiling of food as due to microorganisms and developed process to remove them
  - Maintained that microorganisms also responsible for disease: germ theory
  - Vaccination—anthrax and rabies

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).

Conflict with Berthelot

- Berthelot attributed fermentation to a soluble ferment (enzyme):
  - "It is a matter of knowing if the chemical change, brought about in all fermentations, cannot be resolved in terms of a fundamental reaction, brought about by a defined special principle, of the class of soluble ferments… One has to know how to isolate it, i.e. to ascertain the special conditions under which the soluble ferment is secreted in larger amounts than those under which it is consumed."

Pasteur's Retort

- "Berzelius, Mitscherlich, Liebig, Gerhardt, Monsieur Frémy, Monsieur Berthelot and many other observers ascribed the probable cause of fermentative decomposition to a *catalytic* presence, to use the word of Berzelius, or to a movement imparted by dead matter in the process of alteration. In a word, the mystery was so great that one had to resort as an explanation to downright occult forces." (1879)

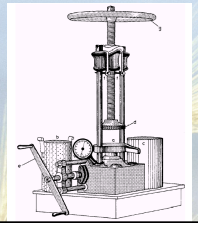
Eduard Buchner (1897): Living cells not necessary

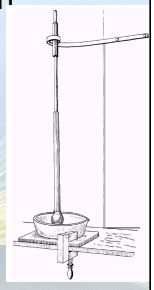
- Buchner was preparing extracts from yeast as part of an attempt to extract extracts for therapeutic purposes and added sugar as a preservative
- Noted the bubbles of gas appearing—fermentation was occurring
- Living yeast cells not required for fermentation
  - It must be due to something in yeast (enzyme means in yeast)

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.

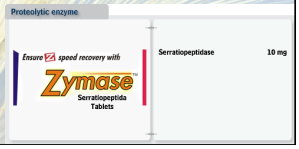
Hydraulic press for producing yeast juice

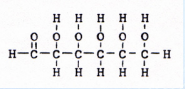




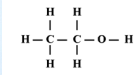
Zymase

- 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).



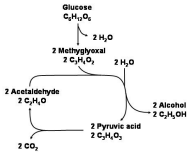


From zymase to fermentation pathway



- Overall reaction: from $C_6H_{12}O_6$ to 2 C_2H_5OH + 2 CO_2
- 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 so it was included in proposed pathways

An early proposal: Neuberg (1913)



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

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

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

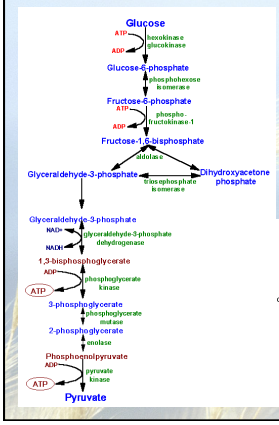
3 $C_3H_4O_3$ → C_2H_4O + CO_2
[Pyruvic acid] [Aldehyde + Carbon Dioxide]

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 phosphates

Reconceptualizing fermentation

- What is the point of fermentation?
  - From one 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 in ATP



Glycolysis (Fermentation) Pathway (1930s)