

## Unit 1: Mechanistic Cell Biology

### 3. Integrating Chemistry into Biology: Biochemistry

#### Phlogiston and Its Demise

- Why do substances burn?
- Why do metals rust?
- Why do animals breathe?
- At the beginning of the 18<sup>th</sup> century Georg Ernst Stahl advanced an explanation for all of these phenomena
  - Phlogiston is a colorless, weightless, tasteless, and odorless element that is 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
- Working within this tradition, Joseph Priestley isolated what we call *oxygen*
  - but viewed it as fully dephlogisticated air



#### From Lavoisier to Physiological Chemistry

- Lavoisier initiated what came to be called the *Chemical Revolution*
  - Reversed what were taken to be elements and compounds by phlogiston chemistry
  - Oxygen, hydrogen, carbon, nitrogen were now counted as basic elements
- Lavoisier and Laplace extended the ideas to biology: respiration is slow combustion
  - Measured heat generation by melting of ice in a calorimeter
- Treated fermentation as 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 many potential parts/components of biological mechanisms

- Identified 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
- Named proteins as a particular class of molecules
- Discovered pyruvic acid and aconitic acid
- Introduced the concept and term 'isomerism'
- **Advanced the concept of catalysis (1835), including organic catalysis**

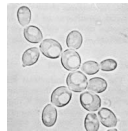
## Components Involved in the Digestion of Food

- Process of making food soluble
  - Prout (1824): gastric juice contains hydrochloric acid
  - Cagniard la Tour and Schwann independently showed that the active component 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



## What About Alcoholic Fermentation? 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 was 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

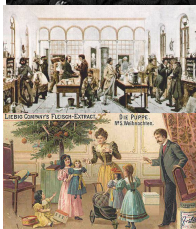
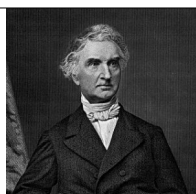
- In the 1820s Friedrich Wöhler had succeeded in synthesizing urea.  
Wrote to Berzelius
  - "I must tell you that I can prepare urea without requiring a kidney of an animal, either man or dog."
- Liebig and Wöhler—satirized the claim that fermentation requires a living cell:
  - Yeast must be creatures, 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)
- For the chemists, the Organism just provide a place
  - It contributes nothing
  - Chemicals are all that matter



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## 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 remove carbon dioxide
- Recognized the role of nitrogen in plant nutrition 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 Wöhler, 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  $\text{CO}_2$  and  $\text{H}_2\text{O}$ 
    - 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 approached plant and animal chemistry in the tradition of Newton: write equations to describe transformations
  - thereby characterize forces in laws without further explaining them

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## Louis Pasteur



- Conducted 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 are also responsible for disease: germ theory
  - Developed the idea of vaccination for anthrax and rabies

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## Clicker Question

What surprising conclusion did Pasteur reach based on his investigation into the formation of butyric acid (butyric acid fermentation)

- A. That it is produced by an inorganic catalyst
- B. That the catalyst (ferment) is an infusorium (little animal)
- C. That these little animals (infusoria) reproduce by dividing
- D. That the infusoria move by gliding over surfaces

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## Pasteur: Life Without Oxygen



- The second surprising conclusion Pasteur reached was that the infusoria he discovered lived without oxygen
  - Indeed, oxygen gas was a poison to them
- We now know that many microorganisms require oxygen free environments
  - Which was the condition in which life evolved on earth
- The creation of molecular oxygen by cyanobacteria was the cause of a mass extinction

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## Pasteur: Agnostic about the Nature of the Ferment



“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).

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## 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.”

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## 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)

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## Discussion Question

Why did Pasteur refer to the proffered explanations of fermentation as “occult forces”

- A. He was convinced that it was sufficient to claim that a whole living organism caused fermentation
- B. Without being able to identify the cause in his microscope, he had no reason to believe in its existence
- C. Without an account of how the process worked, the claims as to what caused it provided no explanation
- D. Other

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## Eduard Buchner (1897): Living Cells not Necessary



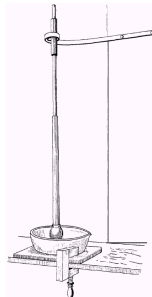
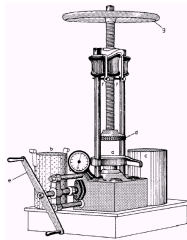
- Buchner was preparing extracts from yeast to use in therapy and added sugar as a preservative
- Noted the bubbles of gas appearing and recognized that fermentation was occurring
- Concluded that living yeast cells are not required for fermentation
  - It must be due to something in yeast (*enzyme* means *in yeast*)

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## Creating Cell-Free Extracts that Perform Fermentation

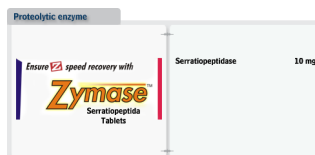
- Not easy to break yeast cells. Buchner added a mixture of quartz sand and diatomite to a mortar and pestle to perform the task.

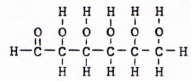
Hydraulic press  
for producing  
yeast juice



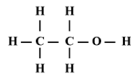
## Zymase

- 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  $\text{C}_6\text{H}_{12}\text{O}_6$  to  $2 \text{C}_2\text{H}_5\text{OH} + 2 \text{CO}_2$ 
  - Clearly not a single reaction
    - Rather, it must proceed through steps
    - Adding or removing chemical groups

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## Discussion Question

How would you go about filling in the pathway from glucose to alcohol?

- Start with sugar, identify simpler substances, identify which ones react, and piece together a pathway using them
- Take known simpler substances and add them to yeast to see which are metabolized and then piece together a pathway using them
- Find ways to stop the reaction at various points and identify which products had been produced and piece together a pathway using them
- Other

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## The Path to Explaining Fermentation

- Researchers began to focus on possible three-carbon intermediates: lactic acid, methylglyoxal, glyceraldehyde, dihydroxyacetone, and pyruvic acid.
- They invoked the principle that a real intermediate will
  - be found in fermenting cells
  - metabolize as rapidly as sugar itself
- Pyruvic acid ( $\text{C}_3\text{H}_4\text{O}_3$ ) satisfied both constraints so it was included in proposed pathways
  - Others didn't, so they presented a challenge

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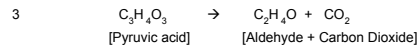
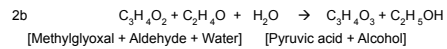
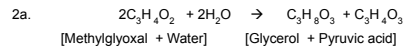
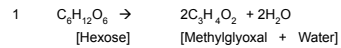
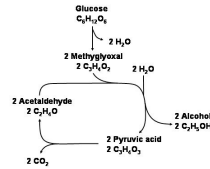
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## An early proposal: Neuberg (1913)



## Two Problems with Neuberg's Proposal

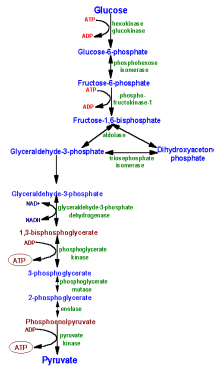
1. Methylglyoxal does not appear in fermentation
2. In order to keep cell-free fermentation going, phosphates must be added (discovered by Harden and Young in 1903)
  - Neuberg's proposal has no role for phosphates

## Figuring Out the Mystery of Phosphate

- Working on lactic acid fermentation in muscles, Embden observed phosphoric acid produced in equimolar proportions
  - Muscle preparations metabolized Harden and Young's hexosediphosphate
- Embden advanced a visionary hypothesis—all the intermediates in lactic acid fermentation were phosphorylated
- But why?
  - Discovery of two other phosphorylated compounds
    - Phosphocreatine
      - proximate agent for energy required in muscle contraction

## Reconceptualizing Fermentation

- Discovery of adenosine triphosphate (ATP) around 1930
  - Seemed to be an intermediary between glycolysis (fermentation) in muscles and muscle activity
- The hypothesis was advanced that phosphates play a central role in fermentation generally
  - Creation of a (high-energy) phosphate bond that captures the energy released in fermentation
- Focus now turned to identifying the oxidation reactions which liberated energy
  - that was then captured in ATP



## Two Views of Fermentation

- A reaction that produces useful products
  - Especially alcohol
  - Everything else, including ATP, is a waste product
- A reaction that provides energy to the organism
  - ATP is not a waste product but the main product
- The shift from the first to the second involves a shift from focusing just on the reactions involved to the role the reactions play in the life of the organism