Context. This is a supplementary handout for a course in introductory organic and biochemistry, using the textbook R J Ouellette, Organic Chemistry - A Brief Introduction, 2/e, 1998. Specific book references below are to this book.

There are two reasons for including (and assigning) references to Ouellette here. The most important is to emphasize to the class that many of the issues here have been brought up before. Second, as a practical matter, it makes it easier for me if I do not have to include material that is otherwise readily available.

A. Overview

In recent chapters we have looked at some classes of biochemicals, those organic chemicals found in and characteristic of living systems. For example, Ch 15 dealt with the amino acids and their biological polymers, the proteins. From time to time, we have also looked at individual biochemical processes. For example, Sect 8.8 included the biological hydrogen carrier NADH as an example of a reducing agent.

Living systems follow the laws of chemistry. However, there is nothing in our study of chemistry, at the level of general and organic chemistry, that leads to a simple understanding of what life is. Life may be a chemical system, but it is one of seemingly remarkable properties -- including complexity. We now focus on that remarkable chemical system called life. Our goal is to begin to uncover ideas, beyond those we usually deal with in chemistry, that help us understand the chemical aspects of living systems.

What is it that makes a biochemical system worthy of special study? We mentioned complexity -- but in one sense it is not complex. There are about ten million organic chemicals, but only some tens of thousands of biochemicals. A basic set of biochemicals, enough to make a quite good living system, would include a few hundred “small” chemicals -- the building blocks. Even if we included each kind of polymer molecule (protein, etc), there would be only a few thousand chemicals. So what is this biochemical complexity all about? Think about it, and we will discuss it in class.

We now examine “metabolism”: the biochemical interconversions of molecules. A major goal is to establish a general “purpose” and plan for metabolism -- to establish a “big picture”. We will focus on the flows of carbon, nitrogen and energy in biological systems. It is important that you emphasize the logic of this material; it is easy to get bogged down with details. You should be able to follow major figures, but you aren’t expected to write out (for example) the citric acid cycle. Biochemistry is not just a list of chemicals or reactions; try to develop a sense of the “big picture”.

Metabolism is not addressed as an explicit topic in Ouellette. Some aspects of it are in the book, at various places. As a result, this handout and class coverage will be the major presentation of this topic. Some sections below contain references to Ouellette or to web sites that are “required” for that topic.

B. The "big picture" and the detail -- key conceptual points

As we discuss metabolism we will go back and forth between the big picture and the detail. As "big picture", I will show you charts that summarize "all" of the reactions in a cell, on one large piece of paper. On the other hand, we will look at specific biochemical reactions, or sets of reactions called "pathways"; we will focus on the citric acid cycle as one example of such a pathway. Both the big picture and the detail are important. In a sense, however, the big picture is more important. The individual reactions, alone, are not biology. The metabolic chart, showing hundreds of reactions, in interconnected pathways -- that is biology. The metabolic chart is too complex to digest at once, so we focus on small parts of it. But when we do that it is important not to lose sight of the big chart. The individual reactions or even individual pathways are "biological" only when they are considered in the context of the big picture.

The metabolic charts embody important concepts of what biology is. The relationship of individual reactions or pathways to the big picture is always important.

> (If you would like to see a metabolic chart online, or would like to obtain a copy of one of the charts I show, see Sect M, below.)

C. Enzymes

C.1. Reading assignment

On the Internet…

The online Worthington **Introduction to Enzymes**, listed below under Computer resources, Sect M, is a good presentation of enzyme basics. *It is "required" reading for this topic.*

From Ouellette…

- Review Ch 2 Sect 10, with a particular goal of understanding the role of catalysts, as in Fig 2.7.
- p 433, subsections "Enzymatic hydrolysis" and "Enzymatic end group analysis". The purpose for us is to establish that different proteases (protein-degrading enzymes) have different specificities. (You need not remember any of the specifics here.)
- p 441, subsection "Quaternary structure". Last paragraph talks about shape changes in proteins, and allostery. Specifically, discusses O_2 binding to hemoglobin.
- Essay, p 464, discusses drugs. One part of this, on sulfa drugs as enzyme inhibitors, is referred to in this section.

C.2. What are enzymes, and why do we use them?

A simple answer to "what" is that enzymes are biological catalysts. They catalyze the thousands of reactions that occur in living systems. At the start of Ch 15 Ouellette lists several roles for the proteins discussed in that chapter; one major role is serving as enzymes. Most enzymes are proteins. (Most? See Sect C.9, below.)

More interesting is the "why". You will sometimes hear that enzymes are needed because most biological reactions are too slow to proceed without them. That is true, but really misses the point. As we go through the rest of this section, think about how enzymes, by controlling individual reaction rates, determine the overall flow of metabolites -- the "big picture" -- in the cell. Simply put, the enzymes control what happens.

We will briefly review the role of catalysts, then look in general at how proteins serve as catalysts. One important consideration for biological catalysts is their specificity. And another important issue is controlling their activity.

C.3. Catalysts

The background for understanding proteins as enzymes (catalysts) is the topic of reaction rates. As noted above (C.1), you should review the relevant sections of Ouellette as needed on reaction rates and catalysts.

One way to speed up a reaction is to raise the temperature. But organisms are isothermal (more or less); raising the temperature is not an appropriate way to get over the activation energy barrier in biology.

A way to speed up reactions at constant temperature is to add a catalyst. A catalyst -- by definition -- is a substance that speeds up a reaction without itself getting changed. Typically, it works by lowering the activation energy. The reactant and product molecules have the same energy as they did without the catalyst, but there is now a smaller activation energy -- or barrier -- between them.

We have looked at several organic reactions during this course. In some cases we noted that a catalyst was required. Go back and look over the reaction summary for almost any chapter; catalysts are often shown, sometimes specific ones, sometimes just general. But these are reactions which really aren't very interesting or useful without catalysts.

C.4. Enzymes as catalysts -- general

Most reactions in biology require a catalyst, and most of the catalysts are proteins. We call these macromolecular catalysts enzymes. Ouellette has introduced examples of enzyme catalysis (e.g., p 226, p 314).

Enzymes allow biological reactions to proceed at body temperature. But that is only the beginning of the story. Our cells contain a few thousand molecules (we might try to list them). To a first approximation, they are all mixed together in our cells (though there is some compartmentalization; not an issue here, but see An et al, 2008). We don't want them all reacting together; we want certain reactions to proceed, and other similar reactions not to proceed. And at different times we want different reactions to proceed. We want control over what reactions proceed. Enzymes are a critical part of that story. Enzymes not only speed up reactions; they also and perhaps more importantly determine which reactions occur.

Key points about enzymes… specificity, and the control of enzyme activity. These are discussed in Sections C.5 & C.7, respectively, below. The intervening section, C.6, discusses some general properties of enzymes.

C.5. Enzymes and specificity

Enzymes catalyze chemical reactions, but they are very specific for what they catalyze. Enzyme specificity is (one key part of) what keeps biochemistry orderly.

> Example. The first step in metabolizing glucose (Glc) is to form a phosphate ester, at the 6-position. But there are lots of -OH groups in Glc. How do we get the phosphate onto the 6-position? The enzyme achieves that by holding both the glucose and the phosphate donor (ATP, as we will see later) "just right". It holds them so that the phosphate can be transferred from the ATP to the 6-position of the glucose. The phosphate doesn't go to the 3-position. Further, the enzyme will put a phosphate on Glc, and perhaps some other sugars, but not most other compounds. That is, the enzyme is specific for the reactants and for specific positions on the reactants.

Lock and key model. A simple view of enzyme specificity is that the enzyme shape fits the shape of the molecules it reacts with (called substrates). We discussed how proteins have specific shapes (Ch 15); the idea that the enzyme shape is complementary to its substrate is a simple extension. This lock and key model is a useful idea, but it is an oversimplification.

Induced fit model. A more complex view is that the proper cavity for the substrate is created as the substrate binds. Protein shapes are due to weak interactions, such as dipole forces and hydrophobic interactions. Therefore, proteins are flexible -- and enzyme shapes are flexible. The precise 3D shape of a protein depends on the precise conditions. Enzyme shape can be altered by many things, including binding to substrates and to other molecules. The idea that the complementary binding site on the enzyme is created during substrate binding is called the induced fit model. (See Koshland, 2004, for some history of the idea.)

The distinction between the lock and key and induced fit models isn't important at the moment. Both share the key feature that the enzyme-substrate interaction is specific, due to contacts between the complex protein and the substrate molecule. The lock and key model is easier to imagine; the induced fit model is more realistic, in that it takes into account the flexibility of proteins, whose shape is due to weak interactions.

How specific are enzymes? Well, that varies. We noted that the first enzyme of glucose metabolism, called glucokinase, is specific for phosphorylating the 6-position of D-glucose. The specificity for the 6-position is virtually absolute. The specificity for D-glucose compared to L-glucose is virtually absolute. However, the enzyme will phosphorylate some other D-hexoses. The details vary with specific enzymes. [D-mannose, the 2-epimer, may be phosphorylated well; D-galactose, the 4-epimer, less well. There is also a hexokinase, which generally phosphorylates a variety of sugars at the 6-position.]

> Kinase? A kinase is an enzyme that transfers a phosphate group to some molecule. Most commonly, ATP is the phosphate donor. For example, glucokinase phosphorylates glucose, using ATP. Hexokinase phosphorylates hexoses, using ATP. A protein kinase phosphorylates a protein, using ATP; this is an important type of reaction in biochemistry, as the phosphorylation modulates the shape -- and hence the activity -- of proteins. (See "kinase" in the Glossary at my web site.)

One aspect of the specificity is chiral specificity. In Ch 6 we noted that chiral molecules are distinguished by other chiral molecules in chemical reactions. Fig 6.11 lays the groundwork for enzymes distinguishing chiral isomers. Amino acids are chiral, so enzymes (made from amino acids) are chiral. Further, most biomolecules are chiral, so it shouldn't be a surprise that the chiral specificity of enzymes is important.

The idea that proteins interact with other molecules by specific shape interactions is general. It is the basis of how enzymes act -- and how they are regulated, Sect C.7. It is also the basis of processes such as antibody-antigen and receptor-ligand recognitions.

> See Cornish (2006), and other references listed with it, for some work on developing or improving enzymes. Some of the work involves using our understanding of proteins to develop catalysts "rationally".

C.6. Enzyme properties

Enzyme reactions are chemical reactions. They can be studied like other chemical reactions.

The online Worthington **Introduction to Enzymes**, which is *required* reading, describes some general properties of enzyme catalysis. (The web site for this is listed in Sect M.) These include:

- Activity as a function of enzyme amount [E].
- Activity as a function of substrate concentration [S].
- Activity as a function of temperature (T) and pH.
- Effect of inhibitors, with a distinction between competitive and noncompetitive inhibitors.

Enzyme inhibition can have two contexts, natural and artificial. The former is part of the topic of enzyme regulation, below. The latter includes drugs and toxic agents. For example, sulfa drugs "look like" p-aminobenzoic acid (PABA), which is part of folic acid (p 464). They compete with PABA in making folic acid, which the bacteria must make. Thus sulfa drugs are competitive inhibitors. In contrast, Pb^{2+} ions react with sulfhydryl groups in enzymes, inactivating them. This has nothing to do with the substrate looking like Pb; it's noncompetitive.

See Amábile-Cuevas (2003) for more about antibiotics.

C.7. Enzyme regulation

Our cells don't do the same things all the time, and different cells do different things. That is, enzyme activities vary -- in time and space.

There are two fundamentally distinct approaches to changing enzyme activity:

1. Changing the amount of enzyme that is present. That is, making more or degrading what is present.

2. Changing the activity of existing enzyme.

#1 is the issue of how genes function, including how they are controlled to make the right amount of proteins. Ouellette introduces gene function in Ch 16, which we do not cover. That chapter does not discuss issues of how that gene function is regulated. See Sect N for some things you might pursue on this.

Our interest here is #2, regulating the activity of existing enzymes.

At the simplest level, other molecules modulate enzyme activity. The enzyme can bind its substrate, but it may bind other things, too. The "other" may bind at the same site as the substrate, at a nearby site that overlaps or blocks the substrate site, or at a distant site. These "other" may compete with the substrate for binding, or may change the shape of the enzyme even if they bind at a distant site. In general, such effects can be positive or negative. That is, a molecule may interact with the enzyme and increase or decrease its activity.

Examples of enzyme regulation include natural biochemical processes, where the regulation promotes proper flow of metabolites, and artificial processes, such as toxic agents or drugs. Some examples of unnatural enzyme inhibitors were discussed in Sect C.6, above.

As an example of a natural enzyme inhibition… Bacteria make the amino acid isoleucine (Ile) in several steps. The enzyme at the first step is regulated by the "current" level of Ile. If enough Ile is available, it inhibits the first enzyme in the pathway to make Ile. This should seem "logical". Ile does not look like the substrate for that enzyme; this is a non-competitive inhibition.

Another regulatory effect on protein function is seen with hemoglobin (Hb), the O_2-carrying protein of the blood (p 441). It isn't an enzyme, but the idea is the same. Hb is a tetramer. Each subunit binds one oxygen molecule. Interestingly, when one site binds an O_2, the affinity of a second site for O_2 increases.

These two examples involve distant interactions. The inhibiting isoleucine is not binding at the site for the substrate of the enzyme being regulated; the stimulating O_2 is not binding at the site whose affinity for a second O_2 is increased. Can a molecule that binds at one site affect the shape at another site? Sure. The 3D shape of a protein is its low energy conformation, after balancing all the possible weak bonding interactions. Once some new molecule has bound, we have a new structure. It needs to be re-optimized.

The effect of a molecule binding at one site on the shape at a distant shape is called allostery. Both the Ile and Hb examples above involve allostery. (Ouellette mentions the Hb effect, and introduces allostery, p 441.)

> For a recent overview of allostery, see Changeux & Edelstein (2005).

We have talked about how enzymes are regulated. In a very general sense we have talked about why; different enzyme activities are needed at different times and places. Enzyme regulation is usually logical, but you need to understand the situation. In the Ile example above, the regulation helps to maintain a proper level of this required chemical.

> As another example… In bacteria, enzymes that are needed to degrade the somewhat uncommon sugar lactose are made only if that sugar is present. This example involves changing the amount of enzyme present, rather than regulating the activity of existing enzyme.

C.8. Enzyme classification

⇒ We will not formally discuss this sub-section, and you are not responsible for it. Those going on in biochemistry should at least be aware of the EC system.

Biochemists have classified enzymes into six major groups, based on the type of reaction catalyzed: oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. Except for lyase, the roles of these categories should be apparent from the names. A lyase is an enzyme that catalyses an addition reaction across a double bond, or its reverse elimination reaction. The aconitase enzyme of the citric acid cycle is an example of a lyase; see reactions 1 and 2 in Fig 2, p 18.

An international commission has assigned a unique identifier, called an EC (Enzyme Commission) number, to each enzyme. The first digit of the EC number identifies which of the six groups listed above the enzyme belongs to; other digits identify sub-classes.

> Example: The first enzyme of glucose metabolism catalyzes the transfer of a phosphate group from ATP to glucose, to make glucose-6-phosphate. (This is reaction #17, on p 13, below.) This enzyme, known formally as ATP:glucose

phosphotransferase, is commonly called glucokinase (or hexokinase). (Recall p 6, where "kinase" was introduced.) Its EC number is 2.7.1.1. The 2 indicates it is a transferase; the 7 indicates the subclass phosphotransferase. The third digit, a 1, indicates that the phosphate group is transferred to a hydroxyl group, and the final 1 indicates more specifically that the transfer is to glucose. (Example from p 246 of Lehninger Principles of Biochemistry, 3/e, 2000.)

A list of EC numbers is available from the Sigma Metabolic Pathways page; see Sect M.

C.9. Loose ends

Can enzymes do anything? Can enzymes do the impossible? No. Enzymes catalyze reactions. They speed up the possible. And because of their exquisite specificity, they are selective -- something organic chemists have trouble with.

Are all enzymes in biology proteins? We used to think so -- until about 1980. However, it turns out that some enzymes are RNA molecules, termed ribozymes. This discovery has great implications for theories about the origin of life. It is now considered likely that RNA is a more primitive molecule than protein or DNA, that RNA molecules were the "first" bio-catalysts. But that's beyond us for now. See Curtis & Bartel (2005) and Been (2006) for examples of work on ribozymes.

Spreitzer et al (2005) discusses Rubisco, the inefficient enzyme that plays a key role in photosynthesis.

D. Energy

An energy supply is necessary because making a biological system from its subunits is energetically unlikely (non-spontaneous, endergonic, $\Delta G > 0$, $K_{eq} < 1$). Energy gained from burning (combusting, oxidizing, catabolizing) our food is used to "drive" biosynthetic (anabolic) reactions.

The connection between energy sources and energy uses is not direct. The energy gained from burning the food is "captured" in "energy carriers" such as ATP and NADH. These energy carriers are then used to power energy-requiring reactions. Thus these energy carriers couple energy-yielding and energy-requiring reactions.

In the next section we introduce these "energy carrier" chemicals, which play key roles as intermediates. We also introduce other types of carriers. Then, in Sections F-I, we examine, at various levels of detail, how biologically useful energy is captured from glucose.

E. Carriers

E.1. Reading assignment

The following sections in Ouellette are "required":

- Essay, Redox reactions in biochemistry, pp 47-48. Introduces role of NAD and FAD.
- Sub-section, Reduction of carbonyl compounds, pp 231-233. Gives an example of the role of NADH, comparing it to hydrogenating agents commonly used by organic chemists. Includes structure of the "active" part of the molecule. The Essay on p 230 also shows the role of NAD in oxidation of alcohols.
- p 314, top half of page. Mentions a reaction involving the H carrier NADPH.
- Essay, Thioesters are Nature's active acyl compounds, pp 348-9. Discusses acetyl CoA, and includes its complete structure.
- Essay, Esters and anhydrides of phosphoric acid, pp 354-5. Introduces "high-energy" phosphate anhydrides, and specifically the full structure of ATP. Discusses the phosphorylation of glucose to yield glucose-6-phosphate.

  Erratum. Last paragraph in left column. First sentence is not quite correct; the statement is not true for the esters of simple phosphoric acid. All subsequent discussion is fine, and should make this clear.

- Sub-section, Biochemical condensation reactions, p 357. Shows the role of acetyl CoA in introducing acetyl groups into the citric acid cycle.
- Sub-section, Active transport, p 383. Discusses the use of ATP to provide energy for transport of molecules across the cell membrane.

The following parts of Ouellette Ch 16 are "recommended". You are responsible for them only in how they help enrich or reinforce the basic material, discussed above or in the handouts. You are not responsible for the new material in them. Specifically, you are not responsible for nucleic acid or protein synthesis.

- Sect 16.2. Since all the carriers discussed above contain adenine nucleotides, reading a section that focuses on these may be useful. Remember that the adenine of the carrier molecules is the same adenine (=A) found in the genetic polymers DNA and RNA. ATP, the energy and phosphate carrier, is also a direct precursor for making RNA. (See Jordan, 2007, for an addition to the list of adenine forms of vitamins.)
- Sub-section, Amino acids are activated for reaction, p 463, with its Fig 16.11. This shows one more example of the role of ATP as an energy carrier, in activating the amino acids for protein synthesis.

E.2. Overview

We introduced the idea of energy carriers in Sect D. These molecules, such as ATP, carry energy from a source to where it is needed. ATP couples energy-yielding and energy-requiring processes.

It is simpler to use common carriers than to directly couple primary energy sources to each need. This indirect coupling through common carriers allows for modular processes. Burning any new food source requires only coupling its metabolism to ATP production; introducing a new energy-requiring process requires only coupling it to ATP utilization. Without the use of the common carriers, each energy-requiring process would have to be directly connected to all possible energy sources.

> Analogy… Think about using coal to run the lights at home. You could do it, but it is much more convenient to have a centralized facility at which the energy from coal is captured in a common form, such as electricity, which can be used to run the lights, and other appliances.

ATP is perhaps the best known carrier, but there are many others in biochemistry. The following parts of this section introduce some of them. The important point is to begin to appreciate the central role of carriers. You are not responsible for remembering the structures of the carrier molecules.

E.3. Electron carriers (H carriers)

Review Sect 2.4, on oxidation-reduction (redox) reactions, as necessary. Remember that redox reactions are fundamentally defined as electron transfer reactions, but that looking at transfer of H is often equivalent and convenient.

In biochemistry the energy story is closely intertwined with redox stories, as we will see while going through glucose metabolism in the following sections. A simple view is that biologically useful energy comes from the oxidation of food.

It is important to distinguish H and H^+. The latter is the familiar "proton" that is released by acids; it has nothing to do with the current discussion. Redox reactions fundamentally involve electrons. When we refer to a change in the H count, we are referring to H atoms, H with one electron -- sometimes shown as H· to be explicit. No, such H atoms do not exist free, but the H carriers we discuss are as much electron carriers as H carriers. Note that H_2, a common reducing agent in organic chemistry, contains two H atoms (H·) bonded together; H_2 has 2 electrons. The following equation describes these equivalencies; it is not intended to describe any specific event.

$$H_2 = H\text{-}H = H{:}H = 2\ H\cdot = 2H^+ + 2\ e^- \qquad (10)$$

> Equations are numbered starting with 10. The numbers 1-9 refer to reactions in the citric acid cycle, Fig 2, p 18, in Sect G.

One of the biological electron carriers is flavin adenine dinucleotide, abbreviated FAD. In the reduced form, the FAD carries two more H atoms; it is now $FADH_2$.

$$FAD + 2\ H\cdot \rightarrow FADH_2 \qquad (11)$$

Equivalently, we might also say that it carries two H^+ ions plus 2 electrons.

$$FAD + 2\ H^+ + 2\ e^- \rightarrow FADH_2 \qquad (12)$$

Eqns 11 & 12 are reversible; $FADH_2$ can give up its H. We thus see that the $FADH_2$ can be thought of as an H carrier. That statement is intended to be equivalent to saying it is an electron carrier.

The other major electron (or H) carrier is nicotinamide adenine dinucleotide, a cation properly abbreviated NAD^+. In the reduced form, it carries two additional electrons, but only one additional H. The second H appears as a free H^+ ion.

$$NAD^+ + 2\ H\cdot \rightarrow NADH + H^+ \qquad (13)$$

Or, equivalently

$$NAD^+ + 2\ H^+ + 2\ e^- \rightarrow NADH + H^+ \qquad (14)$$

The precise way NAD carries 2 electrons is more complex than for FAD. But as a practical matter, this is not important for us. For convenience, I will usually refer to the oxidized and reduced forms simply as NAD and NADH.

Another electron (or H) carrier is NADP. This has the same general structure as NAD, but has an extra phosphate group on it. For our purposes, NAD and NADP are equivalent; however, each individual enzyme usually requires one or the other specifically. Ouellette mentions NADP on pp 47 & 314.

> The H carriers FAD and NAD contain the common vitamins riboflavin and niacin, respectively, as part of their structures.
>
> Chemicals such as NAD, small molecules that are required for an enzymatic reaction, are called cofactors. See the Glossary at the web site for more.

E.4. Energy carriers

The most famous of the energy carriers is ATP, adenosine triphosphate.

What does it mean that a molecule is an energy carrier? The phosphate groups in ATP are linked by acid anhydride linkages, which are relatively unstable. That is, the hydrolysis of such an anhydride linkage yields energy. (Recall Ch 12, e.g., Sect 6.)

$$ATP + H_2O \rightarrow ADP + P_i + \text{energy} \qquad (15)$$

In this equation, P_i is a biochemical shorthand for "inorganic phosphate". The energy released is about 7.3 kcal/mol, under standard conditions.

As an example of how this energy can be used to drive an unfavorable reaction, consider the phosphorylation of glucose (the first reaction of glucose metabolism):

glucose + $P_i \rightarrow$ glucose-6-phosphate + H_2O (16)

This reaction requires 3.3 kcal/mol; it is unfavorable. But if we couple reactions 15 and 16, we get

glucose + ATP $\rightarrow$ glucose-6-phosphate + ADP (17)

Algebraically, that equation is the sum of equations 15 and 16. Biochemically, the coupling is done by an enzyme (glucokinase) transferring the phosphate group from the ATP (anhydride) to the glucose (ester). Note that H_2O no longer appears in the coupled equation.

The energy of this combined -- or "coupled" -- reaction is the sum of the energies for the separate reactions, -7.3+3.3 = -4.0 kcal/mol. Thus the energy of ATP hydrolysis has been coupled to the phosphorylation of glucose. Recall Fig 12.3, which showed that making an ester from an anhydride is a favorable reaction.

(Ouellette introduces this reaction in the Essay, p 354.)

An important point when we talk about energy carriers is that the energy comes from reactions. What is of interest is the energy released in a particular reaction. Talking about energy "in" ATP is a shorthand, which can be confusing.

All chemicals carry energy. We identify ATP as an energy carrier because of the central role it plays in metabolism. Glucose carries energy. Glucose is "burned" and much of its energy of combustion is used to make ATP. That ATP, then, is used to drive biological processes that require energy. ATP, or more specifically those anhydride linkages in ATP, serve as intermediates to carry energy from its "ultimate source" (glucose metabolism) to where it is needed (e.g., moving muscles, or driving unfavorable reactions).

Other compounds similar to ATP also serve as energy carriers. We will see GTP = guanosine triphosphate, in the citric acid cycle. ATP and GTP are examples of nucleoside triphosphates, collectively denoted as NTP. They all have the same kind of phosphoric acid anhydride linkages; that ATP, and to some extent GTP, are the major energy carriers is an arbitrary fact of biochemistry. (If there is some evolutionary reason for the choice of these particular NTPs as the major energy carriers, we do not know what it is.)

E.5. Phosphate carriers

The same nucleoside triphosphates (NTPs) that serve as energy carriers also serve as phosphate carriers. For example, the phosphorylation of glucose during glycolysis uses the terminal phosphate of ATP. This reaction was discussed above, to illustrate energy coupling and the use of ATP as an energy carrier. But it is also an example of the use of ATP as a phosphate carrier. (York & Hunter, 2004, have recently described the use of inositol pyrophosphates as phosphate carriers.)

E.6. Acetate (acyl group) carriers

In many biochemical pathways, acetate is carried on the carrier molecule called Coenzyme A (CoA), which is a thiol. Acetate is coupled to the CoA by a thioester linkage. Recall that thioesters are somewhat higher energy than regular esters (Ouellette, p 332).

> Coenzyme A is a complex chemical (p 349). One part of it is pantothenic acid, sometimes considered to be one of the B vitamins.

When relevant, CoA is sometimes written CoA-SH, and the coupled compound as acetyl-SCoA.

Other acyl groups can also be carried on CoA, by the same type of thioester linkage. For example, in the citric acid cycle, Sect G, we will see succinyl CoA.

E.7. Nitrogen carriers

We will briefly discuss nitrogen metabolism in Sect J. We will see that glutamate is a carrier for amino groups, the most common cellular form of N.

F. Glucose metabolism -- overview

Glucose is one common food. We eat glucose, and metabolize it as a source of carbon and energy. We will discuss glucose metabolism as an example.

> Starch is a polymer of glucose (p 321); starch that we consume is hydrolyzed to the monomer sugar. Common table sugar is sucrose, a disaccharide of glucose and fructose (p 319). The first step in metabolizing sucrose is hydrolyzing it to its monomers. Half of that product is glucose; the other half is fructose, whose metabolism is very nearly the same as for glucose.

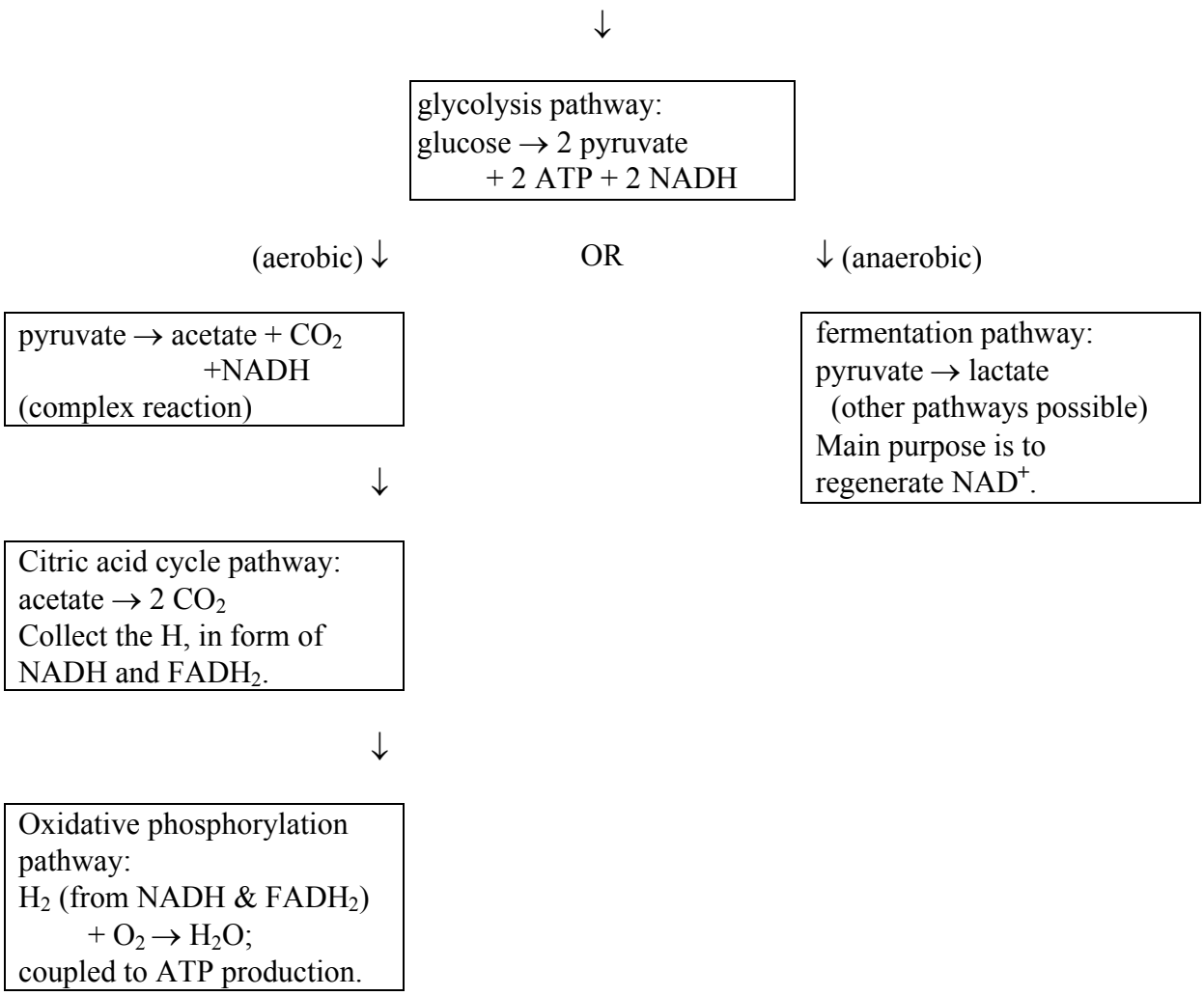
In the rest of this section, we will briefly outline glucose metabolism, as summarized in Fig 1, p 15. This Fig, like the big metabolic charts, can be thought of as the "big picture". Then, in Sect G, we will discuss one part of glucose metabolism, the citric acid cycle, in detail.

Glucose enters cells, and is metabolized in a pathway called glycolysis. The first step is phosphorylation, which converts it to a charged compound that is less likely to leak out; this is reaction 17, which we discussed earlier as an example of the role of ATP as an energy and phosphate carrier. Glycolysis (in several steps) breaks the C_6 sugar down to two molecules of pyruvic acid, a C_3 compound. Some energy is released in this limited oxidation. Two molecules of the energy carrier ATP are made per molecule of glucose in glycolysis. Further, two molecules of the "redox carrier" (electron carrier) NADH are made.

What happens after glycolysis depends, broadly, on whether or not aerobic metabolism is

possible. If so, then the pyruvate is converted to acetate, in the form of the acetate carrier acetyl CoA. The acetyl CoA continues into the citric acid cycle, discussed in the next section.

Fig 1. Main pathways of glucose metabolism.



Both the pyruvate → acetate conversion and the citric acid cycle result in the production of more NADH. The H of the NADH, whether from the citric acid cycle or from earlier steps, is oxidized by O_2, in a complex reaction sequence known as oxidative phosphorylation, discussed in Sect H; each NADH results in the production of multiple ATPs. Suffice it for now that oxidative phosphorylation is the major pathway for making the energy carrier ATP in aerobic cells.

> Note that the preceding paragraphs introduce the roles of three of the carrier molecules that were described in Sect E.

If aerobic metabolism is not possible, the cell needs to regenerate the NAD^+. One way to do

this is by reducing the pyruvic acid, to lactic acid. Thus we have a lactic acid fermentation -- a process that yields only two ATP per glucose, plus two molecules of lactic acid. Another fermentation pathway yields ethanol. We will not say anymore about fermentation.

If the glucose is consumed completely through the aerobic metabolism, the overall chemical result is

glucose + oxygen → carbon dioxide + water + energy; (18)

$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$ + energy. (19)

This is exactly the same equation as if we burned sugar in ordinary complete combustion. Once again, biochemistry is chemistry. The same reaction occurs, the same result happens -- including the same amount of energy.

As we look at the details of such a biochemical process, a major question should be to distinguish what is "biological" about the process. Simply listing some chemical steps per se does not distinguish it from ordinary combustion.

Macroscopically, we might observe that biological combustion appears to be done at constant temperature, whereas ordinary combustion is dominated by release of heat. (We can quibble about exactly what the first part of that means, but to do so would miss the point for now.)

Biological combustion is done in a series of small, discrete, carefully controlled steps. Common carriers are used as needed to carry such things as electrons (or H atoms), acyl groups, phosphate groups. By carefully carrying out the oxidation/combustion in small discrete steps, almost one electron at a time, the biochemical process can capture a significant fraction of the energy of combustion in the form of a chemical energy carrier that is useful to the cell in powering other cellular activities. That energy carrier is ATP.

As we go through some parts of biological glucose combustion in detail, emphasize the broad principles of stepwise metabolism and the use of carriers. Try to see how many of the individual steps involve familiar organic chemistry. Try to avoid being overwhelmed by the number of steps, and by all the complexity of the big picture, the big metabolic charts.

> Glycolysis is discussed in greater depth in a separate handout, which is largely in the form of a worksheet; see Sect K, below. A web site for glycolysis is listed in Sect M.

G. Citric acid cycle

G.1. Reading assignment

The following parts of Ouellette discuss reactions of the citric acid cycle, and are "required":

- p 226 bottom.
- p 357, "Biochemical condensation reactions".

G.2. Overview

The citric acid cycle is a pathway in which acetate is oxidized to CO_2. The acetate comes from glucose, in the form of acetyl CoA, as discussed in Sect F. (Acetate, as acetyl CoA, also comes from breaking down fat; we will not discuss that.) The oxidation is carried out by electron (H) carriers, such as NAD and FAD. The electrons (H atoms) that are removed in these oxidations are then carried to the oxidative phosphorylation pathway (Sect H, below), where the H atoms are reacted with O_2, yielding energy -- much of which is captured to make the biological energy carrier ATP.

> The citric acid cycle is also known as the tricarboxylic acid (TCA) cycle, or the Krebs cycle.

There are two broad goals in discussing the citric acid cycle. One goal is to fit this pathway into the "big picture". The other goal is to examine this pathway in some detail, to see how individual biochemical steps are carried out. In so doing, one general message is that the reactions are ordinary organic chemical reactions. Another general message is to see the role of the biological carriers, introduced in Sect E. The citric acid cycle is the only pathway that we will examine in such detail; it is important in its own right, but it also serves as an example of a pathway for us.

G.3. The pathway

Fig 2, p 18, shows the pathway, with structures of all the main compounds. Each step is catalyzed by an enzyme. We will not discuss the enzymes per se, but simply recognize that almost all biological reactions are catalyzed by enzymes that determine the specificity of the reaction (Sect C).

> You may want to have multiple copies of Fig 2, to mark up in various ways. You can get more copies from the web site.

G.4. The big picture

Sect F, including Fig 1 (p 15), shows how the citric acid cycle fits into the bigger picture of glucose metabolism. The sugar is broken down to C_2 units (in the form of acetyl CoA) prior to the citric acid cycle. In the citric acid cycle, the C_2 units are fully oxidized to CO_2. A small amount of the energy of this oxidation is captured in the energy carrier GTP (similar to and equivalent to ATP). But most of the energy is captured in the H carriers NADH and $FADH_2$. The H from these H carriers is later oxidized with O_2, yielding considerable ATP; this process is called oxidative phosphorylation, and is introduced in Sect H, below. In terms of energy metabolism, the oxidation of H's captured during the citric acid cycle is the major energy source for the cell.

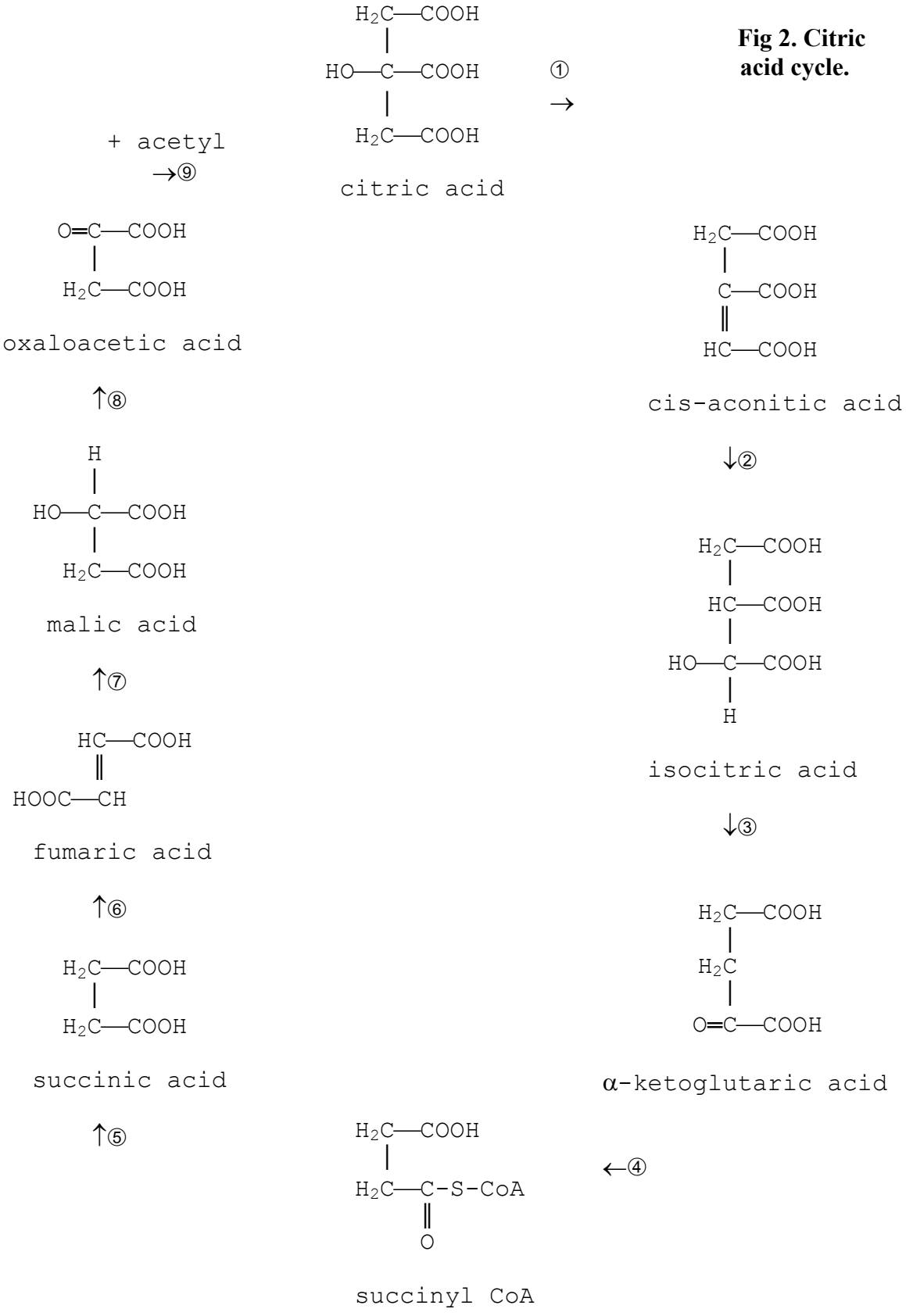


Fig 2. Citric acid cycle.

In practice, some of the citric acid cycle intermediates also serve as sources of cellular biochemicals. We introduce the idea that metabolic pathways are branched in Sect G.6.

G.5. The details

In class we will go through much of this pathway in detail. As preparation for this, I encourage you to look at the chemicals in the pathway, and try to see how each compound differs from the preceding one. That is, try to describe what is happening at each step in organic chemical terms. I should caution you that some of these changes are fairly complex, but many are simple. So look through the entire pathway, and see what you can identify.

I will help you get started by posing some questions here. Try to answer each question yourself, as you read through this, by examining the pathway. Then go on and read the following discussion.

Reactions 1 and 2. Start with citric acid. The first major "goal" is to oxidize the alcohol group. Why is that a problem? What is accomplished, overall, in steps 1 + 2? What is the strategy? That is, in organic chemical terms, what happens in reactions 1 and 2?

PAUSE. Stop reading at this point, and go to the pathway. Try to analyze the above questions yourself before reading on. The issues here are all basic organic chem.

CONTINUING… Citric acid is a tertiary alcohol. We know that 3° alcohols cannot be oxidized; no H is available on the alcohol C. The net result of reactions 1 + 2 is to convert the 3° alcohol to a 2° alcohol; the latter can be oxidized. The strategy is to carry out an elimination (dehydration) on the original alcohol, creating a double bond, then hydrating the double bond. In effect, water is removed, turned around, and put back. Both the elimination and addition reactions are familiar to you. Note how all aspects of this discussion should strike you as familiar organic chemistry: the relative oxidizability of various alcohols, and the reactions that interconvert -OH and C=C.

> Both steps 1 & 2 are carried out by a single enzyme, called aconitase. The intermediate, cis-aconitate, remains bound to the enzyme. In fact, it is often omitted in drawing the citric acid cycle.

Reaction 3. There are two changes that happen in reaction 3. One is a familiar reaction type; the other is not, but you should still be able to describe (though not necessarily explain) what happened. What are these two changes?

PAUSE.

CONTINUE. 1) The secondary alcohol is oxidized to a ketone, a familiar organic chemical reaction. 2) There is a loss of one C. We started with a C_6 compound, and now have C_5.

Regarding the oxidation… This involves removing two H atoms. Where do you think these H's go? Apply your knowledge of biochemical principles, introduced earlier in this handout.

Regarding the loss of a C… What compound do you think was released?

PAUSE.

CONTINUE. The oxidation transfers the H to a biochemical H carrier, NAD or FAD. You have no way to predict which specific one would be used in this case, but you should begin to recognize the general issue, the use of these H carriers. In this case, NAD is used, giving NADH. More precisely, $NAD^+ + 2\ H\cdot \rightarrow NADH + H^+$ -- as shown in Eqn 13.

The C loss produces CO_2. You have no direct basis for saying this, except that if you look at isocitric acid and α-ketoglutaric acid, you should see that one of the -COOH (the middle one) is now -H. This might suggest to you that the other product is simply CO_2. This reaction is known as decarboxylation. We have not discussed it before, but it is a known type of organic chemical reaction. In this case, CO_2 release in the citric acid cycle should also make sense as part of the complete combustion of glucose.

At this point, instead of going through the reactions in sequence, let's look for some features.

What other reaction in the citric acid cycle produces CO_2?

PAUSE.

CONTINUE. Reaction 4. $C_5 \rightarrow C_4$.

Does this reaction also involve oxidation of the main organic compound? (Careful. Don't be distracted by the CoA. In fact, it might be simplest to think of α-ketoglutaric acid to succinic acid.)

PAUSE.

CONTINUE. Yes. Look at the "bottom" carboxyl group of α-ketoglutaric acid. It is attached to a C with a carbonyl group, a ketone carbon. Loss of the CO_2 per se leaves an aldehyde group here. But succinic acid has a carboxyl group, so there must be an oxidation. (That the succinate first appears as a thioester really doesn't affect this point; it just hides it a bit.)

Thus reaction 4, like 3, is an oxidation. It also produces an NADH.

What other reactions are oxidations, and produce 2 H atoms?

PAUSE.

CONTINUE. Reactions 6 and 8. Reaction 6 produces an $FADH_2$, and reaction 8 produces an NADH. (It is not obvious which H carrier should be used in each case.)

Now that we have surveyed the entire citric acid cycle for oxidation steps… How many molecules of the H carriers are produced during one trip around the cycle? Which ones?

PAUSE.

CONTINUE. Each round of the citric acid cycle produces 3 NADH and 1 $FADH_2$.

We have seen two reactions in which the number of C is reduced. Which reaction increases the number of C? What type of reaction is this? (Best is to think of the reaction as having two distinct steps, each of which corresponds to a well-known reaction type.)

PAUSE.

CONTINUE. Reaction 9 involves reacting a C_2 compound with a C_4 compound, to get a C_6 compound. The C_2 compound is in the form of a thioester, acetyl CoA, but we ignore that in our C count; after all, the CoA is just a carrier for the acetate group, and it is the acetate that matters. The key reaction is an aldol condensation; The $-CH_3$ of the acetyl group, which is α to a carbonyl, adds across the keto carbonyl group of the oxaloacetate. This is followed by hydrolyzing the thioester. Ouellette describes this reaction sequence on p 357.

Have we missed anything important? Well, there is one more bit of energy captured. Reaction 5, the hydrolysis of the thioester succinyl CoA, is coupled to the formation of GTP:

$$\text{succinyl CoA} + \text{GDP} + P_i \rightarrow \text{succinate} + \text{GTP} + \text{CoA} \qquad (20)$$

GTP is similar to ATP, both in chemical form and its role as an energy carrier (briefly noted in Sect E.4). In fact, phosphate groups can easily exchange among these related compounds, such as:

$$\text{GTP} + \text{ADP} \rightleftarrows \text{GDP} + \text{ATP} \qquad (21)$$

This "direct" production of a nucleoside triphosphate is called substrate-level phosphorylation. This term distinguishes this type of ATP production from that coupled to respiration, called oxidative phosphorylation (Sect H).

> If you find something else that seems important or interesting in this pathway, ask me about it.

G.6. Branches in the citric acid cycle

So far we have presented a pathway as if it were a single linear sequence of reactions, with no connections to any other pathway (except at the beginning and end). That is a simplification, which allows us to look at one thing at a time. Even a quick glance at the big metabolic chart shows you that pathways are interconnected. We will briefly note some specific examples of this in Sect J.

H. Oxidative phosphorylation

We will not examine this pathway in detail. However, we need to discuss its role briefly to complete the story we have started.

The oxidative phosphorylation pathway takes the H produced in the citric acid cycle (and other sources), and burns that H. The H comes to this pathway in the form of the biological H carriers, NADH and $FADH_2$. The H's are burned -- reacted with O_2 to form H_2O. This produces energy, of course, and the biological process of burning H captures much of that energy in the form of ATP.

> As another view of this process, we could say that the electrons in the electron carriers NADH and $FADH_2$ are used to reduce O_2 to H_2O.

For the purposes of bookkeeping, it is useful to know that each NADH delivered to the oxidative phosphorylation pathway yields 3 ATP. Each $FADH_2$ yields 2 ATP. We will use these numbers in the next section.

> The numbers 3 ATP per NADH and 2 ATP per $FADH_2$ are somewhat arbitrary. Neither theory nor experiment are entirely clear; perhaps the best current values are 2.5 for NADH and 1.5 for $FADH_2$. Since we are not discussing oxidative phosphorylation in any detail, we will not try to deal with this uncertainty. In any case, the nominal numbers are intended as "best case".
>
> Some supplementary materials on oxidative phosphorylation are listed in Sect M. They include animations of electron transport.
>
> A key enzyme of oxidative phosphorylation is the enzyme that actually makes ATP. It is usually called ATPase (after its reverse reaction). This complex enzyme is embedded in the membrane, and it rotates with each cycle. For more

on this fascinating enzyme, including movies directly observing the rotation, see Rondelez et al (2005), van den Heuvel & Dekker (2007), and Sect M.

In eukaryotic cells, oxidative phosphorylation is carried out in the mitochondria.

Several references in Sect L discuss other aspects of energy metabolism. Others were listed for Ch 13.

I. An energy budget for glucose metabolism

Table 1, below, summarizes biological energy production from the complete oxidation of glucose. We have outlined all steps shown here (Sect F), but considered only the citric acid cycle in detail (Sect G). Thus, for the citric acid cycle, you should understand where these numbers come from. (The relationship between ATP and H carriers such as NADH was discussed in Sect H.)

Table 1. ATP yield from glucose metabolism.

Step(s)	"Energy" produced (per glucose)	ATP/glucose
Glycolysis	2 ATP (net) -2 in early steps +4 in later steps 2 NADH	2 6 (or 4)*
Pyruvate→acetate	2 NADH	6
Citric acid cycle	2 GTP 2 $FADH_2$ 6 NADH	2 4 18
TOTAL		38 (or 36)* ATP/glucose

Some things to note:

1. Most of the ATP is actually produced by oxidative phosphorylation, "burning" H that was removed during various earlier steps, and carried to oxidative phosphorylation by NADH or $FADH_2$.

2. Most of that H comes from the citric acid cycle.

3. Organisms growing anaerobically, without benefit of oxidative phosphorylation, gain only the ATP that is produced directly, by substrate level phosphorylation, in glycolysis. In this case, the NADH produced during glycolysis is recycled without energy capture; Sect F. Thus the overall energy yield in anaerobic metabolism (fermentation) is 2 ATP/glucose.

4. * The NADH from glycolysis is produced in the cytosol, rather than in the mitochondria. In some cases, it costs 2 ATP to transport this NADH, or its equivalent, into the mitochondria. Thus the total glucose yield becomes 36 ATP/glucose.

Rich (2003) briefly explores some of the numerology of our energy needs.

J. Nitrogen metabolism

For animals and many common bacteria, nitrogen metabolism is relatively simple. They (and we) consume N largely in the reduced form, ammonia or amines. They use it in that same reduced form. Thus nitrogen metabolism consists largely of moving reduced N around.

In fact, a major class of enzymes of N metabolism is aminotransferases, which carry out transamination reactions. Example:

pyruvate + glutamate $\rightarrow$ alanine + α-ketoglutarate (22)

I encourage you to draw out all those structures, so you can see the amino transfer.

The general transamination reaction is:

α-ketoacid$_1$ + amino acid$_2$ $\rightarrow$ amino acid$_1$ + α-ketoacid$_2$ (23)

In both equations 22 and 23, note that the exchange is between a keto group (which is oxidized, compared to an alcohol group) and an amino group (the fully reduced N group). Of course, in this transamination, there is no net redox reaction. Glutamate is commonly involved in these reactions, thus leading to the suggestion that it can be considered an N carrier (Sect E.7).

Reaction 22 also begins to illustrate another important aspect of the "big picture". Biochemical pathways are branched (Sect G.6). Eqn 22 shows that amino acids are biosynthetically related to compounds in the energy-yielding pathways. Specifically, we see that the amino acid alanine is one step away from pyruvate, and that the amino acid glutamic acid is one step away from the citric acid cycle intermediate α-ketoglutarate.

Question. What other amino acid can be made simply by transamination to a citric acid cycle intermediate?

PAUSE.

CONTINUE. Aspartic acid, from oxaloacetate. The question asks about a simple transamination, so you want to look for an α-ketoacid. Change the keto to an amino, and see whether you have made a standard amino acid.

With a protein-rich diet, the carbon skeleton of excess amino acids, after having the amino group removed, can go into the energy-yielding pathways. Or, if amino acids are in short supply, some of the C flowing through the energy yielding pathways can be removed to make amino acids.

Excess N is secreted. The first step is to deaminate glutamic acid, producing ammonia:

glutamic acid $\rightarrow$ α-ketoglutarate + NH_3 (24)

As with the transamination reactions above, this involves interconversion of reduced and oxidized groups. However, in contrast to the transamination reaction, Eqn 24 shows a net oxidation. Not surprisingly, then, a biological oxidizing agent is involved. A more complete form of Eqn 24 is:

glutamic acid + NAD^+ + H_2O $\rightarrow$ α-ketoglutarate + NH_3 + NADH + H^+ (25)

Because ammonia itself is rather toxic, the N is transferred to less toxic N-rich molecules, such as urea or uric acid, for excretion.

Some organisms can use ammonia as nitrogen source. For example, common lab bacteria such as E. coli are often grown with NH_3 or NH_4^+ as the N source. One way to use this ammonia is by a reaction that is essentially the reverse of reaction 25.

The topic of N changing oxidation states is beyond our scope. This includes nitrogen fixation, the use of atmospheric N_2, carried out by some bacteria. Plants commonly use nitrate, NO_3^-, as the N-source, first reducing it to ammonia. Microbes carry out a variety of N transformations, which are interesting and important in the context of global N balance.

K. Other handouts for this topic; homework

The following handouts are for your use in studying metabolism. They are intended as good practice, to help you develop the ideas. You are not responsible for remembering the content, but for using the ideas.

The Glycolysis worksheet will guide you through the details of the initial steps in standard glucose metabolism. Recall Sect F. A web site for glycolysis is listed in Sect M.

The Pathways quiz will challenge you with a novel metabolic diagram, and guide you to extract useful information from it, using your general understanding of metabolism.

> These are also available at the web site, along with this main handout, on the Metabolism page.

These two supplementary handouts, along with the dialog in Sect G, should serve as useful homework exercises for you. (There is also a short dialog in Sect J; we may not get to this topic.)

L. Further reading

Books

The following two are for "one-semester" versions of biochem. Perhaps useful for some; less overwhelming than the traditional biochemistry books, such as Lehninger, Stryer or Voet.

- T McKee & J R McKee, Biochemistry; The Molecular Basis of Life. 3/e, 2003, McGraw-Hill. (Title varies for older editions. If you want to find this, suggest that you do an author search. Older books called "Biochemistry" by the same authors are apparently the same basic book.)
- H R Horton et al, Principles of Biochemistry, 4/e, 2006, Prentice Hall.

I have a page of book suggestions for general reading posted at my web site. Some of the books listed there are relevant to current material. Briefly, they include:

- L Guarente, Ageless Quest - One scientist's search for genes that prolong youth.
- F M Harold, The Way of the Cell - Molecules, Organisms and the Order of Life.
- J Prebble & B Weber, Wandering in the Gardens of the Mind - Peter Mitchell and the making of Glynn.
- N Lane, Power, Sex, Suicide: Mitochondria and the Meaning of Life.

See my web page for more information; browsing encouraged. And contributions to the list are welcomed.

Articles

> Some of the FR for Ch 13 (Lipids) and Ch 15 (Proteins) are also relevant here. Also see the web page of FR on Medical Topics.

B J Koebmann et al, The glycolytic flux in Escherichia coli is controlled by the demand for ATP. J Bacteriol 184:3909-3916, 7/02. (Also see news story: S Oliver, Metabolism: Demand management in cells. Nature 418:33, 7/4/02.) Regulation of cellular metabolism is a major issue. There are hundreds of reactions, and everything has to happen at just the right level. Here they provide evidence that the activity of glycolysis is controlled to a large extent by demand -- by how much energy is needed (rather than by levels of individual enzymes in the pathway). They do the test by providing an artificial waste sink for ATP; turning on that ATP-wastage increases flow through glycolysis, in an attempt to compensate. This result would seem to be consistent with the emerging idea that biochemical networks are robust -- that the results are not very sensitive to specific details along the way. Of course, one might argue that this should be so, but it is not the simplest view. Also see Schilling et al (2002).

C H Schilling et al, Genome-scale metabolic model of Helicobacter pylori 26695. J Bacteriol 184:4582-4593, 8/02. Helicobacter pylori is a bacterium closely associated with, and probably causing, stomach ulcers. This paper is an example of genomics work; it involves computer modeling of the organism's metabolism, based in part on the genome sequence as well as available biochemistry. Some Helicobacter metabolism is somewhat unusual, but it can be seen as variation on the standard metabolism that we discuss. Also see Koebmann et al (2002).

P Rich, Chemiosmotic coupling: The cost of living. Nature 421:583, 2/6/03. Concept Essay. In this brief essay, Rich gives an overview of our energy needs. Lots of fun numbers, such as… a person turns over about 65 kg of ATP each day, using about 14,000 m^2 of surface area of the inner mitochondrial membrane to make it. Operating a human is very energy intensive, and the energy apparatus is subject to error and decay.

C F Amábile-Cuevas, New antibiotics and new resistance. Amer Sci 91:138, 3/03. A good general-audience article on the continuing battle of antibiotics vs antibiotic resistance. Many antibiotics are enzyme inhibitors.

W B Frommer et al, Plant science: Hexokinase, Jack-of-all-trades. Science 300:261, 4/11/03. News. Hexokinase, the enzyme that phosphorylates sugars, is also part of a signal system that transmits information about sugar levels, integrated with other physiological inputs, to the nucleus. In work discussed here, using mutants of the simple model plant Arabidopsis, they show that the catalytic and regulatory functions are distinct.

T Kasahara & T Kato, Nutritional biochemistry: A new redox-cofactor vitamin for mammals. Nature 422:832, 4/24/03. PQQ was first discovered in bacteria barely 20 years ago. Dietary deficiency of PQQ leads to growth deficiencies in mice. Here they identify a specific mouse enzyme that requires this new redox factor. They thus suggest that PQQ should be added to the list of B vitamins.

R M Anson et al, Intermittent fasting dissociates beneficial effects of dietary restriction on glucose metabolism and neuronal resistance to injury from calorie intake. PNAS 100(10):6216-6220, 5/13/03. This was a rather big news story. One treatment that really does seem to delay aging, in a wide range of organisms, is caloric restriction. Here they show that alternating days of eating and fasting, with a total food intake that is approximately normal, leads to some of the benefits of caloric restriction. This work is with mice. An intriguing result. Also see Couzin (2004), below.

E Ruiz-Pesini et al, Effects of purifying and adaptive selection on regional variation in human mtDNA. Science 303:223, 1/9/04. Humans have variation in their mitochondrial genes, just as in nuclear genes. This article suggests -- quite tentatively -- that some of the variation in mitochondrial genes and function is due to selection by climate. Briefly, the idea is that those who live in very cold climates may have mitochondria that are less efficient, in the usual sense, and generate more heat.

J Couzin, Research on aging: Gene links calorie deprivation and long life in rodents. Science 304:1731, 6/18/04. News. See Anson et al (2003), above, for some background. The work discussed here seems to implicate a particular gene in mediating the effect of caloric restriction on aging. The gene was first recognized in yeast, but the current work suggests a linkage in mice. Also see Guarente's book, on my web page of Book suggestions.

L Pellerin & P J Magistretti, Neuroscience: Let there be (NADH) light. Science 305:50, 7/2/04. News. The common view is that neuronal activity is tightly linked to glucose consumption. The work discussed here shows that it is more complex. They provide evidence that glucose is metabolized to lactate, by glycolysis, in the nearby astrocytes. The lactate, then,

is shuttled to the neurons, where oxidative metabolism continues. Key to tracking this down was following the NADH, by sophisticated fluorescence microscopy.

D E Koshland, Crazy but correct. Nature 432:447, 11/25/04. A "Turning point" essay on the origins of the induced fit model, with discussion of the skepticism that greeted his new idea.

J D York & T Hunter, Signal transduction: Unexpected mediators of protein phosphorylation. Science 306:2053, 12/17/04. News. We have discussed ATP and the related nucleotide triphosphates as phosphate carriers. Here they discuss recent work showing that inositol pyrophosphate also serves as a phosphate carrier.

T Burmester, Physiology: A welcome shortage of breath. Nature 433:471, 2/3/05. News. The work suggests that one aspect of the insect respiratory system is designed to restrict oxygen access (rather than to provide oxygen). If this interpretation is valid, it emphasizes the toxic aspect of oxygen.

Y Rondelez et al, Highly coupled ATP synthesis by F1-ATPase single molecules. Nature 433:773, 2/17/05. More about how the ATPase of oxidative phosphorylation (Sect H) works. The natural role of the ATPase is to make ATP, but it is easier to study the reverse reaction and most work has been with that reaction. Here they set up a system to show how rotation, which they drive with magnetic tweezers, is coupled to ATP synthesis. Also see van den Heuvel & Dekker (2007) and Sect M.

J B Andersen et al, Physiology: Postprandial cardiac hypertrophy in pythons. Nature 434:37, 3/3/05. The metabolism associated with digesting food requires oxygen. Pythons eat only occasionally, hence have bursts of high metabolic rates -- 40-fold higher than fasting rates. This paper shows that they gain 40% heart muscle mass within 48 hr of feeding.

D K Smith, A supramolecular approach to medicinal chemistry: medicine beyond the molecule. J Chem Educ 82:393, 3/05. This article is a nice overview of molecular interactions, both as they are involved in disease processes and in drug actions. Examples include the aggregation of proteins (Alzheimer's disease and CJD); drug delivery systems (cyclodextrins): complexes that better deliver hydrophobic drugs; and drugs designed to be targeted to enzyme sites (Relenza, an anti-flu drug).

J-P Changeux & S J Edelstein, Allosteric mechanisms of signal transduction. Science 308:1424, 6/3/05. Review. Changeux (formerly of UC Berkeley) was among the originators of the idea of allostery, 40 years ago. Over time, the importance of protein flexibility and distant interactions has become increasingly recognized.

R A Miller, Biomedicine: The anti-aging sweepstakes: Catalase runs for the ROSes. Science 308:1875, 6/24/05. We have mentioned the problem of oxygen toxicity, due to ROS = reactive oxygen species (such as superoxide ion, O_2^-). Catalase is the enzyme that breaks down one ROS, hydrogen peroxide. The work discussed here shows that increased catalase activity extends the life span of mice -- if it is targeted to the mitochondria. The targeting result emphasizes the importance of getting antioxidants to the right place. Whether the result here has any implications for humans is speculative at this time.

D Deamer, A giant step towards artificial life? Trends in Biotechnology 23(7):336, 7/05. Deamer proposes 12 conditions that must be met if we are to make artificial life. Interestingly, 11 of them have been achieved. Of course, it is a big leap to integrate them together. A fascinating little article!

L Tong, Acetyl-coenzyme A carboxylase: crucial metabolic enzyme and attractive target for drug discovery. Cell Mol Life Sci 62:1784-1803, 8/05. Review. In the biosynthesis of fat, one key player is malonyl CoA, the C_3 unit. This is made by carboxylation of acetyl CoA, catalyzed by the enzyme acetyl CoA carboxylase (ACC). Mice deficient in one form of ACC eat more but gain less weight than wild type mice. The effect may be partly due simply to loss of an enzyme needed to make fat. However, it is probably also due to relief of inhibition of fatty acid breakdown, by the malonyl CoA. Whatever the explanation, the enzyme is an attractive target for a drug. This work relates to Ch 13 (lipids) as well as to the current topic (enzyme regulation, metabolism).

R J Spreitzer et al, Phylogenetic engineering at an interface between large and small subunits imparts land-plant kinetic properties to algal Rubisco. PNAS 102(47):17225-30, 11/22/05. A fascinating enzyme is ribulose-1,5-bisphosphate carboxylase, often called RuBisCO. This is the enzyme that actually "fixes" CO_2 in photosynthesis. RuBisCO is probably the most abundant protein on earth. One reason it is so abundant is that it is extremely inefficient -- sometimes referred to as the "most incompetent" enzyme known. Here they explore the differences between plant and algal RuBisCO enzymes, with the hope that such information might ultimately lead to improvements. Also see Thauer (2007).

E A Curtis & D P Bartel, New catalytic structures from an existing ribozyme. Nature Struct Mol Biol 12:994, 11/05. An important aspect of biology is how new variation arises from what already exists. Does this hold for the RNA world? Here they show that novel ribozymes can arise with only modest sequence variation from existing ribozymes. Also see Been (2006).

J Henderson et al, The EPAS1 gene influences the aerobic–anaerobic contribution in elite endurance athletes. Hum Genet 118:416-423, 12/05. From the abstract: "EPAS1 is a gene involved in complex oxygen sensing. … Since EPAS1 has a role as a sensor capable of integrating cardiovascular function, energetic demand, muscle activity and oxygen availability into physiological adaptation, we propose that DNA variants in EPAS1 influence the relative contribution of aerobic and anaerobic metabolism and hence the maximum sustainable metabolic power for a given event duration." Also see MacArthur et al (2007).

V W Cornish, Biological chemistry: Catalytic competition for cells. Nature 440:156, 3/9/06. News. Discussion of an experimental approach to studying evolution of enzymes. "Ways of evolving proteins, and assessing the vast numbers of variants needed to identify those with novel enzymatic activity, are themselves evolving. Oil droplets containing basic cell machinery provide a promising approach." Also see Robertson & Scott (2007) and Jiang et al (2008) for other items on making better enzymes.

P G Falkowski, Evolution: Tracing oxygen's imprint on earth's metabolic evolution. Science 311:1724, 3/24/06. We consider O_2 fundamental to our lives, yet also recognize that there is anaerobic life. In fact, the early earth was (nearly?) oxygen-free, and early life was anaerobic. So how did the atmosphere become oxygen-rich? From the biological process of

photosynthesis. This short and readable "Perspective" article gives a nice overview of our emerging understanding of the interwoven stories of the biology and geochemistry of oxygen.

1) N Lane, Mitochondrial disease: Powerhouse of disease. 2) T M Embley & W Martin, Eukaryotic evolution, changes and challenges. Nature 440:600 & 623, 3/30/06. The first is a "news feature" discussing the role of mitochondria in disease. The second is a review article on the origin of eukaryotic cells, with an emphasis on the origin of mitochondria. The importance of mitochondria in human diseases, with perhaps a key role in aging, is being increasingly recognized. Genes for mitochondrial function are found both in the mito and in the nucleus, so the genetics of mito diseases can be confusing. As to the evolution, it is now well accepted that the mito itself is derived from a bacterium that "took up residence" inside another cell -- a phenomenon called endosymbiosis. But the details are unclear, and are getting less clear. For a while it was thought that some "primitive" eukaryotes which seemed to lack mito might be evolutionary intermediates: organisms with a eukaryotic cell but not yet with mito. But the recent recognition that such eukaryotes contain hydrogenosomes or mitosomes, now all thought most likely to be degenerate mito, leaves us with no clear pathway to modern eukaryotic cells. Also note Lane's book, listed at top of this FR section.

S J Benkovic & S Hammes-Schiffer, Biochemistry: Enzyme motions inside and out. Science 312:208, 4/14/06. News, about a study of how short range thermal motions within an enzyme help promote H transfer, and achieve an extraordinary rate of catalysis. Also recall Schotte et al (2003) and Huang & Montelione (2005), both in Ch 15 FR.

M Yoshizawa et al, Diels-Alder in aqueous molecular hosts: Unusual regioselectivity and efficient catalysis. Science 312:251, 4/14/06. Organic chemists marvel at the specificity of enzymes. This paper discusses the development of an organic chemistry catalyst that shows considerable specificity. The article discusses the design issues.

A G Dyer et al, Behavioural ecology: Bees associate warmth with floral colour. Nature 442:525, 8/3/06. One product of metabolism is heat. As warm-blooded animals, we regulate heat production. This article discusses plants that heat their flowers -- to provide a nice place for the bumblebees that pollinate them. Also see Berg et al (2006).

B S Khakh & R A North, P2X receptors as cell-surface ATP sensors in health and disease. Nature 442:527, 8/3/06. Review. "P2X receptors are membrane ion channels activated by the binding of extracellular adenosine triphosphate (ATP). For years their functional significance was consigned to distant regions of the autonomic nervous system, but recent work indicates several further key roles, such as afferent signalling, chronic pain, and in autocrine loops of endothelial and epithelial cells. P2X receptors have a molecular architecture distinct from other ion channel protein families, and have several unique functional properties." One of those roles is sensing a full bladder. As the bladder fills and stretches, ATP leaks out of the epithelial cells. The extracellular ATP activates a receptor, which leads to a "pain" signal to the brain.

F Berg et al, The Uncoupling Protein 1 gene (UCP1) is disrupted in the pig lineage: A genetic explanation for poor thermoregulation in piglets. PLoS Genetics 2(8):e129, 8/06. If we could just burn our food without capturing the energy, we could eat more with less weight gain. A way to avoid energy capture would be to somehow dissipate the energy from NADH

oxidation, avoiding coupling it to ATP formation. In fact, “uncoupling proteins” (UCP) allow just that. Mice have them, in brown fat. Humans have them, but only in infancy. Pigs do not have them, and this paper explores the genetic basis of that defect. Free online at: http://genetics.plosjournals.org/perlserv/?request=get-document&doi=10.1371/journal.pgen.0020129
Also see Dyer et al (2006).

M D Been, Molecular biology: Versatility of self-cleaving ribozymes. Science 313:1745, 9/22/06. This news article introduces two papers in this issue. One is about the mechanism of a ribozyme; they show that it is modulated by a small molecule -- just as is common for protein enzymes. In fact, the idea of riboswitches -- regulatory RNA molecules whose shape is affected by binding a ligand -- is now well accepted. In this case, the ligand seems to be part of the active site of the ribozyme, providing a group needed for catalysis. The other paper looks for ribozymes in the human genome -- and finds them. One is related to the hepatitis delta virus; perhaps it was the origin of that virus. Also see Curtis & Bartel (2005).

C B Saper, Biomedicine: Life, the universe, and body temperature. Science 314:773, 11/3/06. News. Is 37° C, our “normal” body temperature “good”? An interesting question. In the work discussed here, they trick mice into having a somewhat lower body temperature than usual. The cool mice live longer. Interesting.

M Freeman, Structural biology: Enzyme theory holds water. Nature 444:153, 11/9/06. News. Hydrolytic enzymes need water. Simple enough, but in recent years several proteases have been discovered that work inside a membrane, in the apparent absence of water. Now it seems that they carry their own water with them. Examples of intramembrane proteolysis include: sterol regulatory element binding proteins, which respond to cholesterol levels in the membrane and are released if the cell senses it needs more; presenilin, an enzyme that is, in part, responsible for the development of Alzheimer’s disease, but which also has normal roles in development.

F Jordan, Adenosine triphosphate and thiamine cross paths. Nature Chemical Biology 3(4):202, 4/07. News. We have noted that several vitamins and cofactors involve adenosine. Now, add a new one to the list. This news story discusses the discovery of a new form of thiamine (vitamin B1): adenosine thiamine triphosphate (AThTP). The implications of this discovery are not clear, but they did find it in organisms ranging from bacteria to mammals.

A Gibbons, Paleoanthropology: Food for thought. Science 316:1558, 6/15/07. News. A large brain is a distinctive feature of humans. Development of the brain is very energy intensive. However, overall energy needs of a developing human are not unusually high. How can that be? One argument is that the mother provides the extra energy needed for brain development of the newborn child. Another idea is that some other aspect of humans is less developed than in other mammals; there is now considerable evidence that might be the digestive tract, which seems to be relatively small in humans. A new twist on the story is that perhaps it was learning to cook meat, providing us with more food that is easy to digest, is what allowed the gut to “wither” a bit -- thus allowing the expansion of the brain. In this news feature, Gibbons discusses these and related ideas.

M G L van den Heuvel & C Dekker, Motor proteins at work for nanotechnology. Science 317:333, 7/20/07. Review. The ATPase of oxidative phosphorylation is an example of a motor protein, in which energy is coupled to motion of a biological object. Muscle proteins are another example, as are flagella and the proteins involved in intracellular transport of cargo along microtubules. Can these biological motors be exploited by man? So far, we are "playing" with them, but have not done anything really useful. This article is a good overview of these motor proteins, and their potential. Also see Rondelez et al (2005).

M P Robertson & W G Scott, Biochemistry: Designer enzymes. Nature 448:757, 8/16/07. News. The work discussed here involves developing a new enzyme in the laboratory, by a lab process analogous to natural selection. The key in this lab system is to connect the enzyme activity to its gene. Also see Cornish (2006) and Jiang et al (2008) for other items on making better enzymes.

J Stubbe, Computational biochemistry: Models of transition. Nature 448:762, 8/16/07. News. The work discussed here led to the identification of the substrates for an enzyme by computational approaches. The structure of the enzyme was known, and its general type of reaction predicted by comparison with of known enzymes. Then, by commuter modeling, they docked a long list of possible substrates to the enzyme, to see which ones were likely substrates. Biochemical tests confirmed much of their prediction. This is an interesting approach to determining what an enzyme does. This news story puts it in perspective.

D G MacArthur et al, Loss of ACTN3 gene function alters mouse muscle metabolism and shows evidence of positive selection in humans. Nature Genetics 39:1261, 10/07. Genetic differences in the muscle protein α-actinin-3 have been associated with athletic performance. Presence of the active protein is important for sprinters; absence of the active protein is associated with endurance. Here they study the role of this protein in a mouse model system. They show that loss of the protein leads to a metabolic shift toward the more efficient aerobic pathway -- as expected for endurance. Also see Henderson et al (2005).

R K Thauer, Microbiology: A fifth pathway of carbon fixation. Science 318:1732, 12/14/07. News. Carbon fixation is the process of incorporating CO_2 into organic material. The most common pathway is the Calvin cycle, involving the Rubisco enzyme (e.g., Spreitzer et al, 2005). However, among the microbes there are other ways. This news story summarizes them, including a report of a newly discovered pathway. It also discusses some evolutionary aspects of these multiple pathways.

L Jiang et al, De novo computational design of retro-aldol enzymes. Science 319:1387, 3/7/08. One great challenge in understanding protein structure and function is to design new enzymes. This paper reports some progress. They describe their design logic, as well as their results. Fewer than half of their designed products had the desired activity, few worked well, and none worked as well as common natural enzymes. On the other hand, they found that the designed proteins did have structures very close to what was predicted. And they think they learned some principles about enzyme design, such as the importance of water in the mechanism. Overall, this paper represents significant progress in our understanding of enzymes. Also see Cornish (2006) and Robertson & Scott (2007) for other items on making better enzymes.

H R Christofk et al, The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. Nature 452:230, 3/13/08. Cancer cells often have elevated levels of glycolysis. This paper shows that this is probably due to an altered version of the enzyme pyruvate kinase being expressed in the cancer cells. (Pyruvate kinase is the enzyme that converts phosphoenolpyruvate to pyruvate; that is, it is named for the reverse reaction. See step 9 of the glycolysis pathway in the "Glycolysis worksheet" handout.) If this finding is significant, it could suggest new therapeutic approaches.

S An et al, Reversible compartmentalization of de novo purine biosynthetic complexes in living cells. Science 320:103, 4/4/08. We sometimes start with a simple view of the cell being a bag of enzymes. However, it really is more complex than that -- much more complex. There is much organization in the cell. Some aspects of that are fairly obvious, such as enzymes bound to the membrane, or in a defined compartment such as the nucleus. Beyond that, we are increasingly recognizing more and more cellular organization, mediated by the cytoskeleton. Another phenomenon is that enzymes working on one task may cluster together, allowing the product of one enzyme to be efficiently transferred ("channeled") to the next enzyme. Here they examine the complex of enzymes that makes purines, and show that the complex dissociates when not in use.

A J Kirby & F Hollfelder, Biochemistry: Enzymes under the nanoscope. Nature 456:45, 11/6/08. News. A nice discussion of the importance of very small distances in determining how enzymes work. Enzyme catalysis depends on good binding to a key intermediate in the reaction, called a transition state. Distances on the order of a few picometers -- much less than the length of an ordinary covalent bond -- matter.

M. Computer resources

(See web page for details and links.)

Required. The web site for Worthington Biochemical Corp contains a nice **Introduction to Enzymes**, which is a "required" reading assignment for this course.
http://www.worthington-biochem.com/introBiochem/beginBiochem.html

Metabolic charts. Several options for obtaining a chart, either a web chart or a paper version, are listed on my Intro Organic/Biochem Internet Resources page, under Metabolism. The Metabolic Pathways page at Sigma also includes additional resources on metabolism. There are several animations of metabolic processes, and a link to a list of enzymes by EC (Enzyme Commission) number.

Other

A good source of the complete glycolysis pathway. You can use this to check yourself if you do Part 1 of the Glycolysis worksheet (my supplementary handout), or you can just use it as a source of the pathway.

An extensive tutorial on oxidative phosphorylation is included in a fine collection of tutorials at Washington Univ (St Louis) that generally aim to show practical aspects of freshman chemistry. The tutorial "Energy for the Body: Oxidative phosphorylation" includes a quite complex animation of the electron transfer process. Of course, while at the Tutorials site, look around for others that may interest you.

Electron transport and ATP formation. Dr Thomas Terry, Univ of Connecticut, has made available a couple of his pages on these processes, including some nice animations.

The final enzyme in the oxidative phosphorylation sequence is the ATPase. This enzyme actually rotates in the membrane as it works to make ATP. This entry lists two sites that show diagrams of this complex enzyme. The first shows an animation of the enzyme rotation; the second shows a movie of the rotation, directly observed by attaching a visible propeller to the enzyme. Both are parts of more extensive sites on metabolism. Also see Rondelez et al (2005) and van den Heuvel & Dekker (2007).

This site is for a course in Anatomy and Physiology, but it has a remarkably wide ranging set of "Animations, Movies & Interactive Tutorial Links" for biology and chemistry. Of most direct relevance at the moment would be the section on Cellular Respiration. But I encourage you to browse this whole site; I found the quality to be quite high.

For fun, two sites featuring songs about metabolism are listed on the web page.

N. Beyond this course

Logically, the next topic after Proteins might well be to consider how proteins are made -- biologically. Ouellette introduces this in Ch 16 (Nucleic acids). This topic is the heart of what is commonly called Molecular Biology or Molecular Genetics.

You might want to look at some of these introductory books …

- D P Clark & L D Russell, Molecular Biology made simple and fun. 3/e, 2005.
- J D Watson et al, Recombinant DNA: Genes and Genomes -- A Short Course. 3/e. 2007.
- L Gonick & M Wheelis, The Cartoon Guide to Genetics. 1991.

Sections of my web site include Biotechnology in the News (BITN), aimed at the general audience, and Molecular Biology.