

Chapter XX

The Chemistry of Biological Systems

A. Introduction

We have worked our way through the chemistry associated with each of the common functional groups of organic chemicals. We have even viewed the chemistry associated with molecules bearing several functional groups, wherein there are intramolecular interactions among those functional groups. Thus, we are ready to view complex systems of chemistry, such as those associated with biological processes. Unfortunately, it is often considered that biological systems are entirely separate from those of ordinary chemical processes. However, the reality is that biological systems actually consist of *chemical* processes, simply involving molecules that are multifunctional with stereochemical precision. It is important that we understand such biological processes in chemical terms that we can view them systematically and see what factors are involved in their normal operation, as well as to understand the things that can “go wrong” with them. It is to this end that we now look at several important processes of biological systems from such an organic chemistry point of view, using the concepts we have developed to this point.

In doing so we will look at two particularly important biological processes for our detailed consideration. These are processes involved in intermediary metabolism (processes occurring within biological cells), those specifically being *glycolysis* and some aspects of fatty acid metabolism.

B. Glycolysis

Glycolysis (literally, the breakdown of sweet material) is the intracellular process that converts D-glucose into pyruvate with the associated production of adenosine triphosphate (ATP). The overall scheme of glycolysis is shown in Figures XXX.1 and XXX.2

It is not of particular importance at the moment for us to contemplate *how* we get D-glucose into the cell, or what its ultimate origin is. It could be introduced to the organism directly in the form of D-glucose, or by the degradation (hydrolysis) of a more complex carbohydrate (*e.g.* the relatively simple disaccharide D-fructose, or a more complex polysaccharide such as starch). Suffice it to say that D-glucose can be provided to the organism in one form or another and enter the cell wherein it undergoes glycolysis.

Reactions are shown using a standard method with biological processes, wherein enzymes (catalysts of the processes, consisting of peptide units with other associated units) involved are indicated in *italics*. Additional reactants and products (other than the materials being transformed or produced) are generally shown with curved arrows off the main arrows indicating the reaction of particular interest. In **bold** are indicated each of the reaction processes that are considered in detail in further discussion.

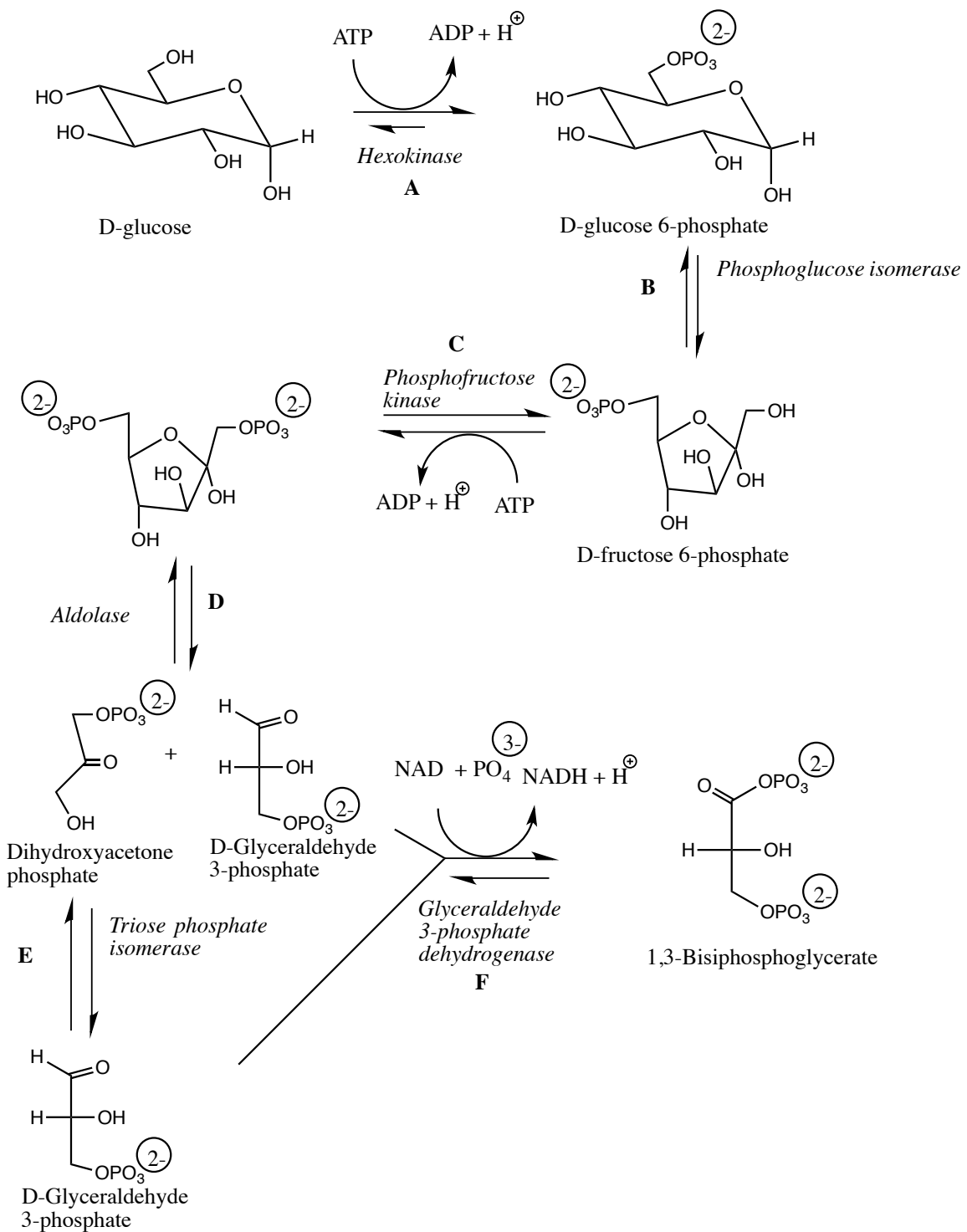


Figure XXX.1

This shows the initial processes of glycolysis, the cleavage of a single molecule of D-glucose.

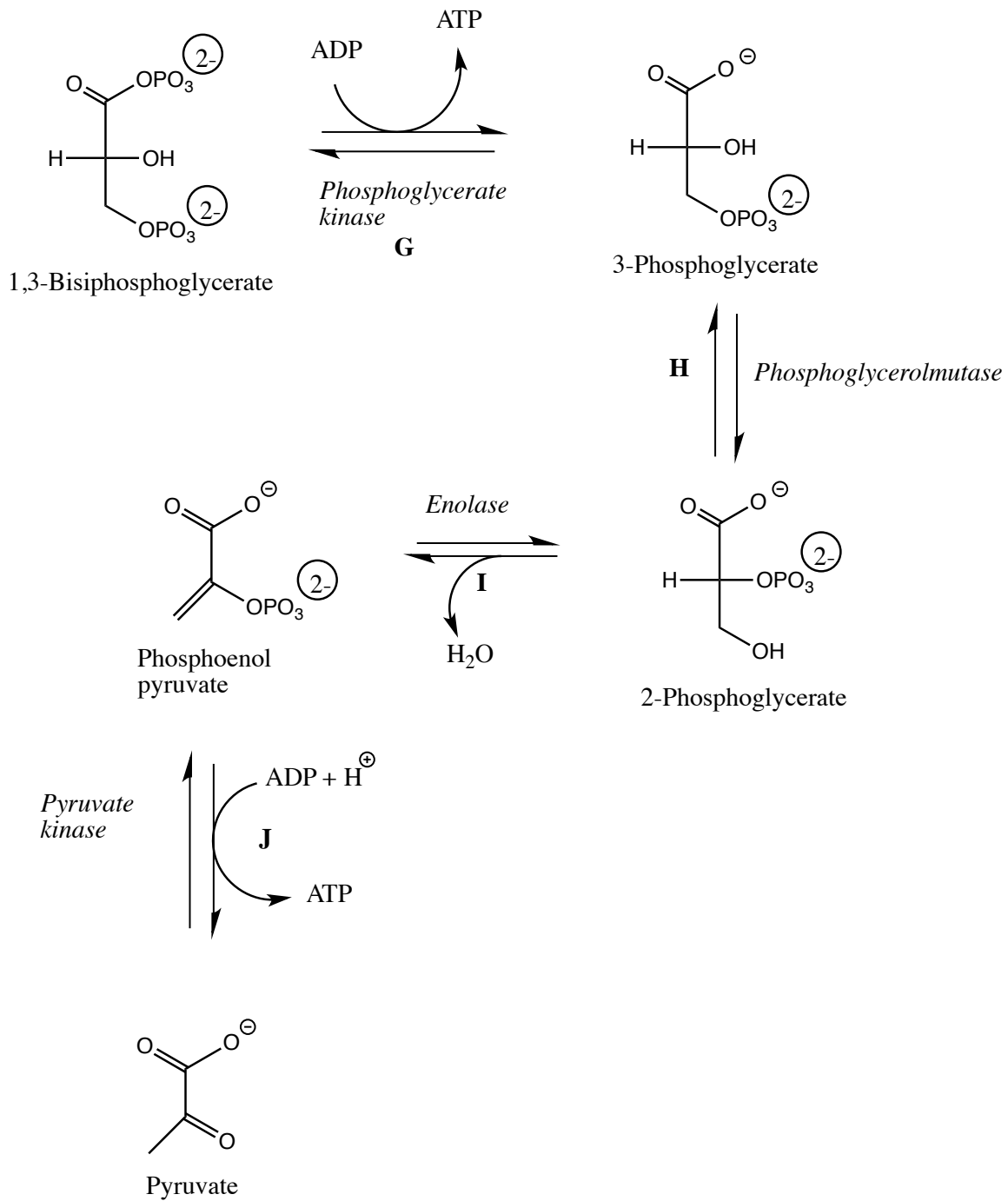


Figure XXX.2

This indicates the latter stages of glycolysis. It should be noted that one molecule of

D-glucose ultimately leads to *two* units of pyruvate.

Now, let us consider progressing through these individual reactions that make up the total process of *glycolysis*. Remember, an *enzyme* is simply a catalyst, an agent that lowers the activation energy (by one means or another) between reactant and product, and facilitates *the attainment of equilibrium*. A catalyst does not change the position of equilibrium, but simply makes it easier for equilibrium to be attained. Thus, both the forward and reverse reaction processes are equally facilitated by a catalyst. As the *reverse* process follows exactly the reverse path of the *forward* process, we need consider in detail only one direction, that of the *forward* reaction process leading to “product” as we talk about the overall process of glycolysis.

Individual reactions **A** and **C** are simply phosphorylations and may be considered together as having the same fundamental nature with the reactants. (The catalysts will be different – both are noted as *kinases* – but perform similar functions on slightly different substrates.) The actual reactions (without showing the involvement of the enzymes) are reactions of an anhydride (ATP – adenosine triphosphate) with a primary alcohol to form an ester of the acid derived from the anhydride. In this sense, these two reactions are entirely understood through our prior considerations of the formation of carboxylate esters with carboxylic anhydrides and alcohols. Our anhydride now, however, is an anhydride derived from phosphoric acid rather than an anhydride derived from a carboxylic acid. The process for **A** (without association of the catalyst) is shown in Figure XXX.3, and for **C** is shown in Figure XXX.4. We can determine the details of the process using specific isotopic labeling of the terminal phosphorus in ATP as well as through the use of oxygen isotopic labeling at the terminal phosphorus site of ATP that it is indeed the terminal P site that becomes esterified with the primary hydroxyl group, and that it does so with backside attack and inversion of configuration at that phosphorus site, typical of ordinary S_N2 processes.

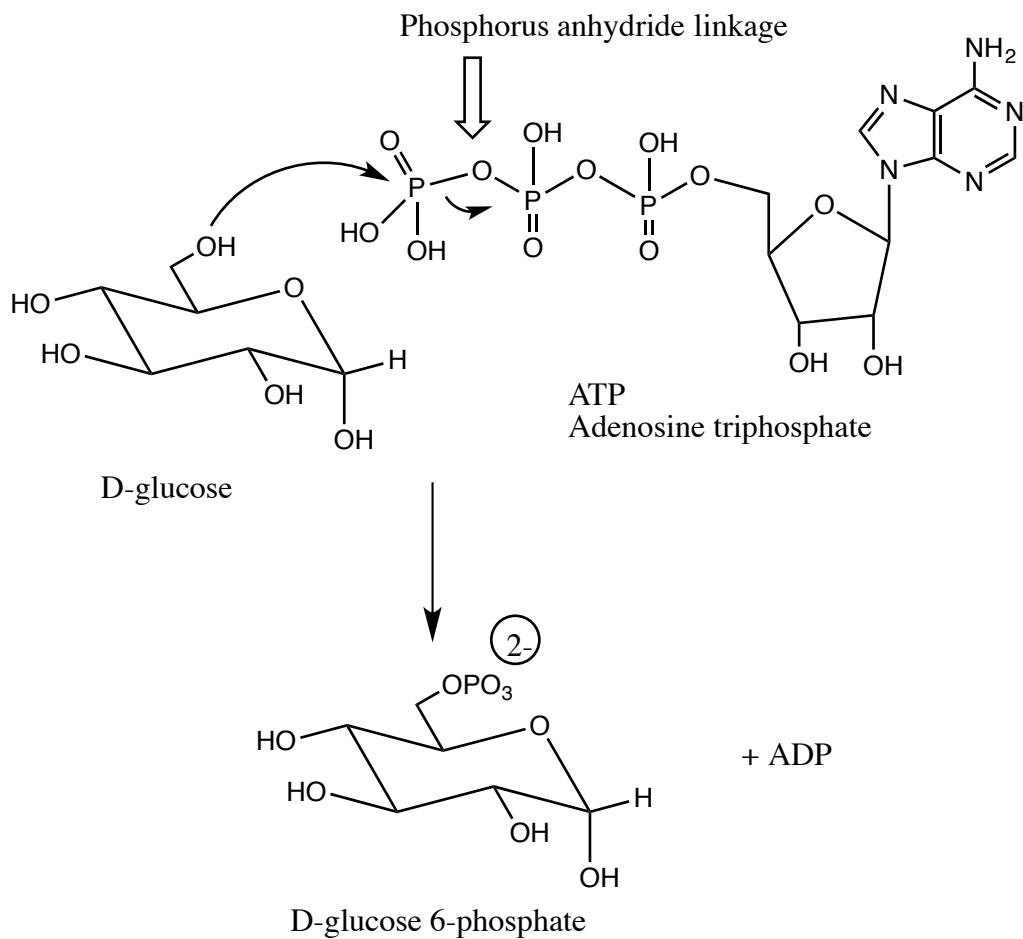


Figure XXX.3

There are actually *two* anhydride type linkages in each ATP molecule. For the phosphorus ester formation process only one of these anhydride linkages is actually used, that being the distal site. The other anhydride linkage remains in the by-product, the ADP.

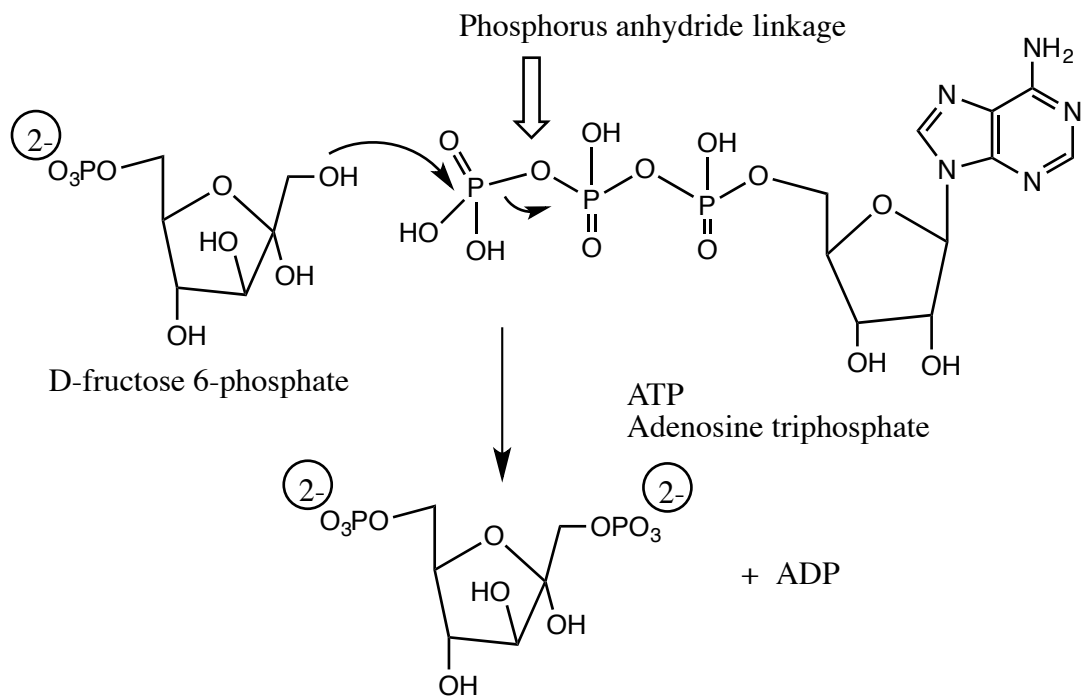


Figure XXX.4

Once the D-glucose 6-phosphate has been converted to the D-fructose 6-phosphate (note that the fructose system is in a furanose ring form) a similar phosphorylation occurs utilizing another unit of ATP, again a primary hydroxyl site being phosphorylated using the distal phosphorus site of the ATP. There remains an unused anhydride linkage in the ADP by-product.

It should be noted that Reactions **G** and **J** are simply the reverse of phosphorus ester formation (phosphorus ester cleavages involving kinases as the catalysts and producing ATP from ADP), thereby being the same as the reverse of the processes noted as **A** and **C**. Thus, these processes are all to be understood as chemically of the same nature.

Moving on to a consideration of the isomerization process indicated as reaction **B** in the overall glycolysis scheme, we look at the conversion of D-glucose 6-phosphate to D-fructose 6-phosphate. This reaction we also recognize from our earlier consideration of carbohydrate chemistry. In consideration of regular carbohydrate chemistry we observe that D-fructose is a *reducing sugar* in spite of the fact that it is a ketone rather than an aldehyde. We note this situation to be the result of the conditions for determination of reducing sugar status involving an aqueous basic medium that allows the glucose species to proceed through an ene-diol structure, isomerizing from an aldose to either an epimeric aldose a ketose. When using a ketose (fructose) epimerization to either of two aldoses is possible. Here we are simply observing a derivative of an aldose epimerizing to a ketose type structure. For the system with a 6-position hydroxyl having been phosphorylated (process **B**), this conversion is illustrated in Figure XXX.5.

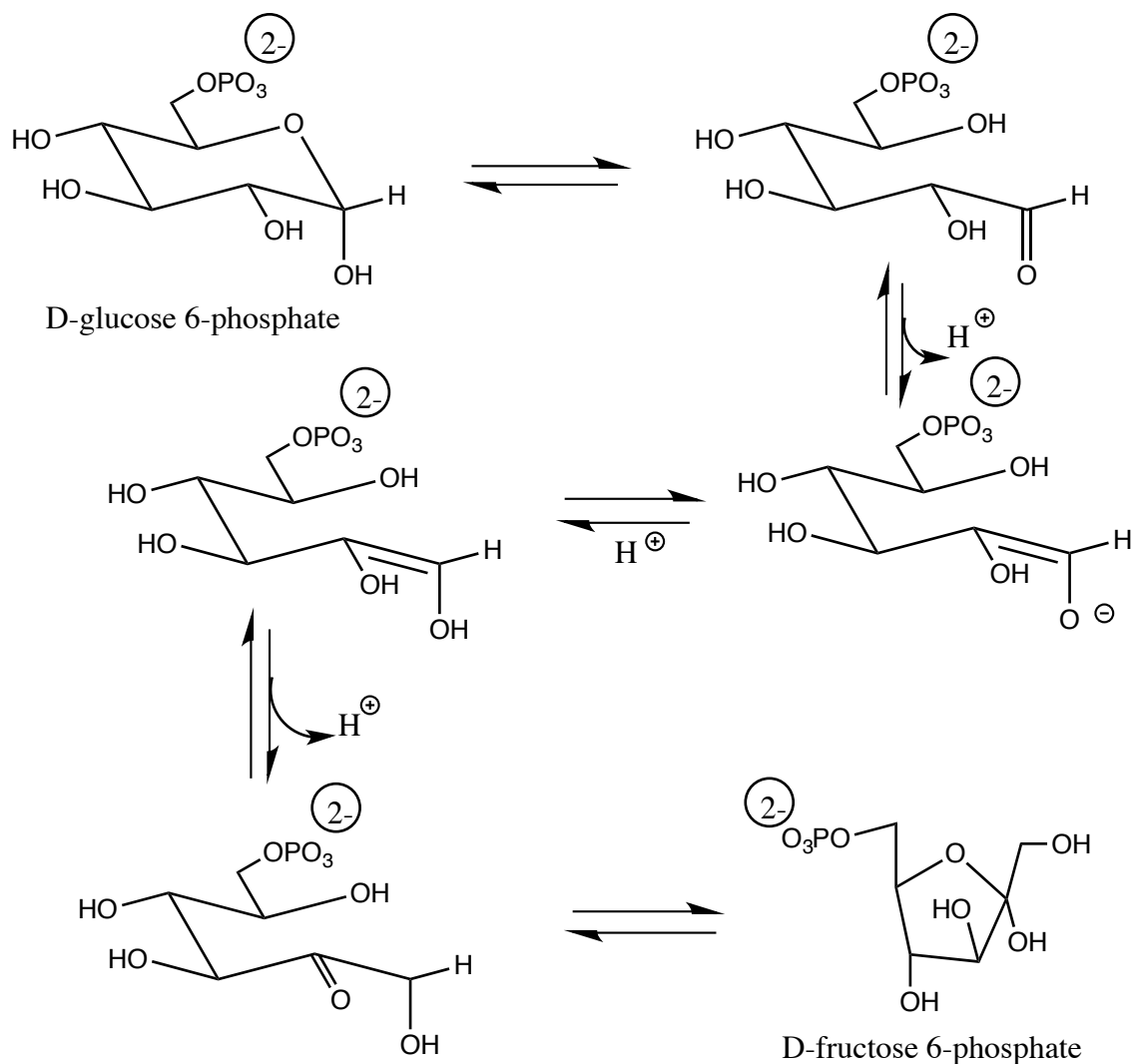


Figure XXX.5

The epimerization of the D-glucose 6-phosphate (in a pyranose ring system) to D-fructose 6-phosphate (in a furanose ring system) is illustrated proceeding through an ene-diol structure (as shown in the center-left of the Figure).

We can now contemplate what is known as the *aldolase reaction* in this sequence (process **D**) which is simply a retro-aldol condensation (looking in the forward direction and an aldol condensation in the reverse – Remember: catalysts speed both forward and reverse reactions; they simply allow an equilibrium to be established more quickly than without their action.) This is broken down in Figure XXX.6 illustrating the reverse of an aldol condensation, wherein an enolate anion attacks a carbonyl carbon site to cause an addition reaction. Note the carbon-carbon bond of the five-membered ring that is broken in this retro-aldol condensation (indicated by the heavy line) and carbon atoms involved as the carbonyl carbon of the aldol condensation (*) and the enolate carbon attacking site (#).

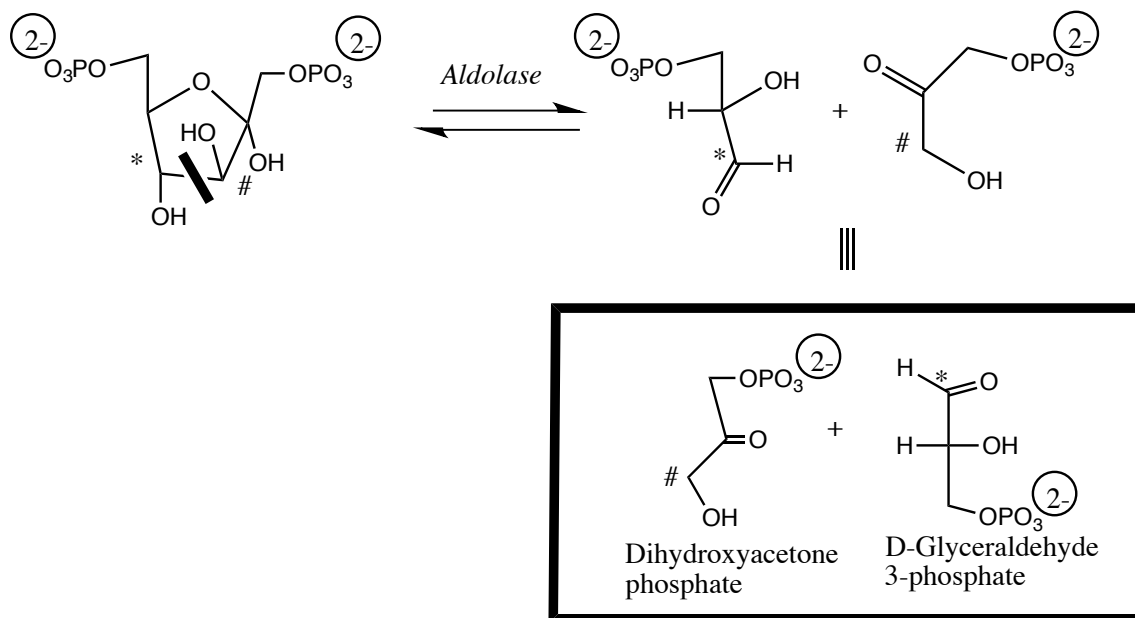


Figure XXX.6

The splitting of the original six-carbon unit (after several preparative reactions) into two three-carbon components is shown here. Initial carbons and their ultimate fates are shown by # and * symbols.

The involvement of the aldolase in this retro-aldol reaction occurs with the opening of the hemiketal (which can occur at all pH) and its reaction with an amino group of a lysine residue of the aldolase to form an imine. It is the protonated imine in which the carbon-carbon bond breaks yielding the D-glyceraldehyde 3-phosphate and the charge stabilized protonated form of the imine of the enolate anion of the dihydroxyacetone phosphate. This is illustrated in Figure XXX.7.

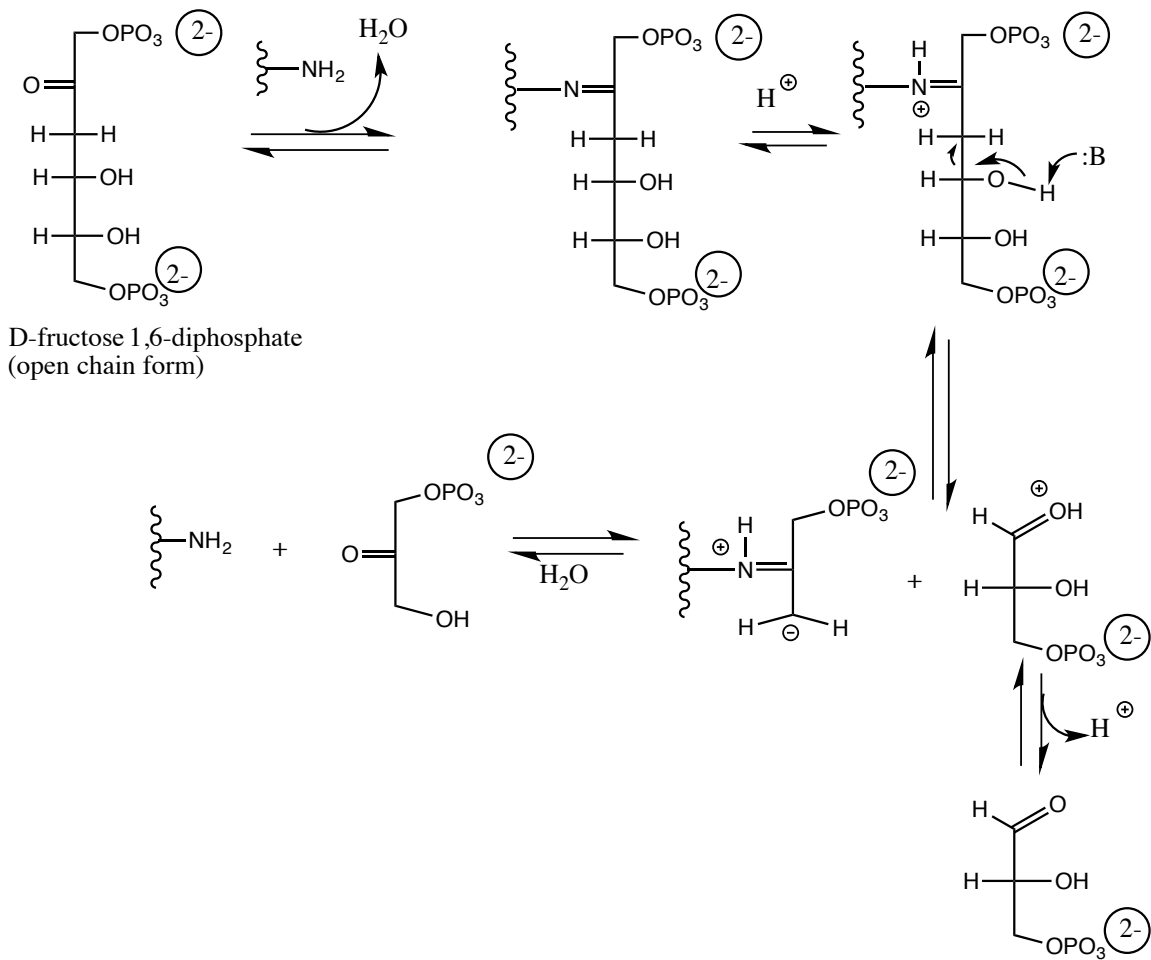


Figure XXX.7

Here we see details of the process of splitting the six-carbon components into two three-carbon components.

The reaction involving *Triose phosphate isomerase* (process **E**) is simply another ketose/aldose isomerization proceeding through an enediol intermediate as we previously saw with process **B**. This reaction produces the second unit of D-glyceraldehyde 3-phosphate from the original glucose ring system, as illustrated in Figure XXX.8.

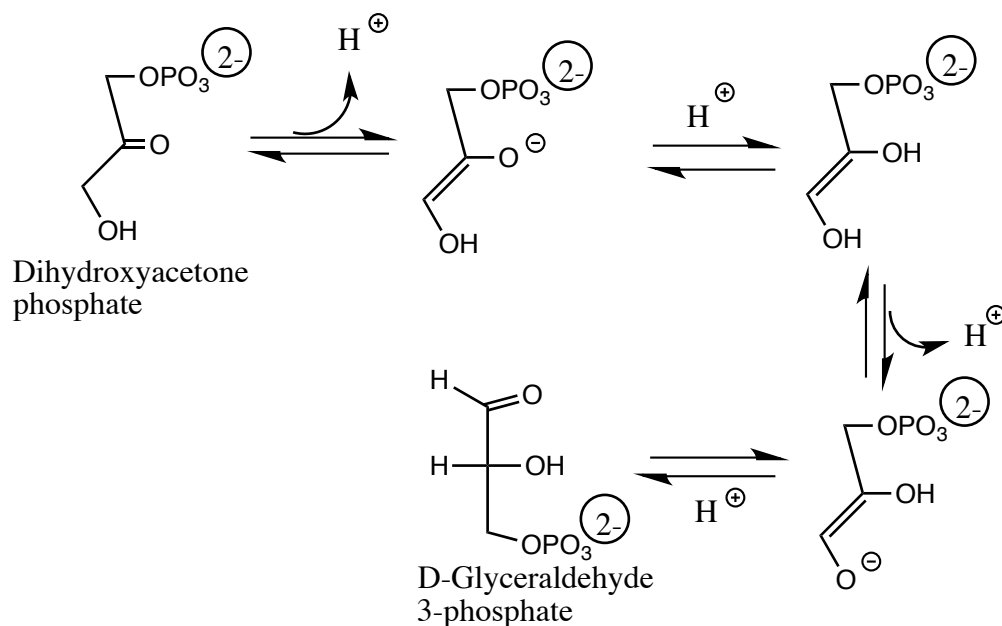


Figure XXX.8

Here we again see an epimerization process, the dihydroxyacetone phosphate being converted into a second unit of D-glyceraldehyde 3-phosphate.

The reaction process **F** is a combination of redox (the NAD is reduced to NADH, picking up the hydrogen from the aldehyde carbon of the D-glyceraldehyde 3-phosphate) and phosphorylation to form a mixed anhydride (involving both phosphoric and a carboxylic acid). While the hydrogen is removed from the aldehyde carbon, the phosphate oxygen adds to that carbon generating the mixed anhydride. The overall reaction occurs in a stepwise manner, the aldehyde carbon initially undergoing an addition reaction with the thiol functionality of a cysteine residue of the enzyme. The NAD acts as an oxidizing agent (itself being reduced to NADH) on the modified thio-acetal, yielding an activated thio-ester of a carboxylic acid. This activated thio-ester is thus susceptible to a displacement reaction (addition-elimination) by phosphate yielding the mixed anhydride, as shown in Figure XXX.9.

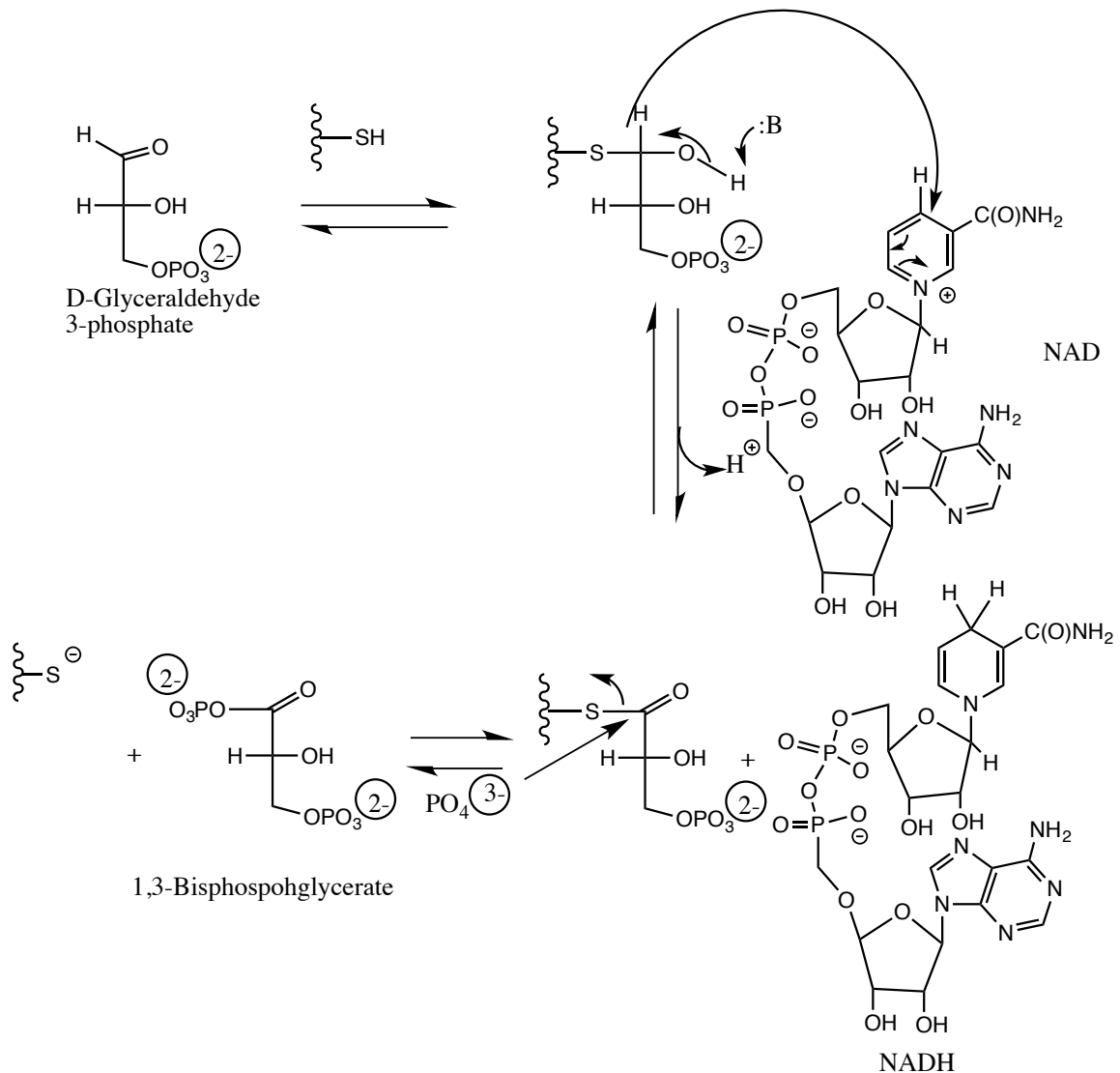


Figure XXX.9

This oxidation and subsequent phosphorylation generates a new mixed anhydride. It is a complex process. The D-glyceraldehyde 3-phosphate (each of the two such molecules generated previously) interacts with a cysteine residue of the enzyme to form an intermediate that is oxidized using the NAD (shown to the right side of the Figure). Arrows indicate the electrons involved in transfer of the hydrogen from the original aldehyde site of the D-glyceraldehyde 3-phosphate molecule to the pyridinium ring of the NAD. The product of that, in addition to the oxidized molecule that is then attacked by phosphate, is the reduced material NADH with *two* hydrogens attached to one carbon of the pyridine-like ring of NADH.

There remain two steps for the review of the complete glycolysis process. The first of these, process **H**, is a transesterification intramolecularly. The phosphate ester linkage is transferred from the 3-position hydroxyl oxygen to the adjacent 2-position hydroxyl oxygen. Such migrations of phosphate functionalities among adjacent hydroxyl groups are common in phospholipid systems where they can occur quite rapidly. With the involvement of the enzyme phosphoglycerolmutase the process occurs even more

rapidly. This may be shown simply as a positionally-enhanced attack of a hydroxyl group on phosphorus to yield a new ester with a previously esterified hydroxyl group now freed, as shown in Figure XXX.10.

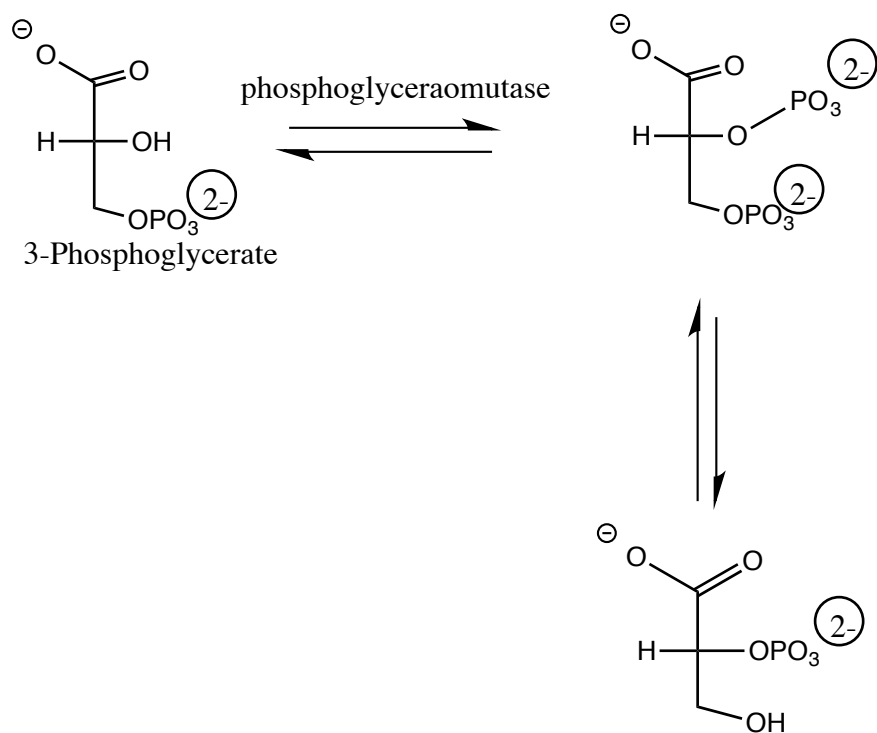


Figure XXX.10

The “movement” of the phosphate linkage from the terminal site to the internal site actually proceeds through the diphosphate, as shown. This is a highly reactive species and does not survive. The phosphate unit that becomes ultimately attached to the internal position *is not* the same phosphate unit that was initially attached to the terminal position. Actually, the 3-phosphoglycerate initially reacts with a phosphorylated histidine site of the enzyme, transferring the phosphate to the 2-position oxygen of the substrate. After an allosteric shift of the enzyme, the phosphate at the 3-position oxygen of the diphosphate intermediate is transferred back to the original histidine site of the enzyme, leaving the 2-phosphoglycerate product.

Finally, we need to consider the conversion of 2-phosphoglycerate to phosphoenol pyruvate, process **I** of glycolysis. This overall is a dehydration process. This process is unusual *not* in the sense that it is a dehydration, but rather that it is a process that leads from a simple ester to a highly reactive species, a type of mixed anhydride. One may not initially think of phosphoenolpyruvate as an anhydride, but one must contemplate the species that are connected: phosphoric acid, and an enol, enols being significantly more acidic than ordinary alcohols (remember their relationship to phenols, which are significantly more acidic than ordinary alcohols, and the appearance of enolic hydrogens in ^1H NMR spectra far downfield, indicating their deshielded character and

relative acidity.) So, phosphoenol pyruvate is really an anhydride (the elements of water missing from between two acid components).

Enolase is a metalloenzyme that uses two bound magnesium ions to hold the carboxylate ion site of the 2-phosphoglycerate in position while the free amino group of a lysine residue attaches to and removes the 2-position hydrogen yielding an electron-deficient site on the changing 2-phosphoglycerate in an E1cb-type mechanism while the carboxylic acid site of a glutamic acid residue associates with the 3-position hydroxyl and removes it to complete the dehydration. This is illustrated in Figure XXX.11.

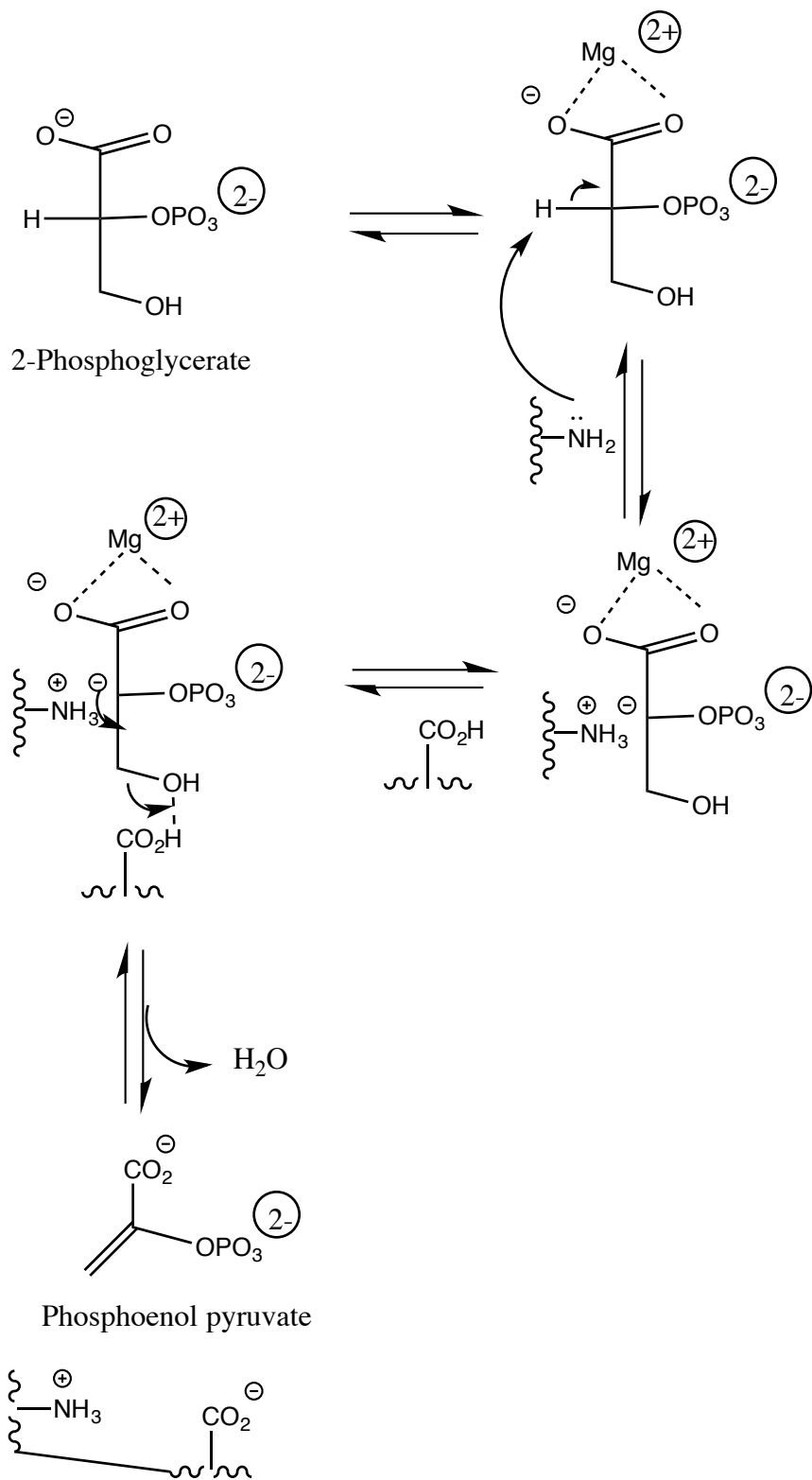


Figure XXX.11

Note that the magnesium site (there are actually two of them involved in holding the 2-phosphoglycerate to the enzyme) is also connected to both of the other sites of the enzyme involved, *i.e.* the lysine residue (source of the amino site picking up the proton

from the 2-position) as well as the glutamic acid residue (picking the hydroxyl from the 3-position).

The only considerations remaining in the glycolysis pathway are stereochemical. In process **E**, that involving *triose phosphate isomerase* in the conversion of dihydroxyacetone phosphate to D-glyceraldehyde 3-phosphate, the original ketonic carbon site which is transformed into a hydroxyl site is so transformed stereospecifically yielding the a stereogenic site of *S* absolute configuration. The intermediate ene-diol picks up the hydrogen at the 2-position carbon *only* from the *si* face of the trigonal planar 2-position carbon site (last step in Figure XXX.8). With this stereochemical route, the product is only the D-glyceraldehyde 3-phosphate.

There is one other stereochemical note worthy to be mentioned here, although it may seem that it has nothing to do with the actual glycolysis products or intermediates. This has to do with the production of NADH in process **F**. The structures of NAD (also known as NAD⁺) and NADH are shown again in Figure XXX.12.

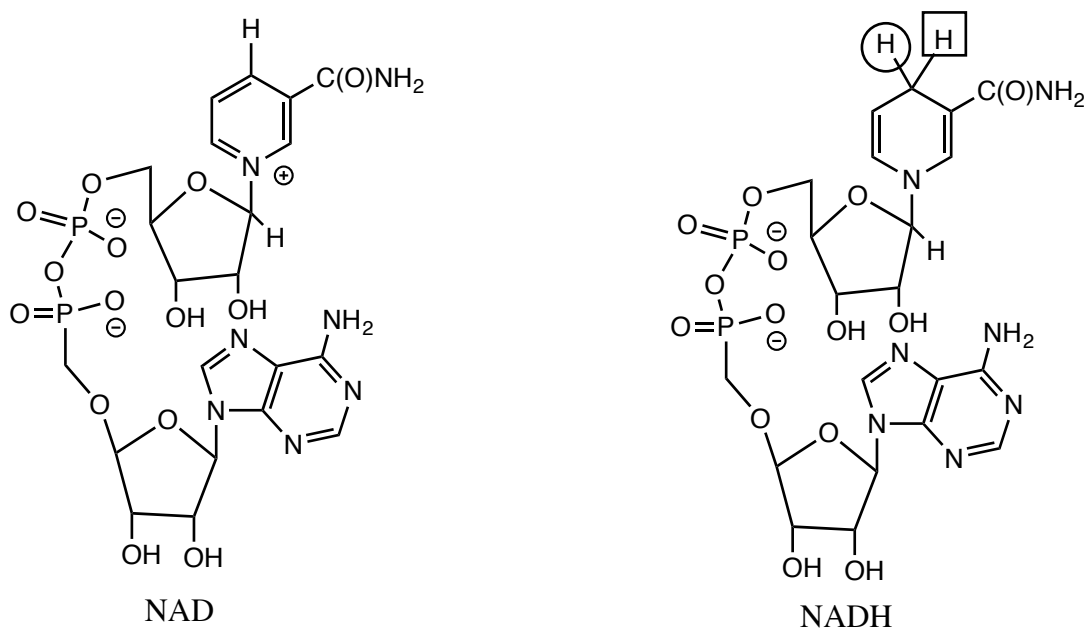


Figure XXX.12

Structures of NAD and NADH. The NAD (shown on the left) is the oxidized form (which can serve as an oxidizing agent) with the reduced form (NADH) that can serve as a reducing agent is shown on the right.

Note the two hydrogens in NADH that are attached to the pyridine ring system. One is marked by an enclosing circle while the other is marked by an enclosing square. These two hydrogens are *not* equivalent. They are diastereotopic, and as such are distinguishable quite easily even by reagents that are only prochiral with which they might interact. (The hydrogen to the rear, enclosed in the square, is noted as being Pro *S*, while that enclosed in the circle is noted as being Pro *R*.) The specifics of stereochemistry involved are dependent on the enzyme involved (these are involved in numerous

biological redox reactions). However, for a particular enzyme process, only one is ever involved; the other remains firmly attached to the NADH or NAD species. For example, in the process **F** under consideration here, only one site is occupied by the hydrogen picked up from the intermediate (see Figure XXX.9) thioacetal. For the reverse process with the same enzyme (glyceraldehyde 3-phosphate dehydrogenase) it is *this same hydrogen site only that is transferred to the 1,3-bisphosphoglycerate generating the D-glyceraldehyde 3-phosphate*.

C. Fatty Acid Metabolism

The breakdown of fats in cells is a critical process providing required energy for the operation of living cells. Such fats constitute an efficient storage medium for energy, which is obtained when the fats are degraded within the cell. Fats are both anhydrous (not associating with water) and a highly reduced form of organic molecule (as opposed to glucose within which the carbon atoms are in a more highly oxidized condition) allowing a greater amount of energy to be gained from their degradation.

The general structure of natural fats, along with the structure of a specific example of a natural fat, are shown in Figure XXX.13.

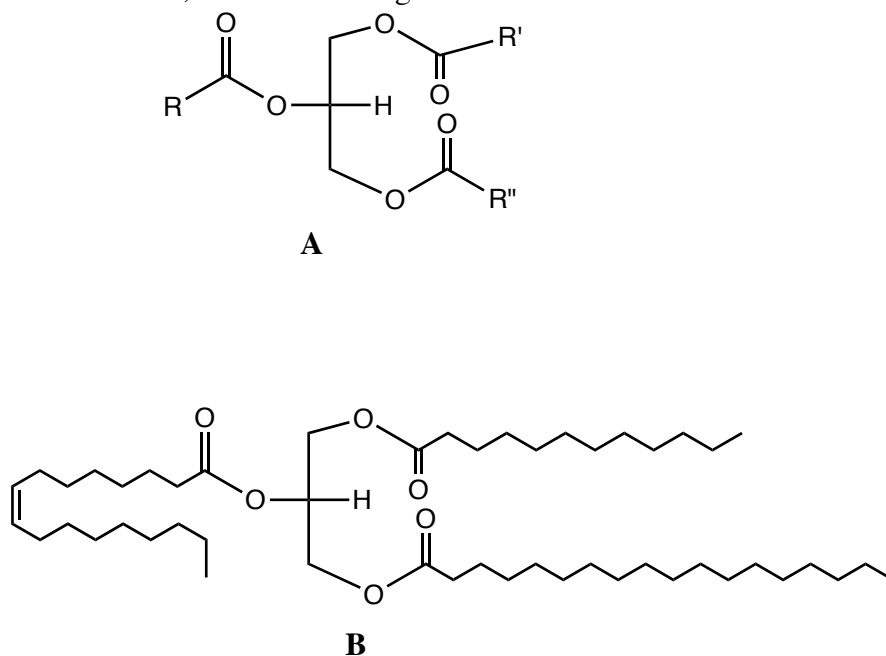


Figure XXX.13

The general structure of a natural neutral storage fat (**A**) is shown at the top, the structure being a tri-acylated form of glycerol. Each of the groups R, R', and R'' are hydrocarbon groups, possibly containing one or more alkene linkages (always *cis*). A specific fat is shown as (**B**) wherein the acyl groups are (from top to bottom) dodecanoyl (lauroyl), *cis*- Δ^9 -octenoyl (oleoyl), and octadecanoyl (stearoyl).-

The beginning of the degradation of fats involves the enzyme catalyzed hydrolysis of the fat to generate along with the glycerol, the three fatty acids, as shown overall in Figure XXX.14. The enzymes facilitating such hydrolyses are known as

lipases. There exist in cells a variety of lipases, and it is not in our current interest to consider in detail their nature. Rather we will focus on the degradation of the resultant fatty acids once the fat has been hydrolyzed.

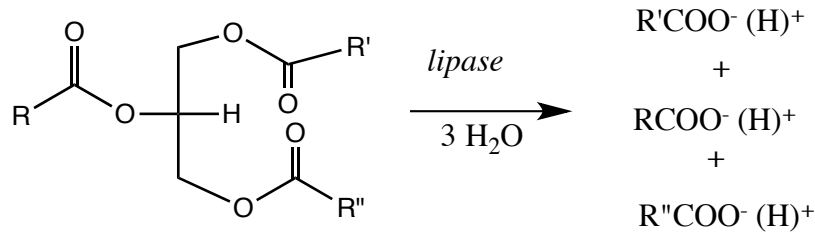


Figure XXX.14

Overall lipase facilitated breakdown of a fat (a triacylglycerol molecule) in the acyl components with glycerol as a side-product.

A critical factor in the degradation of fatty acids is *coenzyme A*. (Note – a *coenzyme* is a non-peptide compound that is necessary for the operation of an enzyme.) The specific coenzyme known as coenzyme A (the *A* refers to *acetylation*) has the structure shown in Figure XXX.15

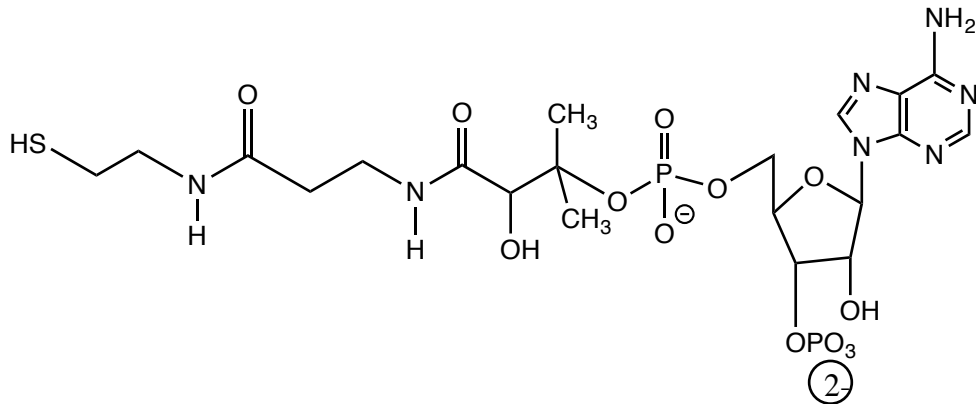


Figure XXX.15

Structure of Coenzyme A. Note the critical sylvhydriyl group at the terminus that is involved in reaction with the fatty acids in their degradation. This sulfhydryl group ultimately forms an activated thioester

The ATP is involved initially in this process by reaction with a fatty acid unit (we'll use dodecanoate in our consideration here, although it is completely analogous for the other fatty acids) to form pyrophosphate with the dodecanoate in our consideration here, although it is completely analogous for the other fatty acids) to form pyrophosphate along with the *acyl-CoA*. Note that there are *two* phosphorus anhydride units in ATP, the reaction here involving a displacement of pyrophosphate from the partial phosphorus ester/anhydride site most closely attached to the ribose ting system, as shown in Figure XXX.16.

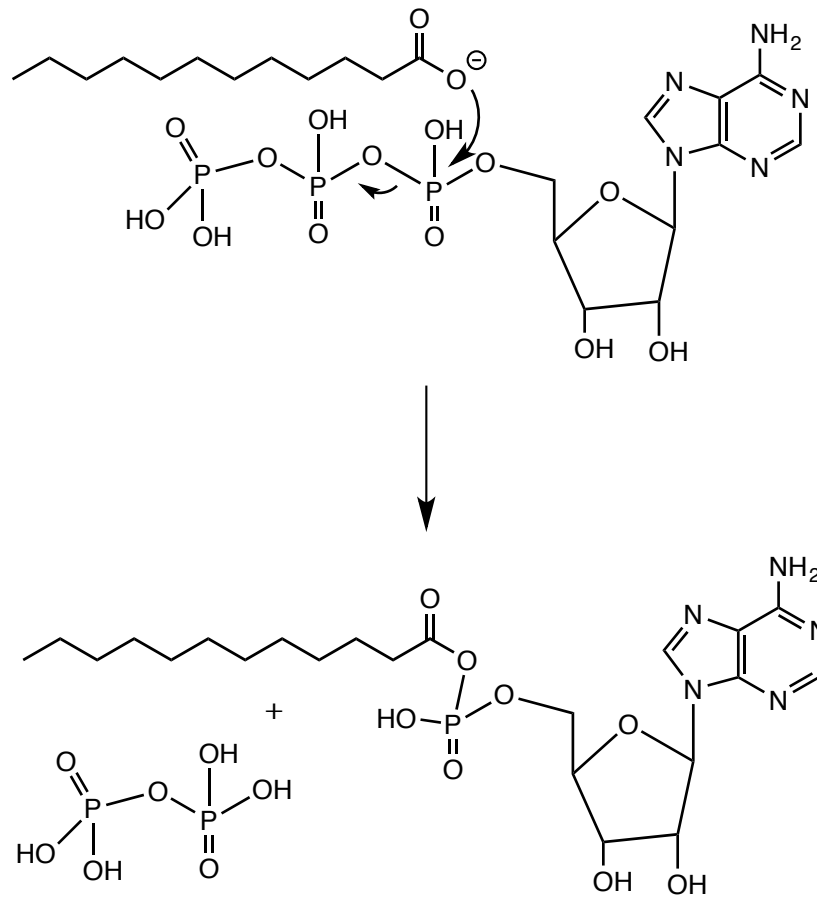


Figure XXX.16

Formation of the acyl adenylate by reaction of the fatty acid with ATP releasing pyrophosphate. This reaction, and the subsequent reaction of the resultant acyl adenylate (a mixed anhydride of a carboxylic and a phosphoric acid) are catalyzed by *acyl CoA synthetase*. Note that the attack of the carboxylate here is *not* at a classical phosphorus anhydride site (as we have seen earlier), but rather is at the phosphorus involved in both an ester linkage and an anhydride linkage.

The sulfhydryl group of the Coenzyme A now reacts with the mixed anhydride linkage of the acyl adenylate *on the carboxylate side of the mixed anhydride* to generate the thioester acyl CoA, as shown in Figure XXX.17.

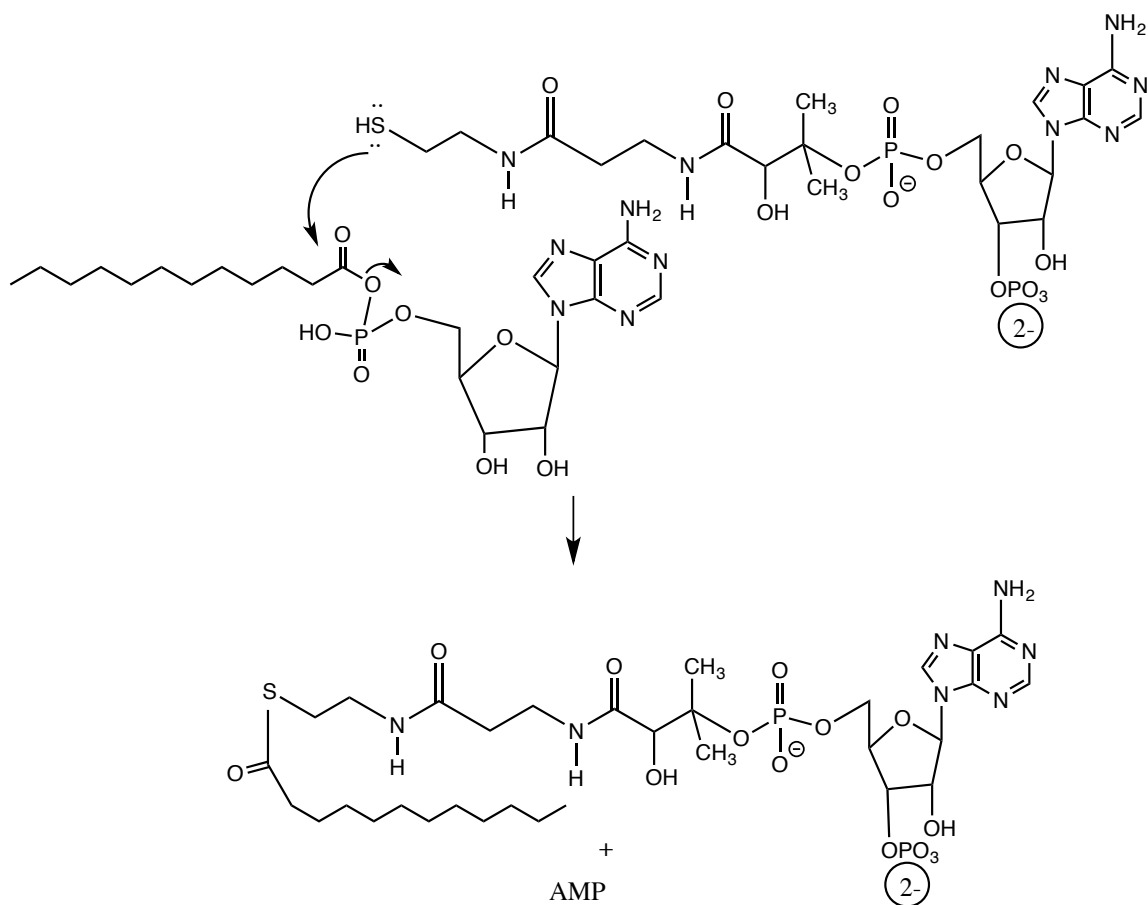


Figure XXX.17

Formation of *acyl Co A*. The sulfhydryl group reacts with the mixed anhydride linkage (carboxylate/phosphate) of the acyl adenylate to generate a new thio-ester linkage as found in the *acyl Co A*, along with AMP as a by-product.

The chain shortening of the fatty acid now can begin with the oxidation of the fatty-acyl function at the sites α, β relative to the carboxylate carbon site. This oxidation (dehydrogenation) occurs through interaction with flavin adenine dinucleotide (see Figure XXX.18) which picks up two hydrogens (formally protons) along with a pair of electrons from the α, β site of *acyl Co A*. The reactive site of the flavin adenine dinucleotide (FAD) is the isoalloxazine ring system rendering each of the “side” rings aromatic in the product (FADH₂). Thus we have a driving force for the proceeding of this reaction. This is illustrated in Figure XXX.18.

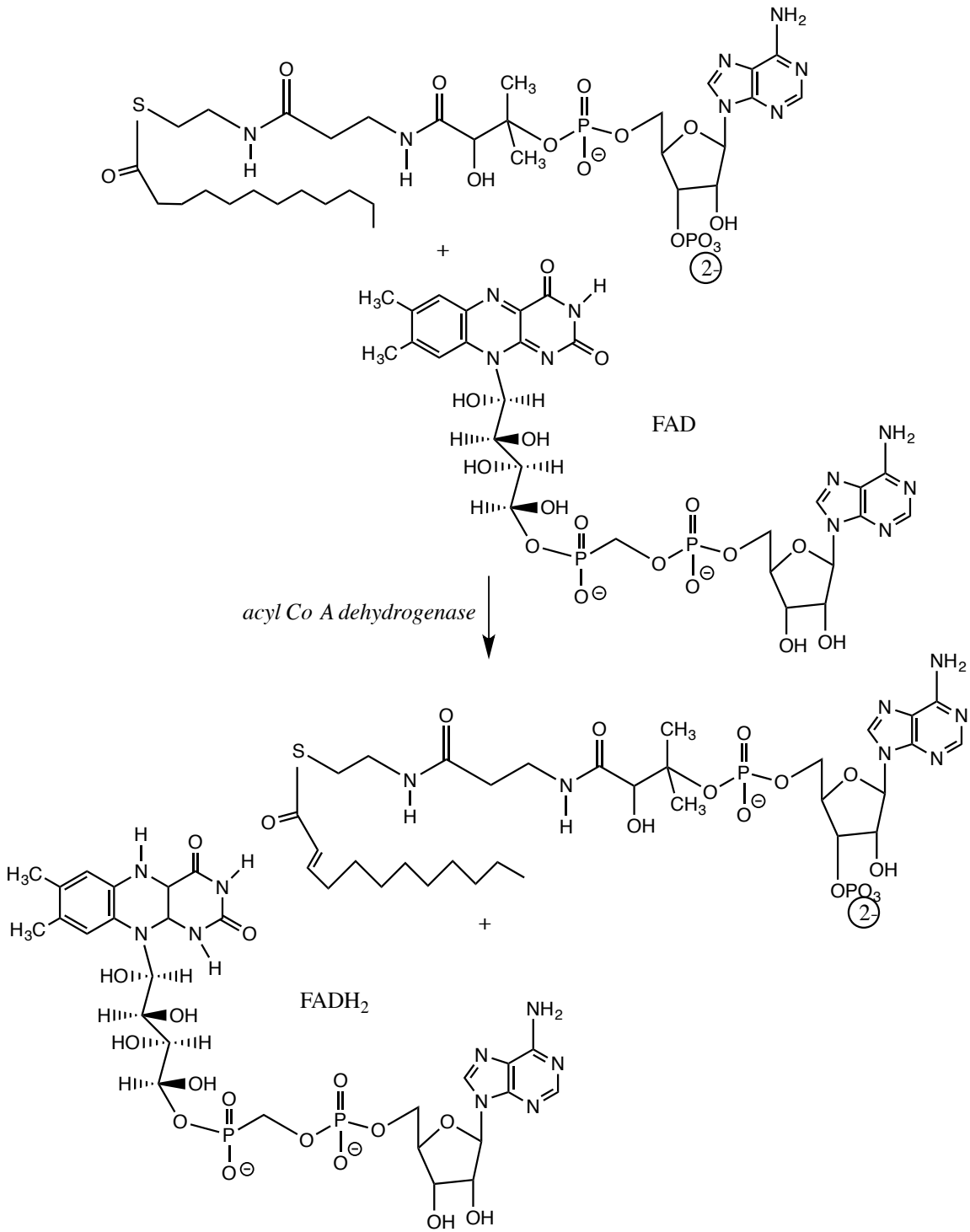


Figure XXX.18

XXX1

Dehydrogenation of an acyl Co A species involving flavin adenine dinucleotide and acyl CoA dehydrogenase. It should be noted that the acyl Co A product that is generated now

has an alkene linkage conjugated with the carboxylate C=O linkage, and that the alkene linkage is *trans*.

The new *unsaturated* acyl Co A species is now capable of undergoing *hydration* of the alkene linkage, stereospecifically and regiospecifically. Regiospecifically, the hydroxyl group is placed on the 3-position of the acyl group (with a hydrogen placed on the 2-position site) with the 3-position specifically having the *S* absolute configuration. This reaction is facilitated by the enzyme enoyl Co A hydratase, and is illustrated in Figure XXX.19.

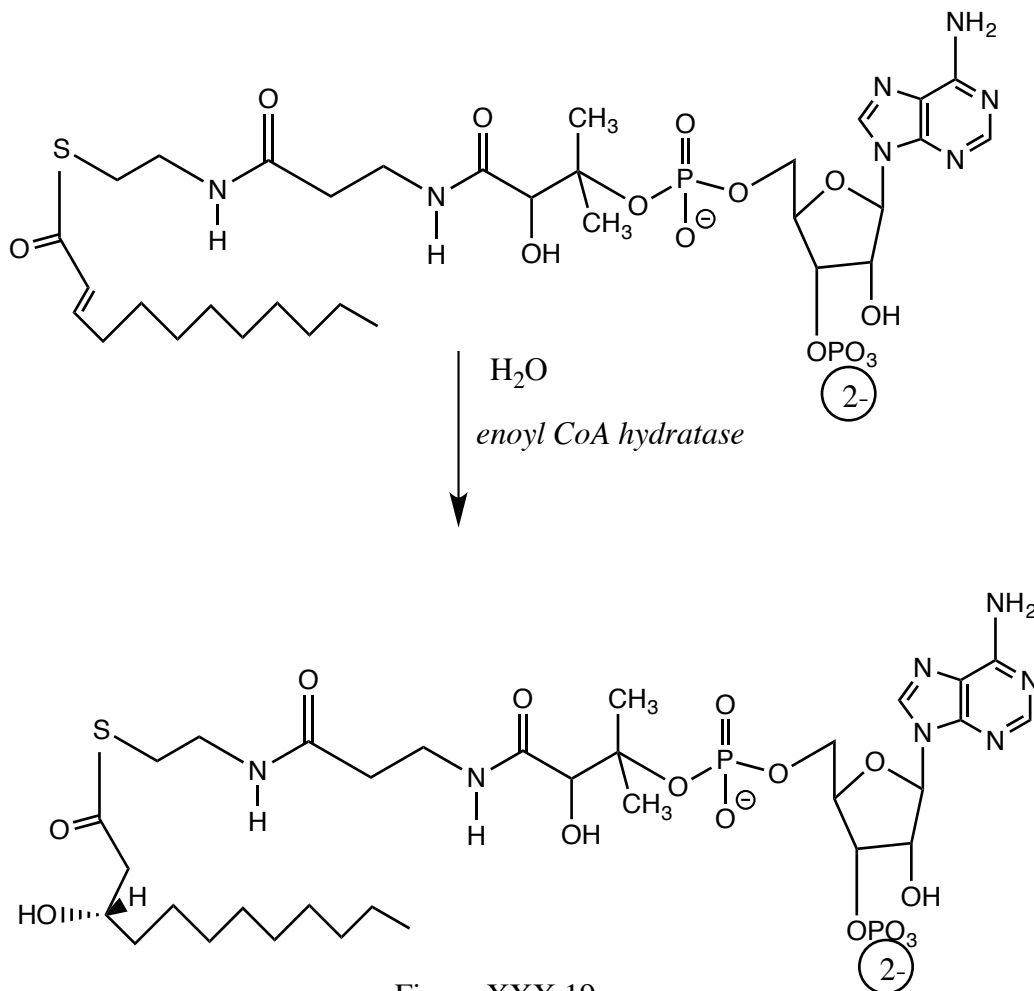


Figure XXX.19

The product of the reaction, the hydrated acyl CoA, has the *S* absolute configuration at the hydroxyl-bearing carbon (position 3 of the acyl chain). This configuration is often referred to as an *L* configuration using the older notation system derived from carbohydrate chemistry.

The stereospecificity of the hydration is lost in the following step in which the secondary alcohol is oxidized to a carbonyl linkage (generating a β -keto-ester) in a reaction facilitated by the enzyme commonly known as *L*-3-hydroxyacyl CoA dehydrogenase for which the actual oxidizing agent is NAD, as shown in Figure XXX.20.

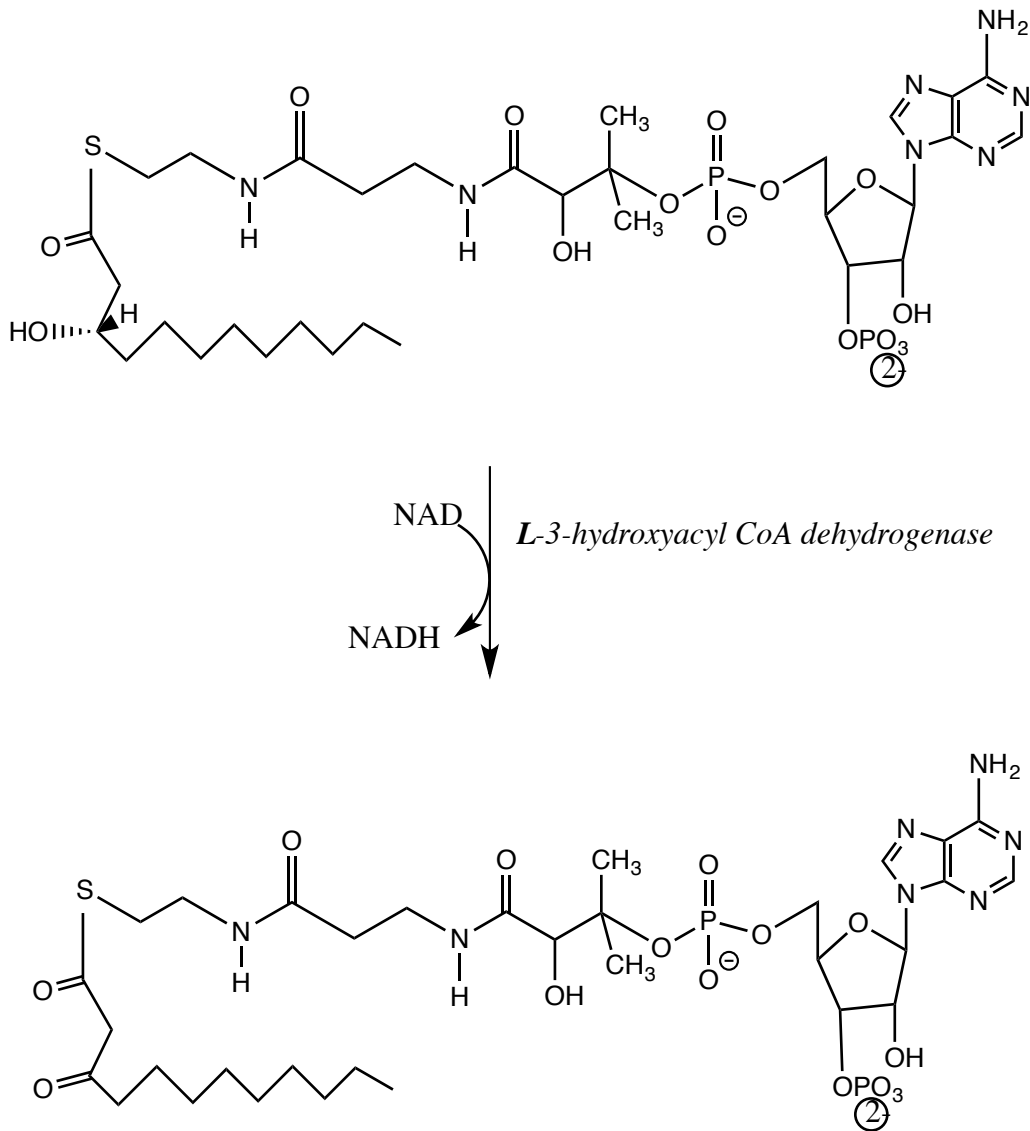


Figure XXX.20

Oxidation of the β -hydroxy thio-ester to a β -keto thio-ester. The intervening steps (dehydrogenation, hydration, followed by oxidation) have been preparing the acyl Co species for shortening of the chain length.

Once the system has been oxidized to the β -keto-thioester, it can be attacked at the thio-ester site by a molecule of CoA (facilitated by the enzyme β ketothiolase) which generates acetyl Co A, and, most importantly, a fatty acyl-Co A that *has been shortened from the original fatty acid molecule by two carbon atoms*. Thus the fatty acid units can be continually decreased in length by two carbon atoms through each cycle of the process, ultimately being degraded to acetate. This is illustrated in Figure XXX.21.

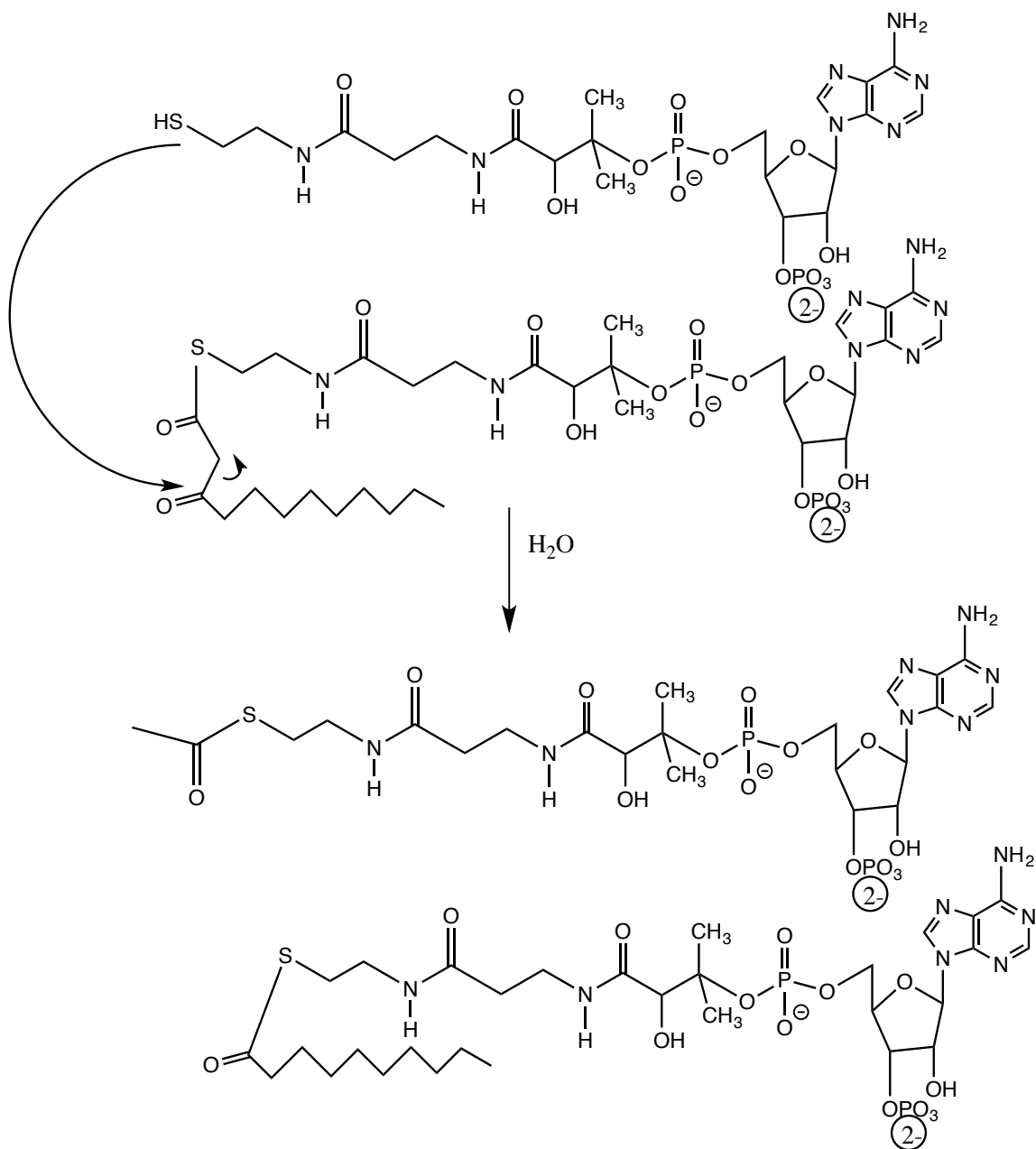


Figure XXX.21

Chain shortening of the fatty acyl chain resulting in the generation of acetyl Co A (top structure in products) and an acyl Co A species shortened by two carbon atoms from the original fatty acyl function.(see Figure XXX.17). As the now shortened fatty acyl CoA molecule is recycled through the steps of Figures XXX.17 through XXX.20, each fatty acyl function is successively shortened by two carbon atoms in each cycle until all is degraded to acetyl Co A. The acetyl Co A thus produced is converted into a number of species of use in the operation of cells.