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1973
ADDISON-WESLEY PUBLISHING COMPANY, INC.
ADVANCED BOOK PROGRAM
Reading, Massachusetts

London • Amsterdam • Don Mills, Ontario • Sydney • Tokyo

PREFACE

This book is not written for the expert in the field, but for the person who wishes to obtain a grasp of the basic principles and reactions underlying the conversion of foodstuffs into energy. It is the outgrowth of a series of lectures given to first-year medical students in which an attempt was made to put together a coherent story of the various aspects of energy metabolism. Though these reactions are dealt with in nearly all textbooks of biochemistry, they are usually so dispersed throughout the book that the student loses the thread of the total story. In this volume the basic reactions involved in biological oxidations at the substrate level are given first. For the sake of simplicity in presentation, these reactions are divided into three categories: dehydrogenations, decarboxylations and "make-ready." The latter term is applied to all reactions in which alterations in a metabolite occur that lead to a product that can undergo dehydrogenation and/or decarboxylation. The reactions involved in the conversion of glucose and fatty acid to CO_2, H^+, and electrons are presented from this viewpoint, with the acceptance of the electrons and some of the H^+ by those coenzymes that are termed primary electron acceptors. The passage of electrons from these primary electron acceptors to oxygen over the electron transmitter system and the generation of high-energy phosphate then follows.

A brief chapter is devoted to the localization within the cell of the reactions involved in biological oxidations, and to some of the problems posed by membrane barriers. A chapter entitled "Energy Storage" deals with the reactions involved in the conversion of glucose to glycogen and fatty acids. In this chapter the pathways for the formation of TPNH used in fatty acid synthesis are presented. A final chapter deals with energy mobilization and glucogenesis. In the reactions involved in the breakdown of glycogen and fat to supply the energy needs of the body, the role of hormones and cyclic AMP in these mobilization processes is stressed.

CONTENTS

CHAPTER 1

Biological Oxidations and the Basic Pattern of the Process

The energy demands of the living organism are met mainly by the combustion of glucose and fatty acids. The equations that depict the overall reactions of these processes are

	R.Q.	Kcal/g
$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$	1.0	4.1
$C_{16}H_{32}O_6 + 23O_2 \longrightarrow 16CO_2 + 16H_2O$	0.7	9.5

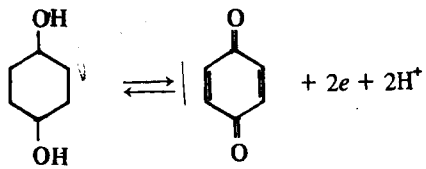
where palmitic acid is used as a representative of fatty acids. In each of these reactions energy is released, energy which originally had its source in the sun and which was trapped in these foodstuffs as chemical energy by the photosynthetic reactions of plants. The energy released on the combustion of these substances is expressed above in terms of kilocalories per gram generated as heat. Though these values are used in expressing the energy needs of the human body for dietary purposes, they are not exact from the standpoint of the energy yield to the animal. This is because the living cell is not a heat engine and can make use only of the free energy released in these reactions. However, the heat production values are close approximations of the free energy release and serve to indicate that fatty acids, per unit weight, are a far better source of energy than glucose. The ratio of CO_2 produced to the oxygen consumed in these two reactions, the respiratory quotient (R.Q.), differs. This fact permits one by measurement of the O_2 consumption and CO_2 production of the living organism to estimate the relative amounts of the two substances that are undergoing oxidation and hence the calories or energy consumption under any given metabolic state.

The overall equations given above for the oxidation of glucose and palmitic acid might be interpreted to indicate that oxygen reacts directly with these substances to yield CO_2 and H_2O. In the living cell this is not the case. In essence the process involves the transfer of electrons from the foodstuff to

oxygen in a stepwise fashion over a chain of compounds. Each participant in this chain constitutes an oxidation–reduction system; it is oxidized by the removal of electrons and is reduced when it accepts electrons. The simplest form of such a system is that represented by the interconversion of ferrous and ferric ions:

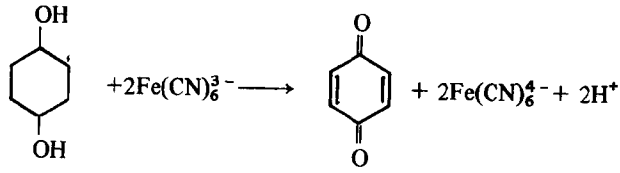
$$Fe^{++}(X) \rightleftharpoons Fe^{3+}(X) + e$$

Here (X) represents a substance with which the iron may be chelated, and e represents an electron which must be accepted or donated by another substance for the reaction to proceed. Since one electron is involved, it is termed a one-electron system. A second and more common type of oxidation–reduction reaction in biological processes is that represented by the oxidation of hydroquinone to quinone:



In this case two electrons and two hydrogen ions are removed during the oxidation, and the system is termed a two-electron system. Each oxidation–reduction system is characterized by an oxidation–reduction potential which is expressed as the voltage difference at a fixed temperature between a solution containing equal amounts of the oxidant and reductant of the system and some agreed-upon standard oxidation–reduction system. The pH of the solution must be specified when H^+ is involved in the reaction, as, for example, the hydroquinone–quinone system, since the potential of such systems will vary with pH. In the case of $Fe^{++} - Fe^{3+}$ systems, which play an important role in biological oxidation, it should be noted that the potential of the system will vary markedly depending on the nature of (X).

The extent and direction of interaction between two oxidation–reduction systems can be predicted if their potentials are known. For example, let us suppose that we are dealing with the ferri–ferrocyanide system ($X = CN^-$). At pH 7.0 and 30°C the potential of this system is +0.425 volt as measured against the standard system, H_2 (1 atm) : H^+ (1.0 normal). The hydroquinone–quinone system under the same condition yields a potential of +0.275 volt. We can write then the following equation:

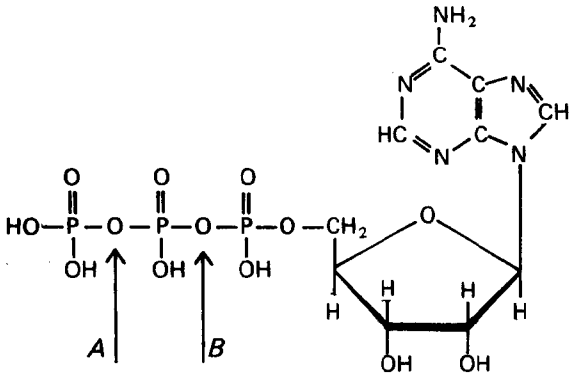


Since the potential of the ferro–ferricyanide system is positive to the hydroquinone–quinone system, we can predict that ferricyanide will remove electrons from hydroquinone and the reaction will proceed in the direction of the arrow. If 1 mole of hydroquinone is mixed with 2 moles of ferricyanide, we can also predict that the potential of the resulting mixture will be halfway between that of the two systems reacting, or +0.350 volt and that over 99% of the two starting materials will have undergone an exchange of electrons.

In examining the series of oxidation–reduction reactions that occur in biological oxidation, it is convenient for the purposes of our presentation to classify them as shown in Fig. 1-1. The first step is the removal of electrons and hydrogen ions from the metabolite and the acceptance of the electrons by what will be termed a primary electron acceptor. As shown in the diagram, it is at this step that all the CO_2 produced in biological oxidations is formed. A small amount of the total energy to be released in the overall pathway may be converted into high-energy phosphate ($\sim$P) at this step. This process will be referred to as phosphorylation at the substrate level. The second step in this presentation will be the passage of electrons from the primary electron acceptor into the chain of oxidation–reduction systems constituting the electron transmitter system (E.T.S.) The hydrogen ions removed simultaneously from the substrate with the electrons do not follow hand in hand with the passage of electrons over the E.T.S. to oxygen. In some cases for each electron transferred a hydrogen ion is simultaneously taken up, in others only one hydrogen ion for each two electrons, and in others none. An attempt is made to represent this fact in the diagram by the dotted lines. The bulk of the high-energy phosphate formed in the overall process is produced in step II. In this process, referred to as oxidative phosphorylation, energy equivalent to 8000 to 9000 calories and provided by an oxidation–reduction reaction is captured by the conversion of adenosine diphosphate (ADP) and inorganic phosphate (P_i) to adenosine triphosphate (ATP):

$$ADP + P_i \longrightarrow ATP$$

Adenosine triphosphate has the following structure:



Adenosine Triphosphate (ATP)
Hydrolysis at *A* or *B* yields 8000 to 9000 calories per mole (high-energy phosphate bond). Hydrolysis at *A* produces inorganic phosphate (P_i) and adenosine diphosphate (ADP). Hydrolysis at *B* yields pyrophosphate (PP_i) and adenosine monophosphate (AMP) (adenylic acid).

It is to be noted that all the oxygen consumed is reduced to water. At rest a human being of average size consumes approximatley 250 ml of oxygen per minute. This amount of oxygen will yield about 25 ml of H_2O per hour, or 600 ml day. Not all this water is net gain, since, as will be seen later, some water is consumed by the metabolic pathways involved in its production. In some species this water formed by oxidative processes plays a major role in the economy of the animal. For example, birds excrete very little water as compared to man and need a much smaller water intake to supplement the water produced by oxidative processes and that consumed with their food. Animals that inhabit the desert also rely heavily on water generated by meta bolic processes.

In later chapters there will be presented the specific reactions by which substrates (glucose, fatty acids, and their metabolites) donate electrons to a primary electron acceptor and produce CO_2. Before considering these reactions it will be helpful in understanding them to point out certain basic principles which will be encountered. For this purpose it is convenient to divide these reactions into three categories: (1) dehydrogenation, (2) decarboxylation, and (3) make-ready.

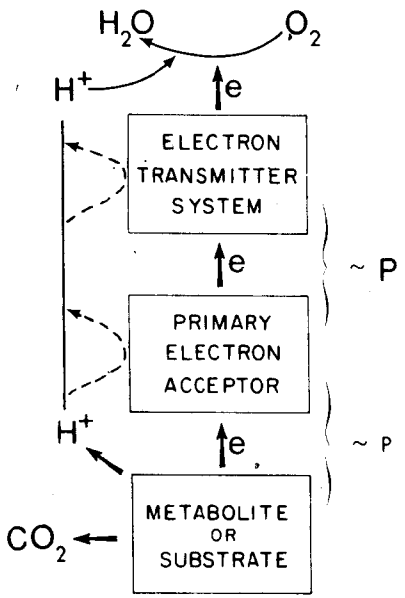


FIG. 1-1. The basic pattern of biological oxidations. The overall process depicted here is sometimes called the respiratory chain. This term and the term electron transport system are also used to describe the chain of reactions encompassing the primary electron acceptors and what herein is called the electron transmitter system. The word transmitter is employed to describe this system because its components, unlike the primary electron acceptors, are not in true solution and hence the passage of electrons through this system resembles to some extent the passage of electrons over a wire.

Dehydrogenation

All oxidations at the substrate level may be called dehydrogenations, since they involve the removal of two hydrogen atoms or, what is the equivalent, two hydrogen ions and two electrons. Two main types of chemical grouping are discernible in the dehydrogenation of substrates. First is the grouping –CHOH–; as an example we may use the dehydrogenation of lactate to form pyruvate:

$$CH_3 \cdot \underline{CHOH} \cdot COO^- \rightleftharpoons CH_3 \cdot \underline{CO} \cdot COO^- + 2H^+ + 2e$$

The reaction is written here as a half-reaction, and of course it will not proceed unless a substance, here called the primary electron acceptor, is available to accept the electrons. As we shall subsequently see, the hydrogen ions may be also passed on to this acceptor or released into the environment. An enzyme must be present to catalyze the reaction; enzymes of this type are called dehydrogenases and are named for the substrate from which hydrogen ions and electrons are removed. In the example given, this would be lactate dehydrogenase. It should be noted that the reaction is reversible and that the enzyme also catalyzes the reduction of pyruvate and thus might be also called pyruvate reductase. The second type of chemical grouping frequently encountered in biological dehydrogenations is $-CH_2 \cdot CH_2-$. As an example of this reaction we may use the conversion of succinate to fumarate:

$$COO^- \cdot \underline{CH_2 \cdot CH_2} \cdot COO^- \rightleftharpoons COO^- \cdot \underline{CH = CH} \cdot COO^- + 2H^+ + 2e$$

The main point to be emphasized is that the oxidation of all substrates always involves the removal of two hydrogen ions and two electrons.

Decarboxylation

All the CO_2 produced in biological oxidations is formed by a process called decarboxylation. This may be represented in its simplest terms as the conversion of a carboxyl group to CO_2 in the following manner, where R represents the rest of the substrate:

$$R \cdot CO \cdot COOH \longrightarrow R \cdot C\overset{O}{\underset{H}{=}} + CO_2$$

In all the reactions to be considered later this process occurs *pari passu* with a dehydrogenation reaction, and a stepwise separation of the two processes on an experimental basis is difficult. No specific enzyme other than the dehydrogenase may be needed for the decarboxylation reaction to occur, though the presence of metal ion may be essential. As will be seen later, the actual production of a free aldehyde group, $R \cdot C\overset{O}{\underset{H}{=}}$, does not take place.

Make-Ready

This term has been coined as a convenient means to designate a reaction or a series of reactions in which a substrate undergoes alteration in preparation for a dehydrogenation. In many cases such reactions are a means for producing in a substrate either a –CHOH– or a $-CH_2 \cdot CH_2-$ grouping where none existed before. The simplest example of such a reaction is the addition of H_2O. For example, the fumarate produced by the dehydrogenation of succinate in the reaction given above can be made ready for another dehydrogenation by the addition of water, namely:

$$COO^- \cdot CH = CH \cdot COO^- + H_2O \rightleftharpoons COO^- \cdot \underline{CHOH} \cdot CH_2 \cdot COO^-$$

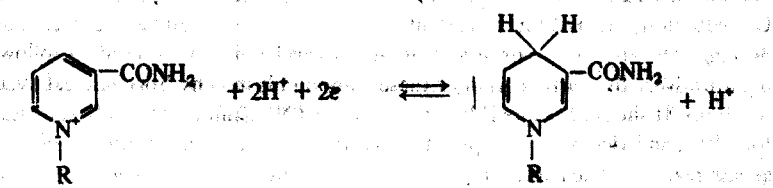
There is thus formed the grouping –CHOH–, which is susceptible to a dehydrogenation step. A specific enzyme is required for such a process, and in the case of fumarate this enzyme is called fumarase.

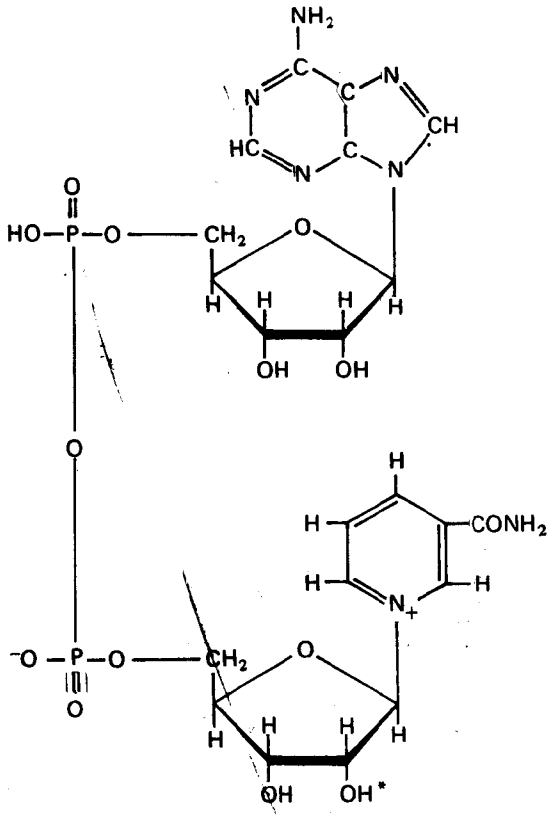
Primary Electron Acceptors

The acceptors of electrons from substrates fall mainly into two types – the so-called pyridine nucleotides and the flavin nucleotides. As a general rule, a pyridine nucleotide acts as the primary electron acceptor when the substrate grouping undergoing oxidation is –HCOH–. When the grouping undergoing dehydrogenation is $-CH_2 \cdot CH_2-$, then a flavin nucleotide is usually involved as a primary acceptor.

Two pyridine nucleotides are known which function as primary electron acceptors. Diphosphopyridine nucleotide (DPN^+), also called nicotinamide adenine dinucleotide (NAD), is usually employed as an electron acceptor when the electrons are to be subsequently handed over to the electron transmitter system for the generation of high-energy phosphate. The other, triphosphopyridine nucleotide (TPN^+), also called nicotinamide adenine dinucleotide phosphate (NADP), is largely employed when substrate electrons are to be used for synthetic purposes. An example of this use will be encountered when the conversion of glucose to fatty acids is considered.

The part of these coenzymes that functions in the acceptance of electrons is the nicotinamide portion which is derived from the vitamin nicotinic acid. The reaction may be written in the following manner:





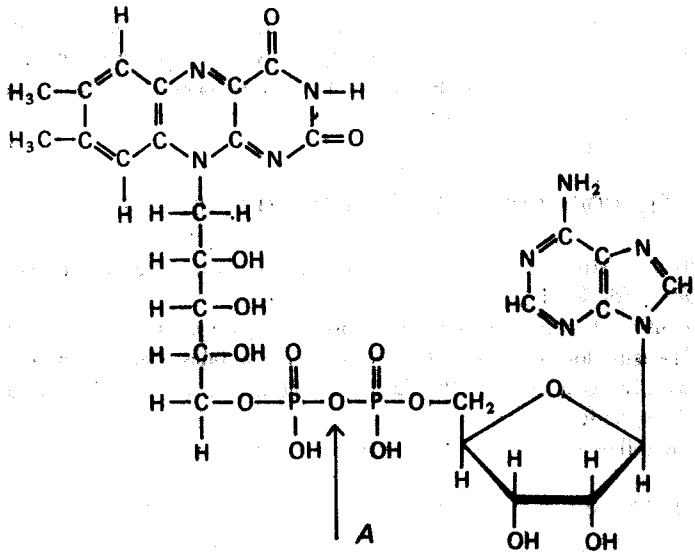
Diphosphopyridine Nucleotide (DPN^+)

Triphosphopyridine nucleotide (TPN^+) is formed when the OH^* group is replaced by $-OPO_3H^-$.

The reaction is written in this form to emphasize the fact that, though both electrons are accepted, only one of the two hydrogen ions removed from the substrate becomes attached to form the reduced pyridine nucleotide. One of the hydrogen ions is released and temporarily at least must be absorbed by the buffering capacity of the cell. Hence the reduced forms of the pyridine nucleotides are represented by the abbreviations DPNH and TPNH, or NADH and NADPH. On reduction, the pyridine nucleotides develop an absorption band centered at 340 mμ. The appearance or disappearance of this band may be used to follow quantitatively the participation of these compounds in dehydrogenase-catalyzed reactions. It should be noted that, in general, DPN^+ cannot effectively substitute for TPN^+, and vice versa, as a primary electron acceptor in a specific dehydrogenase reaction. The additional phosphate grouping on the adenylic acid portion

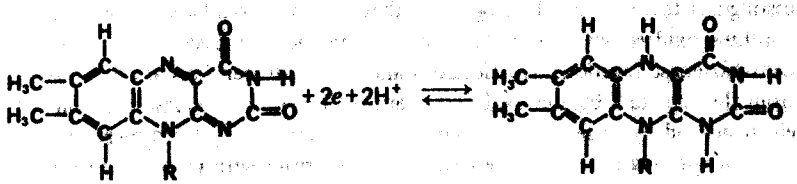
of TPN^+ would appear to be the determining factor in this marked specificity.

The other type of primary electron acceptor, the flavin nucleotides, are usually participants in a reaction when a $-CH_2-CH_2-$ grouping in the substrate is to be dehydrogenated. Two flavin nucleotides are known – flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD).



Flavin Adenine Dinucleotide (FAD)
Hydrolysis at *A* yields flavin mononucleotide (FMN) and adenylic acid (AMP).

The flavin nucleotides contain the vitamin riboflavin, and it is the isoalloxazine ring of this vitamin which acts as the electron acceptor in the following manner:



Here two electrons and two hydrogen ions are accepted by the yellow oxidant to form the reduced colorless form. As in the case of the pyridine nucleotide, the reaction therefore may be measured spectrophotometrically.

The pyridine and flavin nucleotides in most biological oxidations accept electrons and hydrogen ions directly from the substrate. An exception occurs when the substrate is an α-keto acid – for example, pyruvate or ketoglutarate. Here a sulfhydryl compound, lipoic acid, acts as intermediate between the substrate and what we have called the primary electron acceptors. This situation will be dealt with in more detail later.

The reaction between the primary electron acceptors and the substrate occurs only in the presence of the dehydrogenase that is specific for the substrate. In the case of a pyridine nucleotide there is generally no firm attachment between it and the dehydrogenase. We shall therefore write such reactions, for example, as follows:

$$CH_3CHOH{\cdot}COOH + DPN^+ \rightleftharpoons CH_3CO{\cdot}COOH + DPNH + H^+$$

it being understood that a specific dehydrogenase participates in the reaction. The DPNH (or TPNH) formed is thus capable of existing free in solution and may be utilized in conjunction with another dehydrogenase to effect a reduction. The situation is different for the flavin nucleotides. Here a firm attachment usually exists between the flavin nucleotide and the protein that catalyzes the specific reaction. We shall therefore write reactions of this type as involving a flavoprotein (FP). For example in the reaction

$$COOH{\cdot}CH_2{\cdot}CH_2{\cdot}COOH + FP \rightleftharpoons COOH{\cdot}CH = CH{\cdot}COOH + H_2FP$$

FP is succinate dehydrogenase and represents both the enzyme and the electron acceptor. Unlike DPNH, it is not possible for H_2FP to react as a reductant for a number of metabolic intermediates.

Direct oxidation of a reduced pyridine nucleotide by oxygen occurs very slowly at best and is not of importance in biological processes. Some reduced flavoproteins resemble DPNH or TPNH in that they react poorly with oxygen and are therefore to be classed as dehydrogenases – for example, succinate dehydrogenase. Other reduced flavoproteins react rapidly with oxygen, and to distinguish them from dehydrogenases they are called oxidases – for example, xanthine oxidase, glucose oxidase, and D-amino acid oxidase. However, as in a dehydrogenase reaction, two electrons and two hydrogen ions are removed from the substrate by the oxidase to form H_2FP. In the oxidase reaction the electrons and hydrogen ions are handed on directly to O_2 to form H_2O_2 instead of forming H_2O as is the case when they react with O_2 through the mediation of the electron transmitter system. The energy released in an oxidase reaction therefore is presumably wasted, since high-energy phosphate production appears to occur only when the flow of electrons to O_2 is by way of the electron transmitter system.

The amount of oxygen consumed in oxidase reactions is not a significant percent of the total O_2 consumption of living cells. Of more importance is the fact that the H_2O_2 generated can be detrimental to the cell if allowed to accumulate. The cell has two means for the disposal of H_2O_2. First, the enzyme catalase accelerates the breakdown of H_2O_2 to water and oxygen.

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

Second, peroxidases catalyze reactions in which two electrons and two hydrogen ions are removed from a substrate and are made to react with H_2O_2 to produce two molecules of water.

$$R{\cdot}H_2 + H_2O_2 \longrightarrow R + 2H_2O$$

Both catalase and peroxidase are enzymes that contain iron phorphyrin groupings.

CHAPTER 2

Fatty Acid Oxidation: Citric Acid Cycle

The bloodsteam transports both glucose and fatty acids to the tissues to supply their energy requirements. On entering the tissue both substances must be made ready for dehydrogenation by processes that require the expenditure of energy. It may be said that it is necessary to prime the pump in order to activate the process by which energy release from these substances is achieved. Hence reactions of this type will be included under the category "make-ready." Activation of both glucose and fatty acid is accomplished by reactions involving ATP in which either a phosphate group or adenylic acid from ATP becomes linked to the substrate. Enzymes that catalyze reactions of this type are termed kinases. Since the reactions for the initial steps in fatty acid oxidation are the simplest, we shall take them up first. They fall into the two categories of reactions which we have chosen to label make-ready and dehydrogenation.

The preparation of fatty acids for oxidation involves the use of coenzyme A. This coenzyme is composed of β-mercaptoethylamine (*A*), attached in amide linkage to the vitamin pantothenic acid (*B*), which in turn is bound in ester linkage to adenosine 3′-phosphate 5′-pyrophosphate (*C*). The abbreviation CoA·SH or CoA will be used for coenzyme A.

The reactions involved in the oxidation of fatty acids to acetyl CoA are given in Fig. 2-1. Reaction 1, classified as a make-ready reaction, involves the formation of a thioester linkage between the fatty acid carboxyl group and the SH group of coenzyme A. In the first step the linkage between the fatty acid and AMP involves the phosphate group of adenylic acid and forms the high-energy bond $-C(=O)-O-P(=O)(OH)-O-$. The pyrophosphate (PP_i) which is produced undergoes hydrolysis to inorganic phosphate by the action of a pyrophosphatase. In the second step the SH group of coenzyme A replaces adenylic acid in the ester linkage to form a thioester, $-C(=O)-S-CH_2-$ which is also a high-