Human biochemistry

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by way of its influence on the corresponding enzyme level, as discussed below.

Cellular precursor (substrate) concentration

This usually entails the passage of the precursor of either exogenous or endogenous origin through the cell membrane into the cytoplasm. Regulation may occur at this step, since transport of essential nutrients through the cell membrane usually involves either active transport (p. 14) or facilitated diffusion by way of a transport protein (p. 14). An example is the active transport of glucose through cellular membranes. In fact, a primary role of the hormone, insulin, in regulating carbohydrate metabolism (p. 427) appears to be the facilitation of the active transport of glucose.

Cellular enzyme levels

In general, the regulation of the cellular concentration of an enzyme is the relatively slower, long-term *coarse control* of metabolism. It represents a dynamic balance between the genetically regulated intracellular biosynthesis of the enzyme, on the other hand, and its removal from the cell or degradation in the cell, on the other.

Intracellular enzyme formation may be *induced* by the substrate itself, or by certain hormones (p. 383), notably the steroid hormones, or possibly even by intermediates of the pathway. The first or "key regulatory enzyme" of a pathway is usually the one primarily induced, although succeeding enzymes of the pathway may also be induced simultaneously, particularly if their structural genes are located adjacent to that of the first enzyme on the chromosome (p. 54).

A number of examples of substrate induction of enzymes are cited in this text; one is the induction of the enzyme tyrosine transaminase (p. 328) at the level of transcription (p. 58) by the adrenal corticoid hydrocortisone (p. 411). Pertinent is the fact that some 2 to 3 hours, a relatively long time, is required for the enzyme to attain a maximal cellular level. A similar period of time is required for the cellular enzyme concentration to decrease to a low base-line level after the hormonal stimulus is withdrawn. Hence, this type of metabolic regulation is typically a slower, longer-acting control and is appropriately termed a "coarse control."

A second type of genetic regulation of cellular enzyme levels is the *repression* of enzyme formation (p. 121), typically by the end product of the pathway, the so-called feedback inhibition. A classical example is the inhibition of the porphyrin-heme biosynthetic pathway by its end product, heme (p. 782). Heme apparently acts as a "corepressor" (p. 121). When the amount of the end-product decreases below a required level, its "need" would be signaled by its disappearance as a corepressor. The repressor effect thus would be negated and the biosynthetic pathway would again be "turned on," provided, of course, that sufficient substrate were available.

Still another factor involved in determining the cellular level of an enzyme is its *degradation*, or removal from the cell. For a number of enzymes this process occurs rather rapidly, even within 2 to 3 hours. Indeed, some enzymes have a half-life of only 1 to 2 hours as determined by the use of radioactively labeled enzyme preparations.

Cellular enzyme activity

This type of regulatory mechanism is the rapid, usually short-term acting, *fine control* of metabolic pathways. The increase or decrease in the *activity* of the enzyme in this case results from a rapid *conformational* change in its structure,

Formation of tissue proteins

The steps involved in the biosynthesis of proteins from amino acids in the animal organism have been considered in detail in Chapter 5. This is the *major* route for the utilization of amino acids, since about 75% of the amino acids metabolized in the normal human adult are used for this purpose. The reason, of course, is the constant destruction of body proteins by wear and tear, the loss in excreta, desquamation of cells, and other minor losses. A large proportion of the "endogenous" amino acids derived from tissue breakdown are recycled, however, as indicated in Fig. 10-1. Present estimates are that at least 140 gm. of amino acids are contributed to the amino acid pool in an adult human subject daily by the turnover of tissue proteins. Isotopic studies of the turnover rates of different tissue proteins support these estimates. The half-life (T/2) of liver proteins, for example, is approximately 10 days. Plasma proteins also have a T/2 of about 10 days; muscle proteins, 180 days; and collagen, considerably longer. Some proteins, on the contrary, have much shorter T/2 values. The proteins of the intestinal mucosal cells turn over very rapidly, in a few days, and the T/2 of protein hormones and enzymes is also very short. The T/2 of insulin has been estimated as 6.5 to 9 minutes (p. 427). The average T/2 of the total body proteins

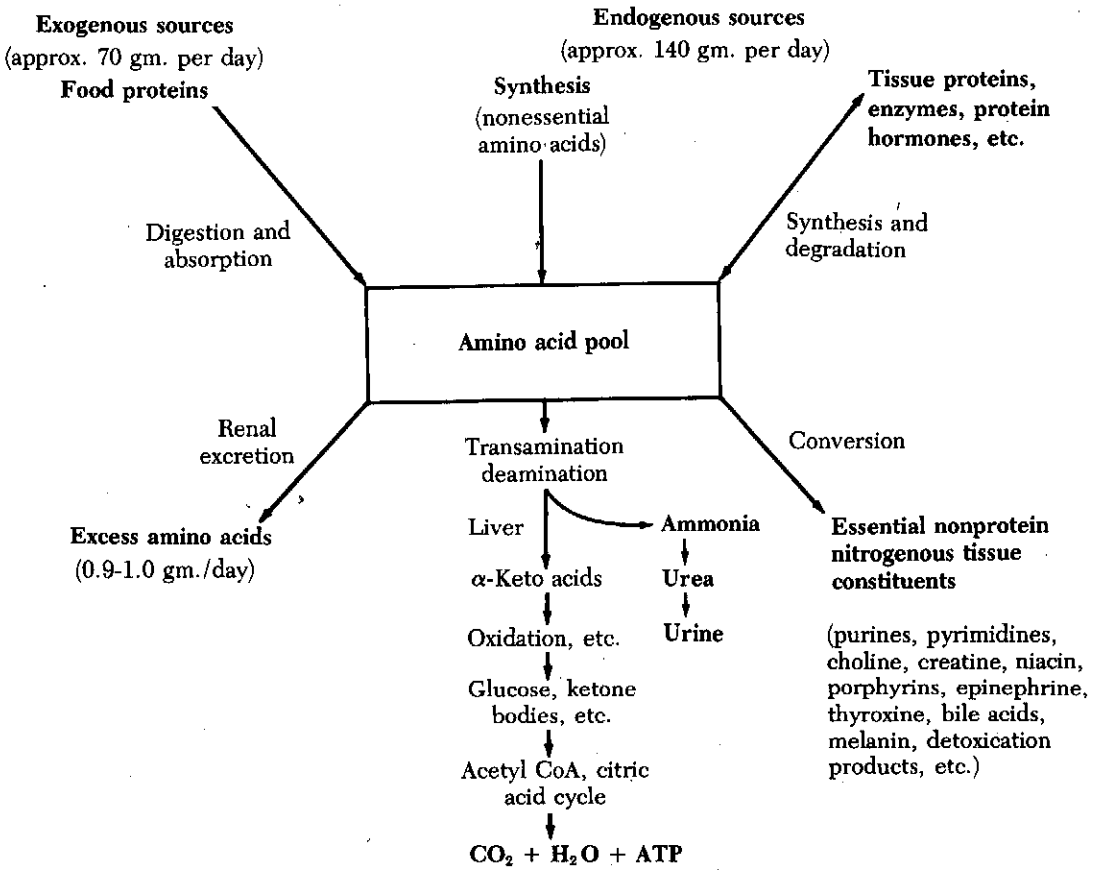


Fig. 10-1. Scheme of general paths of amino acids in metabolism (average man).

Chemical changes within the large intestine

Although the large intestine does not secrete any significant amount of body fluid but is rather a reabsorptive and an excretory structure, biochemical processes occurring in its lumen can perhaps be appropriately considered at this point.

The biochemical processes that go on in the large intestine are attributable mostly to the activity of the myriads of microorganisms that live and die there. These enter the tract with food and saliva and may survive passage through the stomach since the hydrochloric acid is not always present in bactericidal concentration. Consequently, some living, microorganisms pass into the small intestine and begin to multiply as the reaction becomes favorable. However, even near the ileocecal valve the intestinal contents do not contain large numbers of such organisms. At this point are present some undigested food residues, unabsorbed secretions, e.g., bile and pancreatic juice, and cell detritus. Analysis of this material shows it to have about the same amounts of nitrogen, fat, and carbohydrate (based on dry weight) as normal feces, but it is not like feces. The pH is about 5.9 to 6.5.

Feces

In the large intestine, such materials as just described are transformed into feces. A number of enzymes are possibly present in the secretion of the mucosa of the large gut, but digestion by them is generally believed to be of little importance. This secretion is alkaline and viscid and undoubtedly tends to bring the contents over to the alkaline side. The conditions for bacterial growth (particularly anaerobic) are excellent: there is warmth, darkness, little oxygen, an almost neutral medium, and food material in a semisolid condition. The organisms flourish, utilize the food materials, transform them into their own protoplasm, multiply, and die. In fecal material, from one fourth to one half of the dry matter is made up of living and dead bacteria. Water is absorbed by the mucosa and the characteristic consistency results.

In the newborn infant the first fecal discharge is termed meconium. This is a dark brownish green semisolid material. It consists of intestinal and biliary secretions that have accumulated in the large intestine from the fourth fetal month on. Meconium continues to be passed for the first 3 or 4 days after birth and accounts for much of the loss of weight that occurs during this period. Usually, with the ingestion of milk, a gradual change to the usual type of infant feces is seen. These are greenish yellow in color and have an acid reaction. The approximate general composition of stools of the infant and of the adult is given in Table 13-2. In the feces of infants, there is very little protein but rather large amounts of fat, fatty acids, and soaps.

Adult fecal material is normally brown, varying in color with fat and water, which lighten the color, and bile pigments, which darken it. About 80 to 170 gm. of feces are eliminated per day. The composition varies greatly. Feces contain

Action of microorganisms on carbohydrates and lipids

Table 13-2. General composition of stools (in percent)

	Stool of breast-fed infant	*Stool of adult*
Water	85	75
Organic solids	13	20
Ash	1	5

Fiber

Other nonutilizable carbohydrates are the indigestible polysaccharides—cellulose, lignin, agar-agar, gums, etc. These constitute a large part of the fiber of food, the indigestible fraction that gives bulk to the feces. Food must contain such substances, for a dearth of them tends to produce constipation. The tendency in modern civilization has been toward a refinement of food, with a lessening in the amount of the indigestible parts of grains, fruits, and vegetables. On the other hand, an overabundance of fiber can lead to irritation of the intestinal mucosa. The physical presence of the indigestible material, or the distention of the colon that it causes, is not the sole factor stimulating peristalsis. Certain intestinal bacteria that are capable of decomposing hemicelluloses and mixed polysaccharides, with the production of lower volatile fatty acids, along with the hygroscopic nature of carbohydrates and of some other products resulting from the action of microorganisms, have a stimulating peristaltic effect and so induce bowel movement. Lignin and cellulose, which also escape digestion, have less effect than the hemicelluloses and mixed polysaccharides in this respect. In food tables this fraction is usually termed "fiber." Thus the total carbohydrate of a food is not utilizable for energy by the body.

The fiber portion is valuable in other respects, however, as indicated by current investigations. Epidemiologic studies in man as well as experiments in animals indicate that such pathologic conditions as diverticular disease of the colon, cancer of the colon, and coronary heart disease may be related to a lack of dietary fiber. Lack of food fiber has also been implicated in such widespread ailments of Western civilization as hernias of the gastrointestinal tract, hemorrhoids, gallbladder disease, appendicitis, varicose veins, and even obesity. There is considerable experimental evidence that food fiber has a hypocholesteremic effect. Studies indicate that fiber *binds* bile acids and possibly cholesterol itself in a nonabsorbable complex, thus increasing the fecal excretion and thus draining plasma and tissue cholesterol levels. Further studies are needed to clarify and establish the role of fiber in foods. Meanwhile, however, the foregoing current investigations support the wisdom of including in the daily diet of normal adults "at least four or more servings each of whole-grain cereals, fruits, and vegetables."

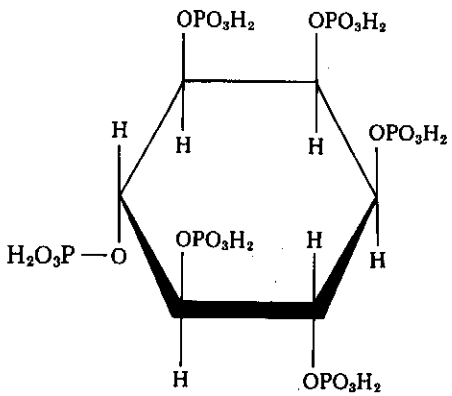
After absorption, the monosaccharides utilized are converted to glucose. The glucose then is utilized either directly or indirectly after it has been converted to glycogen or to fat. It is in these two forms that excess carbohydrate is stored in a number of tissues and organs. Since a fraction of the protein molecule may be transformed into sugar, this fraction also may become part of the glycogen and fat stores of the body (Chapters 8 and 9).

The 1974 recommended dietary allowances include no definite value for carbohydrate because of insufficient data. However, the consensus is that at least 100 gm. of carbohydrate are needed per day by the normal adult to avoid ketosis, excessive protein catabolism, and other undesirable metabolic responses. The suggestion also has been made that at least for a limited period of time a diet containing 100 gm. of carbohydrate, 100 gm. of protein, and 100 gm. of fat, possibly with vitamin supplementation, would be satisfactory as a reducing diet for an average normal man.

low phosphorus and normal calcium. An empiric index for determining whether a child is rachitic is the product of the serum phosphorus and serum calcium (in milligrams per 100 ml.). If the index is below 30, rickets is present or will develop, but not if it is above 40.

Phosphorus is found in those foods containing phosphoproteins, nucleoproteins, phospholipids, and glycerophosphates, as well as the inorganic phosphates, chiefly calcium and sodium. Since quantitatively the greatest proportion of the phosphorus is used to form the bone salt, which is largely calcium phosphate, evidently the phosphorus intake should bear an optimal relation to the calcium intake. The foods richest in calcium are also richest in phosphorus, namely, milk, cheese, and beans. Eggs, cereals, fish, and meats are also high in this element.

Phosphorus is present in foods also as phytates. In fact, a large proportion of the phosphorus of vegetables is in this form. Although the exact structure of these salts is not known, phytates are quite insoluble mixed calcium and magnesium salts of phytic acid, which in turn is a hexaphosphate of inositol. Available evidence indicates that phytic acid and its compounds interfere with the absorption of calcium, zinc, and iron from the intestinal tract. Unrefined cereals are rich in phytates, but white flour contains little. Hence the phytate problem is not serious in the United States; but in areas of the world where unrefined cereals form a large part of the diet and little calcium is consumed, the interference with calcium absorption may result in serious deficiencies of calcium, including the development of so-called "cereal rickets." The phosphate and inositol of these substances are, for the most part, unavailable nutritionally. However, slight digestion may be accomplished by gastric juice and somewhat more by intestinal phosphatases.



Phytic acid

Requirement of phosphorus. The recommended dietary allowance for phosphorus (p. 510) has been established on the basis of an approximate 1 : 1 relationship with calcium. The recommended daily allowance for both men and women is 800 mg. For infants and children the values range from 240 to 800 mg.; for teen-agers the value is 1200 mg., depending on age (see p. 510). The amount during pregnancy and lactation should be increased to 1200 mg. per day. The recommended 1 pint or more of milk per day for the adult and 1 quart for the

ure to decarboxylate oxidatively leucine, isoleucine, and valine. The urine acquires an odor resembling that of maple sugar (p. 320).

Porphyrins are sometimes found in urine, giving it a red color if sufficient amounts are present. The urine may fluoresce a brilliant vermillion color in ultraviolet light. Porphyrins are found in urine in the porphyrias, congenital diseases in the biosynthetic pathway of heme (p. 784). Porphyrins may also appear in the urine temporarily after the administration of certain drugs, e.g., sulfonamides and sulfonal, or in alcoholism or lead poisoning, or after excessive exposure to ionizing radiations. The porphyrinuria in these instances usually disappears with the cessation or removal of the causative agent.

Tyrosinosis is an exceedingly rare anomaly in which the aromatic amino acids are eliminated as tyrosine or hydroxyphenylpyruvic acid (p. 330).

A summary of some of the more common or biochemically unique hereditary metabolic disorders is given in Table 16-12 (see also Table 10-2). Where known, the specific enzyme defect and the characteristic metabolite appearing in the urine in abnormal amounts are also given. Obviously the list does not include those metabolic derangements in which there is a *tissue* accumulation of a metabolite rather than an increased urinary excretion. As discussed earlier, hereditary disorders of this type include the glycogen storage diseases (p. 229), the various lipidoses (p. 289), and those in which there are alterations in the biosynthesis of body proteins (e.g., the hemoglobinopathies and aberrations in the formation of various plasma proteins).

Detoxication

It was stated on p. 473 that the quantities of toxic products absorbed from the large intestine are not very great, and even these small amounts are detoxified. The process is commonly called detoxication and is appropriately considered at this point because the detoxified products are excreted mainly in the urine. The term "detoxication" is a misnomer, from one point of view at least. The detoxified product is sometimes more toxic than the original substance. "Biotransformation" has been suggested as a preferable term.

The primary purpose of the detoxication process is to convert the toxic substance to a more *polar* compound, which is thus *less lipid soluble*. The object is to decrease the permeability of the polar compound through lipid membranes, thus protecting the cell interior; the object is also, if possible, to increase the water solubility and hence the excretion of the compound in the urine or bile or intestinal secretions.

A summary of the typical pathways for the hepatic detoxication of a variety of classes of chemical compounds is presented in Table 16-13. As stated previously, the detoxified substance is excreted primarily in the urine. This desirable result is effected by mechanisms that the body ordinarily uses in its normal metabolic processes; i.e., detoxication mechanisms are probably not specific in regard to toxic substances absorbed from the bowel nor indeed for any substances simply because of their toxicity, but rather they are directed toward particular types of substances that arise in cellular activities. Some of these substances happen to be harmful and may be rendered less so by the chemical transformations resulting from the usual activities of enzymes on definite chemical groups or linkages. Hence it is not surprising to note, on the one hand,

Table 16-13. Typical pathways of hepatic detoxication*

Acids	Aldehydes	Alcohols	Ketones	Esters	Amines	Nitro compounds	Aromatic compounds	Phenols	Arylhalides	Pyridine derivatives

that these detoxication systems operate against poisonous substances of whatever origin or however introduced and, on the other hand, that the operation of some of these detoxicating systems does not necessarily imply or guarantee that a nontoxic or even a less toxic substance will be produced.

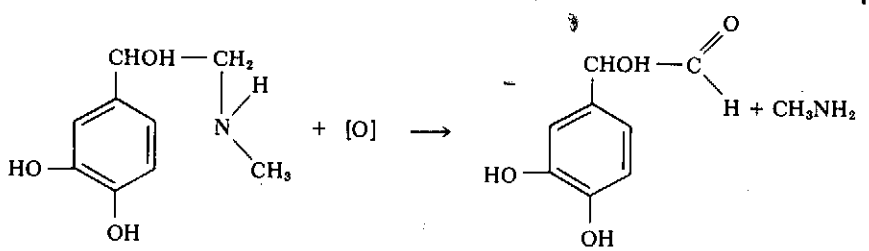
Detoxication reactions have a direct bearing on the pharmacologic reactions of drugs. Because of this, it is important to list several factors that influence the detoxication process: (1) the specific chemical structure of the compound being acted on, (2) the dose of the compound in relation to the weight of the organism, and (3) the species of the animal metabolizing the compound. A realization of this last factor is of prime importance in the pharmacologic evaluation of a drug. It emphasizes that drugs tested on animals cannot be administered to the human being with absolute assurance of safety since detoxication routes may vary in different animal species (including the human being). Thus phenylacetic acid is detoxified by conjugation with glycine in the rabbit and dog, by conjugation with ornithine in the bird, and by glutamine in man and the chimpanzee. Other factors are age, sex, environmental conditions, e.g., temperature and barometric pressure, heredity (alkaptonuria), concurrent administration of other compounds, and the physiologic state of the organism. Further details can be obtained from textbooks on pharmacology.

Most detoxications occur in the liver.

Oxidation. Oxidation usually occurs first and sometimes is followed by conjugation. Indole is an example (p. 335). It is first oxidized to indoxyl, which is then conjugated with sulfate. Some substances can be completely decomposed by oxidation. Ethyl alcohol, in moderate amounts, can be oxidized by the body to carbon dioxide and water. The fat that methyl alcohol yields intermediate toxic products, formaldehyde and formic acid, in the same kind of oxidation, emphasizes the fact that these reactions are general metabolic mechanisms that may fall short of total detoxication.

Aliphatic amines are completely oxidized by the body. An enzyme, amine oxidase, that accomplishes this has been found to occur in brain and other tissues. In the case of butylamine, a product of the reaction is acetoacetic acid, which is, of course, a normal metabolite. There is present in liver, intestine, and other tissues a similar enzyme that catalyzes the oxidative deamination of epinephrine and related amines.

The reaction is as follows:



Epinephrine

It was pointed out on p. 258 that phenyl-substituted fatty acids are oxidized by β-oxidation, losing two carbons at a time in the process. The final products

COLLOIDAL STATE

In 1861, Graham classified all substances into two categories, depending on the ability of the substances to pass through parchment and similar membranes. Since those substances the diffused readily were the ones that easily crystallized, e.g., copper sulfate, sucrose etc., he designated them "crystalloids." Those that did not pass through, e.g., gelatin, starch paste, glue, etc.., were considered to be noncrystallizable and were called "colloids," from the Greek word meaning 'glue.' These terms continue to be used, although we now are able to crystallize many of the colloids, and the crystalloids can be converted to a colloidal from. The modern concept of these differences is based on the size of the particles dispersed in the water or other medium. Colloidal particles are large; they cannot pass through the pores of ordinary parchment or collodion membranes. However, they are not large enough to settle out by gravity, as suspensions do, or to float at the top of the medium, as imperfect emulsions do. In true solutions, so-called crystalloidal solutions, the mixture is homogeneous; the constituents are present in the molecular or ionic state and are uniformly distributed throughout and among the molecules of water or other solvent. Colloidal systems are heterogenous; i.e., there are two *phases*—the finely divided particles and the medium in which they are suspended. By "phase," we mean a physically distinct portion of matter. The particles are called the *dispersed phase*, and the medium, usually a fluid, is the *dispersion medium*. Both phases may be solids, liquids, or gases, with a single exception: it is not possible to have a colloidal dispersion of a gas in a gas. Smoke is a solid dispersed in a gas, fog is a liquid dispersed in a gas, and froths and foams are gases dispersed in liquids. We are more concerned with liquids dispersed in liquids, liquids dispersed in solids, and solids dispersed in liquids.

The size of the particles in colloidal systems is generally stated to be from 1 millimicron (mμ) to 100 mμ, but arbitrary limits at either end cannot be set. In fact, the tendency is to place the upper limit somewhat higher, such as at 500 mμ (0.5 μ). A millimicron is one millionth of a millimeter (0.000001 mm.). Particles having smaller diameters than 1 mμ are molecular or ionic, and if much above 100 to 500 mμ, they are coarse enough to settle out. The smallest colloidal particles, therefore, are but little larger than crystalloidal molecules, and the largest ones are nearly the size of the particles in a suspension.

Colloidal particles may be removed from the dispersion medium by forcing the fluid, under pressure, through an appropriate membrane. This is termed *ultrafiltration*. By using membranes of varying porosity, it is possible to separate different colloids from each other and to estimate the size of colloid particles. A colloid particle is often termed a *micelle* (p. 902).

Ultracentrifugation is another method of removing colloid particles. By centrifuging at a very high speed, the dispersed phase may be separated from the dispersion medium. Substances in true solution cannot be separated from their solvents by these two methods. Still another procedure is electrophoresis (p. 959).

A simpler method than any of those described is dialysis. This will be discussed later in the chapter.

Types of colloids

Colloids may be grouped into two main classes, depending on their ability to take up the dispersion medium. The *lyophilic colloids* (emulsoids) have a

Surface tension may be measured in a number of ways, perhaps most conveniently with a stalagmometer. This is a pipette of special design with a capillary tube ending, permitting a measured amount of fluid to flow out drop by drop. The number of drops depends on the size of the drops, which, in turn, varies with the surface tension. A comparison with the number of drops of pure water permits one to calculate the surface tension of the solution. Surface tension is expressed as ergs per square centimeter or dynes per centimeter. For accurate work, great precautions of cleanliness must be taken, since small amounts of some substances alter the surface tension materially. Soaps, oils, proteins, and salts of the bile acids reduce the surface tension of water, whereas sodium chloride tends to increase it. These and similar effects aid in explaining some physiologic actions, e.g., fat digestion and absorption. Substances that reduce surface tension accumulate in the surface film and are said to be adsorbed, whereas the reverse is true of those that increase surface tension. There is a stalagmometric method for the determination of bile acids in bile that has been used as a liver function test, based on the fact that an important function of the liver is the secretion of bile acids. The du Noüy tensiometer is another device for determining surface tension. In this, a light metal ring is set on the surface of the fluid under examination. As the ring is raised, a film of the fluid clings to it. The amount of force required to pull the ring off and break this film is a measure of the surface tension and can be exactly and conveniently measured.

DIFFUSION, OSMOSIS, AND DIALYSIS

Diffusion. If a strong solution of a salt, e.g., copper sulfate, is placed in a glass vessel and a layer of distilled water is carefully poured over it, the blue copper sulfate rises gradually into the colorless water until finally the entire body of fluid has the same color. This process is called diffusion. The velocity with which it occurs depends on the size of the particles of the substance in solution. Thus Prussian blue, being composed of large particles, diffuses more slowly than does copper sulfate. Higher temperatures also speed up the process. It should be observed that diffusion involves the passage of substances, in true solution or in colloidal solution, through the fluid in which they are suspended. In the many fluids of the body, within cells, during secretory activity, diffusion must be constantly occurring.

Osmosis. Osmosis is the passage of a solvent through a semipermeable membrane. Such a membrane is permeable only to the solvent, not to the solute, i.e., the substance in solution. The classic experiment of Pfeffer illustrates the point. He precipitated copper ferrocyanide in the walls of an unglazed porcelain jar by filling the jar with potassium ferrocyanide solution after immersing it in a solution of copper sulfate. Such a film of copper ferrocyanide permits water to pass through but does not allow certain soluble substances, e.g., sugars, to do so. Consequently, when such a jar, fitted with a glass tube into which the liquid can rise, and filled with sugar solution, is placed in distilled water, the water passes through the semipermeable membrane into the sugar solution until the column of diluted sugar solution is no longer increased. The pressure that

ENERGY TRANSDUCTION

One of the fundamental problems of biochemistry is the mechanism by which energy is transduced in living systems. It is common knowledge that "energy" is in some way obtained by the "burning" (oxidation) of food. Extensive research has shown that the locus of this oxidation is in cellular organelles, the *mitochondria* (p. 202)—sometimes appropriately called the "powerhouse" of the cell. The mitochondrion thus serves in the transduction of oxidative energy into a special form of chemical energy, ATP. The chloroplast with its quantasomes serves a similar function in plants (p. 8).

Carbohydrates are the chief source of energy in the animal organism, being converted ultimately to carbon dioxide and water with the formation of ATP. This is a stepwise process involving the degradation of glucose by glycolysis or alternate pathways to pyruvic acid and lactic acid and then to acetyl CoA for final oxidation by way of the citric acid cycle and an electron-transport chain as discussed earlier. Acetyl CoA is also formed by the oxidation of the fatty acid moiety of fats and is likewise oxidized by way of the citric acid cycle and the electron-transport chain. Moreover, most of the amino acids are converted into keto acids, e.g., pyruvic, oxaloacetic, α-ketoglutaric, and enter into the same pathways for final oxidation, as described in Chapter 10. Hence, the citric acid cycle has been termed the "final common pathway" of metabolism. A consideration of the mechanisms of oxidations in living matter, termed "biologic oxidations," is now relevant.

Oxidations

The term "oxidation" is applied to protoplasmic oxidations in the same senses as in nonvital oxidations—i.e., combination with oxygen or removal of hydrogen, in any case, *a loss of electrons*. In some of the complex reactions, the transfer of electrons may be difficult to express, particularly in our present state of knowledge. It is perhaps unnecessary to remark that every oxidation must be accompanied by a reduction. It might be more pertinent to note that biologic oxidation in itself cannot be a source of energy. Oxidation involves an increase in valence, i.e., a removal of electrons, which requires energy. Indeed, it is the oxidation-reduction reactions involved in the transfer of electrons from hydrogen through intermediary acceptors, to be described later, and ultimately to oxygen, that yield the energy released in cellular oxidations.

Although not all oxidations involve oxygen, nevertheless oxygen is a vital requirement. Anaerobic organisms may and do perform oxidative reactions in the absence of oxygen, and even aerobic organisms are able to do so under some circumstances. However, a human being cannot live in an oxygen-free atmosphere for more than about 3 minutes. It is probable that most, if not all, human tissues require oxygen for the completion of their vital oxidations, even though the intermediate stages may go on in its absence.

Remember that molecular oxygen is incapable of oxidizing *biologic* substances outside the body at body temperature except to a very slight extent. Within the body such oxidations are occurring constantly. It is therefore evident that the body must possess means for making such reactions possible at such comparatively low temperatures. The two problems before us are (1) how the cell brings about oxidation of its substrates at the low temperature of the body and (2) how the energy derived from the oxidation of this substrate is utilized

without being dissipated as heat. These are complicated reactions, many of them chain reactions, of such a nature that the oxidations are in small steps and are controlled. In this way the energy evolved may be used physiologically.

Energy relationships

The degradation of biologic substrates in the living cell by the process of biologic oxidation involves changes in which compounds of higher levels of energy go to products having lower levels of energy. Not all the energy evolved is available for work. The thermodynamic function $\Delta G°$ is a measure of the maximum work available from a reaction occurring at constant pressure and constant temperature. $\Delta G°$ is also related to the equilibrium constant of a reaction as given in the following equation:

$$\Delta G° = -RT \ln K$$

where R is the gas constant, T is the absolute temperature, *ln* is the natural logarithm, and $\Delta G°$ is the standard free energy change.

Reactions that have a negative $\Delta G°$ are spontaneous, and reactions that yield energy are *exergonic*. However, a reaction, which may be thermodynamically spontaneous, may not occur by itself at any appreciable rate. For a reaction to occur, the energy that is needed to convert the reactants to the form in which they can actually react must be supplied. This is the *energy of activation*. In biologic systems the function of enzymes may be to lower the activation energy and thus make possible the multitude of reactions at the rates that are seen in biologic systems. In other than biologic systems, many of these same reactions occur only at high temperatures.

Chemical reactions that require energy for their occurrence are termed *endergonic* and are representative of anabolic reactions. Keep in mind that the catabolism or breaking down of physiologic substrates has as its prime purpose the maintenance of anabolic reactions, as typified in growth and the performance of biologic work. Such endergonic reactions must, of course, be driven by the utilization of part of the energy of an exergonic reaction. The only mechanism available for such a transfer of chemical bond energy from one reaction to another is by the utilization of *a common reactant of both reactions*. Consider Reactions I and II below as being two steps in the overall reaction $A \rightleftharpoons C$, or $A \rightleftharpoons B \rightleftharpoons C$. K is the equilibrium constant, and $\Delta G°$ the free energy change between the initial compound and its product, both at standard concentrations.

$$\text{(I) } A \rightleftharpoons B;\ (K = 0.01,\ \Delta G° = -RT \ln K = +2470 \text{ cal.})$$
$$\text{(II) } B \rightleftharpoons C;\ (K = 1000,\ \Delta G° = -RT \ln K = -4110 \text{ cal.})$$

Since Reaction I is endergonic, it will proceed to the left unless the concentration of B is less than 1% of the concentration of A. However, since Reaction II is exergonic, it will proceed to the right until the concentration of B is less than 0.1% of C. Thus B will be removed from the coupled reactions as fast as it is formed from Reaction I. Consequently, Reaction I can proceed to the right. Energetically, the overall reaction $A \rightleftharpoons C$ may be considered in terms of its K and the $\Delta G°$ values.

$$K = K_1K_2 = (0.01)\,(1000) = 10$$
$$\Delta G° = (2470 - 4110) = -1640 \text{ cal.}$$

Thus the reaction proceeds to the right until the concentration of C is 10 times that of A, and we have a net exergonic reaction. Another principle, demonstrated in this typical example, is the relationship between the endergonic or driven reaction and the exergonic or driving reaction. The driven reaction must precede the driving reaction.

In biologic systems, phosphate compounds occupy the unique position of being the common reactant in a multitude of reactions. Most particularly is this function seen in ATP and other so-called "high-energy" compounds. These are discussed in Chapter 8.

Terminology. The biochemical agents involved are *enzymes, coenzymes,* and *hydrogen acceptors* or *carriers.* Those enzymes that act on the substrate and make possible the removal of hydrogen from it are called *dehydrogenases;* those that act on oxygen and cause it to take part in an oxidative chain are termed *oxidases*. These enzymes are rather specific; there are many dehydrogenases and a number of oxidases. Hydrogen carriers or acceptors are defined as compounds that, by virtue of their ability to be oxidized and reduced, function in the transport of hydrogens or electrons from tissue metabolites to oxygen or some other oxidizer. In general, they are also of a complex protein nature but are characterized by particular prosthetic or active groups that make possible their specific functions. Certain coenzymes, i.e., NAD^+ and $NADP^+$, which are discussed more fully in Chapter 6 are dissociable from their protein enzyme fractions but usually do not function independently as carriers.

Methods of study. Since the respiration of tissues involves the utilization of oxygen, either immediately or eventually, naturally the primary method of evaluation of such activity should involve a direct measurement of oxygen uptake. The development by Warburg of a microrespirometer provided the basic tool for investigation in this field. Although many variations of his apparatus have been devised, the techniques originally described by him are still widely used. Fig. 20-3 is a diagram of this apparatus.

Oxygen utilization in a system can also be determined by the use of an *oxygen electrode*. This device is somewhat similar to a pH electrode except that it detects and determines the concentration of free oxygen gas dissolved in the medium. In general, measurements can be made on four main types of test materials: (1) tissue slices, (2) tissue homogenates (fine minces), (3) subcellular fractions obtained by differential centrifugation, and (4) isolated components of the enzyme systems to be studied. Certain intrinsic limitations exist in each of these test materials. The purer the chemical system under study, the less may be inferred as to the direct participation of the system in intact tissues; the results obtained with tissue slices often fail to disclose the complexity of the enzyme systems involved since only the end results are seen.

Investigation of the dehydrogenase activity of tissue slices, homogenates, or isolated enzyme systems has also been accomplished by means of the Thunberg technique. Here measurements are made of the rate of anaerobic decolorization of methylene blue. The methylene blue functions as a hydrogen acceptor, and the velocity of its reduction is thus a measure of the activity of the respiratory enzymes.