CHAPTER 5

REVIEW OF BIOCHEMISTRY

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INTRODUCTION

In some respects the life process resembles a gyroscope. It is in metastable equilibrium requiring a continuous energy input to function in a structure carefully designed and built to accommodate the system. Yet, despite the intrinsic delicacy of its operation, it is remarkably able to cope with and recover from stresses that would cause alterations in its equilibrium. Living systems are incredibly complex and require an exquisite integration of processes to fulfill the requirements of energy production, structural formation and maintenance and homeostasis — the maintenance of the status quo. Biochemistry provides the scientific discipline to accommodate much of our present knowledge of life. It is convenient to review the subject from the viewpoint of the divisions that have been enumerated. To show how normal biochemical processes may be affected by chemical agents, examples of biochemical pathology will be cited, as well as clinical applications of biochemistry in the detection of occupational disease.

Energy Production

The fundamental reaction by which energy is produced in the body may be written:

$$3O_2 + 2(-CH_2-) \rightleftharpoons 2CO_2 + 2H_2O + energy$$
(Equation 1)

This is an oxidative process by which oxygen has been added to the fuel source, ($-CH_2-$) to produce the waste materials carbon dioxide, water and energy. As in other energy transformations, the total amount of energy available is independent of the path by which the change occurs. It depends only upon the difference between the free energy of formation of the reactants on the left and the products on the right.

Although the chemical change in the energy-producing system can be written as depicted, the actual source of the available energy originates in the relative positions of electrons in the orbital structures of the atoms involved in the reactions. At one end of the spectrum of electron movement we recognize that an atom may release an electron altogether. The change
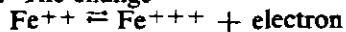
$$Fe^{++} \rightleftharpoons Fe^{+++} + electron$$

represents such an oxidation of Fe^{++} to Fe^{+++} with release of an electron. Since such a process can occur only with the simultaneous acceptance of the electron by another moiety, an oxidation is coupled by a reduction. In biological systems, for example, the oxidative loss of the electron from the Fe^{++} structure is frequently coupled with acceptance by oxygen according to the reaction

$$electron + 1/2 O_2 + H^+ \rightleftharpoons OH^-$$
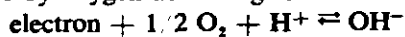
In this case the oxygen atom has been reduced by acceptance of the electron. The total process

$$Fe^{++} + 1/2 O_2 + H^+ \rightarrow Fe^{+++} + OH^- + energy$$
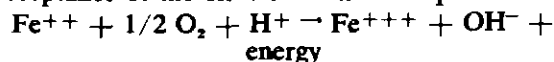
represents a transformation from reactants on the left to products on the right at a lower energy level.

The statement of the reaction involved in the major biological source of energy (equation 1) represents a less extreme movement of electrons than that described for the iron atom. In essence the formulation suggests that the movement of an electron pair from its essentially equidistant point between the carbon and hydrogen atoms in the structure

$$-\overset{\textstyle H}{\underset{\textstyle H}{C}}-$$
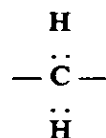
closer to the oxygen atoms in the product structures

$$:\overset{..}{O}:: \quad C \quad ::\overset{..}{O}: \quad and \quad :\overset{..}{O}: \quad H$$
$$\overset{}{\underset{\textstyle H}{}}$$
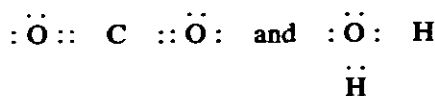
involves a decrease in the energy levels of the reactants and products. The available liberated energy can be usefully trapped by the organism. The energy-trapping mechanism must be one which provides the potential for the conversion of the chemical-bond energy of oxidation to heat, electrical, mechanical or new formulations of chemical bond energy by means of specialized transducers.

To fulfill its function in living systems the overall reaction of energy production implies the availability of oxygen, the fuel substrate ($-CH_2-$), the removal of waste products (CO_2, H_2O, and other materials) and a system for control of energy made available by oxidation. These conditions may not prevail due to injuries related to the industrial environment. Silicosis may impair oxygen transport from lung to blood; carbon monoxide may combine with hemoglobin to prevent it from carrying oxygen. The cells may not have access to substrate when the cell membrane is poisoned: thus glucose cannot move into the cell due to inactivation of the phosphorylating enzymes. Waste products cannot be removed when kidney function is damaged by toxic agents such as mercury, uranium, and phenolic substances.

Oxygen Metabolism

Life ceases within minutes when the continuous supply of oxygen is interrupted. A responsive integrated physical, mechanical, hydraulic and

chemical system provides this essential element. The diffusion of oxygen from the air inspired in the lungs to the tissues, where it is utilized, is facilitated by a sequential decrease in its partial pressure. The pO_2 of approximately 158 mm in the inspired air decreases to about 103 mm in the alveolar spaces of the lung, 100 mm in the arterial blood, and 37 mm in the peripheral venous blood subsequent to tissue utilization. The mechanical system of the muscle-controlled collapsible lung provides for the volume flow of oxygen containing air. The hydraulic arrangement of the heart pump and blood vessels allows for the fluid movement of the blood which serves as the oxygen transport medium. In the blood the biconcave doughnut-shaped erythrocyte (red cell) serves as a package for the oxygen transporting protein hemoglobin. This cellular bundle has manifest advantages in protecting and controlling the delivery system.

Hemoglobin

From the viewpoint of the biochemist, the protein hemoglobin provides an intriguing example of the evolution of a molecular structure adapted to a specific function.

The total molecule, with a molecular weight of approximately 64,000 is made up of four subunits of about 16,000. Each subunit has two essential components. One is a polypeptide chain of somewhat more than 140 linearly condensed amino acids. Associated with each polypeptide chain is a planar iron-porphyrin complex, heme, which serves as the oxygen binding moiety. The heme structure fits into a cavity of the polypeptide. In simplified diagramatic outline, the hemoglobin structure can be visualized as illustrated in Figure 5-1. In order to most effectively fulfill its biological oxygen transport function within the red cell package, the molecule of hemo-

globin has been carefully designed. It is globular in shape so that it provides maximum volume in the least space. High solubility of the protein is maintained by a large number of charged groups on the surface of the molecule. These tend to attract and hold polar water molecules close to the protein. The hydrophilic shell helps to keep hemoglobin in the aqueous phase.
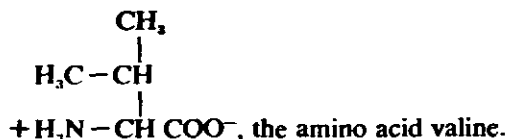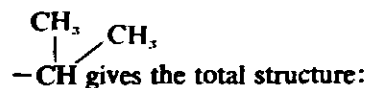
During a normal lifetime, a human will produce five different types of polypeptides which will pair to form four different kinds of hemoglobin. Alpha and epsilon polypeptides appear first in ambryonic life followed by alpha and gamma chains in the foetus. With birth, the gamma chain production ceases, to be replaced by the combination of alpha and beta chains of hemoglobin A_1, the major component of adult hemoglobin. Starting with birth, small quantities of a second hemoglobin, A_2, made up of alpha and delta chains are to be found in the erythrocyte. The ultimate arrangement in space of the polypeptide chains is dependent upon the sequence of the polymerization of the alpha amino acids of which they are composed. In the typical amino acid structure:
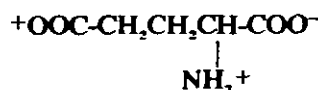
$$\overset{\displaystyle R}{\underset{\displaystyle H}{+H_3N-\overset{|}{\underset{|}{C}}-COO^-}}$$
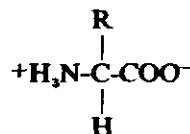
the R group can represent a variety of possible substituent groups. When it is a hydrogen atom, the total structure would be

$$\overset{\displaystyle H}{\underset{\displaystyle H}{+H_3N-\overset{|}{\underset{|}{C}}-COO^-}}, \text{ the amino acid glycine.}$$
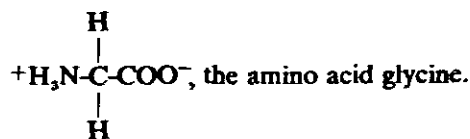
An uncharged hydrophobic lipid group such as:

$$\underset{-CH}{\overset{CH_3}{\underset{|}{\diagdown}} \diagup CH_3} \text{ gives the total structure:}$$

$$\overset{\displaystyle CH_3}{\underset{\displaystyle +H_3N-CH}{H_3C-\overset{|}{CH}}} COO^-, \text{ the amino acid valine.}$$

When the substituent groups are ionizable, as in glutamic acid:

$$\underset{\displaystyle NH_3^+}{^+OOC-CH_2CH_2CH-COO^-}$$

$$+H_3NC \text{ lysine.}$$

$$\underset{\displaystyle NH_3^+}{^+H_3N(CH_2)_4-CH-COO^-}$$
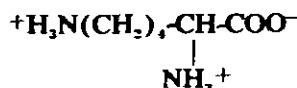
The polymer resulting from linkage of the amino acids through alpha amino and carboxyl ends will have points of charge extending from the chain of this primary backbone structure:

Figure 5-1.   Hemoglobin Structure

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$$R \qquad R$$
$$\text{- - - NH-CH-CONH-CH-CO- - -}$$

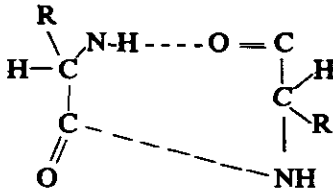The subsequent arrangement in space of the peptide chain depends upon interactions between groupings. The bonding of a hydrogen atom of the peptide linkage between nitrogen and a reactive peptide oxygene atom, (a "hydrogen bond"):


can lead to coiling of the chain in the shape of an alpha helix. This has the overall appearance of a straight cylinder with the peptide backbone wound in a spiral. In addition to the hydrogen bonds, the availability of appropriately placed oppositely charged, "R", side chain groupings can assist in the maintenance of a specific three-dimensional conformation of the structure.

$$\text{-COO}^{-}\text{- - - NH}_3{}^{+}$$
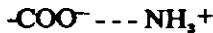
Sharp bends in the chain are made possible by the linkage of the cyclic amino acid proline
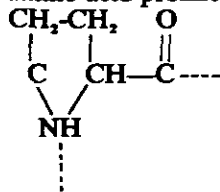
More definite intrachain linkage is achieved, for example, by covalent bonding of sulfur atoms from reactive cysteine amino acids:
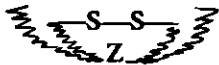
In the case of the hemoglobin polypeptides made up of approximately 140 amino acid residues, a significant contribution to the structure arises from the interaction of the uncharged hydrophobic R side groupings. These interact by Van der Waals surface forces to provide an essentially uncharged internal cavity from which water molecules are excluded and into which a "heme" oxygen binding structure carefully fits.

Hemoglobin is packaged in the erythrocyte with a variety of enzymes and other substances which play a supporting role in its function and survival. The red blood cell, in humans, has lost its original nucleus; has no mitochondria — those "powerhouses" of the tissue cells—yet uses energy. It has a limited capacity for synthesis, and wear and tear will limit its life span. The term "the rancid red cell," applied to aging cells, seems appropriate; although it functions in oxygen transport, too much oxygen can injure the cell. The anti-oxidant, Vitamin E, is one of its safeguards. Normally, alpha tocopherol, the principal E Vitamin is adequate as supplied in the usual diet, but special conditions such as high oxygen pressure warrant supplementation. Hence the astronauts' diet includes a proprietary orange drink with a high content of this vitamin.

Oxides of nitrogen as well as more complicated nitrogen compounds — amines, nitro compounds, the sulfa drugs — either directly or through their metabolites can oxidize the iron of hemoglobin to the ferric state. In most people, fortunately, the enzymes of the cell can regenerate hemoglobin by reducing the ferric iron. However, there exists a substantial population in which this process functions poorly. Infants do not have fully developed methemoglobin reducing systems. Drinking water standards for nitrate nitrogen take cognizance of this. There are persons who by reason of genetic defects are inordinately susceptible to methemoglobinemia from contact with a variety of common drugs and chemicals such as naphthalene, sulfas, anti-malarials, nitro and amino compounds. The frequency of such individuals is relatively high (ca. 10%) among American Negroes). Its significance in industrial hygiene and its relation to glucose-6-phosphate dehydrogenase deficiency has been noted in the literature. A smaller frequency of the population are known to exhibit hemoglobin variants — sickle cell, type M, Portland, etc. These variants arise from substitution of different amino acids in the molecule. The variant hemoglobins lack durability. Chemical stress may cause irreparable damage, with anemia resulting. Lists of substances known to induce methemoglobinemia and hemolytic anemia appear in the references.

Other Proteins

As in the case of hemoglobin, the three-dimensional structure of the vast array of proteins found in the body is determined by the sequence of the amino acids of their primary structure. The extent of their helical structure, charge interaction, cross-linking and other secondary and tertiary structural characteristics flow from this factor. In general, the proteins dissolved in the body fluids are globular in shape. A notable exception is the plasma protein fibrinogen which is long and narrow. As in the case of hemoglobin, its structure is in keeping with its function in blood clotting. The straw-shaped fibrinogen molecule readily forms a mat to trap the formed elements of the blood to form a clot at the bleeding point.

Other proteins of the blood plasma include albumin, the major constituent of the plasma proteins. Albumin provides a regulatory influence on the fluid balance of the blood through its osmotic effect. The globulin proteins of the plasma in general provide the potential for immunologic defenses. Upon exposure of the organism to antigenic foreign proteins or small molecules linked to protein (haptens) the immune defenses of the body produce a protective protein antibody able to react and neutralize the antigen. The globulin fraction of plasma proteins, especially the gamma globulins, provide this defensive capability. Structural proteins, such as collagen in connective tissue, are generally long and linear. Collagen is a triple-stranded counter-twisted triple helix. Keratin of skin, hair and nails is constructed of single peptide chains of alpha helices counter-twisted

into bundles of triple chains. This structure provides both strength and flexibility.

A deficiency of the serum protein, α-1 antitrypsin, has been found in some persons with chronic respiratory disease. The deficiency has been correlated with emphysema and a genetic dependence established. Experimental evidence indicates proteolytic enzymes released from injured cells may exacerbate the damage unless suppressed by antitrypsin. Studies of coal miners have yielded controversial conclusions. The frequency of antitrypsin deficiency, which is highest among persons of North European ancestry, makes it a factor to consider in investigations of emphysema and respiratory diseases. The measurement of serum antitrypsin is routinely carried out in many clinical laboratories, although usually in relation to other diseases.

Enzymes

Enzymes, the catalysts of the body, are also proteins. As for other proteins their three dimensional structure or conformation is the consequence of the sequence of the amino acids in their primary structure. The ordered geometry of the enzymes in space provides specific sites at which the substrate molecules upon which they act may become fixed. As a consequence of the localization of the substrate at the active site of the enzyme the energy required to initiate a subsequent reaction is decreased. This decrease in activation energy means that a larger fraction of the molecules will have sufficient energy for reaction even at body temperature, as compared to relatively extreme conditions of pH and temperature required for similar reactions in vitro. The reaction rate will consequently increase. Enzymes thus increase the speed of the reaction. Nearly any influence which changes the shape of the enzyme molecule will influence its ability to function as a catalyst. Modifications in the *hydrogen ion concentration (pH)* of the environment will influence the charge distribution of the enzyme surface and may thus alter the shape of the enzyme or modify the ability of charged substrates to approach and be affixed to the active site. Characteristically enzymes are found to be most effective at an optimal pH. Other influences such as the concentration of charged particles in the medium may also influence the enzyme surfaces. Thus the *ionic strength* of the solution is significant. The *ambient temperature* is important because with increasing temperature the substrate molecules have increasing energy. More of the molecules are capable of reacting and the rate of the reaction increases. On the other hand the enzyme protein also is susceptible to the influence of an increase in temperature and may be inactivated (denatured) with loss of catalytic capacity. The positive and negative effects balance at a point of optimal temperature. For many enzymes this is body temperature.

The catalytic role of enzymes is critical in the performance of metabolism. Factors which influence their rate of reactivity markedly alter body function. The *availability of substrate molecules* is clearly a limiting consideration. When substrate molecules are present in such high concentration

that they continuously occupy all the active catalytic sites of the enzyme the reactions may proceed at *maximal velocity* (V_{max}). On the other hand, the removal of substrate by alternative competitive pathways of reaction, or the presence of molecules in the medium which compete for the active catalytic site may slow a given enzymatic reaction in a sequence of reactions to the point at which it becomes the rate limiting step in an overall metabolic process. Other forms of inhibition are known. When, for example, a component of the medium other than the substrate can attach to the enzyme surface in such a way as to alter the configuration of the active site, it may simultaneously decrease the catalytic activity of the enzyme. This *"allosteric inhibition"* provides a mechanism for the control of enzyme activity and with it a method of process control in the cell. An additional control mechanism involves the accumulation of the products of a given reaction or sequence of enzymatic reactions. Since many systems operate at initial and final energy levels which are not widely separated, the pileup of product may be sufficient to slow or halt the progress of the reaction. This *feedback inhibition* can be exerted at a single enzymatic step or in a chain of reactions.

The structure of an enzyme and hence its catalytic activity may be modified by other influences. In the body these proteins are subjected to a continuous process of breakdown. This may occur by oxidation and hydrolytic scission (proteolysis) of the peptide chains.

External influences such as the presence of toxic metals in the body can interact with active catalytic enzyme sites or react elsewhere with the molecule to render it ineffective. Interruption of the function of a critical enzyme can have overwhelming toxic effects for the organism.

A classic example of this, and of so-called "lethal synthesis," occurs when fluoracetate is metabolized in the citric acid cycle (Figure 5-2): fluocitrate is formed, which bonds irreversibly to the enzyme aconitase, rendering the system inoperable.

While many enzymes function as a single protein entity, a number require the presence of other co-factors and activators. The vitamins, especially those of the B-vitamin group, are in this category. Thiamine, biotin and lipoic acid are co-enzymes frequently associated with enzymes required for the addition and removal of carbon dioxide from substrates. Nicotinamide, riboflavin, and ascorbic acid have roles in energy transfer associated with oxidation/reduction reactions. Pantothenic acid is an essential component of the enzyme complex, coenzyme A, involved in the metabolism of acetyl groups. Vitamin B-12, cyancobalamin, has the trace metal cobalt as an integral portion of its structure and is a component of the enzyme system which is concerned with the metabolic handling of a one-carbon unit. Folic acid, another member of the B-vitamin family, has a related function.

In the absence of an adequate nutritional supply of the vitamins, the function of the enzymes of which they are components is severely compromised. The resulting metabolic malfunction
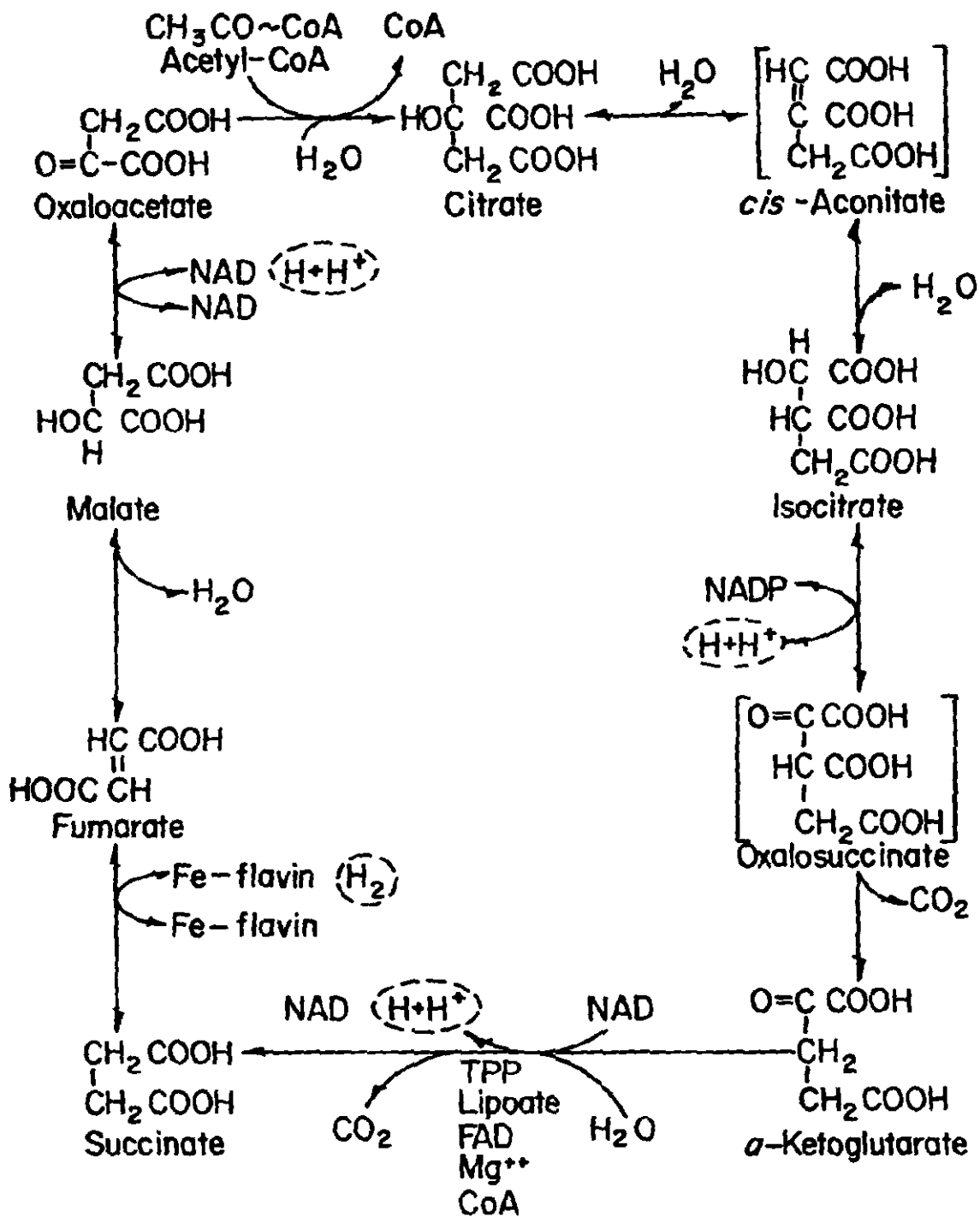
Figure 5-2. Steps in the Tricarboxylic Acid Cycle

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finds expression many ways. Tiredness, lack of energy, anemia, loss of weight, loss of appetite and a host of subclinical and, in the acute stages, overt clinical manifestations can occur.

Cobalt has been mentioned as a component of the total enzyme systems involved in the metabolism of single carbon units. Other metals fulfill related functions as enzyme co-factors. Calcium and magnesium have prominent roles in hydrolytic reactions. Copper, iron and molybdenum serve for the purposes of oxidation/reduction systems. Zinc occurs in hydrogen transport enzyme systems, in the hydration of carbon dioxide to form carbonic acid and, as does manganese, in enzymes involved in the cleavage of peptide bonds. With the exception of calcium and iron, nutritional deficiencies are rare for these trace elements. It is generally the fact that the intestine provides a barrier to the excess accumulation of the metals. On the other hand the nutritional intake of iron and calcium may be marginal for some portions of the population. Women during the childbearing years are subjected to continuous iron loss in menstrual bleeding. Their nutritional replacement of this loss is frequently insufficient to maintain homeostasis. At times of rapid skeletal growth in children, during periods of lactation and in older age for both women and men the usual intake of calcium may also be marginal or insufficient.

Types of Enzymes. In the course of the previous discussion some examples have been cited of the types of reactions catalyzed by enzymes. A systematic grouping on this basis would include the 1) *oxidoreductases*, enzymes concerned in oxidation/reduction reactions 2) *the transferases*, enzymes which bring about the movement of a molecular grouping from a substrate to a recipient 3) *the hydrolases*, which are involved in cleavage of bonds by the addition of the elements of water 4) *the lyases*, a group of enzymes whose function is the cleavage of a segment of a molecule 5) *the isomerases*, which catalyze the rearrangement of the molecular framework and 6) *the ligases*, which bring about the combination of molecular structures by covalent linkage.

The isolation of enzymes of distinctive structure but which perform the same catalytic function is a subject of considerable interest. These *isoenzymes* by virtue of their variable cellular distribution and modified responsiveness to controlling mechanisms, are able to provide enhanced modulation of the complex integration of the reactions which occur in metabolism. The fact that organs of the body and consequently the tissues and cells of which they are composed may have specialized functions is a matter of considerable import for the diagnosis of disease. When liver tissue is destroyed as in acute hepatitis, the breakdown of the liver cells releases cellular enzymes to the plasma. By measuring the plasma enzyme activity of the liver transferases such as glutamic-pyruvic transaminase and the liver dehydrogenases such as lactic dehydrogenase, it becomes possible for the physician to obtain a biochemical index of the cellular destruction. Muscle tissue damage, as in acute myocardial infarction or in the wasting diseases of muscular dystrophy can be monitored

by the activity level of the phosphate group transfer enzyme, creatine phosphokinase. Differentiation between damage to the liver or the heart can be made by the identification in plasma of the respective lactic dehydrogenase isoenzyme.

Because of their essential functions the enzymes are vulnerable points of attack by external influences. Toxic agents from the environment as well as drugs used for diagnosis and therapy can induce marked changes in the entire organism by alteration of the rates of enzyme reactivity.

Measurement of the activity of the enzyme cholinesterase has been very useful in surveillance of exposure to anti-cholinesterase insecticides. The enzymes in the blood, like that in the nervous tissue, split acetyl choline into acetic acid and the base, choline, but other substrates may be used in following the reaction. Depression of enzyme activity below normal is an indicator of response to the agent.

In other cases serum enzymes may be increased, rather than depressed, as a result of toxic injury to cells or organs. There are five isoenzymes of lactic dehydrogenase occurring in serum. These may be separated by electrophoresis, using standard clinical laboratory equipment. They are designated by number according to their rate of migration, and their proportions reflect, in large degree, their tissue of origin. The heart contributes much of LDH isoenzyme No. 1, the liver mostly LDH isoenzyme No. 5. Recent work on animals, and a few cases of mercury exposure of workmen suggests that an increase of LDH isoenzyme No. 5 over normal proportions may serve as an indicator of liver injury from exposure to inorganic mercury.

Protein Synthesis

Under normal conditions the continuous breakdown of protein is matched by protein biosynthesis. The individual stays in nitrogen balance in that nitrogen constituents provided in the diet are matched by nitrogen excretion in feces and urine. During periods of growth the increase in cell mass requires that protein synthesis be accelerated. More nitrogen is retained than is excreted. The individual is in a state of positive nitrogen balance.

Whether for replacement or for growth the continuous need for protein synthesis is met by an exquisitely coordinated mechanism in the cell. The problems to be solved are formidable. The flow of requisite structural components, the amino acids, must be maintained and controlled in the cell environment. The transfer of the amino acids from the plasma across the cell wall requires a specific transport mechanism and a supply of energy. Once within the cell the amino acids need to be selected and arranged in the proper sequence so that when final linkage takes place between their adjacent amino and carboxyl groups, the resulting polypeptide chain will have the exact sequence requisite for its biologic and biochemical function. To provide an inkling as to the dimensions of the problem, consider that thousands of proteins of specific structure may be required, that variation in sequence of amino acids may result in uncountable structural modification, and that

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the initiation and termination of the synthetic events must be completely controlled if the cell is to avoid death by atrophy or by the uncontrolled overgrowth of cancer.

The somewhat more than two dozen individual amino acids required for protein synthesis have their original source in the dietary intake. When ingested in the form of food proteins, they are cleaved in the intestine by the proteolytic enzymes of the pancreas, (trypsin, chymotrypsin and an array of peptidases) to the constituent amino acids which are then actively absorbed across the intestinal wall into the plasma. While the processes of metabolic transformation can convert most of the amino acids from one structural form to another, a number can be provided only from food sources. These "essential amino acids" include valine, methionine, threonine, leucine, isoleucine, phenylalanine, tryptophan and lysine. For adequate nutrition a protein intake of between 1 to 2.5 g/kg/day from a variety of foods including meat, eggs, milk and plant sources is considered requisite. The lower value provides for normal tissue replacement in the adult while the higher value is needed for the rapidly growing infant.

Following absorption from the intestine, the amino acids circulate in the plasma for utilization directly in cellular protein synthesis or metabolic conversion. The uptake of amino acids by the cell involves their specific "active transport," an energy requiring process, across the cell membrane. While the detailed mechanism of membrane transport is not clarified, it is established that in some instances the process is controlled by an initial attachment of hormones to specific receptor sites on the cell membrane. This triggers the subsequent events which bring about the cellular synthesis of proteins.

Blueprints for Proteins

Protein synthesis starts with the stimulation of the cell nucleus to read an appropriate portion of its stored genetic information in its macromolecular double-stranded desoxyribonucleic acid (DNA) and produce from this template a messenger ribonucleic acid (mRNA) which will serve as the information source for protein synthesis in the cell cytoplasm. The mRNA moves from the nucleus to the cytoplasm and affixes to the ribosomes located in the fine structure of the cell sap. At this point the amino acids of the cytoplasm are selectively activated using available energy from the "high energy" chemical bond of adenosine triphosphate (ATP) to attach to a carrier transfer ribonucleic acid (tRNA). The activated amino acid is then delivered to the ribosome where it is attached to the template of the messenger RNA. Depending upon the nature of the code of the RNA the various activated amino acids are tied to the ends of the growing peptide chain to form the linear peptide. The tRNA, having delivered its specific amino acid, returns to the cytoplasm for reloading of an amino acid. The processes which signal the start of protein synthesis and its completion at the end of the peptide chain are not clearly understood as yet for ·mammalian cell systems. It appears though that a regulatory code provides for the start and stop signal of protein biosynthesis.

The remarkable capability of living things to transmit hereditary information resides in the unique structure of the nucleic acids. Their building blocks are the nucleotides composed of the sequence: base-sugar-phosphate. The bases are derived from the purine and pyrimidine classes of compound by minor functional group modifications (Figure 5-3). The sugars in the nucleotides are either of the ribose or 2-desoxyribose structure with the nucleotide assembly linked by way of a phosphate ester. Not only does the nucleotide serve as a common structural unit in the nucleic acid but also in an isolated unit as a co-factor of many enzyme systems to be later discussed. The linkage of nucleotides to form a strand of nucleic acid is through the combination of a phosphate of one nucleotide to the sugar of a second. In this way the nucleotide bases extend horizontally from the linear chain in the same way that the rungs of a ladder are tied to the frame. In actuality two chains of nucleotides associate with the bases in apposition and are linked through hydrogen bonds. The total system would be approximated by visualizing the rungs of the ladder to be cut in the center of each but held together so that they still had a ladder appearance. A further complication is that the ladder instead of being in one plane is twisted in a right-handed helix. In order for this structure to serve as an information mechanism it unfolds so that a single strand of nucleotides is exposed to the environment. Synthesis of a new strand now takes place by the linear alignment of complementary bases to those of the original strand. The lineup of bases in the newly formed nucleic acid (mRNA and tRNA) provides for the specific ability of the new structure to selectively pick a given amino acid from the environment for protein synthesis on the ribosomal surface. Protein synthesis can be influenced by environmental factors. Inhalation of vanadium pentoxide alters the content of the amino acid cystine in the hair of rats and the fingernails of workmen. The lungs of coal miners with emphysema contain more of the fibrous tissue protein, collagen, than do normal lungs or abnormal, but not emphysematous, lungs. Protein synthesis can be altered or stopped by exposure of man to environmental factors and this can result in enzyme induction or repression, misdirected or uncontrolled protein synthesis. These changes then manifest themselves as clinical changes, lesions or death.

Hormones

Hormones are defined as a class of endogenous compound effective in low concentration in controlling or modifying metabolic processes at a distant receptor. Their activity may be exerted on a target cell to induce metabolic change directly, or they may serve to cause the production of a second hormone which in turn controls cellular function, or a given hormone may have both types of end result. The hormones originating in the pituitary gland in response to "releasing factors" have generally been divided into two groups depending upon their anatomical source. Hormones of the anterior lobe include the gonadal active follicle stimulating hormone (F.S.H.), the luteinizing hor-

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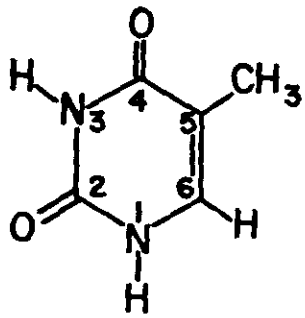
Thymine

PYRIMIDINES
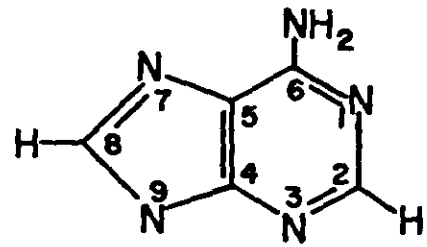
Adenine

PURINES

Cytosine

Guanine

Adenosine Triphosphate

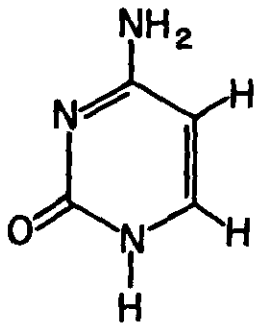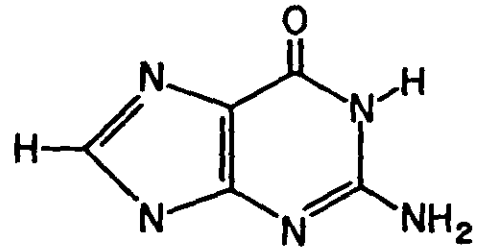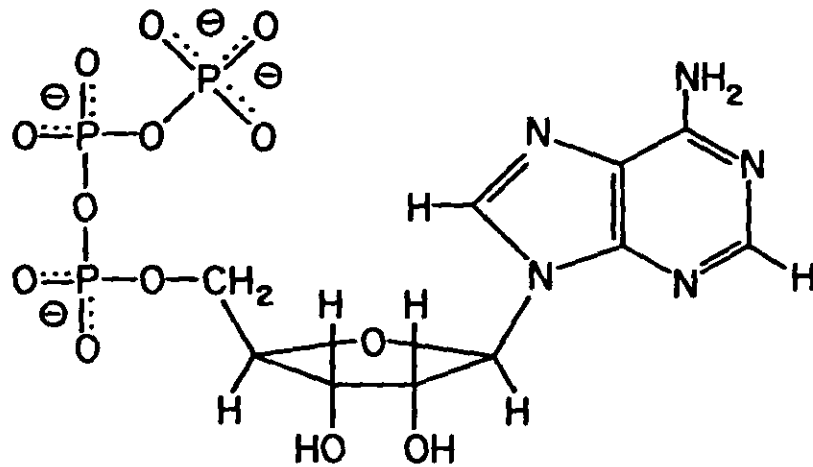Figure 5-3.   Typical Pyrimidine, Purine and Nucleotide Fragment

mone (L.H.), and prolactin whose major effects act directly upon their specific target cells. In addition, the anterior pituitary also produces other protein hormones which may also have more general metabolic effects. *Thyroid stimulating hormone* acts directly upon the thyroid gland to induce the capture of plasma iodide by the gland, its incorporation into a protein thyroglobulin, scission of the protein to yield a second amino acid hormone *thyoxine* which circulates in the plasma partly bound to a carrier protein, thyroid binding hormone. Thyroxine acts as a potent regulator of cellular metabolism inducing a marked increase in the rate of oxygen utilization simultaneously with a sharp increase in cellular metabolism. The *growth hormone* of the anterior pituitary is especially effective in inducing protein synthesis in early development. Increase in cell mass, development of the long bones and accelerated utilization of carbohydrate are among its noteworthy effects. The primary effect of the *adrenocorticotropic stimulating hormone* (ACTH) is to induce the synthesis of the steroid hormones of the adrenal cortex. Two hormones of the posterior pituitary have regulatory functions. *Oxytocin* and *vasopressin* are peptides of eight amino acids each. The major effect of the former is to cause contraction of smooth muscles. Vasopressin has a significant action on the kidney inducing salt and water retention.

Although several dozen intermediates and steroid metabolites have been isolated from the adrenal cortex, two compounds represent the major hormonal products of the gland. *Cortisol (hydrocortisone)* is elaborated upon the stimulus of ACTH by a biosynthetic pathway which starts with the two-carbon acetate unit. Successive combinations of three such units lead to a six-carbon intermediate which is then degraded to the five-carbon isoprenoid structure. Condensation of three five-carbon units leads to the C-15 farnesol moiety which in turn doubles to form the 30-carbon linear squalene structure. It is of interest that these intermediates in the pathway of mammalian biosynthesis also occur in the plant world and lead to the familiar essential oils. Cyclization of squalene produces the condensed four-ring structure of the steroid nucleus and degradation of the side chain produces the C-27 sterol, cholesterol. When the nutritional circumstances of the individual provide a greater supply of C-2 acetate units than can be utilized for energy or biosynthetic turnover the excess is converted into fats, including cholesterol. The combination of excessive lipid intake, especially saturated fats, and a sedentary and stressful life style is associated with high concentrations of cholesterol in the plasma and with atherosclerotic plaque deposition in the vascular system. Individuals in this category are high risk possibilities for coronary disease.

Although not universally accepted, some evidence suggests that carbon monoxide and carbon disulfide may elevate cholesterol and promote plaque formation. Vanadium compounds have been found to inhibit cholesterol synthesis in animals and man; however, after some time the orig-

inal effectiveness disappears. The additional metabolic degradation of cholesterol in the adrenal gland produces *cortisol*. This steroid hormone provides the stimulus for a biochemical response to stress. It induces conversion of amino acids to glucose and stimulates the adipose storage areas of lipids to release fatty acid for transport to the liver for utilization as an energy source. The second major steroid hormone of the adrenal cortex is *aldosterone*. The role of this hormone is to assist in the control of the excretion of salt and water. When the adrenal is destroyed as in Addison's disease, the accelerated loss of salt and water can have rapidly fatal consequences. Other steroid hormones include *progesterone* produced by the corpus luteum and the placenta in pregnancy to maintain the uterine cellular structure, *estradiol* formed in the ovary and responsible for the development of secondary female sexual characteristics and *testosterone*, the sex hormone in the testes responsible for analogous processes in the male. Mention should be made of the important hormone *epinephrine* of the adrenal medulla. It is another means for biologic response to stress in that its production and distribution in response to a neural signal causes constriction of the blood vessels with increase in blood flow, release of glucose from the liver to the plasma and fatty acid from the lipid stores. All these responses provide an added capability to meet emergency contingencies. Several other glands provide significant factors for metabolic control. The pancreas is the source of the protein hormones, *insulin* and *glucagon*, which exert a direct control over the glucose level of the blood. Insulin is released from the pancreas upon elevation of blood sugar after a meal or for other reasons. By incompletely understood mechanisms the hormone accelerates the transfer of the sugar from the circulation to the cells where it may be stored as the polymer glycogen until required. Glucagon on the other hand, is elaborated when blood sugar levels fall below the normal range. Its major biochemical effect is to cause breakdown of glycogen and release of glucose to the circulation. *Parathyroid* hormone formed in the parathyroid gland and *thyrocalcitonin* a product of the thyroid gland are involved in the maintenance of calcium homeostasis. In response to a decrease in the normal circulating level of calcium, the released parathyroid hormone produces a sequence of biochemical responses whose net result is to elevate the concentration of plasma calcium. Hormone induced breakdown of bone cells provides calcium and simultaneously phosphate which is cleared by the kidney by hormone induced phosphaturia. Secondary conservation of calcium occurs at the kidney simultaneously with increased absorption at the intestine. An elevation of plasma calcium is followed by increased secretion of thyrocalcitonin producing enhanced deposition of the element on the skeletal surface and fall in circulating calcium levels. The biochemical balancing of the two hormones provides a fine adjustment for homeostasis of plasma calcium which is basically maintained by interaction of plasma calcium with the mineral reserves of the bone. For these hor-

mones and most others described above recent investigations have established a common sequence of events leading to their biochemical consequences. At the target cell hormone specific receptor sites on the cell membrane are stimulated to activate the membrane enzyme adenyl cyclase. In turn the enzyme converts adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP) which in the intercellular milieu initiates the cellular events characteristic of the hormonal response. The formulation of this "second messenger" concept has provided a framework for further study of the intriguing question of the mechanism of the profound effects of trace amounts of hormonal substances. In essence the overall picture is one of an amplification system in which a trigger mechanism provokes a significantly enhanced response. An interesting correlative change that occurs with the membrane fixation of most hormones is the release and cellular uptake of calcium ion. This event has been invoked to explain the electrical changes observed in the membrane upon hormonal stimulus.

Hormone production or function is known to be altered by some metals and organic compounds.

Lead interferes with thyroid hormone production; the synthesis of epinephrines depends on adequacy of copper for an amine oxidase. It has been reported that a deficiency of norepinephrine was associated with treatment for alcoholism with antabuse; this compound is known to bind to copper. Chromium has been found to be an essential element, notably for its role in glucose metabolism. It is apparently an adjunct to insulin. Some elderly diabetics have been benefited by administration of chromium.

The Heme Porphyrin Structure

While the globin protein serves as the structural framework, it is the iron-porphyrin combination which is responsible for the molecular transport of oxygen. At the time of synthesis of hemoglobin in the young red cell all components, the globin protein, the porphyrin structure and the iron atom must be at the right place at the right time.

The biosynthesis of the porphyrin molecule starts with the amino acid glycine which couples with activated succinic acid in the presence of an enzyme catalyst to yield, after elimination of CO_2, delta aminolevulinic acid:
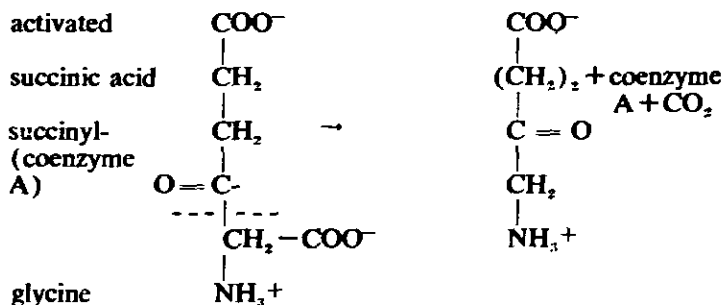
Two molecules of delta aminolevulinic acid join asymetrically to form porphobilinogen in the presence of an enzyme catalyst:
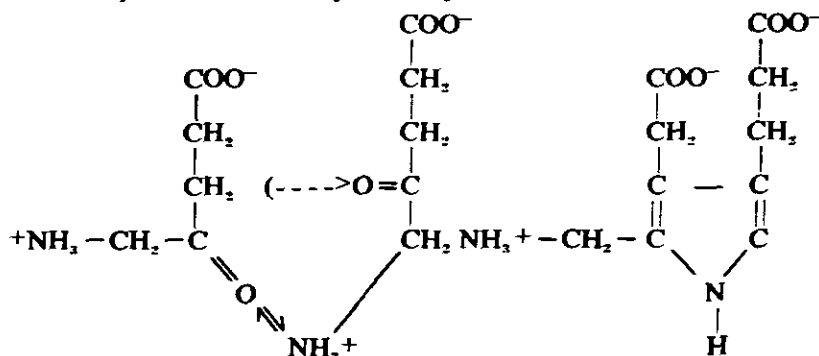
and four molecules of porphobilinogen link in linear fashion and then ring close to form the important intermediate structure uroporphyrinogen. By selective loss of carbon dioxide and hydrogen atoms, this is converted to the essential structure protoporphyrin IX (Figure 5-4).

Insertion of the iron atom completes the assembly of the heme structure for junction with the globin to form hemoglobin.

Porphyrin Chemistry

Certain aspects of the heme structure are of special significance to its oxygen transport func-

tion. The planar structure of the molecule permits it to slip readily into the cavity of the globin protein. It is held there by several forces. The anionic charges of the porphyrin carboxyl side chains are linked to points of positive charge in the globin protein. The conjugated double bonds of the porphyrin confer aromatic character upon the structure with consequent availability of pi electrons. These interact with analogous aromatic structures strategically located in the cavity wall. The iron atom, linked by coordinate bonding to four nitrogen atoms of the porphyrin has two coordinating bonds available for additional linkage. One is
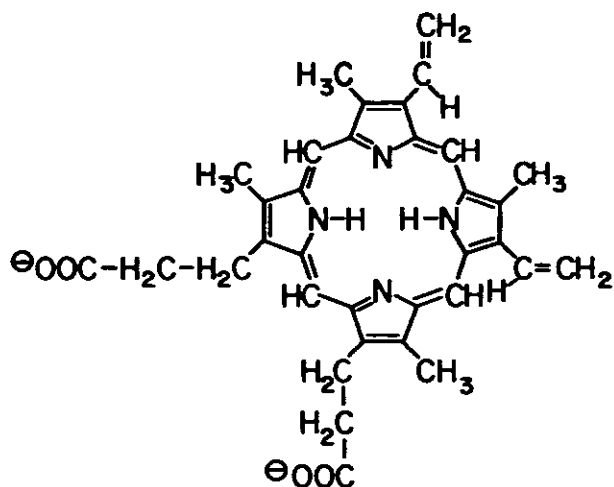
Figure 5-4. Structure of Protoporphyrin IX

utilized for linkage to a histidine amino acid of the globin structure and the sixth linkage is available for the reversible binding of oxygen.

In addition to the heme structure of hemoglobin, porphyrins are essential in the processes of energy production. In the cytochrome molecules, they perform the task of electron transport from one energy level to another. Subtle changes in the structure, as by conversion of the $-CH=CH_2$

$$\text{side chains to} \quad \overset{\overset{\displaystyle H}{|}}{\underset{\overset{\displaystyle |}{OH}}{-C-}} CH_3, \text{ and alterations in the}$$

structure of the combining protein serve to convert the molecule from its role of oxygen to electron transport.

Breakdown of the porphyrin structure involves cleavage of the ring to form a linear *tetrapyrrole* followed by scission of the tetrapyrrole to a dipyrrole structure. The accompanying color changes of the molecules provide the green pigment of bile and then the clay brown pigment of feces. When liver damage or obstruction inhibits the catabolism or excretion of the bile pigments, they appear in the blood and skin as the yellow color of jaundice.

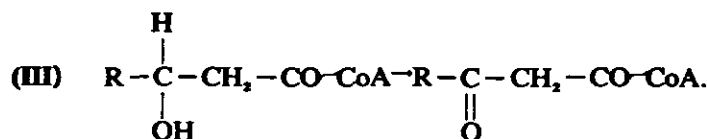Abnormalities of porphyrin metabolism are common to a number of industrial health problems. Delta aminolevulinic acid accumulates in urine in lead poisoning and special chromatography columns are commercially available for assaying its urine content. Lead also increases the urinary coproporphyrin; this substance was once regarded as criterion for lead poisoning. As noted, accumulation of porphyrin waste products may cause porphyria or bilirubinemia unless the liver functions to convert them into less toxic, excretable compounds. The induction of liver enzymes, by DDT, to enhance bilirubin detoxication, has been noted.
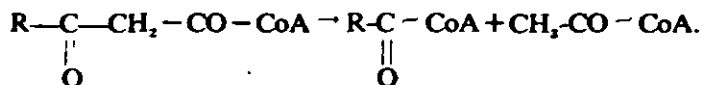
Metabolism of Lipids

While carbohydrate and lipid can largely replace each other in the human diet some lipid appears essential to supply not only dietary palatability but also the highly unsaturated fatty acids that can not be produced by metabolic interconversions. The high caloric value of lipids also makes this class of nutrient a valuable energy storage reservoir. Upon ingestion the lipids along with other dietary nutrients are emulsified in the stomach and pass to the upper intestine where the bile acids, originating in the liver by catabolism of cholesterol, assist in the stabilization of the dispersed nutrients. The dietary lipids are then cleaved by the pancreatic lipases to yield fatty acids as well as mono and diglyceride scission products of the nutrient triglycerides. In the mucosal cells of the intestine a separation and reshuffling of the lipid constituents takes place. Short chain fatty acids proceed by way of the portal circulation to the liver while the longer chain fatty acids are resynthesized into triglycerides. Dietary cholesterol is esterified to a great extent with unsaturated fatty acids during intestinal cellular transport. The lipids which move into the lymphatic circulation after absorption do so in the form of small droplets called chylomicrons. These are stabilized by a coating of protein which inhibits their tendency toward agglomeration. The lymphatic drainage is discharged into the circulation at the thoracic duct. Lipids are then picked up by adipose tissues or are metabolized by the liver. The course of lipid metabolism in the liver or peripheral tissues involves a process of sequential degradation by which the fatty acid chains are reduced two carbons at a time to yield acetyl coenzyme A. This common catabolic end point serves as the primary fuel source of the cell. The mechanism by which the catabolic sequence occurs is of some interest. In the first step the fatty acids are activated by the use of ATP bond energy to form their acyl thiol coenzyme A esters. In this form they are dehydrogenated to yield the alpha-beta unsaturated compounds (Reaction I).

(I) $R\text{-}CH_2-CH_2 CO\text{-}CoA \rightarrow R-CH=CH-CO\text{-}CoA$

and the reduced flavin nucleotide. The latter compound feeds into the mitochondrial electron transport system for capture of the available energy in the form of the ATP high energy bond.

In a second step a hydrolase enzyme adds a molecule of water across the double bond to produce a beta hydroxyl acyl derivative (Reaction II).

(II) $R\text{-}CH=CH\text{-}CO\text{-}CoA + HOH \rightarrow R-\underset{\overset{\displaystyle |}{OH}}{CH}-CH_2-CO\text{-}CoA.$

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In the third step the hydroxyl group is oxidized by a dehydrogenase enzyme with a nicotinamide co-factor to produce the corresponding keto acid (Reaction III).

$$\textbf{(III)}\quad R-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle OH}{|}}{C}}-CH_2-CO-CoA \rightarrow R-\overset{\overset{\displaystyle }{\|}}{\underset{\underset{\displaystyle O}{}}{C}}-CH_2-CO-CoA.$$
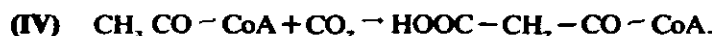
The reduced nicotinamide co-factor proceeds to surrender the hydrogen to mitochondrial oxidation for additional energy capture as the ATP bond.

In a final step of the process the fatty acid is cleaved to yield a molecule with two less carbon atoms with the simultaneous formation of an acetyl coenzyme A compound.

$$R-\overset{\|}{\underset{O}{C}}-CH_2-CO-CoA \rightarrow R-\overset{\|}{\underset{O}{C}}-CoA+CH_3-CO-CoA.$$

The acetyl coenzyme A produced in this reaction feeds into the tricarboxylic acid (TCA) cycle. The residual fatty acid derivative is ready for further degradation by another two carbon units. The reverse process of fatty acid synthesis accounts for the fact that nutritional excesses can convert dietary constituents to fat. Acetyl co-enzyme A by mediation of a biotin cofactor enzyme temporarily adds a molecule of carbon dioxide to form malonyl coenzyme A. (Reaction IV).

$$\textbf{(IV)}\quad CH_3CO-CoA+CO_2 \rightarrow HOOC-CH_2-CO-CoA.$$

This in turn adds a second acetyl coenzyme A unit, loses carbon dioxide and ends as a C-4 keto acid, CH_3-CO-CH_2-CO CoA. By reversal of previous reactions, essentially, the body produces a C-4 fatty acid, butyric acid, CH_3-CH_2 — CH_2 — CO CoA. Repetition of the procedure results in chain elongation to form the longer chain acids. It is interesting to note that the process of fatty acid anabolism is not identical with the catabolic route. This difference provides the organism with the advantage of multiple points of metabolic control. The final step in the synthesis of triglyceride is the addition of the activated fatty acid to phosphorylated glyceride to form the final product. In addition to the storage of lipids in adipose tissue the materials of this category have an essential structural role in cell membranes. The generalized structure of these compounds consist of a diglyceride coupled through a phosphoric ester to a nitrogenous constituent. In the formula R — PO_3^- CH_2 CH_2 NH_3^+ as for cephalin for example, the structure has a highly hydrophobic fatty diglyceride, R, head with a charged ionic polar nitrogenous tail. The net result is that the molecule orients itself in an aqueous medium with the polar group in the water phase and the lipid structure oriented in the opposite direction. When combined with cholesterol and proteins these phospholipids provide the structure of the cell membrane which allows for remarkable specificity and selectivity for the passage of small molecules. Modification in structure by substitution on the nitrogen atom provides for the multiplicity of the class of phosphatides. Attachment of the carbohydrate inositol yields the inositides.

Substitution of the glyceride fatty acid by aldehydes yields the family of plasmalogens.

The prostaglandins are an interesting family of lipid substances. Although fatty acids, chemically, they are tissue and cell hormones functionally.

The analgesic effects of aspirin are ascribed to its inhibition of prostaglandin synthesis.

Metabolism of Carbohydrates

Dietary sugars and starches provide most of the carbohydrate in human nutrition. The starch macromolecule is hydrolyzed in the intestine by the pancreatic enzyme amylase. After cleavage to glucose this monosacchoride and others present in the food are absorbed across the mucosal surface of the intestine. For the most part the hexose sugars such as galactose and fructose are converted to glucose either during absorption or subsequently in the liver. After transport through the portal blood from intestine to liver the glucose is either utilized in the liver as an energy source, polymerized for storage as glycogen or proceeds through the peripheral circulation as part of the glucose supply to the tissues. Two major pathways characterize the catabolism of glucose. The first is the process of glycolysis by which glucose is converted anaerobically to pyruvic acid or further to lactic acid. The second alternative sequence, the pentose phosphate shunt, is an aerobic degradation of glucose which subserves certain specialized needs of the organism.

Glycolysis starts with the energy requiring phosphorylation of glucose to yield its 6-phosphate. Rearrangement of the molecule by enzymatic isomerization yields fructose 6-phosphate which is further phosphorylated to produce fructose-1, 6-diphosphate. This latter reaction is also endogenous in its requirement for an energy source. After phosphorylation at the ends of the molecule, scission takes place in the center to produce two phosphorylated C-3 units, phosphoglyceric aldehyde and phosphodihydroxyacetone. Enzymatic isomerization converts the latter to the former compound. In essence, then, one six-carbon sugar is converted to two three-carbon sugars. In the next stage of glycolysis the aldehyde group is converted to an acid with some of the
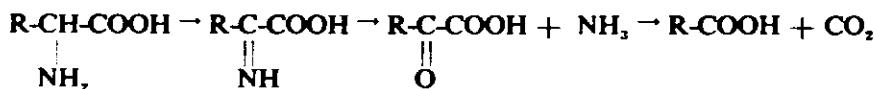
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released energy trapped in the form of the reduced nicotinamide co-factor. In turn, the reduced co-factor feeds into mitochondrial oxidation to provide usable energy in the form of the high energy ATP bond. The glycolytic process continues with shift of the phosphate from its position at the end to the center of the molecule. The 2-phosphoglyceric acid loses a molecule of water to produce the enol phosphate which in turn relinquishes the phosphate to produce pyruvic acid. Under the usual conditions of oxygenation the pyruvic acid is oxidatively decarboxylated to yield carbon dioxide and acetyl coenzyme A. During vigorous muscular exercise the pyruvic acid is reduced to lactic acid which is released to the plasma for return to the liver. The glycolytic sequence thus starts with a six-carbon sugar, glucose, traps some of the decrease in free energy in the form of metabolically useful reduced co-factor or in the bond energy of ATP and provides four of its six carbons as acetyl coenzyme A for further metabolism.

The second major pathway for metabolism of glucose is oxidative, requiring oxygen for the first step. An enzyme, glucose-6-phosphate dehydrogenase, utilizing nicotinamide-adenine dinucleotide phosphate, (NADP), as a co-factor converts the glucose-6-phosphate substrate to the corresponding acid. The resulting decrease in free energy from the starting material to the reaction products is partly held in the form of the reduced nucleotide to be utilized elsewhere in the body for anabolic purposes, for example, the reductive steps involved in lipid biosynthesis depend on the availability of NADPH. Decarboxylation of the gluconic acid produces a five carbon pentose sugar. In an intricate series of recombinations and scissions the five-carbon pentose is eventually degraded to carbon dioxide. In contrast to muscle tissue which metabolizes glucose almost entirely by the Embden-Myerhof glycolytic path, the oxidative sequence of the pentose shunt occurs in other cells, especially liver and erythrocytes, as an alternative although less significant mode of carbohydrate breakdown. The significance of this pathway is that it offers a means for the body to provide the pentoses needed for nucleic acids, yields NADPH needed for a number of anabolic tasks, and offers an alternate means for interconversion of carbohydrates as well as the breakdown of glucose. The reversal of carbohydrate breakdown can occur from any metabolite which is convertible to pyruvic acid. Such possible sources include the lipids and the proteins. Thus most foodstuffs can ultimately yield storage carbohydrate in the form of liver and muscle glycogen. At some points in the glycolytic and glycogen synthesizing pathways the reaction energetics is highly unfavorable for anabolism. At such points alternative steps circumvent this problem. For reversal of glycolysis one such step is the conversion of pyruvic acid to phosphoenolpyruvate. The problem has been solved by addition of carbon dioxide to pyruvate, to form oxalacetate followed by conversion of the ketoacid to phosphoenolpyruvate in a coupled reaction. While the breakdown of glycogen to glucose-1-phosphate is catalyzed by the complex group of phosphorylase enzymes the synthesis of the storage polymer follows an alternative pathway. Glucose-6-phosphate is coupled to the nucleotide uridine which serves as a carrier of the saccharide in the form of uridine diphosphate glucose (UDPG). In this form the glucose is available as well for interconversion to other sugars such as galactose and the amino sugars. The latter form a significant component of the mucopolysaccharides, a complex structural polymer especially of connective tissues.

Protein Metabolism

Amino acids derived from proteins can enter into the mainstream of energy production by elimination of the nitrogen of the amino group and oxidative conversion of the product to a fatty acid derivative. These reactions, which occur primarily in the liver, may be depicted in the following stages:

$$R\text{-}CH\text{-}COOH \rightarrow R\text{-}C\text{-}COOH \rightarrow R\text{-}C\text{-}COOH + NH_3 \rightarrow R\text{-}COOH + CO_2$$

$$\underset{NH_2}{|} \qquad \underset{NH}{||} \qquad \underset{O}{||}$$

The ammonia produced in the sequence is combined with carbon dioxide to yield the excreted waste product urea, NH_2CONH_2 (Cf. Figure 5-5). For the adult on a usual mixed diet approximately 20-30 g/day of urea will be formed and excreted through the kidney into the urine. Uric acid is the end product of purine metabolism in man.

Metabolism of Acetyl Coenzyme A

The conversion of proteins, carbohydrates and lipids to the two-carbon acetyl unit in the form of its coenzyme A combination makes this a focal point of energy metabolism. By means of the tricarboxylic acid (TCA) cycle, the two carbons of the acetyl group are converted to carbon dioxide. The difference in the free energy levels of the acetyl group and its product carbon dioxide is held temporarily by the conversion of the oxidized form of the nicotinamide co-factor (NAD^+) to the reduced state, NADH. The steps involved in the TCA cycle consist of a series of enzymatic condensations, redox reactions, and decarboxylations summarized in Figure 5-2. The net result of the total process is the elimination of the acetyl group in the form of CO_2 and the formation of reduced co-factors for energy trapping in mitochondrial oxidation.

Mitochondrial Oxidative Phosphorylation

Part of the energy released by catabolism is made available to the body in the form of heat. For all other purposes, however, it must be harnessed in a way which will permit its utilization in subsequent coupled energy requiring reactions. This is achieved in the process of mitochondrial oxidative phosphorylation. The oxidation process is controlled by subdivision of energy release into incremental steps. At appropriate points in the reaction chain, energy available from oxidative change is used to couple inorganic phos-
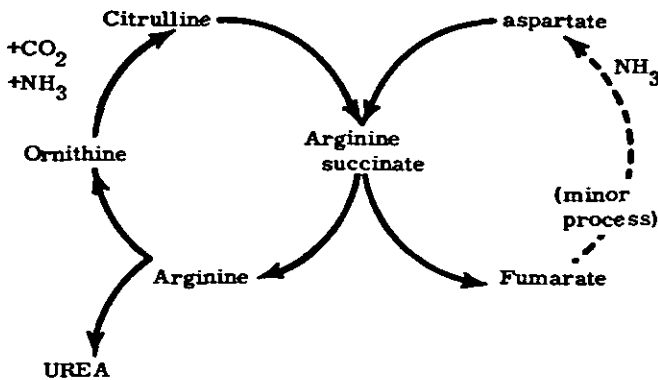
Dawkins MSR, Rees KR: A Biochemical Approach to Pathology. London, Arnold, 1959.

Figure 5-5. Urea Cycle

phate to adenosine diphosphate (ADP) to form the important energy storage form adenosine triphosphate (ATP). The reaction: $ADP + Pi \rightarrow ATP$ requires approximately 8000 Cal/mol to form the phosphate anhydride bond. Conversely when the ATP molecule is coupled in an appropriate enzyme system with an energy requiring reaction it is able to make available the 8000 Cal/mol of its "high energy" phosphate bond. In an appropriate system this energy can be utilized for new chemical bonding, electrical, or mechanical energy.

Steps in Mitochondrial Oxidative Phosphorylation

The free energy change ($-\Delta F'$) represented by the change from reactants to products, can be measured in calories or recalculated in terms of the change in electrode potentials, (E_o'), expressed in volts since the two terms are related by the expression: $\Delta F' = {}^{-}\Delta E'_o nF$ where n represents the number of electrons (or hydrogen atoms) involved and F is the Faraday (96,487 coulombs). From this relation it can be calculated that a difference of 1 volt between the E'_o values when n = 2 represents a change of 46,166 gram-calories. For mitochondrial oxidative phosphorylation the initial redox step at a value of $E'_o = -0.32$ for the system $NAD^+/NADH + H^+$ ends with the reaction $1/2$ O_2/H_2O at a value for E'_o of $+0.82$. The difference between these E'_o values provides a measure of the total potential energy available to the system. In order to capture and utilize this energy, mitochondrial oxidative phosphorylation takes place in discrete steps with a cascading change in the energetic levels of the system.

Step 1: $NAD^+/NADH + H^+$

In the previously described reactions of the TCA cycle the abstraction of a hydrogen atom to-

gether with its electron has been illustrated (Figure 5-2). The acceptance of the hydrogen atom and electron by NAD^+ represents the reduction of the co-factor coupled with the oxidation of the substrate. This change may be viewed in simplistic terms as a transfer of the potential energy of the donor to the recipient, NADH. The electrode potential of the $NAD^+/NADH + H^+$ system is about -0.32 volts as it operates in the cell.

Step 2:

In the second step of mitochondrial oxidation the coupled reaction occurs by which the reduced nicotinamide co-factor, NADH, transfers its hydrogen and associated electron to a riboflavin co-factor, flavin adenine dinucleotide (FAD). The overall paired reactions may be written as follows:

    a) $NADH + H^+ \rightarrow NAD^+ \; 2H\cdot$

    b) $FAD + 2H\cdot \rightarrow FADH_2$
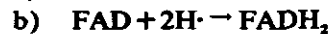
Since the flavoprotein oxidized/reduced couple has an E'_o value of -0.06 volts the reaction represents a difference of -0.26 volts. This change is more than sufficient to provide enough energy to convert adenosine diphosphate and inorganic phosphate to adenosine triphosphate, $ADP + P, \rightarrow ATP$ since the energy requirement is about 8000 Cal, equivalent to 0.15 volts. Thus at this step of oxidative phosphorylation the respiratory chain is able to capture some of the released energy in the form of the reusable high energy bond of ATP.

Step 3:

In the next step of electron transport the reduced flavin nucleotide transfers the hydrogens and associated electrons to the quinoid structure, coenzyme Q. The energy change implicit in the process is only 0.06 volts, insufficient for the formation of a high energy phosphate bond.

    Flavoprotein $H_2 \rightarrow$ Flavoprotein $+ 2H\cdot$

    Coenzyme Q $+ 2H\cdot \rightarrow$ Coenzyme QH_2

Step 4:

From reduced coenzyme Q the electrons are transferred to the iron porphyrin system cytochrome b while the released protons appear in the medium.

    $QH_2 \rightarrow Q + 2$ electrons $+ 2H^+$

    2 cytochrome b (valence $+ 3$) $\rightarrow$

    2 cytochrome b (valence $+ 2$)
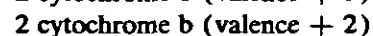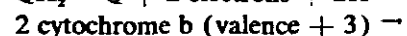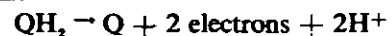
The change in E'_o of approximately 0.26 volts is sufficient to allow for the formation of an additional high energy ATP bond.

Step 5:

Movement of the electrons from cytochrome b to the iron porphyrin cytochrome C_1 involves a change in energy level of only 0.03 volts, insufficient for the formation of an ATP molecule.

Step 6:

The final sequence of mitochondrial oxidation involving the electron transport from cytochrome c to cytochrome a, cytochrome a_3 and finally to oxygen with simultaneous uptake of protons from the medium to form water, is accompanied by a modification in E'_o of a total of 0.73 volts. While this overall change would accommodate the formation of three ATP bonds in fact only one is formed.

The overall process of mitochondrial oxidative phosphorylation, starting with the substrate MH_2 energy source and ending in the transfer of the hydrogen with its electron to form water, provides three ATP high energy bonds of a total seven that are theoretically possible. This is nonetheless a rather efficient mechanism for an energy transducer. One would compare this efficiency of about 43% with conventional systems such as the internal combustion engine or steam turbine and decide that it was rather good.

In summary, the process of energy formation starts with potential substrates from any of the major classes of nutrient proteins, carbohydrates, and lipids. By use of enzyme catalysts, a series of vitamin co-factors, and mineral elements in a synchronized interlocking organized chain, the potential energy implicit in the enzyme substrates is efficiently captured in the form of the chemical bond energy of the ATP molecule for use in energy requiring coupled reactions.

Uncoupling of Oxidative Phosphorylation

Some drugs and toxic agents have the capacity to interfere with the linkage of the energy capturing step of ATP bond formation and the process of electron transport in the mitochondrial electron transport system. As a result the engine continues to run, generates heat, but makes no progress. The transmission has been "uncoupled" from the wheels. As one may anticipate, substrates such as fats are consumed, but ATP bond energy for anabolic purposes is lacking. "Uncouplers" such as dinitrophenol were consequently used for weight reduction many years ago, but have been discarded because of their associated toxicity. Sweating may occur when the uncoupled energy is released, as observed in pentachlorphenol poisoning.

Removal of Wastes

The direct waste products of metabolism are water, carbon dioxide, nitrogen in the form of ammonia and a variety of minor specialized organic catabolites. The excretion of salts is partially regulatory and partially a waste disposal process. The continued processing of all these materials is essential for the functioning of the organism. Water, the major constituent of the body, requires continued input for replacement of the insensible loss of perspiration, in the moisture of the outgoing breath and as a solvent to remove solid wastes by solution in the urine. Control of fluid adjustment by the kidney is achieved by a feedback mechanism triggered by the osmotic pressure of the blood as it flows over the osmorceptors of the kidney and by the sodium content of the blood as it flows through the adrenal cortex. The multiple controlling systems, especially the hormones (particularly aldosterone) of the adrenal cortex and the posterior pituitary hormone vasopressin, integrate the water balance at the kidney level.

The combustion of foods to yield carbon dioxide throws a continuous acid load upon the body. Carbon dioxide, a gas under ambient conditions, is in equilibrium with water to form carbonic acid by the reaction:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$
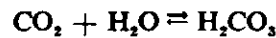
This reaction proceeds slowly under usual conditions but is tremendously speeded in the body by the zinc enzyme of the red cell, carbonic anhydrase. Since the acidity of the blood is carefully maintained at approximately a pH of 7.4, the carbonic acid generated by metabolism is rapidly neutralized to yield the bicarbonate anion, HCO_3^-, which returns to the plasma from the erythrocyte. The hydrogen ion from this reaction is neutralized by the buffers of the blood, notably oxyhemoglobin HbO_2^-, which simultaneously loses oxygen at the tissues and provides for the neutralization of the proton. The reaction $HbO_2^- + H^+ \rightarrow HHb + O_2$ thus simultaneously unloads oxygen at the tissues and provides for the neutralization of hydrogen ion arising from the carbonic acid of metabolism. The process is reversed at the lungs. The oxygenation of hemoglobin forms the stronger acid oxyhemoglobin which in turn liberates a proton for recombination with bicarbonate to form carbonic acid which in turn is converted to CO_2 by erythrocyte carbonic anhydrase to produce the CO_2 exhaled in the expired air. This mechanism for the elimination of carbon dioxide is one of rapid adjustment. The partial pressure (pCO_2) of the blood is constantly monitored by neural receptors which bring about changes in respiration to accommodate to the need for release of metabolic carbon dioxide. One is aware of the slowed breathing of sleep when metabolism is decreased. At such a time the demand for oxygen intake and carbon dioxide elimination is minimal compared to the accelerated breathing during vigorous exercise.

The rapid adjustment of the lungs to acid load is supported by the slower fine modulation at the kidney. One of the major excretory components of the urine is its phosphates. It will be recalled that phosphoric acid has three ionizable groups which function at various points in the acidity scale. The anion pair $H_2PO_4^-/HPO_4^=$ is one which is operative in the maximal acidity range of urine which is roughly from about pH 4.6 to pH close to 8.0. Variations in acid load in the body can be compensated by a mechanism which results in the shift in the phosphate buffer pair by neutralization of the hydrogen ion of the acid. The reaction $HPO_4^= + H^+ \rightleftharpoons H_2PO_4^-$ proceeds to the right and provides a means for the excretion of the acid load in the urine.

The elimination of the nitrogen load of metabolism is essentially by means of its conversion in the liver to urea and excretion of this product in the urine as has been previously discussed. It is clear that damage to the liver will inhibit the detoxification of ammonia through its conversion to urea. Not only does this detoxification mechanism fail with liver damage but so also are other metabolic detoxifications inhibited. Failure of kidney function by damage or disease is equally serious. The accumulation of nitrogenous waste products, azotemia, is usually monitored by measurement of the urea content of the blood. Continued elevation of blood urea offers a poor prognosis of recovery.

BIOCHEMICAL MONITORING

To this point, the authors have presented a body of factual information on the subject of biochemistry. Hopefully, the reader seeks to find how this may be applied to the problems for which he requires solutions.

The first fact that may be obvious from the material presented is that all men are created equal — but different! While genetic categories may be separated, within groups each individual has his own biochemical pattern. This suggests it might be desirable to have a biochemical profile of a worker available for comparison with his subsequent work and medical history. The development of automated procedures in clinical chemistry makes this feasible. An application of biochemical profiling to an industrial health problem is cited in the reading list. Researchers interested in finding sensitive indicators of injury to toxic agents may be intrigued by the pattern depicted in Figure 5-6. This combines data from serum, tissue from lung and adrenals, and leucocyte assays into one picture. Adrenal stress is evident from the elevation of adrenal succinic dehydrogenase: the leucocyte enzymes appear to respond opposite, and to a greater degree, than serum enzymes. One might be led to suspect that leucocyte enzymes may provide a better index of response to injurious exposure than do serum enzymes. Only adequate research will either corroborate or discredit such suspicions. The techniques of leucocyte separation and assays are described in the literature cited.

General monitoring by profiling such as these cases may be useful, but always requires interpretation; a worker may have an alcoholic weekend, or a current infection, and confuse the interpretation.

Monitoring of workers for exposure to metals has been facilitated by the development of the convenient and sensitive methods of atomic absorption spectroscopy.

Assay of cholinesterase activity of blood has been mentioned as useful in monitoring exposure to anti-cholinesterase insecticides. Another type of monitoring involves analysis of urine for the metabolite of the agent to be controlled: for example, measurement of urinary phenol content to evaluate degree of exposure to benzene.

With the prospect that regulatory agencies are aiming at setting biological standards for many organic, as well as inorganic substances, the subject of the next section becomes especially relevant.

DETOXIFICATION PROCESSES

The ability of the body to neutralize potentially damaging materials is remarkable. Heavy metals such as lead are shunted into the skeletal system where they are effectively buried in the bone. This protective process fails when the breakdown of bone, as in fever, may cause a release of the metal in quantity greater than can be handled by the normal slow and low level elimination in the urine and feces. In these circumstances, or when the incoming load is greater than can be effectively han-
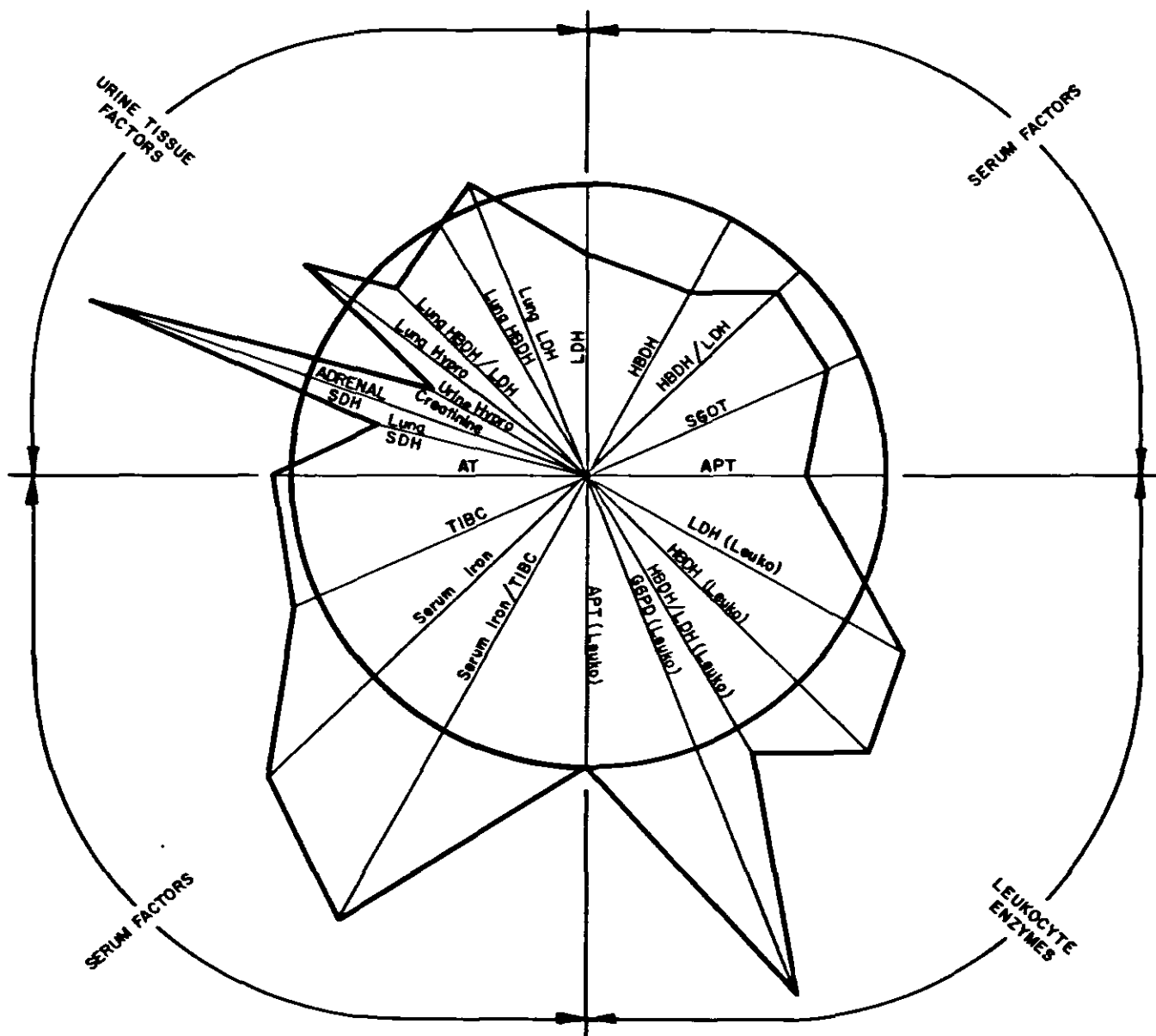
dled, the toxic symptoms of lead poisoning result. In his modification of the environment man has also introduced new factors in the problem. The radio-isotopic elements uranium, strontium and plutonium also are buried in the bone for purposes of detoxication. These elements, however, retain their intrinsic toxicity associated with their continuing radiation. The net long term result is the radiation damage of the surrounding cells and the development of cancer.

Organic compounds are converted, if possible, to forms which can be excreted by the body or are non-toxic. The liver oxidases have a remarkable capability to add an -OH group to otherwise poorly reactive compounds. Aromatic materials such as benzene are converted to phenols. Aliphatic and heterocyclic compounds are hydroxylated to form alcohol derivatives. This mechanism provides a handle by which the organism is further able to convert the compounds to a water soluble product which can be excreted in the urine. Phenols, for example, can then be conjugated with sulfuric acid to form an ethereal sulfate, $ROSO_3H$, derivative. Sulfate derivatives of this kind are readily excreted in the urine. An alternative conjugation is by way of the sugar acids resulting in the formation of a soluble derivative of the form $RO(CHOH)_4 COOH$. Conjugation with amino acids, especially glycine or cysteine, also results in the formation of soluble products that can be cleared through the kidney and eliminated in the urine. Where the toxic agent is susceptible to hydrolytic cleavage, the appropriate enzymes may break them down to their non-toxic component structures. A variety of esterases and proteolytic enzymes are available for the cleavage of amide, peptide and ester bonds. One of the more serious groups of environmental and industrial toxicants is the family of amines, $R-NH_2$. The oxidation of these compounds to aldehydes and acids and their conjugation to more hydrophilic derivatives are frequent modes of their detoxication (Table 5-1). The converse process of reduction, especially of industrial nitro derivatives, $R-NO_2$, provides a mechanism for conversion to more tractable products for elimination.

Despite these ingenious metabolic mechanisms for detoxication, it is clear that the continued pollution of our environment is proceeding with materials in quality and quantity beyond our capacity to handle. Some evidence is available of some increased body burdens of lead and of an accumulation of organic insecticides in our tissues until the last several years. The remarkable biochemical homeostatic mechanisms need help from the technical, political and social efforts which are essential for solution of our critical environment problems.

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URINE TISSUE FACTORS

SERUM FACTORS

SERUM FACTORS

LEUKOCYTE ENZYMES

LDH
Lung LDH
Lung HBDH
Lung HBDH/LDH
Lung Hydro
ADRENAL SDH
Urine Hydro
Creatinine
Lung SDH
AT
APT
HBDH
HBDH/LDH
SGOT
LDH (Leuko)
HBDH (Leuko)
HBDH/LDH (Leuko)
G6PD (Leuko)
TIBC
Serum Iron
Serum Iron/TIBC
APT (Leuko)

Biochemical Profile of Rats (Germ Free)
Exposed to Coal Dust. Control Values as
Reference.
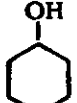
Abbreviations:
LDH — Lactic Dehydrogenase
HBDH — Hydroxy Butyrate Dehydrogenase
G-6 PD — Glucose-6-Phosphate Dehydrogenase
APT — Alkaline Phosphatase
SDH — Succinic Dehydrogenase
AT — Serum Anti-trypsin
TIBC — Total Iron Binding Capacity of Serum
/ — Slant Line Indicates Ratio Value

Courtesy Dr. Larry K. Lowry, Toxicology Section, NIOSH.

Figure 5-6.   Profile, Germ Free, Coal Exposed, Control

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TABLE 5-1   Major Types of Detoxication

| Type	Foreign substance	Detoxication product examples
Methylation $-CH_3$	Inorg. Compounds of As, Te Ring N compounds Certain complex aromatic phenols	$(CH_3)_2Se$ ⬡ $N-CH_3$ ⬡ with OH, OCH_3 $CHOH-CH_2NHCH_3$
Acetylation CH_3CO-	Aromatic Amines Amino Acids (Known exceptions: aromatic amine carcinogens, also aliphatic amines).	⬡ $NHCOCH_3$ $RCHCOOH$ $\mid$ $NHCOCH_3$ e.g., Benzidene – hydroxylated aliphatic amines – aldehydes.
Ethereal sulfate $-OSO_3H$	Phenols (Cyclohexanol glucuronide)	⬡ OSO_3H
Acetyl Mercapturic acid $-SCH_2CHCOOH$ $\quad\quad NHCOCH_3$	Aromatic Hydrocarbons Halogenated Aromatic HC's Polycyclic HC's Sulfonated esters $C_2H_5SO_3-CH_3$ Nitroparaffins $(C_4H_9NO_2)$	$S-CH_2CHCOOH$ ⬡ $\mid$ $NHCOCH_3$ Br C_2H_5 acetyl cysteyl- C_4H_9-acetyl cysteyl-
Thiocyanate	Cyanide, inorganic Organic Cyanides (Nitriles)	$RCNS$
Clycine $-NHCH_2COOH$	Aromatic Acids Aromatic-aliphatic acids Furane carboxylic acids Thiophene " " Polycyclic " " (Bile acids)	⬡ $CONHCH_2COOH$
Glucuronate	Aliphatic (1°, 2°, 3°) and Aromatic Hydroxyl Aromatic Carboxyl	⬡ $OC_6H_9O_6$ (Ether) ⬡ $C=O$ $OC_6H_9O_6$ **(Ester)**
Glucose Hydrazone	Hydrazine " derivatives?	$NH_2N = CHC_5H_8O_5$

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CHAPTER 6

REVIEW OF PHYSIOLOGY

James L. Whittenberger, M.D.

INTRODUCTION

Physiology is the basic biomedical science which deals with function in living organisms. Since form and function cannot be studied apart from each other, anatomy and physiology were undoubtedly two of the earliest biomedical sciences to be developed. As rapid expansion of natural sciences occurred, particularly in the first half of the twentieth century, specialization and fragmentation has led to splitting off of several fields from the parent discipline of physiology — including biochemistry, biophysics, and more recently, molecular and cell biology. For the purposes of this Syllabus, we shall assume that physiology is concerned with the full range of function, from the activities of a living cell to the performance of the whole organism.

A major concern of physiology is the role of environmental factors in influencing function. A fundamental principle of mammalian physiology is that basic intracellular processes can proceed only if the fluid environment surrounding each cell is maintained in a nearly constant state with respect to temperature, oxygen supply, acidity and nutrients. A major role of most systems of the body — respiratory, circulatory, alimentary and excretory for example — is to maintain near-constancy of the so-called internal environment of the body. By contrast, the external environment is variable over wide limits — in temperature, humidity, ionizing and nonionizing radiation energy, barometric pressure, and presence of noxious gases and particles. Interaction between the organism and the external environment is continuous and calls upon various protective and adaptive responses of the organism. In ordinary life and in the usual working environment these adjustments are automatic and unconscious; they put no particular strain on the organism and are a part of healthy existence.

A primary objective of industrial hygiene engineering is to control the occupational environment so that workers will not be exposed to extremes of heat and noise, or to unsafe levels of noxious gases, dusts and fumes. The degree of control needed depends on the type and extent of biologic response that might be induced. Since it is not economical to control the environment completely, it is important to know the nature and extent of biologic response in order to make rational decisions about the extent of control needed in relation to other measures that can be used to protect the health of the workers. For example, it may not be feasible (too costly) to control the heat in the particular environment; however, a biological re-

sponse to heat (that is, a heat stress study curve) can be used to determine a safe level (using temperature and time) of exposure to heat and when the worker should be given a rest period.

The kinds of biologic response to occupational environmental stresses range from harmless physiologic responses (such as respiratory and circulatory adjustments to physical exercise) through a variety of toxicologic manifestations which may include acute diseases such as chemical pneumonias and chronic diseases such as silicosis, or cancer of various organ systems. Thus the responses to environment involve toxicology, pathology and other medical specialties besides physiology; but these sciences cannot be understood without a basic knowledge of physiology and biochemistry.

BASIC CELL FUNCTIONS

The basic unit of all living organisms is an individual cell. Certain principles are common to almost all cells and represent minimal requirements for maintaining the integrity of the cell. Thus a human liver cell and a free-living ameba do not differ much in their means of exchanging materials with the immediate environment, obtaining energy from nutrients, synthesizing proteins, and reproducing themselves.

The difference among cells in the different tissues of the body usually represents a specialization of some one function of the functions common to all cells. Thus the excitability of nerve cells represents a specialization of electrical phenomena common to the membranes of almost all cells; the transport of food molecules across intestinal cells is a specialization of transport mechanisms that are very similar in all cells.

Types of Molecules

Water forms the medium in which all living processes occur, and life as we know it is inconceivable in the absence of water. Sixty percent of the body weight is water, and 80% of the weight of a cell is water. Because so many different molecules can dissolve in water, it is an ideal medium for chemical reactions. Water participates in practically every process in the organism and without this medium we could not have a circulatory system.

Despite the importance of water, the chemistry of living systems is centered about the chemistry of carbon which makes up 45 percent of the dry weight of the body. Carbon atoms can form four separate bonds with other atoms, in particular with other carbon atoms, making possible the formation of large molecules with a variety of

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structures. In combination with hydrogen, oxygen, nitrogen, and occasionally sulfur and phosphorus, carbon compounds make up the major classes of organic molecules in the body — proteins, lipids, carbohydrates and nucleic acids. Proteins constitute about 17% of the body weight — they make up much of the structure of the body as connective tissues and muscle. They catalyze most of the chemical reactions in cells and serve many specific functions, for example, as hormones, carriers of oxygen and carbon dioxide (hemoglobin), and as the principal mechanism of immunity (antibody formation).

Lipids make up about 15% of body weight and because of their relative insolubility in water perform a very important role in the permeability of cell membranes. One class of lipids includes the steroid hormones. Carbohydrates constitute only about 1% of body weight, but supply most of the energy needs of the body. Nucleic acids are even smaller in amount, but include the largest and most specialized molecules in the body — the deoxyribonucleic acid (DNA) molecules which are the blueprints of genetic information in the cell nucleus, and the ribonucleic acid (RNA) molecules which transcribe and carry the information in forms that can be used to synthesize proteins in the cytoplasm.

Energy and Cellular Metabolism

Metabolism refers to the sum of chemical reactions taking place in a living cell or organism; it includes both the process of fragmentation of large molecules into smaller ones and the synthesis of large molecules from raw materials in the cell. Both processes go on simultaneously in different parts of the cell, with hundreds of chemical reactions taking place in an orderly fashion.

That such reactions can take place at normal body temperature is due to the presence of special protein molecules which act as catalysts. Although enzymes are not destroyed by the reactions they catalyze, like all biologic molecules they are in a state of dynamic equilibrium between the rate of breakdown and the rate of synthesis. The cell's ability to control these rates is one mechanism for control of the rates of metabolism within cells. Some enzymes are highly specific, acting on only one type of substrate molecule; others interact with a range of substrate molecules which have a particular type of chemical bond or grouping which is specific for the enzyme. Over 900 different enzymes have been identified and there are undoubtedly many more to be discovered.

THE INTERNAL ENVIRONMENT

Like a free-living single-celled organism in the sea, every cell in the body is bathed in an aqueous medium — the extra-cellular fluid — which in salt composition is not dissimilar to that of the sea (at the salt concentrations which probably obtained when terrestrial life evolved from the sea). The extracellular fluid provides ready access to nutrients and oxygen, serves the needs for waste disposal and stabilizes conditions outside the cell membrane.

The extracellular fluid — the internal environment of the body — consists of two compartments. Eighty percent of it surrounds the cells within tissues in all parts of the body; the remainder is within the vascular system — the liquid part of the blood — the plasma. Since the blood is pumped to all parts of the body, where cells are close to capillaries, there is rapid exchange between plasma and extracellular fluid. Consequently, the composition of the two fluid compartments is very similar, except for the presence of proteins in the plasma. The proteins do not normally pass through the capillary wall and they serve a very useful role in controlling fluid exchange in the capillaries.

Homeostasis

This important concept, first enunciated by Claude Bernard, refers to the relative fixity of the internal environment and the role of many organs and systems in stabilizing the internal environment. The temperature, the concentrations of oxygen, carbon dioxide, nutrients, and inorganic ions — all must remain relatively unchanged in the extracellular fluid. Virtually every system of the body contributes to this stability — the liver adds or subtracts molecules as needed, the lungs delicately adjust the oxygen and carbon dioxide in the blood, the kidneys excrete or absorb the right amount of water and salts, and so on. The activities of tissues and organs are regulated and integrated with each other so that any change in the internal environment automatically initiates a reaction to minimize the change. Thus stability is achieved in the presence of wide fluctuations in activity of the total organism and wide ranges of external environmental conditions.

NEURAL CONTROL MECHANISMS

The development of the human nervous system is one of the most remarkable of evolutionary achievements. The nervous system is a major interface between the organism and the environment; it serves in many of the mechanisms that maintain homeostasis; it controls posture and body movements; it is the seat of subjective experience, memory, language, and the thought processes that characterize human activity.

The fundamental unit of the nervous system is the neuron, which consists of cell body, dendrites, and the axone, which may be several feet in length. Only about 10% of the cells in the nervous system are neurons, the remainder serving mainly as supporting elements.

The connections between nerve cells, called synapses, play a key role in the transmission of impulses in the nervous system; one cell may be directly connected with as many as 15,000 other cells by means of synapses. The basic mechanism of impulse transmission is the action current which results from depolarization of the cell membrane under the influence of chemical or mechanical events. The threshold for depolarization differs in different parts of the neurons and is influenced by the local environment of the cell; this is also true of the synapse. Nerve stimulation or alteration of local ions may be excitatory (lowering threshold for depolarization and action potential generation), or inhibitory (raising the threshold).

Presumably the mechanism whereby impulses are transmitted through synapses involves the release of a chemical at a cell terminal, with rapid diffusion to a reactive site on the second cell. With few exceptions (acetyl choline in the parasympathetic system and norepinephrine in the sympathetic system), the identity of these transmitters is unknown.

There are three functional classes of neurons: 1) afferent neurons which frequently are connected to sensory receptors (touch, taste, smell, sight, etc.) and which transmit information in the form of coded action potentials from the peripheral to the central nervous system; 2) efferent neurons, which transmit action potentials from centers in the spinal cord and brain to skeletal muscle, smooth muscle, or secretory cells in the periphery; and 3) interneurons, which make up 97% of the total and which provide the vast number of interconnections in the central nervous system.

The sensory receptors are of special interest in environmental health for their responsiveness or lack of responsiveness to different kinds of energy in the environment. For example, extremely sensitive receptors in the eye respond to electromagnetic energy in the narrow visible spectrum, but the eye has no receptors which respond to long wave frequencies or to ionizing radiation. The auditory system is highly developed to translate sound energy into nerve action potentials, and smell receptors respond to as little as 4 to 8 molecules of a substance. Other receptors respond selectively to mechanical stresses and to changes in chemical energy. Although receptors in general are adapted to respond to a particular kind of energy, they usually can be activated by other forms applied in sufficient strength. For example, the visual receptors normally respond to light but they can be activated by intense mechanical stimuli, such as a blow on the eyeball.

The Reflex Arc

The reflex arc illustrates many of the components of nervous control. The five components are a receptor, an afferent nervous pathway to carry action potentials to the central nervous system, an integrating center in the spinal cord or brain, an efferent pathway to carry action potentials from the central nervous system, and the effector which is activated.

The receptor detects an environmental change (temperature, pressure, etc.), perhaps by a change of permeability, and alters its signals to the afferent nerve. The integrating center receives input from many receptors and from other parts of the nervous system. The net result of these inputs is an "order" which is transmitted by the efferent pathway to a muscle or gland. If the initial stimulus is cold exposure, the integrating center would be in the brain, and the effector response would be an increase in skeletal muscle tone and constriction of skin blood vessels which would conserve heat by diminishing blood flow. Such responses are automatic and usually not consciously perceived.

Divisions of the Nervous System

Overall the nervous system is divided into central and peripheral portions, the central including both the brain in its several parts within the cranium, and the spinal cord within the vertebral column. Another way of dividing the system is into afferent and efferent components; the afferent includes the pathways from the specialized receptors and from other sensory nerve endings in tissue; the efferent system leaves the brain and spinal cord to transmit impulses to skeletal muscle, cardiac muscle, smooth muscle, and glands.

Autonomic Nervous System

The efferent system to skeletal muscle is the somatic nervous system; the other part of the efferent system is known as the autonomic nervous system. It deserves special attention because of its role in maintaining homeostasis. It provides dual innervation to the heart, the smooth muscle of lungs, blood vessels, intestinal tract, and other organs, and to secretory glands. The dual systems differ physiologically and anatomically and are called the sympathetic and parasympathetic systems. Whatever one division does to an effector organ, the other usually does the opposite; thus action potentials over the sympathetic nerves to the heart increase the heart rate, while action potentials over the parasympathetic fibers decrease it. Dual innervation with nerves inducing opposite effects provides a fine degree of control over an effector organ.

In general the sympathetic system helps the body cope with challenges from the outside environment (increasing flow to exercising muscles, constricting other vessels to sustain blood pressure, increasing metabolism, etc.) whereas the parasympathetic system is more active in digestion and other resting activities. Activation of the sympathetic system is likely to have widespread effects in the body, partly because the adrenal medulla is concurrently stimulated to secrete epinephrine (adrenalin) into the blood.

HORMONAL CONTROL MECHANISMS

A second communications and control system is provided by the glands of internal secretion, which secrete specific chemicals into the bloodstream which then circulates them throughout the body, where they may affect a small number of target cells or in some instances a large number of cells. An example of the former is the effect of thyrotropic hormone from the anterior pituitary, affecting only the cells of the thyroid gland. An example of widespread effect is that of insulin, which increases the entry of glucose into most cells of the body, except in the brain.

Except for maintenance of reproductive activities, the body is capable of functioning without the endocrine glands, including even the anterior pituitary — the so-called master gland. However, the level of function in the absence of hormones is very deficient. Metabolic activities are depressed and resistance to infection and other stresses is much below normal; in addition there are other abnormalities that relate to specific hormones.

A general principle pertaining to hormones is that they are always present in the blood, at concentrations that depend on 1) the rate of production in specific cells, 2) the amount of storage in

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those cells, 3) the rate of release into the blood and 4) the rate of removal from the blood by absorption in a target organ, inactivation, or excretion. The control systems may act on one or more of these factors.

Until recent years, the endocrine systems were studied independent of the central nervous system, although it was recognized that the anterior pituitary was anatomically closely related to the base of the brain (the hypothalamus), and that emotional states could influence the function of the thyroid, the adrenal, and the reproductive glands of internal secretion. Now close functional, as well as anatomic, relationships between the two great communication systems of the body are well established.

In a sense the central nervous system "leads" the major components of the endocrine system by the secretion of "releasing factors" in the hypothalamus. These control the production and secretion of six separate hormones of the anterior pituitary:

1. The thyrotropic hormone regulates the production of thyroid hormone in the cells of the thyroid;
2. The adrenocorticotropic hormone (ACTH) regulates the production of cortisol in the adrenal cortex;
3. The luteotropic hormone controls the production of progesterone;
4. The follicle-stimulating hormone controls the maturation and release of ova from the ovary;
5. The lactogenic hormone (prolactin) controls the production of milk by cells in the breast; and
6. The so-called growth hormone, which has multiple metabolic effects.

In addition to these effects mediated by the anterior pituitary, the hypothalamus secretes two other hormones formerly thought to be produced by the posterior pituitary — the anti-diuretic hormone which regulates the reabsorption of water in the kidney, and the hormone oxytocin, which stimulates contraction of the gravid uterus.

In addition to its central role in regulating the above hormones, the hypothalamus controls the autonomic nervous system, which in turn controls the secretion of epinephrine. There are a few hormones, such as insulin and aldosterone which are not regulated by the hypothalamus or pituitary.

Part of the control of hormone production is the negative feedback effect of the hormone produced by the target organ. Thus thyrotropic hormone from the pituitary stimulates the thyroid cells to produce thyroid hormone, but thyroid hormone, either produced by the organism or administered as a drug, depresses the output of "releasing factor" from the hypothalamus and in turn the output of thyrotropic hormone by the anterior pituitary.

Some of the mechanisms by which hormones act are known; others are not known. They do not create new functions of cells, but are usually found to alter rates at which existing processes proceed by increasing the activity of a critical enzyme (by increasing its production or by activating a stored form) or by altering the rate of membrane transport. For example, one of the actions of insulin is to increase the rate of entry of glucose into cells. Actions of hormones on cells often involve interactions with other hormones; for example, epinephrine causes release of fatty acids from adipose cells only when thyroid hormone is present.

The role of hormones, especially those produced by the adrenal cortex, is important in the recognition of the responses of the body to any kind of stress, be it exposure to toxic chemicals, extremes of heat and cold, heavy exercise, and other stresses. Some effects of a toxic chemical may be direct effects on specific cells while the remaining effects are secondary to increased production of cortisol. Since adrenal cortical hormones play important roles in the inflammatory process, in immune responses, and in diverse metabolic processes, it is clear that a knowledge of hormones is essential to understanding the mechanisms of response to stress.

RESPIRATORY SYSTEM

In common understanding, the respiratory system includes only the lungs and conducting airways. Logically, the term should include the circulatory system as well, since the two systems are jointly responsible for meeting the respiratory needs of the body, providing as they do the mechanism whereby the countless billions of cells are kept in minute to minute contact with the external environment for access to oxygen and for elimination of carbon dioxide.

The basic role of the lungs and related components of the system is to provide the essential conditions under which rapid exchange of oxygen and carbon dioxide can take place between the atmosphere and the blood coming to the pulmonary capillaries. A large surface is needed and this is provided by the estimated area of 70 square meters for an adult male's alveolar surface, where pulmonary capillary blood is in close juxtaposition to the alveolar gas. The rate at which oxygen must be taken into the body (and proportional amounts of carbon dioxide released) varies from about 200 ml/min at rest to 30 times that amount during exhausting exercise. If this wide range of need is to be met without significant change in the internal environment, there must be corresponding changes in the rates at which pulmonary capillary blood is replaced and alveolar gas is exchanged with ambient air; these changes are accomplished by integrated responses of the cardiovascular system so that ventilation and perfusion of the alveolar surface is always closely matched in healthy individuals.

Airways

The conducting portion of the respiratory system appears well-designed as a low resistance pathway for uniform distribution of gases to the alveolar surface, with numerous characteristics which "condition" the air and protect the lungs from at least the largest of infectious or noxious particles in the atmosphere. Some of the protective aspects are as follows:

The convoluted, moist, and richly vascular

mucosa of the nose protects nose-breathers from inhaling particles larger than 5 or 10 microns in diameter; soluble gases are largely removed by absorption, and the inspired air is warmed and moistened (or cooled under hot, dry conditions). In addition, the sense of smell receptors in the upper reaches of the nose may serve a protective role.

The tracheobronchial system is lined with cilia which constantly force toward the larynx a "carpet" of mucus which is secreted by mucosal glands. The mucus carries with it the microorganisms and particles which have impinged upon it, as well as macrophages which move out of the alveoli, often with a burden of material scavenged from the alveolar surface. When the mucus reaches the pharynx it is usually swallowed, but may be expectorated. These "pulmonary clearance" mechanisms play an important role in preventing pulmonary effects from inhalation of dusts, fumes, and other materials of concern in occupational exposure. The cough mechanism is a coordinated pattern of mechanical events that tends to expel foreign materials from the tracheobronchial tree.

Other pulmonary responses occur in response to environmental exposures, but are less clearly protective. Most important of these is the bronchospasm which characterizes the response to many irritant gases. The partial or complete closure of bronchioles certainly impedes access to the alveoli, but it does so at the cost of greatly increased breathing effort and impeded gas exchange in severe cases. An asthma-like response is a normal reaction to many inhaled irritants; it also may represent hypersensitivity of such severity that further occupational exposure must be prevented.

Pulmonary Ventilation

Under precise nervous and chemical control, the respiratory muscles intermittently expand the thorax in such manner that the lungs are continually changing volume. The elastic properties of the lung tend always to empty the lung, but the thorax exerts an opposite effect, except when lung and thorax volumes are large. Consequently, the lung retains a substantial amount of gas even when all respiratory muscles are at rest. In quiet breathing inspiratory muscles contract to enlarge the thorax and lungs; expiration is largely passive. Both frequency of cycling and the volume cycled (tidal ventilation) are variable; respiratory movements can thereby alter the minute volume (tidal volume times frequency per minute) from about 6 liters at rest to over 100 liters during heavy exercise. The ventilation rate is adjusted to maintain the alveolar partial pressure of oxygen and carbon dioxide approximately constant. The increases of ventilation with increasing levels of physical activity are very important in maintaining the homeostasis of the body; such increases may be critical in determining the amount of exposure to noxious or potentially noxious gases or particles in the atmosphere.

Of each breath, at rest, approximately one-third does not exchange significantly with alveolar gas because about that amount of space is accounted for by the conducting airways and the volume of alveoli not perfused by blood. This "dead-space" is normally about 180 ml in adult males and can be substantially increased in certain types of breathing appliances such as gas masks. Small increases in dead-space can be compensated by increased depth of breathing, but excessive dead-space can interfere with the adequacy of respiration.

Work of Breathing

Normally the work of respiratory muscles in ventilating the lungs accounts for a very small part of the total oxygen demand of the body — normally less than 3%. This is not the case when resistance to gas flow in the air passage is increased by bronchospasm or excess of secretions, or when the lung is diseased, as in pneumoconiosis, pulmonary fibrosis, and other occupational or non-occupational lung disease. In such conditions the increase of respiratory work may put a real burden on the whole cardiorespiratory system. Other conditions can also greatly increase the work of breathing, for example, the resistance of breathing through a gas mask, or having to breathe through a tracheostomy tube that is too small.

Ordinarily the respiratory muscles themselves are capable of performing the extra work required in the conditions mentioned above. This would not be the case when respiratory muscles are weakened by diseases such as myasthenia gravis or poliomyelitis, or by exposure to chemicals such as organophosphate pesticides.

Flow of Respiratory Gases

The ventilatory and gas exchange functions of the lungs are linked to the gas exchange needs of the body not only by the mass transport role of the circulatory system but by the peculiar respiratory functions of the blood. If oxygen could be transported only by physical solution in the blood, the kinds of organisms we know could not have evolved. The secret to efficient transport of oxygen (and to a large extent carbon dioxide also) is the red pigment hemoglobin, which combines loosely with oxygen, picking up a full load at the partial pressure of oxygen in the lungs and releasing a large part of the load at the partial pressures pertaining around the capillaries of tissues. Normal blood, by means of its hemoglobin-packed red cells, contains about 20 ml of oxygen per 100 ml blood at the alveolar oxygen pressure of approximately 100 mm Hg, compared to only 0.3 ml per 100 ml blood in physical solution. Under these conditions the hemoglobin is nearly saturated with oxygen. Since the hemoglobin is half-saturated at approximately 25 mm Hg, the blood can give up half its load of oxygen without lowering the pericapillary oxygen pressure to levels that would fail to provide adequate diffusion from interstitial tissue into cells.

Control of Respiration

The precise regulation of breathing to maintain alveolar oxygen and carbon dioxide levels essentially constant in the face of wide changes in body metabolism has always fascinated respiratory physiologists. Several factors are known, some closely interrelated (oxygen, carbon dioxide, and hydrogen ion concentration), but no theory fully explains the most remarkable adaptation of

respiration — the hyperventilation which occurs in proportion to the level of exercise.

Some of the control factors have special importance in certain occupational situations. Excess of carbon dioxide (as in contaminated atmospheres or rebreathing of expired air) is a powerful stimulant, causing 5-to 10-fold increase of ventilation at 5-7% CO_2 breathing. The mechanism may be a direct CO_2 effect on respiratory nerve cells or an indirect effect of increased hydrogen ion concentration in the cerebro-spinal fluid bathing the nerve cells.

In oxygen-deficient atmospheres the respiratory stimulation due to hypoxia is evident, the mechanism involving activation of specific receptor cells in the vicinity of the carotid arteries and the aortic arch. The strength of this stimulus was misjudged for a long time because of the inter-relationships of O_2 and CO_2 effects. Just as high CO_2 is a powerful stimulant, the loss of CO_2 is a potent depressant. Thus the increase of ventilation due to hypoxia eliminates CO_2 out of proportion to metabolic production; the level of CO_2 pressure falls; blood becomes relatively alkaline; and the respiratory drive is inhibited. If there is time for acid-base adjustments to low CO_2, as in acclimatization to high altitude, the depressing effect of low CO_2 disappears and the stimulating effect of hypoxia becomes more evident, with beneficial consequences to the organism in terms of higher partial pressures of oxygen throughout the body.

THE CIRCULATORY SYSTEM

The heart and blood vessels, with the blood contained therein, are the efficient internal transportation system of the body. The system is dual, the right side of the heart pumping blood only to the lungs while the left side pumps the freshly aerated blood to the rest of the body. The normal adult has about five and a half liters of blood; each side of the heart pumps at a rate of about five liters per minute at rest and can increase the output about five-fold during heavy exercise.

Changes of cardiac output involve both frequency of contraction and volume expelled with each stroke. The automatic rhythmicity of the electrical generator of the heart is subject to both nervous and chemical influences. Efferent autonomic nerves from the cardiac control centers in the brainstem can strongly slow or speed up the cardiac beat; epinephrine and drugs can also affect the rate of firing or the speed of transmission through the conducting system.

The stroke output is affected by both intrinsic and extrinsic factors. A fundamental property of heart muscle is that the greater the stretch during relaxation, the greater the energy of the subsequent contraction; thus a speeding up of return of blood for the veins will tend to increase the diastolic filling (increasing the stretch of heart muscle fibers) and automatically increase the force propelling the increased quantity of blood into the arterial system. In addition, the "tone" and force of heart muscle contraction can be influenced by chemicals such as epinephrine and norepinephrine.

The Systemic Arterial System

The arterial system which connects the left cardiac ventricle with capillary beds throughout the body has two basic characteristics: 1) the thick elastic walls of the aorta and other large arteries which enable the system to accept pulses of blood from the contracting ventricles and hold the blood in a high pressure reservoir while it flows off more or less steadily through the peripheral arterioles, and 2) the arterioles themselves, which because of their overall high resistance to flow tend to keep arterial pressure up during cardiac diastole and which, equally importantly, are subject to local control, so that tissues and organs have perfusion rates adjusted to their metabolic needs.

The arterial system contains about 15% of the total blood volume, at pressures which range in a young adult from about 110 mm Hg at the end of cardiac systole to about 70 mm at the end of diastole. This pressure is normally adequate to serve the blood flow needs of all tissues, including the brain when the body is upright. This is not true when there are sudden losses of blood volume (hemorrhage), failures of cardiac output (functional or organic in nature), or when arteriolar tone is sufficiently diminished (as after prolonged bed rest, immobility from other causes, heat exhaustion, and fainting due to various causes).

The local control of arteriolar tone (determining resistance to flow) seems designed largely to protect the brain and heart. During muscular exercise the smooth muscle in arterioles supplying active muscles relaxes allowing greater flow to the muscles; simultaneously there is contraction of smooth muscle in arterioles of other tissues, virtually shutting off flow to organs whose function can be temporarily suspended, such as the digestive organs. Vasoconstriction of this sort never affects flow to the brain or the heart.

In addition to nervous control of local blood flow, other factors can markedly affect arteriolar resistance and hence flow; these include local metabolites such as CO_2, heat, and chemicals like histamine and epinephrine.

Capillary-Tissue Exchange

When blood passes through the arterioles of the systemic circulation, the relatively high resistance is associated with a fall in pressure to about 35 mm Hg at the arterial end of the capillary. The number of capillaries is vast, the aggregate cross-sectional area of capillaries being estimated at 600 times the cross-sectional area of the aorta. This "widening of the stream" into a so-called capillary lake is associated with a greatly decreased flowrate, down to about 0.07 cm/sec. The large capillary area, slow flow, and pressure gradient from capillary to tissue provide ideal conditions for movement of nutrients and oxygen into the extracellular space.

The system also evolved in a way to facilitate return of substances to the capillary lumen. One mechanism relates to net fluid movement at opposite ends of the capillary. Where pressure is abuot 35 mm Hg the hydrostatic pressure forces fluid and its contained electrolytes and nutrients out into

the tissue space. The capillary wall is essentially impermeable to protein molecules, so the proteins continue to exert an osmotic force tending to attract fluid into the vessel. At the venous end of the capillary the filtration pressure has fallen to 15 mm Hg, considerably smaller than the osmotic pressure exerted by the proteins. Therefore net fluid movement is out of the capillary at one end and into the capillary at the other end, the rates tending nearly to balance each other.

A second mechanism, which facilitates gas transfer, is the role of hemoglobin in transporting carbon dioxide. When hemoglobin enters the capillary and gives up part of its load of oxygen it becomes less acid, because oxyhemoglobin is more acid than reduced hemoglobin. This permits hemoglobin to act as a buffer, to absorb some of the carbon dioxide which is higher in the tissue than in the blood.

The Veins

The venous system is the larger caliber, low resistance collecting system that connects the systemic capillary beds to the right side of the heart. About 50% of the total blood is at any time in the veins. Although veins are much thinner than arteries, they are elastic and they do contain smooth muscle. Consequently they are not just a passive collecting system; under nervous influence the overall tone of the system can increase, reducing volume and temporarily increasing venous return to the heart. This serves as a sort of "instant transfusion" to counter the effects of sudden loss of blood.

The Lymphatics

The lymphatics are a semi-independent system of thin-walled vessels that converge into trunks that empty into the large veins in the abdomen or thorax. Lymphatic capillaries start in tissue spaces, where they pick up protein and excess fluid filtered from regular capillaries. They serve a specific transport function for fat droplets absorbed by cells of the intestinal mucosa. The lymphatic systems also plays a role in defense against infectious or noxious materials, since most lymphatic vessels have lymph nodes interposed between tissue spaces and the venous system.

The Pulmonary Circulation

The pulmonary circulation carries the same flow of blood as the systemic circulation but is otherwise quite different. The pulmonary vascular pressures are in general much lower than systemic vascular pressures. Vasoconstriction and consequent local shunting are present in the pulmonary circulation, but much less developed than in the systemic circulation. Diseases of the pulmonary circulation are usually secondary to extensive disease of pulmonary tissue, as in emphysema, fibrosis, and occupational lung diseases.

Integration of Cardiovascular Function

As with other biologic systems, the heart and blood vessels are under nervous and chemical control which tends to stabilize the internal environment. One of the requirements for maintenance of adequate blood flow throughout the body is a sufficient head of arterial pressure. It is, therefore, not surprising to find that any stress tending to

lower arterial pressure (such as hemorrhage, loss of fluids from the body due to severe vomiting or diarrhea, traumatic shock, heat exhaustion) will trigger mechanisms designed to restore blood pressure. These include general arteriolar constriction (except cerebral and coronary arteries), increase in heart rate, and movement of fluid from extracellular space into the blood vessels. The integrated nature of cardiovascular responses to stress often makes it possible to use a simple measurement such as pulse rate to assess the degree of stress (more accurately the degree of response to stress). Thus pulse rate can be used to measure fairly reliably the intensity of exercise or heat stress (assuming given levels of physical fitness or acclimatization to heat).

REGULATION OF WATER AND ELECTROLYTES

As emphasized earlier, the proper functioning of cells in the mammalian body requires near-constancy of the internal environment — the temperature, oxygen supply, nutrient supply, hydrogen ion concentration, and appropriate concentrations of water, sodium, potassium, calcium, magnesium, and other substances. To some extent this constancy can be maintained by internal shifts of elements — from storage reservoirs, or by movement from one compartment to another, as might occur with movement from blood plasma to extracellular fluid to intracellular fluid and the reverse. The real problem is to maintain constancy under the usual conditions of variations of intake and output of a substance. This involves the concept of balance — how is output of water (or sodium, or calcium) controlled so that the "right" amount is retained when intake is varied?

The balance for water, as an example, involves a daily intake of about 2.5 liters in a normal adult under average conditions: 1 liter from food, 0.3 L from metabolic conversions, and 1.2 L from drinking water or beverages. An equal amount is lost each day — by urination 1.5 L, by evaporation from lungs and skin 0.9 L, and the remainder by sweating or in feces.

The major role in regulating body water, electrolytes and other substances is performed by the kidneys. These small organs, weighing less than 1% of total body weight, receive nearly one-quarter of the output of the heart in serving this regulatory role.

The kidney serves its unique function by a combination of three processes. Each microscopic unit of the kidney (or nephron) includes a capillary tuft (glomerulus) which filters plasma into a surrounding space (Bowman's capsule), which is the beginning of a tubule. The tubule processes the glomerular filtrate in complex ways before joining other tubules which ultimately constitute the system for carrying urine to the exterior of the body.

The glomerular filtrate contains all the components of plasma except proteins, which are generally too large to pass through capillary walls and capsule membranes. The amount of filtrate is large — about 180 liters per day for both kidneys — and if most of it were not reabsorbed the

body would be in a precarious position in a matter of minutes. A simple calculation will show that the entire plasma volume passes through the glomeruli some 60 times a day. Fortunately the "wanted" substances are reabsorbed to a high degree, if not totally. Ninety-nine percent of the water is reabsorbed by the tubules, together with 99.5% of the sodium and 100% of the glucose, compared to about 50% for urea, a waste product. The reabsorption involves both active and passive transport. Sodium is reabsorbed by an energy-consuming process which requires the presence of aldosterone, an adrenal cortical hormone. Water reabsorption to some extent follows sodium reabsorption, but is also very much influenced by the anti-diuretic hormone produced in the hypothalamus. Glucose is actively reabsorbed by a process which normally returns all the filtered glucose to peritubular capillaries, but the process can be overloaded if blood glucose is elevated above a certain limit. The excess of sugar filtered into the glomeruli then escapes into the urine, as is frequently the case in diabetes.

The third renal mechanism is of particular importance in toxicology, because many foreign substances are eliminated in the urine by secretion from tubular cells. Like tubular reabsorption, tubular secretion involves both active and passive transport. Of the many substances secreted, only a few, such as hydrogen ion and potassium, are normally found in the body. How mechanisms evolved for transporting the large numbers of foreign substances is a mystery.

Analysis of the balance of water and many ions, combined with study of the ways these substances are handled by the kidney, shows that the concentrations of most of the ions which determine the properties of the extracellular fluid are regulated primarily by the kidneys. Thus the kidneys are not "glorified garbage disposal" units for elimination of nitrogenous wastes so much as they are guardians of the minute to minute composition of all the body fluids and electrolytes.

THE DIGESTIVE SYSTEM

The function of this system is to accept raw materials in the form of food stuffs, minerals, vitamins and liquids in the external environment, to prepare such materials for absorption, and to absorb them into capillaries or lymphatics for distribution to the body. The system also has a limited role in excreting materials from the body.

Since the digestive tract is largely a tube open at both ends, in a real sense the contents of the gastro-intestinal tract remain exterior to the body. In order to gain access to the body, ingested materials must run a gamut of extreme acidity in the stomach and a series of enzymatic attacks starting in the mouth and extending to the large intestine. Some large molecules such as cellulose remain unaltered and are excreted in the feces. Other large molecules which could not otherwise pass through membranes are broken down into constituent amino acids or monosaccharides which are readily absorbed.

Almost all digestion and absorption of food and water takes place in the small intestine. The volume of fluid absorbed is far greater than that taken in as food and beverages. The latter average about 800 gm of food and 1200 ml of water/day in adults. To this is added 1.5 L of saliva in the mouth, about 2 L of acidic gastric secretions, 2 L of pancreatic and biliary secretions and 1.5 L of intestinal secretions; this adds up to about 8.5 L absorbed per day, with only 0.5 L passing into the large intestine.

Most of the carbohydrate ingested is in the form of starch. The starch is split to disaccharides by the amylases from saliva and from the pancreas. Further splitting into monosaccharides is brought about by enzymes in the small intestinal mucosa, after which the sugar molecules are actively transported into the blood.

Proteins are broken down first into peptides, then into free amino acids which are actively transported across intestinal cells. In adults very little protein is absorbed as such, but in the newborn child proteolytic enzymes are absent; thus a newborn child can absorb protective antibodies from its mother's milk.

Most digestion of fat occurs in the small intestine from the combined actions of pancreatic lipase and bile salts secreted by the liver. The latter act primarily as emulsifying agents. Fatty acids are resynthesized to triglycerides in the intestinal cells, whence they are secreted into the lymphatics as small lipid droplets.

The large intestine has relatively little capacity to absorb or secrete. Water and sodium continue to be absorbed from contents of the large intestine. The remaining contents are largely desquamated epithelial cells from the small intestine, bacteria, and the indigestible residue of food. Contrary to popular opinion, there is no absorption of toxic materials from unexpelled contents of the large intestine.

REGULATION OF ENERGY BALANCE

The energy released in the breakdown of organic molecules in the body performs biologic work (muscle contraction, synthesis of new molecules, or active transport) or appears as heat in the cells. The biologic work done by skeletal muscles in moving external objects (or raising the body to a height by walking uphill) is considered external work. The internal work done by skeletal muscles, by heart muscle and by other tissues appears ultimately as heat. Thus all energy utilized in the body is converted to heat, except during growth and during periods of net fat synthesis, when energy is being stored.

The metabolic rate is the total energy expenditure, measured in kilocalories, per unit time. During fasting conditions, when there can be no net energy storage, the rate of metabolism can be measured either directly or indirectly. The direct method is simple in theory but difficult in practice — it consists of measurement of total heat produced per unit time when the individual is enclosed in a whole-body calorimeter. The easier method is to measure the rate of oxygen utilization, assuming that fats, carbohydrates, and proteins are being utilized in a constant ratio, and that the oxygen-heat equivalents are the same for the three

classes of compounds. These assumptions are sufficiently accurate for most purposes, one liter of oxygen being required for the combustion of approximately 4.8 kilocal. of fat, protein, or carbohydrates.

The oxygen utilization of a healthy adult under standard conditions of rest, 12 hours fasting and a relaxing environment corresponds to a heat production of 40 kilocal. per hour per square meter of surface area — about equal to the heat production of a 100 watt light bulb. Metabolic rate is higher in the young than in adults and is lower in females than in males of equal weight. In late adulthood the resting metabolic rate declines, for reasons which are not clear. The ingestion of food increases metabolic rate by 10 to 20%, due to a specific action of protein and not due to increased activity of the digestive processes.

The above factors influence the standard metabolic rate by amounts which are trivial compared to the effects of physical activity. Since hard physical work can increase metabolic rate by about fifteen-fold it is most important that the average level of physical activity be considered in estimating the energy requirements of the body.

Two hormones also have a very significant effect on metabolic rate: epinephrine release, as occurs during emotional or physical stress, may cause a rapid increase of oxygen utilization by as much as 30%; thyroxin administration, or release of the hormone from the thyroid, causes a slower but very prolonged increase of oxygen utilization which affects all cells of the body except those of the brain.

Control of Food Intake

Normal adults live for long periods with an essentially constant body weight. This must mean that food intake exactly balances the internal heat produced and the external work done. There is no net storage or loss of energy from fat sources. A number of theories have been developed to explain this adjustment — the levels of blood sugar or fat storage affecting appetite centers in the hypothalamus, and other possibilities. The role of specific afferent inputs to the hypothalamic centers has not been well-defined. Clearly there are influences from higher brain centers as well. One of the most intriguing relationships is that between levels of physical activity and food intake. It is generally known that high levels of physical activity are accompanied by increased appetite and increased food intake to keep body weight constant. A few studies have suggested that this relationship does not hold below certain levels of activity that in a sense are "optimal"; below these levels an inverse relation holds — the more sedentary an individual becomes the more likely he is to overeat.

Obesity

When the mechanisms which govern the balance between energy intake and energy expenditure are malfunctioning, the most likely result is "overeating" and obesity. This is often called America's No. 1 health problem because obesity is so prevalent and mortality rates are about 50% higher in the overweight than in those of standard weight. If obesity, because of the obvious importance of social and economic factors in its etiology, is considered an environmental health problem, it must share with cigarette smoking a ranking as two of the most important environmental health problems in this country.

As in many other diseases and conditions predisposing to disease, there are undoubtedly multiple factors in the etiology of obesity. Experimental obesity has been produced in rats by injuries to the hypothalamus. Obesity is associated with certain endocrine disorders. There are undoubtedly many "constitutional" factors which influence the metabolism and storage of foodstuffs and which presumably make one person more "susceptible" to obesity than another. Ultimately, however, the defect is failure of food-intake control mechanisms to adjust to energy expenditure over a long enough period of time for obesity to develop. Often the predominant cause seems to be psychological or psycho-social.

Regulation of Body Temperature

With the evolution of mechanisms for maintenance of a constant body temperature, the mammals and birds achieved freedom from the marked extremes of temperature that characterize the atmosphere near the surface of the earth. The nearly constant temperature, around 90-103° F in mammals and around 108° in birds, greatly facilitates the action of enzymes in the chemical processes which go on continuously in cells.

The body temperature is in fact variable over a substantial range — from about 96° F in early morning after sleep to as much as 104° (rectally) during heavy exercise. Such excesses of temperature quickly return to about 98° when exercise is over. In fever the temperature may be equally high or higher, but in this case the heat control mechanism ("thermostat") is set at a higher level. Under extreme heat stress, the control mechanisms may fail and the temperature reach 107° or higher. At such temperature the brain is severely affected and death may ensue (heat stroke).

The usually quoted body temperature of 97.6-98.6° F is about the diurnal range of normal oral temperature. Deep body temperatures are a degree or so higher and skin temperatures are several degrees lower.

The body temperature is a measure of the balance between heat gain (metabolism, incident radiation from warm bodies) and heat loss. With changes in heat gain or heat loss, adaptive changes take place to keep body temperature essentially constant. In cold exposure, heat gain could theoretically be increased by secretion of epinephrine and thyroid hormone; this rarely if ever occurs. Heat gain is in fact increased by increases in skeletal muscle tone, involuntary shivering, and voluntary muscular activity. Heat loss is curtailed by vasoconstriction of blood vessels supplying the skin and by various behavioral adjustments such as changing body contours to reduce surface area, increasing insulation by use of clothing, and seeking shelter.

More important environmental exposures in industry are heat stresses from excessive tempera-

ture and humidity and from radiant heat load. These exposures, and the physiologic and behavioral responses thereto, will be considered in a separate chapter.

DEFENSE MECHANISMS
AGAINST INFECTION

At least as important as physical and chemical factors in the environment are the hosts of microbiologic agents in the environment. Two classes of microorganisms are of particular concern to man — the bacteria and viruses. Most bacteria are complete cells, capable of reproducing themselves. The viruses, essentially nucleic acid cores in a protein coat, lack the enzyme machinery for energy production and the ribosomes necessary for protein synthesis. They can survive only inside other cells, whose metabolic mechanisms they utilize.

The first line of defense against microorganisms is the complex of anatomic and microchemical barriers provided by external and internal body surfaces. The skin, with its thick layers of cells and secretions, is almost impervious to microorganisms. The mucous membranes contain secretions which inhibit bacterial growth and they usually flow toward the exterior, as in the case of tracheobronchial mucus.

When microorganisms gain access to the body, they may multiply and produce toxins, producing the signs and symptoms of an infection. The mechanisms for controlling growth or killing microorganisms and for neutralizing toxins involve the formation of antibodies, the action of complement, and the activities of phagocytic cells. These phenomena are interrelated.

Most of the phagocytic cells are either in the blood stream (white blood cells) or are closely associated with the vascular and lymphatic systems, including lining cells of bone marrow, liver, spleen and other lymphoid tissue. Despite the great importance of these cells, there is little knowledge of the mechanisms controlling their production. Most bacterial infections result in prompt increases of circulating granulocytic cells; viral infections tend to increase lymphocytes, but decrease other white blood cells.

Antibodies are specialized plasma proteins capable of combining chemically with the specific antigens which induced their formation. Most antigens have molecular weights greater than 10,000; however, smaller molecules may act as antigens after attaching themselves to proteins of the host cells. The antigens involved in infection are usually bacterial toxins or proteins of the microorganism's surface. Components of almost any foreign cell can act as antigens.

The plasma proteins known as complement are normally present in plasma and are not induced by antigens. The complement assists in killing bacteria, after an antibody has combined with its specific antigen in the wall of a bacterium. The complement apparently kills the bacterium after damaging the wall at the site of the antigen-antibody complex. Complement and antibodies also predispose bacteria to phagocytosis by phagocytes in the vicinity.

In addition to antibody formation and phagocytosis, a third defense mechanism plays a significant role in resistance to viral infections. This is the formation of the protein *interferon* in response to a viral infection of a particular cell. Unlike antibodies, interferon is not specific; all viruses stimulate production of the same interferon, and interferon inhibits the growth and multiplication of many different viruses. At present there is no way of using this information to improve treatment of virus infection.

Allergy

Allergy is an acquired hypersensitivity to a particular substance — an antigen-antibody reaction that results in cell damage. The term is usually reserved for the response to nonmicrobial antigens. The addition of complement to the antigen-antibody complex probably triggers cell damage and inflammatory response. The puzzling feature is why the response is so inappropriate to the antigen stimulus. Symptoms are often localized to the surface exposed, for example the respiratory response to aero-allergens such as ragweed pollen. Generalized allergic responses may also occur with widespread liberation of histamine to produce hives, extensive skin eruption, bronchospasm, rapid heart rate and hypotension. In extreme examples, death can occur, as for example, from the sting of a single bee.

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