occurs in two forms, dextrorotatory and levorotatory, and of these only the dextrorotatory form has biological activity. Since free pantothenic acid can readily be obtained only as a sirupy, gummy mass, it is usually converted to the calcium salt, which is a white powder and the form in which the synthetic product is supplied.

Since it is an amide, pantothenic acid is readily hydrolyzed by heating in either acid or alkaline solution. Hydrolysis results in complete destruction of the vitamin activity. It is rather stable to boiling in neutral aqueous solutions, although it is destroyed by long heating at 120°C.

It appears that pantothenic acid is not extensively destroyed by ordinary cooking of food. Losses of approximately 50 per cent may, however, occur if the cooking water from vegetables is discarded.

Occurrence

Yeast, liver, egg yolk, and rice polishings are very rich sources of pantothenic acid, while dairy products, whole cereals, muscle meats, green leafy vegetables, and certain other vegetables like cauliflower and sweet potato, may be classed as good sources. Fruits and egg white are low in pantothenic acid.

The assay of foods for this vitamin is based on the growth response of chicks when fed the test material. A bacterial method very similar to the one described above for riboflavin has also been developed. The human requirement for pantothenic acid has not yet been determined, but it has been suggested that about 10 mg. per day is adequate.

PYRIDOXINE (VITAMIN B_6)

Physiological function

Rats receiving an inadequate supply of this vitamin develop a dermatitis, which makes its appearance in a characteristic manner. The paws and tips of the ears and nose are first affected, becoming red and swollen. The area immediately surrounding the nostrils becomes bare, and there may be a nasal discharge. The administration of pure pyridoxine materially improves the condition of the rat, but even more striking improvement results from the use of certain fats, especially those which supply the so-called "essential fatty acids." The relation between the physiological action of these fatty acids and pyridoxine is not yet clear. It may well be that both are required for the normal nutrition of the rat. Neither black-tongue, pellagra, nor chick dermatitis is cured by pyridoxine. It has been shown, however, that pyridoxine is required by dogs, swine, pigeons, and chickens, and several reports indicate that it is also important in human nutrition. Deficiency symptoms that have

been encountered in various animals include a type of anemia and fits resembling epileptic seizures in human beings. Pyridoxine deficiency in man has been observed in a number of cases of pellagrins who still were not completely well after receiving nicotinic acid, thiamine and riboflavin. Symptoms noted in such patients were nervousness, irritability, abdominal pain, weakness, and difficulty in walking. These symptoms were quickly relieved by the use of synthetic pyridoxine.

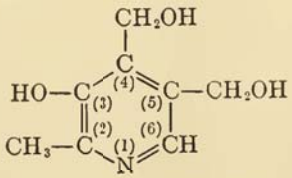
Chemical nature

Pyridoxine, or vitamin B_6, was first isolated as a pure chemical substance in 1938, and during the next year it was prepared synthetically.

Courtesy of Merck & Co., Inc.

Fig. 9-12. Pyridoxine.

The chemical nature of this vitamin is best expressed by its structural formula:

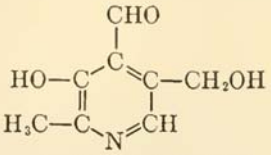


Pyridoxine, $C_8H_{11}NO_3$

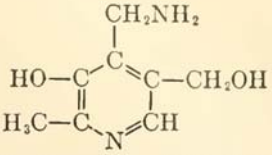
Note that it is related to nicotinic acid in that it is a pyridine derivative. The name pyridoxine is derived from the chemical name for this substance,

which is 2-methyl-3-hydroxy-4,5-di-(hydroxymethyl)-pyridine.

Two closely related substances, pyridoxal and pyridoxamine, are represented by the following formulas:

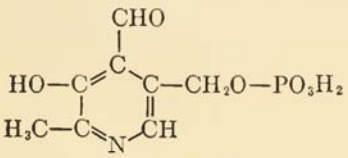


Pyridoxal, $C_8H_9O_3N$

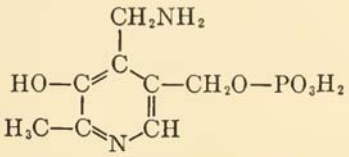


Pyridoxamine, $C_8H_{12}O_2N_2$

These substances have about the same vitamin B_6 activity for animals and for yeast cells as pyridoxine does, but are several thousand times more effective for certain bacteria. A phosphorylated derivative, pyridoxal phosphate, functions as a coenzyme for enzyme systems present in many bacteria, which break down amino acids into the corresponding amines by removing carbon dioxide from the carboxyl group of the amino acid (p. 321). It is therefore called a codecarboxylase. Both pyridoxal phosphate and pyridoxamine phosphate function as coenzymes in certain transamination reactions (p. 343) and may, therefore, be called cotransaminases.



Pyridoxal phosphate



Pyridoxamine phosphate

Pyridoxal phosphate also serves as a coenzyme for the enzyme system involved in the synthesis of tryptophan by a certain mold species (*Neurospora crassa*). It is, therefore, quite clear that the B_6 vitamins play important roles in both the decomposition, interconversion, and synthesis of amino acids in living cells.

Pyridoxine is stable to heat, alkalies, and strong acids, but is rather easily attacked by oxidizing agents. As yet little work has been done on its destruction during the cooking of food. No reliable figure for the human requirement is available, but a tentative value of 1.5 mg. per day has been suggested.

Occurrence

Pyridoxine is present in yeast, bran and embryo of cereal grains, meats, milk, and leafy vegetables. The amount of this vitamin in a number of common foods is given in Table 9–5.

Table 9–5

Pyridoxine content of common foods

	Milligrams per 100 g. edible portion
Beef, lean	0.40
Beef, liver	0.73
Bread, white	0.30
Bread, whole wheat	0.70
Cabbage	0.29
Carrot	0.19
Chicken, dark meat	0.20
Lamb, leg of	0.38
Milk, whole	0.20
Oatmeal	0.25
Pork loin	0.60
Potatoes, white	0.16
Yeast, dried brewer's	5.5

BIOTIN

This member of the vitamin B complex is a substance which has been variously known as "coenzyme R," "vitamin H," "biotin," and the "anti-egg white injury factor." It was first obtained in pure form and given the name biotin in 1936 by Kögl, who was studying it as one of the vitamin-like substances required for normal yeast growth.

Physiological function

The feeding of biotin brings about the cure of a nutritional disease which develops when rats, chickens, or human beings consume large amounts of raw egg white. This "egg white injury" disease is primarily a dermatitis, characterized in the rat by swelling and inflammation of the skin, especially around the mouth, and by loss of hair. The disease is actually an induced biotin deficiency caused by the combination of the biotin normally present in the food with a particular protein, *avidin*, present in raw egg white. When so combined, biotin cannot be absorbed and utilized by the animal organism. Cooked egg white on the other hand is perfectly safe in the diet, since heating to 100°C. destroys the ability of avidin to combine with the vitamin.

Although the above facts demonstrate that biotin is an indispensable nutrient, it has not been possible to produce the "egg white injury" disease in rats by feeding them diets extremely low in biotin. Apparently a sufficient supply of the vitamin to meet the needs of the animal is synthesized by bacteria in the intestinal tract. However, this deficiency can be produced in the chick without the use of raw egg white. Like-

wise many of the lower organisms such as yeasts, bacteria, and fungi do require biotin for normal development. No biotin deficiency has been observed in human beings consuming their customary diets. The daily intake of biotin on an average diet ranges from 25 to 50 μg., and the urine and feces together may contain from two to five times these quantities.

Courtesy of the S. M. A. Corporation.

Fig. 9-13. Biotin.

Biotin appears to function (possibly in the form of a coenzyme, although none has yet been identified) as a catalyst for one of the reactions of the citric acid cycle (p. 330):

$$CO_2 + CH_3—CO—COOH \longrightarrow HOOC—CH_2—CO—COOH$$

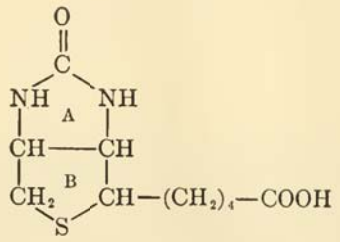
Pyruvic acid — Oxalacetic acid

Further, certain lactobacilli which normally require biotin grow well without it if oleic acid is supplied instead. This observation indicates some kind of a metabolic relationship between these two substances, perhaps participation of biotin in the biosynthesis of oleic acid. The vitamin is also required for deamination by bacterial cells of serine, threonine, and aspartic acid.

Chemical nature

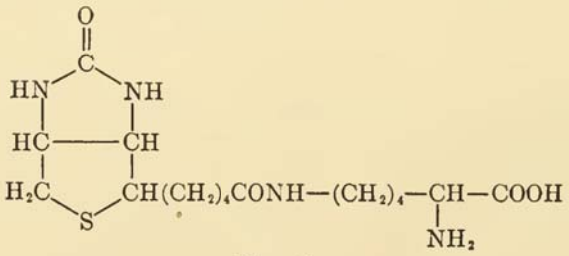
Biotin has a two ring structure with a side chain attached to one of the rings. It is an acid, as is indicated by the carboxyl group in the side chain. Note the urea-like structure in one of the rings (*A*) and the

presence of sulfur in the other ring (*B*). Biotin and thiamine are the only vitamins that contain sulfur.



Biotin, $C_{10}H_{16}O_3N_2S$

Although it is readily destroyed by such oxidizing agents as hydrogen peroxide, biotin is, in general, a very stable substance. It is not affected by light, strong acids such as normal HCl or H_2SO_4, nor by exposure to a degree of heat greater than that encountered during ordinary cooking operations. However, it is destroyed by strong alkali. In many tissues it appears not to exist in a free state, but in combination with some cell constituent, presumably protein. This view is supported by the recent isolation from autolyzing yeast of *biocytin,* a peptide-like combination of biotin and the amino acid, lysine. Note that the linkage is through the *epsilon* amino group of lysine.



Biocytin

Nothing is known as yet regarding the amount of biotin needed by human beings. However, the quantities required by various lower organisms are so extremely minute that it must be regarded as one of the most highly active substances known. Its effect on yeast growth, for example, can still be detected at dilutions of 1:300,000,000,000.

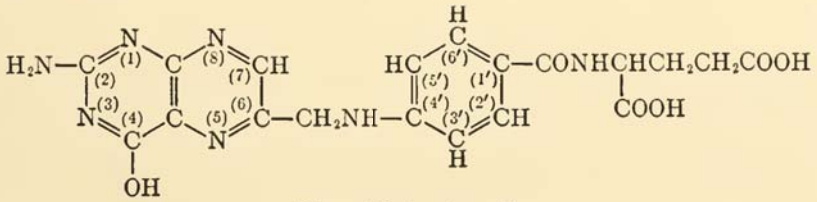
PTEROYLGLUTAMIC ACID

This vitamin was first observed in connection with studies on the nutritional requirements of lactic acid bacteria. An impure preparation from liver, designated as the "norite eluate factor," was shown to be necessary,

in addition to previously known vitamins, for the normal growth of these organisms. The effective substance present in such preparations was later found to be identical with "factor U" and "vitamin M," which at that time were still unidentified, but were recognized as dietary essentials for chicks and monkeys, respectively. Other investigators, working with various experimental animals, proposed still other names for vitamin-like substances which eventually turned out to be pteroylglutamic acid, or closely related compounds. These names included "vitamin B_c," "factor R," "factor S," "folic acid," "*S. lactis* R factor or SLR factor," "liver *L. casei* factor." The term folic acid is still in use, but should now be replaced by the proper chemical names (see below).

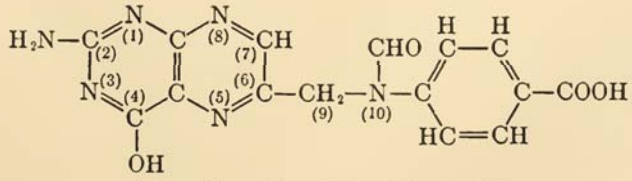
Chemical nature

Pteroylglutamic acid is a complex substance made up of three parts, glutamic acid, para-aminobenzoic acid (p. 254), and a pterin, chemically linked together:



Pteroylglutamic acid

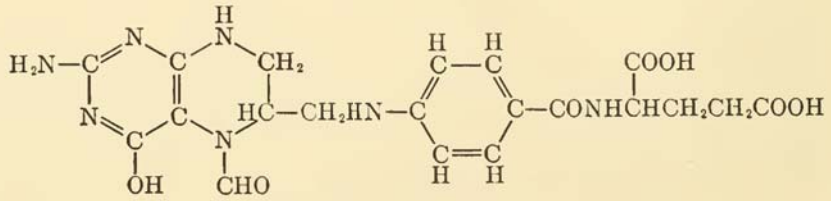
This substance is identical with the "liver *L. casei* factor," vitamin B_c, and folic acid. The name *folacin* was proposed in 1949 by the American Institute of Nutrition as a synonym for folic acid. The "fermentation *L. casei* factor" is very similarly constituted except that three glutamic acid residues are present. In this form, also called *teropterin*, the second and third glutamic acid residues are linked to the preceding one through the *gamma* carboxyl group rather than through the *alpha* carboxyl (see p. 131). This is the same type of peptide linkage as is found in glutathione. Still another form, vitamin B_c conjugate, contains seven glutamic acid residues. The SLR factor, or *rhizopterin*, contains no glutamic acid at all but bears an aldehyde or formyl group on the nitrogen atom in position 10:



Formylpteroic acid (or rhizopterin)

The glutamic acid derivative of rhizopterin, formylpteroylglutamic acid, or formyl folic acid, has been prepared synthetically and found to possess the typical vitamin activity of other members of this group. It probably also occurs naturally. Rhizopterin itself, however, does not relieve the symptoms of folic acid deficiency in higher animals.

Very recently a substance needed for normal growth of the bacterium *Leuconostoc citrovorum* (the so-called "citrovorum factor") has been found to be closely related to formylpteroylglutamic acid, from which it can be obtained by reducing and heating. The product, named *folinic acid* by one group of investigators and *leucovorin* by another, has been shown (Consulich, *et al.*, Pohland, *et al.*) to have the following formula:



Folinic acid (or leucovorin)

In many tests folinic acid possesses higher activity than other members of the folic acid group. It may be the metabolically active (coenzyme) form of this vitamin, or at least it may be more closely related to the coenzyme than pteroylglutamic acid itself.

Physiological function

This vitamin is essential for a wide variety of living organisms, and, in fact, is probably needed by all living cells. The outstanding deficiency symptoms in higher forms (mammals, birds) are anemia, leucopenia (a reduced number of white blood cells), weight loss, oral lesions, and diarrhea. In the chick the deficiency also results in abnormally poor feathering.

That several human diseases are the result of a lack of pteroylglutamic acid or related substances is indicated by the improvement which follows their administration. The best example is sprue, a disease characterized by macrocytic anemia (enlarged red blood cells), leucopenia, glossitis (inflammation of the tongue), diarrhea with large amounts of fatty material in the feces, weight loss, and poor absorption of food from the intestine. Daily doses of 10 mg. of pteroylglutamic acid or of the triglutamate, teropterin, result in prompt relief of these symptoms. Related conditions described as nutritional macrocytic anemia and macrocytic anemia of pregnancy are similarly benefited. Pernicious anemia patients are benefited somewhat, but the improvement is temporary and incomplete, in contrast to the effects of vitamin B_{12} (see below).

In all of the above diseases the administration of relatively large daily doses (about 4 g.) of a simple pyrimidine compound, namely thymine (p. 155), has an almost equally beneficial result. From this and other evidence it seems probable that the biological function of pteroylglutamic acid is concerned with the biosynthesis of thymine and other components of nucleic acids. Teropterin has been claimed to relieve pain in advanced cases of human cancer and to retard the growth of tumors in experimental animals.

Food sources and requirements

The pteroylglutamic acids are rather sensitive substances which may be quite largely destroyed during the cooking of foods. Losses of 50 to 90 per cent have been reported in meats cooked in different ways. Vegetables kept for three days at room temperature lost 20 to 80 per cent, and large losses occurred during canning. When a solution of the pure vitamin was placed in bright daylight for 8 hours, 88 per cent was destroyed.

According to Toepfer and co-workers a number of common foods may be grouped as follows, on the basis of the milligrams of folic acid which they contain per 100 g. of dry weight: Over 1.0, brewer's yeast, chicken liver, asparagus, broadleaf endive, broccoli, leaf lettuce, spinach; 0.4–1.0, most of the other leafy greens, liver, blackeye peas, dried beans, soy flour; 0.1–0.4, other vegetables except root vegetables and a few fruits; 0.03–0.1, root vegetables, most fresh fruits, grains and grain products, nuts, lean beef; 0.03 or less, eggs, milk, meats (other than beef), poultry.

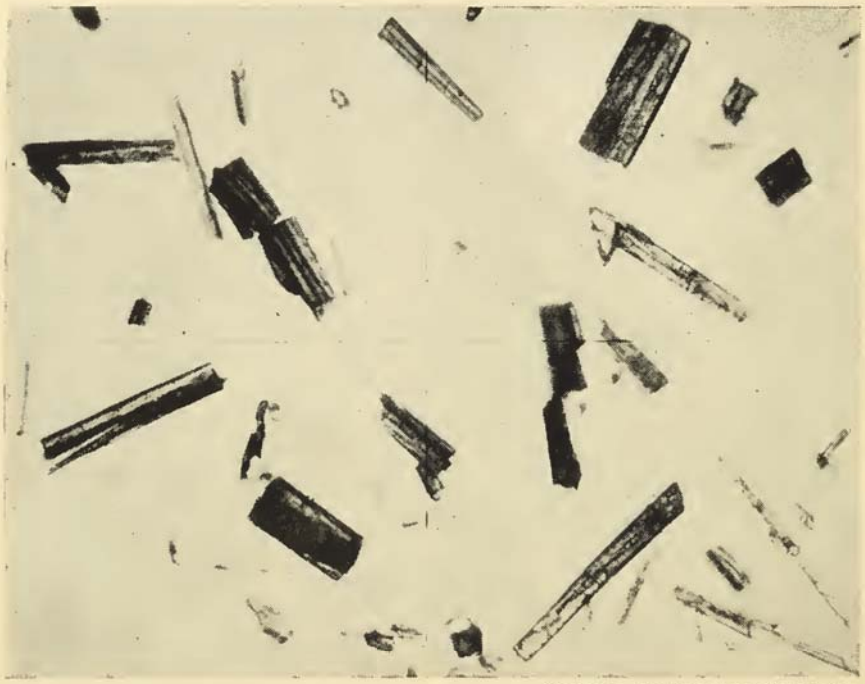
The amount of pteroylglutamic acid normally required by human beings has not been established. Various animal species need 0.005 to 0.06 mg. per kilogram of body weight per day.

VITAMIN B_{12}

It has long been recognized that liver and suitable extracts prepared from liver contain some substance which is effective in the treatment of pernicious anemia, a serious, wasting disease of man, which if untreated is invariably fatal. Many efforts to isolate and identify the "antipernicious anemia factor" in liver have been made. With the discovery of pteroylglutamic acid and the observation that it is effective in curing certain pathological blood conditions, it seemed that the long-sought substance might have been found. However, continued treatment of pernicious anemia patients with pteroylglutamic acid proved disappointing, since the initial improvement did not last and was often followed by severe neurological complications.

Finally, in 1948, a red crystalline substance was isolated from liver

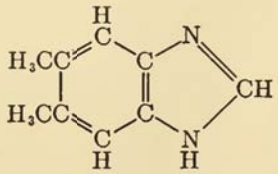
which proved to be effective against pernicious anemia in amazingly small doses (Fig. 9–14). The new material, designated *vitamin* B_{12}, contained 4.4 per cent cobalt, 2.3 per cent phosphorus and had the formula $C_{61-64}H_{86-92}N_{14}O_{13}PCo$ (molecular weight about 1350). Although the



Courtesy of Abbott Laboratories.

Fig. 9–14. Vitamin B_{12} crystals ($\times$ 200).

complete structure is not yet known, several fragments of the molecule, including 5,6-dimethyl benzimidazole, have been identified after acid hydrolysis. Surprisingly, this substance itself showed full vitamin B_{12} activity for rat growth when tested in 5 mg. daily doses. Other hydrolysis



5,6-Dimethyl benzimidazole

products identified are propanolamine ($CH_3CHOHCH_2NH_2$) and a phosphorylated derivative of the 5,6-dimethylbenzimidazole (ribose-3-phosphate attached to the N at position 1). Vitamin B_{12} also contains a

cyanide group (CN) bound in a coordination complex with the cobalt atom, which can be replaced by Cl, SO_4, OH, SCN, or other groups to produce analogs of the natural substance. The analog containing the water molecule has been called vitamin B_{12a} and is apparently identical with another preparation provisionally designated B_{12b}. Brink and co-workers have suggested that the B_{12} molecule, except for the cyanide group, be called *cobalamin*. By this nomenclature, vitamin B_{12} would be named *cyano-cobalamin* and B_{12a}, *hydroxo-cobalamin*. All of these various forms of the vitamin have approximately the same kind and amount of biological activity.

Physiological function

In the short period since its isolation vitamin B_{12} has acquired exceptional practical importance because of its demonstrated usefulness in pernicious anemia and related diseases, in livestock feeding, and in human nutrition. Its absence from the tissues of the body is apparently the specific cause of pernicious anemia. Injection of as little as 1 μg. per day dramatically alleviates the symptoms of this disease. It is less effective when given by mouth because pernicious anemia patients lack some substance ("intrinsic factor") in the gastric juice which protects vitamin B_{12} and favors its absorption. Small doses of vitamin B_{12} are also effective in sprue and other macrocytic anemias. See Plate IV opposite p. 223.

It has been known for many years that animal protein supplements (*e.g.*, meat scraps, dried whey, etc.) used in livestock feeding contain some factor necessary for growth of animals fed only plant proteins. This unknown substance was called the animal protein factor (APF). Vitamin B_{12} is certainly the chief and, perhaps, the only component of APF. Because of its high APF potency, it is now widely used in animal feeds. Availability of vitamin B_{12} has made possible the use of larger proportions of the relatively cheap plant protein concentrates (soybean, linseed, cottonseed meals), which are more plentiful than those from animal sources, and has thus been a boon to livestock production.

The vitamin B_{12} used in feeds is obtained almost exclusively from fermentation sources, and especially as a by-product of the fermentations which produce such antibiotics as aureomycin, terramycin, and streptomycin. It was noted that crude B_{12} concentrates from these sources gave greater growth responses in some species than could be accounted for by their B_{12} content. The extra effect was traced to the antibiotics still present as impurities in the concentrates. This discovery has opened new vistas in the science of nutrition, since by use of this combination faster growth rates have been achieved than had previously been considered optimal on the best mixtures of natural foods. The effect is

shown by a wide variety of antibacterial agents and is probably due to destruction of intestinal microorganisms which otherwise compete with the animal for essential food factors.

Very recently Wetzel et al. have reported that doses of 10 μg. of vitamin B_{12} given daily by mouth to a group of malnourished school children resulted in definite stimulation of growth in 5 of the 11 cases treated. These results establish the existence of human vitamin B_{12} deficiency other than that of pernicious anemia. How extensive this may be remains to be determined by further study, but present indications are that vitamin B_{12} may well prove to have wide applications in human nutrition.

The metabolic function of vitamin B_{12} in the animal body is evidently closely related to that of pteroylglutamic acid (for example, both are effective in certain types of anemia). Specifically, vitamin B_{12} appears to take part in the biosynthesis of nucleic acids and in the formation and use of active methyl groups in the body (for example, in the formation of methionine from homocystine).

Food sources and requirements

As already indicated, vitamin B_{12} is more concentrated in foods of animal origin than in plant products, and relatively large amounts are formed during the growth of many microorganisms. The distribution of this vitamin in various foods, as determined by Elvehjem and co-workers by means of a rat assay method, is shown in Table 9–6. No figure for the normal human requirement for vitamin B_{12} has been established, but 1 μg. per day, if injected, is sufficient to maintain pernicious anemia patients in good condition. This amount is much less than the minimum human requirement of any other vitamin or trace element.

Table 9–6

Vitamin B_{12} content of foods

(Micrograms per 100 g., fresh basis)

Food	*Minimum vitamin B_{12} content*	Food	*Minimum vitamin B_{12} content*
Barley	*	Egg yolk	1.4
Beans	*	Goat's milk	*
Beef, liver	15	Green peas	*
Beef, kidney	20	Horse meat, canned	3.4
Beef, round, cooked	2–3	Mutton	3
Beef, tongue	3	Pork, shoulder	1.1–2
Cabbage	*	Pork, ham	1.2
Cheddar cheese	1.4	Potatoes	*
Chicken liver	11	Tomato juice	*
Cow's milk	Trace	Veal	2

* No measureable amount.

CHOLINE

Physiological function

A lack of choline in the diet of young, rapidly growing rats results in the accumulation of excessive amounts of fat in the liver. There may also be damage to the kidneys, which become discolored from internal hemorrhage. The "fatty livers" are restored to normal by feeding small amounts of choline or of methionine. On the other hand, feeding cholesterol aggravates the condition. Older rats are much less likely to suffer from the symptoms of choline deficiency.

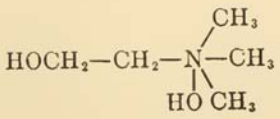
It is supposed that the fatty deposits in the liver are caused partly by a failure of fat transport and partly by a decrease in the normal rate of fat catabolism (that is, transformation into other simpler materials) in the liver. The evidence at present available is consistent with the assumption that neutral fat (that is, glycerides) must be converted into phospholipides before it is transported elsewhere in the body or, if it remains in the liver, before it is catabolized. Since choline is one component of the lecithin type of phospholipides, it would obviously be needed for these purposes. In fact, it has been possible with the aid of radioactive phosphorus to follow the rate of "phospholipide turnover" in the liver, that is, the rate at which phospholipide molecules are formed and removed, and to demonstrate that choline increases this rate. The effect was observed within one hour and was proportional to the amount of choline fed.

Choline is also required for the normal nutrition of chicks and of young turkeys. In conjunction with manganese it prevents the development of a disease of chickens known as perosis, in which the leg tendon slips off from the hock joint as a result of malformation of the bone, and the bird is consequently unable to walk. Normal egg production by chickens is also impaired by a lack of sufficient choline in the diet.

One of the main metabolic functions of choline is to supply "labile" methyl groups for various transmethylation reactions. These are described in Chap. 13.

Chemical nature

Choline is a very strong base, with the following structural formula:



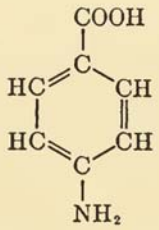
Choline

Like nicotinic acid it had been known to organic chemists and had been obtained synthetically long before its usefulness as a vitamin was discovered. It is very soluble in water and is quite stable to boiling in dilute aqueous solution. Hot alkalies, however, decompose it with the formation of trimethylamine.

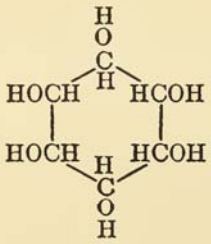
Bound choline in the form of lecithin is present in every living cell, and free choline is likewise very widely distributed in biological materials. At present, no information is available regarding the human requirement for this dietary factor.

OTHER DIETARY FACTORS

There are a number of other factors that have been reported as essential in the diet of experimental animals, but to discuss them in any detail would be beyond the scope of this book. However, two definite chemical substances in addition to those already considered have been shown quite conclusively to belong to the vitamin B complex. These are para-aminobenzoic acid and inositol:



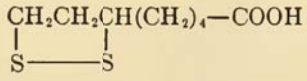
Para-aminobenzoic acid



Inositol

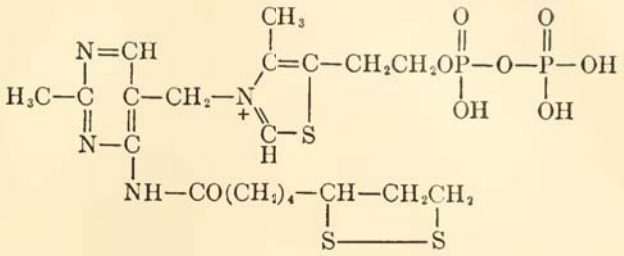
The former is probably used for the biosynthesis of pteroylglutamic acid and owes its vitamin-like activity in certain species to this circumstance. Inositol is required by mice and rats for normal growth and the avoidance of dermatitis and loss of hair. It is not known to be required by human beings.

Another vitamin-like substance needed by certain microorganisms is *lipoic* acid, which has recently been obtained in pure form and found to have the following structure:



α-Lipoic acid or thioctic acid

According to Reed and De Busk it is combined in the living cell with thiamine and phosphoric acid to form *lipothiamide pyrophosphate,* which appears to be a necessary coenzyme for the oxidative decarboxylation of α-keto acids, such as pyruvic acid, during metabolism.



Lipothiamide pyrophosphate

Still another compound, carnitine, has recently been shown by Carter and co-workers to function as a vitamin for a lower animal organism, namely, the larva of the yellow meal worm, *Tenebrio molitor*. These

$$(CH_3)_3N^+CH_2CHOHCH_2COO^-$$

Carnitine

larvae will not grow on synthetic diets containing all the previously known vitamins, but require the addition of supplements such as liver or whey. The effective substance was named vitamin B_T. When iso-

Courtesy of the S. M. A. Corporation.

Fig. 9–15. Pantothenic acid deficiency in the rat. These animals were reared on identical diets except that the one on the left received an adequate supply of pantothenic acid, while the diet of the other was deficient in this vitamin.

lated in pure form, it proved to be identical with carnitine, a compound which had long been known as a constituent of meat extract. It is possible that carnitine functions in the larvae as a source of labile methyl groups (p. 344).

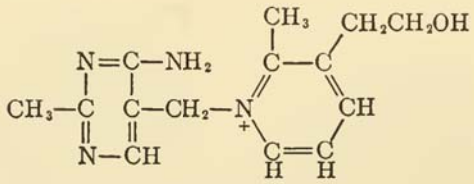
The so-called "antigray-hair factor" may or may not be a definite substance different from the other known vitamins. It is well established that graying of the hair does result from certain nutritional deficiencies in various species of animals, particularly the rat, mouse, dog, and fox.

Deficiencies of pantothenic acid, para-aminobenzoic acid, copper, and biotin have each been reported to cause such graying. However, there is at the present time no acceptable scientific evidence that gray hair in human beings can be restored to its original color by the dietary use of any of these materials, or of any other "gray hair factor."

Other less well-defined factors are vitamin P, which has been reported to correct bleeding caused by weakened capillaries in human beings, vitamin B_{13}, and vitamin B_{14}. A large number of other vitamin-like substances are apparently needed for the normal nutrition of various species of animals, and particularly of microorganisms, but knowledge of their nature and biological significance is too limited to warrant their consideration here.

Antivitamins

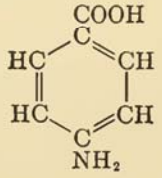
Substances chemically related to certain vitamins interfere with their normal physiological functioning and are therefore called *antivitamins*. For example, mice fed pyrithiamine (a thiamine analog, see formula) develop typical symptoms of thiamine deficiency. Similarly, pyridine-3-



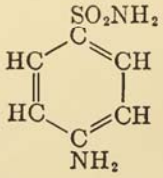
Pyrithiamine

sulfonic acid and glucoascorbic acid act as antagonists of nicotinic acid and vitamin C, respectively. In each case, administration of the vitamin concerned corrects the deficiency, and it appears that the response of the organism depends on the *relative* amount of the vitamin and antivitamin present.

An explanation for behavior of this sort was advanced by Woods and Fildes who found that *p*-aminobenzoic acid (PABA) can counteract the antibacterial effect of the drug, sulfanilamide. They suggested that PABA is an essential metabolite for the bacteria and that sulfanilamide exerts its effect by acting as an inhibitor of the bacterial enzymes concerned with the metabolic use or functions of PABA (*e.g.*, conversion to



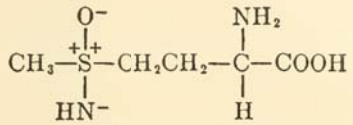
p-Aminobenzoic acid



Sulfanilamide

pteroylglutamic acid). According to this view antivitamins are competitive enzyme inhibitors (see p. 272 for the analogous case of malonate versus succinate).

Substances are also known which act against other types of essential metabolites. For example, *methionine sulfoximine*, a substance produced in flour by a formerly used bleaching agent (nitrogen trichloride) has been



L-Methionine sulfoximine

found to cause "running fits" in dogs by acting as an antagonist of the essential amino acid methionine. In general, such materials are called *antimetabolites*. Many additional examples of antimetabolites are listed by Woolley.

The concept of competition for enzyme surfaces offers a reasonable explanation for the action of antivitamins and other antimetabolites and furthermore may well serve as a guiding principle in the search for new drugs to combat disease. In theory, it should be possible selectively to poison any unwanted organism with a drug patterned after the chemical structure of some metabolite essential for that organism. Injury to the host would be avoided if the metabolite were peculiar to the parasite only.

REVIEW QUESTIONS ON VITAMINS

1. What are vitamins? Name those about the existence of which there is no controversy.
2. Discuss for each of the commonly accepted vitamins: (1) occurrence; (2) symptoms caused by lack of the vitamin.
3. Which vitamins have been obtained in crystalline form? Give briefly the chemical nature of each. Which have been synthesized in the laboratory?
4. Discuss the anti-infective properties of vitamin A.
5. Account for differences in need for vitamin D supplements in northern and southern regions. Why is sunlight transmitted through an ordinary window pane ineffective in preventing rickets?
6. In addition to man, which animals suffer from scurvy? How is the nonsusceptibility of other animals explained?
7. Discuss incidence of the various deficiency diseases in the United States.
8. Which vitamin is formed from a plant pigment? Which from sterols? Which one is particularly susceptible to oxidation?
9. Explain the following terms: (1) "Viosterol," (2) ascorbic acid, (3) pro-vitamin A, (4) riboflavin, (5) calciferol, (6) nicotinic acid, (7) pantothenic acid, (8) folacin.
10. What effect is produced by ingestion of massive doses of vitamin D?
11. What is the nature of the tissue changes responsible for noticeable respiratory trouble in A-deficient animals?
12. List the factors that influence the vitamin D requirement of an animal.

13. Which vitamins are known to function as parts of enzyme systems?

14. Account for the fact that pellagra is much more prevalent in the southern states of America than elsewhere.

15. Correct the following statement: If two samples of milk have the same amount of color, they have the same vitamin A potency.

16. Which vitamins contain N, S, P, Co?

REFERENCES AND SUGGESTED READINGS

Brink, N. G., Kuehl, F. A., Jr., and Folkers, Karl, "Vitamin B_{12}: The Identification of Vitamin B_{12} as a Cyano-Cobalt Coordination Complex," *Science*, **112**, 354 (1950).

Carter, H. E., Bhattacharyya, P. K., Weidman, K. R., and Fraenkel, G., "Chemical Studies on Vitamin B_T Isolation and Characterization as Carnitine," *Arch. Biochem. and Biophys.*, **38**, 405 (1952).

Consulich, D. B., Roth, B., Smith, J. B., Jr., Hultquist, M. E., and Parker, R. P., "Chemistry of Leucovorin," *J. Am. Chem. Soc.*, **74**, 3252 (1952).

Drummond, J. C., "The Nomenclature of the So-Called Accessory Food Factors (Vitamins)," *Biochem. J.*, **14**, 660 (1920).

Eddy, W. H., *What Are the Vitamins?* Reinhold Publishing Corp., New York, 1941.

Eddy, W. H. and Dalldorf, G., *The Avitaminoses*, 2nd ed., The Williams and Wilkins Company, Baltimore, 1941.

Eijkman, C., "An Experiment in Combatting Beri-Beri," *Virchow's Archiv für pathologische Anatomie und Physiologie*, **149**, 187 (1897).

Follis, R. H., Jackson, D., Eliot, M. M., and Park, E. A., "Prevalence of Rickets in Children between Two and Fourteen Years of Age," *Am. J. Diseases Children*, **66**, 1 (1943).

Food and Nutrition Board, "Recommended Daily Dietary Allowances, Revised, 1948," *Nutrition Rev.*, **6**, 319, (1948).

Funk, C., "Etiology of Deficiency Diseases—Beri-Beri, Polyneuritis in Birds, Epidemic Dropsy, Scurvy in Animals (Experimental), Infantile Scurvy, Ship Beri-Beri, Pellagra," *J. State Med.*, **20**, 341 (1912).

Gordon, E. S., *Nutritional and Vitamin Therapy in General Practice*, 3rd ed., The Year Book Publishers, Inc., Chicago, 1947.

György, P. (editor), *Vitamin Methods*, vols. 1 and 2, Academic Press, Inc., New York, 1951.

Harris, L. J., *Vitamins and Vitamin Deficiencies*, P. Blakiston's Son and Company, Philadelphia, 1938.

Harris, P. L., Quaife, M. L., and Swanson, J., "The Vitamin E Content of Foods," *J. Nutrition*, **40**, 367 (1950).

Harris, R. S. and Thimann, K. V. (editors), *Vitamins and Hormones, Advances in Research and Applications*, vols. 1–9, Academic Press, Inc., New York, 1943–1951.

Hopkins, F. G., "The Analyst and the Medical Man," *Analyst*, **31**, 385 (1906).

Kingsley, H. N. and Parsons, H. T., "The Availability of Vitamins from Yeast, IV," *J. Nutrition*, **34**, 321 (1947).

Kögl, F. and Toennis, G., "Isolation of Crystalline Biotin from Egg Yolk," *Z. Physiol. Chem.*, **242**, 43 (1936).

Lewis, U. J., Register, U. D., Thompson, H. T., and Elvehjem, C. A., "Distribution of Vitamin B_{12} in Natural Materials," *Proc. Soc. Exptl. Biol. Med.*, **72**, 479 (1949).

Link, K. P., "The Anticoagulant from Spoiled Sweet Clover Hay," *The Harvey Lecture Series*, **39**, 162 (1944).

Lunin, N., "Concerning the Significance of Inorganic Salts in Animal Nutrition," *Z. Physiol. Chem.*, **5**, 31 (1881).

McCollum, E. V. and Kennedy, C., "The Dietary Factors Operating in the Production of Polyneuritis," *J. Biol. Chem.*, **24**, 491 (1916).

Pohland, A., Flynn, E. H., Jones, R. C., and Shive, W., "The Structure of Folinic Acid-SF, a Growth Factor Derived from Pteroyl-Glutamic Acid," *Abstracts of the 119th Meeting, Am. Chem. Soc.*, Boston, 1951, p. 18 M.

Reed, C. I., Struck, H. G., and Steck, I. E., *Vitamin D*, The University of Chicago Press, Chicago, 1939.

Reed, L. J. and De Busk, B. G., "Lipothiamide Pyrophosphate: Coenzyme for Oxidative Decarboxylation of α-Keto Acids," *J. Am. Chem. Soc.*, **74**, 3964 (1952).

Robinson, F. A., *The Vitamin B Complex*, John Wiley and Sons, Inc., New York, 1951.

Rosenberg, H. R., *Chemistry and Physiology of the Vitamins*, revised reprint, Interscience Publishers, Inc., New York, 1945.

Salcedo, J., Carrasco, E. O., Jose, F. R., and Valenzuela, R. C., "Studies on Beriberi in an Endemic Subtropical Area," *J. Nutrition*, **36**, 561 (1948).

Sebrell, W. H., "Nutritional Diseases in the United States," *J. Am. Med. Assoc.*, **115**, 851 (1940).

Sherman, H. C., *Chemistry of Food and Nutrition*, 7th ed., The Macmillan Company, New York, 1946.

Spies, T. D., *Experiences with Folic Acid*, The Year Book Publishers, Inc., Chicago, 1947.

Sure, B., *The Little Things in Life*, D. Appleton-Century Company, New York, 1937.

du Vigneaud, V., chapter on "Biotin," in *The Biological Action of the Vitamins*, The University of Chicago Press, Chicago, 1942.

Toepfer, E. W., Zook, E. G., Orr, M. L., and Richardson, L. R., *Folic Acid Content of Foods*, Agriculture Handbook No. 29, United States Department of Agriculture, U. S. Government Printing Office, Washington, D. C., 1951.

Wetzel, N. C., Fargo, W. C., Smith, I. H., and Helikson, J., "Growth Failure in School Children as Associated with Vitamin B_{12} Deficiency-Response to Oral Therapy," *Science*, **110**, 651 (1949).

Williams, R. J., Eakin, R. E., Beerstecher, E., Jr., and Shive, W., *The Biochemistry of B Vitamins*, Reinhold Publishing Corp., New York, 1950.

Williams, R. J., Lyman, C. M., Goodyear, G. H., Truesdail, J. H., and Holaday, D., "Pantothenic Acid, A Growth Determinant of Universal Biological Occurrence," *J. Am. Chem. Soc.*, **55**, 2912 (1933).

Williams, R. R. and Spies, T. D., *Vitamin B_1 and Its Use in Medicine*, The Macmillan Company, New York, 1939.

Woods, D. D. and Fildes, P., "The Antisulfanilamide Activity (*In Vitro*) of p-Aminobenzoic Acid and Related Compounds," *Chemistry and Industry* (*London*) **18**, 133 (1940).

Woolley, D. W., *A Study of Antimetabolites*, John Wiley and Sons, Inc., New York, 1952.

Chapter 10

ENZYMES

by G. W. E. PLAUT
ASSISTANT PROFESSOR, INSTITUTE FOR ENZYME RESEARCH
UNIVERSITY OF WISCONSIN

Enzymes may be defined as thermolabile organic catalysts elaborated by living cells and capable of exerting their effects independently of these cells. Certain topics, especially those concerned with digestion and metabolism (Chaps. 11–16), will necessitate mention of these biocatalysts; but nothing will be said there regarding their chemical nature, their mode of action, factors affecting their rate of action, and their other properties.

Occurrence

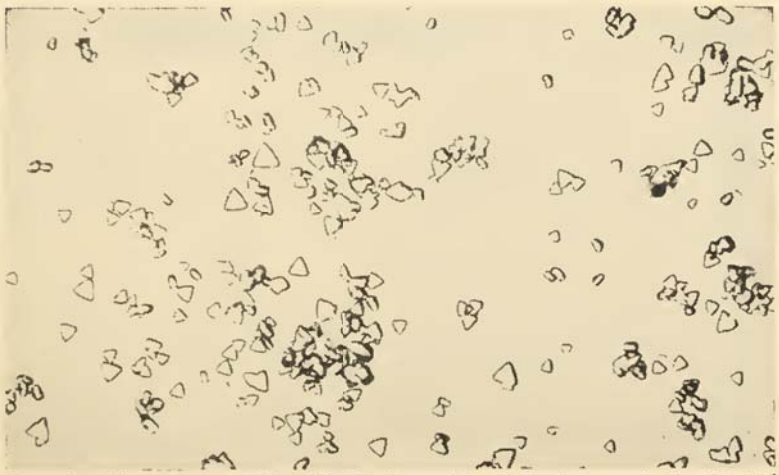
Great numbers of enzymes can be detected in all living cells. If one considers the quantity and diversity of enzymes present in a cell, it becomes evident that *the cell contents must consist largely of enzymes.* Enzymes, such as oxidative enzymes, functioning normally within the cell are usually called *endo-enzymes.* If the usual site of action is outside the cell, as is the case with those involved in digestion, the enzymes are designated *exo-enzymes.*

Chemical nature

All enzymes that have been obtained in a high degree of purity are proteins. Many such enzymes have been obtained in the crystalline state, *e.g.,* urease, catalase, pepsin, trypsin, carboxypeptidase, α- and β-amylases, yellow enzyme, ribonuclease, aldolase, and alcohol-, lactic-, and phosphoglyceraldehyde dehydrogenases. Such enzymes are similar to other proteins in elementary composition, amino acid content, and properties, *e.g.,* color tests, solubility, isoelectric point, thermolability, etc. For example, aldolase recrystallized four to six times was found by Velick and Ronzoni to consist of 18 amino acid residues. Complete accounting of the nitrogen was obtained in the amino acid residues. The number of residues per mole of aldolase (1267) was calculated, and from these data the amino acid formula of aldolase could be expressed as Gly_{105}

Courtesy of Drs. R. M. Herriott and J. H. Northrop and *The Journal of General Physiology.*

Fig. 10-1. Pepsin.



Reproduced by courtesy of Drs. M. Kunitz and J. H. Northrop and *The Journal of General Physiology.*

Fig. 10-2. Trypsinogen.

Ala_{135} Val_{89} Leu_{123} $Ileu_{84}$ $(Cys\text{-})_{13}$ Met_{11} Ser_{88} Thr_{76} Arg_{51} His_{38} Lys_{91} Pro_{69} Phe_{26} Tyr_{41} Try_{16} Asp_{102} Glu_{109}. Glutamic acid is low and valine is high as compared with most proteins, but otherwise there is nothing distinctive about the amino acid content.

Some enzymes are simple proteins, *e.g.*, pepsin, and others are conjugated proteins, *e.g.*, lactic dehydrogenase. The latter has a nicotinamide-containing compound as its prosthetic group. When the enzyme

Courtesy of Drs. M. Kunitz and J. H. Northrop and *The Journal of General Physiology.*

Fig. 10–3. Trypsin.

consists of a specific protein and a prosthetic group, as in the case of lactic dehydrogenase, the protein part is called the *apoenzyme,* and the prosthetic group is called the *coenzyme* of the complete enzyme. See Table 10–1 for examples of other coenzymes.

Classification

Enzymes have been named on the basis of occurrence (*e.g.,* pepsin), the substance (substrate) upon which they act, the products formed by their action, the nature of the linkages broken, or a particularly characteristic type of reaction they may perform (*e.g.,* oxidases). For example, the enzyme which catalyzes the hydrolysis of sucrose has three names; (1) *sucrase,* a name derived from the substrate, (2) *invertase,* so named because the hydrolysis product, an equimolar mixture of glucose and fructose, is called invert sugar, and (3) *α-glucopyrano-β-fructofuranosidase,* a term that indicates the type of linkage broken by the enzyme. The variety in enzyme terminology will be apparent upon examination of Table 10–1. Since enzymes catalyze such an enormous variety of reactions, it is difficult to catalogue them in an exact manner. The classification used in Table 10–1 is empirical, but will serve as a guide to the student for the organization of the material. Like all classifications it is imperfect and subject to change with advancing knowledge.

Table 10–1

An abridged classification of enzymes

Enzyme	*Occurrence*	*Substrate*		*Products*
A. Hydrolases (hydrolyzing enzymes)				
I. Carbohydrases				
1. Glycosidases				
a. Sucrase (invertase)	Intestinal juice, yeast	Sucrose		Glucose, fructose
b. Maltase (α-glucosidase)	Intestinal juice	Maltose		Glucose
c. Lactase (β-galactosidase)	Intestinal juice, bacteria	Lactose	$\xleftrightarrow{Na^+ \text{ or } K^+}$	Glucose + galactose
d. Emulsin (cellobiase, β-glucosidase)	Sweet almonds, yeast, animal organs	Cellobiose Amygdalin		Glucose Glucose, benzaldehyde
2. α-Amylases				
a. Salivary amylase (ptyalin)	Saliva	Gelatinized starch	$\xleftrightarrow{Cl^-}$	Dextrins + maltose
b. Pancreatic amylase (amylopsin)	Pancreatic juice	Gelatinized starch	$\xleftrightarrow{Cl^-}$	Dextrins + maltose
3. β-Amylases				
a. Cereal amylase	Ungerminated barley	Gelatinized starch		Maltose, dextrins
4. Cellulases (probably a mixture of enzymes)	Bacteria, molds, snails	Cellulose		Cellobiose, glucose, etc.
5. Lysozyme	Egg white	Bacterial polysaccharide		Lowered viscosity and turbidity, reducing sugars
6. Hyaluronidase	Bacteria, snake venom, testes	Hyaluronic acid		N-acetyl glucosamine, glucuronic acid
7. Pectinase (probably a mixture of enzymes)	Molds	Pectic acid		Mixture of polygalacturonic acids
II. Esterases				
1. Lipases	Stomach, pancreas, castor bean, bacteria	Glycerides		Glycerol, fatty acids
a. Lecithinase *A*	Rattlesnake venom, bee venom	Lecithin		Lysolecithin, unsaturated fatty acid
b. Lecithinase *B*	Heart, liver, rice bran	Lysolecithin		Choline glycerophosphate, fatty acid

Table 10–1 (Continued)

Enzyme	*Occurrence*	*Substrate*	*Products*
2. Specific esterases			
a. Liver esterase	Liver	Ethylacetate, etc.	Ethyl alcohol, acetic acid, etc.
b. Choline esterase	Nerve and other tissue	Acetylcholine	Choline, acetic acid
c. Cholesterase	Liver, etc.	Cholesterol esters	Cholesterol, fatty acids
d. Chlorophyllase	Green plants	Chlorophyll	Phytol, chlorophyllide
e. Sulfatases	Bacteria, molds, mustard	Esters of sulfuric acid:	
		phenol sulfate	Sulfuric acid, phenol
		chondroitin sulfate	Sulfuric acid, chondroitin
3. Phosphatases			
a. Phosphomonoesterases			
(1) Alkaline monophosphatases (act at pH>7) *e.g.*, nucleotidases	Intestinal mucosa, bone, kidney	α-Glycerophosphate $\xleftrightarrow{Mg^{++}}$	Glycerol + phosphate
		Nucleotides	Nucleosides, phosphates
(2) Acid monophosphatases (act best at pH 4–5)	Plants, fungi, bacteria, some animal tissues	α-Glycerophosphate	Glycerol, phosphate
b. Phosphodiesterases, *e.g.*, ribonuclease, desoxyribonuclease	Animal tissues, snake venom, molds	Nucleic acids, diphenyl phosphate	Nucleotides; phenol and phenyl monophosphate
c. Polyphosphatases	Muscle, yeast, bacteria	Polyphosphates and their esters	Phosphate, pyrophosphate, etc.
d. Phytase	Cereals, molds, intestinal mucosa	Phytates	Inositol, phosphate
III. Amidases (enzymes hydrolyzing —C(=O)—N linkage)			
1. Peptidases			
a. Carboxypeptidases	Pancreatic juice	Peptides having a free carboxyl group	Amino acids
b. Aminopeptidases	Intestinal mucosa	Peptides having a free amino group $\xleftrightarrow{Mn^{++}}$	Amino acids

Enzyme	*Occurrence*	*Substrate*		*Products*
c. Dipeptidases	Pancreatic juice, intestinal mucosa	Dipeptides	$\xleftrightarrow[\text{or } Mn^{++}]{Zn^{++}, Mg^{++}}$	Amino acids
d. Conjugases	Kidney, pancreas	Pteroylglutamic acid conjugates		Pteroylglutamic (folic) acid, glutamic acid
2. Proteinases				
a. Pepsin	Gastric juice	Proteins		Polypeptides, amino acids
b. Trypsin	Pancreatic juice	Proteins		Polypeptides, amino acids
c. Chymotrypsin	Pancreatic juice	Proteins		Polypeptides, amino acids
d. Rennin	Gastric juice	Casein		Paracasein
e. Papains (mixture)	Papaya	Proteins	$\xleftrightarrow{\text{reducing agents}}$	Polypeptides, amino acids
f. Ficin	Fig latex	Proteins		Polypeptides, amino acids
g. Bromelin	Pineapple fruit	Proteins		Polypeptides, amino acids
h. Bacterial proteinases	*Cl. botulinum* *Cl. acetobutylicum* *B. subtilis*, etc.	Gelatin Casein Cereal proteins		Polypeptides, amino acids
3. Other amidases				
a. Urease	Jackbean, soybean	Urea		Ammonia, carbon dioxide
b. Asparaginase	Yeast, animal and plant tissues, bacteria	Asparagine		Ammonia, aspartic acid
c. Glutaminase	Yeast, animal and plant tissues, bacteria	Glutamine		Ammonia, glutamic acid
d. Hippuricase	Animal, plant, and microbial tissues	N-acyl compounds, *e.g.*, hippuric acid		Benzoic acid, glycine
e. Penicillinase	Bacteria, *e.g.*, *E. coli.*	Penicillin		Penicilloic acid
IV. Enzymes hydrolyzing other carbon-nitrogen linkages				
1. Adenase	Liver	Adenine		Hypoxanthine, ammonia
2. Aspartase	Bacteria, plants	Aspartate		Fumarate, ammonia

Table 10–1 (Continued)

Enzyme	*Occurrence*	*Substrate*		*Products*
3. Thiaminase	Fish, clams	Thiamine		Pyrimidine and thiazole (parts of thiamine)
4. Arginase	Liver	Arginine	$\xleftrightarrow{Mn^{++}}$	Urea + ornithine
B. Hydrases (add water to organic cmpd.)				
1. Enolase	Animals, yeast, plants	Phosphoenolpyruvate	$\xleftrightarrow{Mg^{++}\text{ or }Mn^{++}}$	2-Phosphoglycerate
2. Fumarase	Animal, yeast	Fumarate		Malate
3. Aconitase	Animal, yeast	Aconitate		Citric and isocitric acids
C. Desmolases (Break or form carbon chains)				
I. Aldolase	Yeast, plants, animals	Fructose-1,6-diphosphate		3-phosphoglyceraldehyde, dihydroxyacetone phosphate
II. Condensing enzyme	Animal	Citrate	$\xleftrightarrow{Co\,A}$	"Acetate" + oxalacetate
III. Carboxylases				
1. α-Keto acid carboxylases				
a. Yeast carboxylase	Yeast	Pyruvate	$\xleftrightarrow{\text{thiamine-pyroPO}_4}$	Acetaldehyde + CO_2
b. Pyruvic oxidase	Animal, bacteria	Pyruvate + O_2	$\xleftrightarrow{\text{thiamine-pyroPO}_4}$	Acetate + CO_2
c. α-Ketoglutaric oxidase	Animal, yeast, bacteria	α-Ketoglutarate + O_2	$\xleftrightarrow{Mn^{++}}$	Succinate + CO_2
2. β-Keto carboxylases				
a. Oxalacetate carboxylase	Pigeon liver, bacteria, plants	Oxalacetate	$\xleftrightarrow{Mn^{++},\,Co^{++}\text{ or }Zn^{++}}$	Pyruvate + CO_2
b. Oxalosuccinic decarboxylase	Animal, plant	Oxalosuccinate	$\xleftrightarrow{Mn^{++}}$	α-Ketoglutarate + CO_2
IV. Amino acid decarboxylases				
1. Histidine decarboxylase	Bacteria, animals	Histidine	$\xleftrightarrow{\text{pyridoxal-PO}_4}$	Histamine + CO_2
2. Glutamic decarboxylase	Bacteria, brain	Glutamic acid	$\xleftrightarrow{\text{pyridoxal-PO}_4}$	γ-Amino butyric acid + CO_2
D. Mutases (Simultaneously oxidize and reduce a molecule)				
1. Intramolecular Canizzaro reaction				

Enzyme	*Occurrence*	*Substrate*		*Products*
a. Glyoxalase	Yeast, liver, muscle, bacteria	Methyl glyoxal	$\xleftrightarrow{\text{glutathione}}$	Lactate
2. Intermolecular Canizzaro reaction				
a. Acetaldehyde mutase	Liver, yeast	Acetaldehyde	$\xleftrightarrow{\text{DPN}}$	Ethanol + acetate
E. Phosphate transfer enzymes				
1. Transphosphorylases				
a. Glucokinase	Animal, yeast, plant	Glucose + ATP	$\xleftrightarrow{Mg^{++}}$	Glucose-6-PO_4 + ADP
b. Phosphohexokinase	Animal, yeast, plant	Fructose-6-PO_4 + ATP	$\xleftrightarrow{Mg^{++}}$	Fructose-1,6-diPO_4 + ADP
c. Creatine phosphorylase	Muscle	Creatine + ATP	$\xleftrightarrow{Mg^{++}}$	Creatine-PO_4 + ADP
d. Phosphoglyceric transphosphorylase	Animal, yeast	3-Phosphoglycerate + ATP	$\xleftrightarrow{Mg^{++} \text{ or } Mn^{++}}$	1,3-Diphosphoglycerate + ADP
e. Myokinase	Muscle	2ADP		ATP and adenylic acid
f. Phosphopyruvate transphosphorylase	Muscle, yeast	Pyruvate + ATP	$\xleftrightarrow[Mg^{++}]{K^{+}}$	Phosphoenol pyruvate + ADP
2. Phosphomutases				
a. Phosphoglucomutase	Animal, yeast	Glucose-1-PO_4	$\xleftrightarrow{\text{glucose-1,6-di}PO_4, Mg^{++}}$	Glucose-6-PO_4
b. Phosphoglyceromutase	Animal, yeast	D-3-Phosphoglycerate	$\xleftrightarrow{\text{1,2-diphosphoglycerate}}$	D-2-Phosphoglycerate
3. Phosphoisomerases				
a. Triosephosphate isomerase	Animal, yeast	Dihydroxyacetone-PO_4		Glyceraldehyde-3-PO_4
b. Phosphohexoisomerase	Animal, yeast	Glucose-6-PO_4		Fructose-6-PO_4
4. Phosphorylases				
a. Glycogen phosphorylase	Muscle	Glycogen and inorganic PO_4		Glucose-1-PO_4
b. Sucrose phosphorylase	Bacteria	Sucrose and inorganic PO_4		Glucose-1-PO_4, fructose
c. Nucleoside phosphorylase	Bacteria, liver	Hypoxanthine desoxyriboside and inorganic phosphate		Desoxyribose-1-PO_4, hypoxanthine

Table 10–1 (Continued)

Enzyme	*Occurrence*	*Substrate*		*Products*
F. Oxido-reduction enzymes				
I. Dehydrogenases				
1. Pyridinoproteins				
a. Lactic dehydrogenase	Animal	Lactate	$\xleftrightarrow{\text{DPN}}$	Pyruvate
b. Glutamic dehydrogenase	Animal	Glutamate	$\xleftrightarrow{\text{DPN or TPN}}$	α-Ketoglutarate
c. Isocitric dehydrogenase	Animal	Isocitrate	$\xleftrightarrow{\text{TPN}}$	Oxalosuccinate
d. Alcohol dehydrogenase	Yeast	Ethanol	$\xleftrightarrow{\text{DPN}}$	Acetaldehyde
e. Phosphoglyceraldehyde dehydrogenase	Animal, yeast, plant	3-Phosphoglyceraldehyde + inorganic PO_4	$\xleftrightarrow[\text{glutathione}]{\text{DPN}}$	1,3-Diphosphoglyceric acid
2. Flavoproteins				
a. TPN-cytochrome *c* reductase	Yeast	TPN + cytochrome $c(Fe^{++})$ + [H]	$\xleftrightarrow{\text{FMN}}$	TPN · H + cytochrome *c* (Fe^{+++})
b. Xanthine oxidase	Milk	Xanthine or hypoxanthine + O_2 or methylene blue	$\xleftrightarrow{\text{FAD}}$	Uric acid + H_2O_2
c. D-Amino acid oxidase	Kidney, *Neurospora*	*e.g.*, D-alanine	$\xleftrightarrow{\text{FAD}}$	Pyruvate + NH_3
d. L-Amino acid oxidase	Kidney, snake venom, molds	*e.g.*, L-phenylalanine	$\xleftrightarrow{\text{FMN}}$	Phenyl pyruvate + NH_3
3. Others				
a. Succinic dehydrogenase		Succinate		Fumarate
II. Oxidases				
a. Cytochrome oxidase	Animal, yeast, bacteria	Cytochrome $c(Fe^{++})$ + O_2 + [H]	$\xleftrightarrow{\text{bound Fe}}$	Cytochrome *c* (Fe^{+++}) + H_2O
b. Tyrosinase	Potato, mushroom	Tyrosine	$\xleftrightarrow{Cu^{++}}$	Oxidized black pigments
III. Activators of H_2O_2				
1. Peroxidases	Horse radish, etc.	Substrate + H_2O_2	$\xleftrightarrow{\text{Fe porphyrin}}$	Oxidized substrate + H_2O
2. Catalase	Many tissues	H_2O_2	$\xleftrightarrow{\text{Fe porphyrin}}$	$H_2O + \frac{1}{2}O_2$
G. Miscellaneous				
1. Transaminases				
a. Glutamic-oxalacetic	Animal, bacteria	Glutamate + oxalacetate	$\xleftrightarrow{\text{pyridoxal-PO}_4}$	Aspartate + α-ketoglutarate

Enzyme	*Occurrence*	*Substrate*		*Products*
2. Polysaccharide-synthesizing enzymes (transglucosidases)	Bacteria	Sucrose		Dextran, fructose
		Sucrose		Levan, glucose
		Maltose		Dextran, glucose
3. Carbonic anhydrase	Blood, gastric mucosa	H_2CO_3	$\xleftarrow{Zn^{++}}$	$H_2O + CO_2$

The table includes some of the best known and most typical enzymes in each class. Enzymes usually occur in many natural materials; hence only one or two good sources are listed under "Occurrence." The substrates given are typical of common ones. In some cases more easily prepared synthetic compounds are used instead of the substrate occurring in nature. The activators needed for some of the enzymatic reactions have been indicated. Where the cofactors needed for the enzyme action are known, arrows have been placed between the substrate and products and the activators or coenzymes indicated.

Nature of enzyme action

It has been stated that enzymes are biological catalysts. This means that they are agents which affect the *rates* of metabolic reactions. However, although they greatly affect the speed of reactions, they do not influence the *extent* of the chemical change concerned, that is, they do not influence the final position of chemical equilibrium. The latter is determined by the nature (particularly the energy content) of the reacting substances and the products formed (see Chap. 16). The rates of metabolic reactions, however, are all-important for living organisms, since they must be able to utilize foods fast enough to keep up with their metabolic needs. For example, the same amount of glucose can be obtained from starch as from cellulose on chemical hydrolysis; yet, while the former will support growth in man, the latter will not. The explanation is that enzymes are present in the human digestive tract which accelerate the hydrolysis of starch, whereas there are none that attack cellulose. The uncatalyzed breakdown of cellulose to glucose is much too slow to be of use to the body. Cattle and other ruminants, however, have in their paunch vast numbers of bacteria which contain enzymes that can break down cellulose to organic acids and thus provide the animal with utilizable food. It follows from the above that any agent or condition which affects the catalytic ability of one or more of the enzymes involved in the metabolism of a vital food will have a profound effect on the development of the whole organism.

Many of the chemical reactions which occur easily in living organisms are difficult to reproduce in the laboratory in good yields and require drastic conditions of pressure, temperature, or pH to proceed at adequate speed; yet these reactions take place under much milder conditions in living cells. Enzymes accomplish this end, since by virtue of their specificity they guide reactions to the desired products, and because they can lower the energy of activation of the reaction (*i.e.*, the energy necessary to get it started).

Mechanism of action

According to the most widely accepted theory an enzyme functions through union with its substrate to form a labile intermediate compound or "enzyme-substrate complex," which in turn decomposes with formation of the end products of the reaction and regeneration of the enzyme. This mechanism can be schematically represented as follows:

$$\text{enzyme} + \text{substrate} \rightleftarrows \underset{\text{complex}}{\text{enzyme-substrate}} \rightarrow \text{product(s)} + \text{enzyme}$$

Since the enzyme-substrate complex is a very labile product and is present for only a very short time, it is difficult to demonstrate its existence. However, in the case of catalase it has been possible to provide evidence for the existence of such an intermediate compound with the aid of very speedy, automatically recorded, electrophotometric measurements.

Factors affecting activity

The speed with which a given reaction proceeds in the presence of an enzyme is influenced by many variables, which include the following: (1) concentration of substrate, (2) concentration of enzyme, (3) specific activators such as coenzymes and metallic ions, (4) temperature, (5) pH, (6) oxidation-reduction potential, (7) ionic strength, and (8) products of the reaction.

When the influence of substrate concentration on the speed of an enzymatic reaction is studied, it is observed that the rate of the reaction increases with substrate concentration up to a certain point beyond which there is no increase in activity. This occurs because all the enzyme eventually is converted into the intermediary compound by mass action, and the limiting speed of the reaction then becomes that of the decomposition of this complex. If under these conditions one doubles the *enzyme* concentration, the rate of reaction will also double, since twice as much enzyme-substrate complex will be available for decomposition.

The rate of most enzyme-catalyzed reactions is increased about 1.2–4 fold by a 10° rise in temperature. This temperature effect is much lower than that observed in the case of many uncatalyzed chemical reactions. For this reason enzymatic reactions proceed at higher speeds at low temperatures than the corresponding uncatalyzed ones. Most enzymes are thermolabile and will lose activity when exposed to high temperatures, *e.g.*, 60°C., over prolonged periods of time.

Acidity also has a profound effect on enzyme activity. Each enzyme in the presence of a certain substrate has a characteristic pH at which its activity is highest. Some enzymes, *e.g.*, pepsin, require an acid medium; others, *e.g.*, trypsin, need alkaline conditions for maximum activity. Most enzymes work best under conditions which are neither strongly oxidizing nor reducing, and, in fact, are frequently inactivated by strong oxidizing or reducing agents.

The effectiveness of some enzymes is influenced by the ionic strength (concentration of ions) of the solution in which they act; this is in addition to the specific effects of various anions and cations. In most cases, enzymes are inhibited by the end products of the reactions which they catalyze.

Specificity

In the study of digestion (Chap. 12) it is noted that fat-splitting enzymes are without effect on carbohydrates or proteins. Neither does an enzyme that hydrolyzes one of the latter attack fats. Even the common disaccharides require different enzymes to effect their hydrolysis. Specificity is frequently due to type of linkage rather than to individual compounds, as is evidenced by the fact that trypsin digests various proteins that differ markedly in composition and size of molecule. Furthermore, emulsin, which causes hydrolysis of many β-glucosides, has no effect on the isomeric α-glucosides; the reverse is true of maltase.

Inhibition

Enzymes are inhibited by a variety of conditions, which have already been indicated under factors affecting activity. In the present discussion attention is focused on the types of inhibition that can be obtained with chemical reagents. There are two main types: *competitive* and *noncompetitive*. If, for example, succinic dehydrogenase is inhibited by malonate (*i.e.*, a soluble salt of malonic acid), a substance which is similar in structure to succinate (the normal substrate of this enzyme), the inhibition can be competitively reversed by increasing the substrate concentration. This means that the amount of inhibition produced depends primarily on the relative amounts of malonate and succinate present. On the other hand, if this enzyme is inhibited by quinone, for example, the activity cannot be restored by an increase in succinate concentration. This is termed noncompetitive inhibition. These phenomena can be visualized if one considers that the enzyme surface has specific points of attachment which fit snugly against groups of the substrate molecule. If an inhibitor is used that is so similar in structure to the substrate that it also can fit into the "mold" on the enzyme surface, it can compete with the substrate for position. However, if an agent is used that changes the enzyme in some way, the substrate can no longer attach to the surface regardless of the amount used. The relation between substrate and inhibitor then becomes noncompetitive.

Various inhibitors have been used successfully for the study of metabolic reactions. If it is desired to study the conversion of α-ketoglutarate to succinate in the presence of other enzymes of the Krebs cycle (see Chap. 13), one can prevent the further metabolism of succinate by the addition of malonate. For additional examples concerned with vitamins, see p. 256.

Some drugs are known to exert their action by inhibition of enzymes. For example, eserine, an alkaloid ($C_{15}H_{21}N_3O_2$) that stimulates the parasympathetic nervous system, inhibits the enzyme choline esterase

which decomposes acetylcholine; as a result, the latter accumulates and causes increased stimulation.

Activation

Zymogens. A number of enzymes are secreted in the form of inactive precursors known as *zymogens.* For each zymogen there is some reagent that can change it into the active enzyme. To illustrate, pepsinogen, the zymogen of pepsin, is slowly converted into active pepsin by hydrogen ions, but it is rapidly activated by pepsin itself; that is, the activation is autocatalytic. Chymotrypsinogen is converted into chymotrypsin by trypsin. The conversion of trypsinogen to the active form is autocatalytic, *i.e.*, by trypsin itself.

Ions. Certain enzymes can be separated into two fractions by dialysis. Either fraction alone is inactive, but upon recombination the activity is restored. The portion of the enzyme that can pass through the membrane has a much smaller molecular weight than the remaining part. This dialyzable portion is considered as a *cofactor* which is necessary for the activity of the total enzyme. In some cases more drastic conditions than simple dialysis must be employed to separate the cofactor from the apoenzyme; for example, treatment with acid in ammonium sulfate solution in the case of certain flavo-proteins. In some enzymes the cofactor is so tightly bound that it has not been yet possible to remove it without destroying the enzyme.

In many cases the cofactor is simply a metallic ion. For example, Mn^{++}, Co^{++}, or Zn^{++} have been found to be activators for certain peptidases. The theory has been proposed that the metal ions form coordination compounds and act as bridges to bring substrate and enzyme together. Certain enzymes have a characteristic *anion* requirement, *e.g.*, salivary amylase is activated by chloride.

Coenzymes. Another group of cofactors are organic compounds which are called *coenzymes.* The study of coenzymes has received much attention by biochemists for the past 20 years, and the chemical structures of many of them have been determined. The cofactors required by several enzymes are given in Table 10–1.

1. *Cocarboxylase.* It has been pointed out previously (p. 227) that thiamine is required for the metabolism of carbohydrates, and particularly of pyruvic acid. The reason for this requirement is that the enzyme which

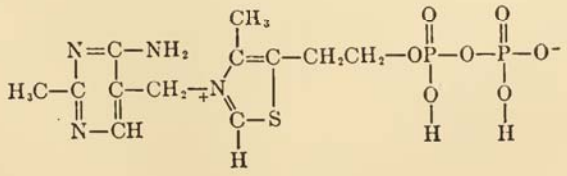


Fig. 10–4. Thiamine pyrophosphate (cocarboxylase).

cleaves pyruvic acid into carbon dioxide and a two carbon fragment (acetaldehyde in yeast) contains thiamine pyrophosphate (cocarboxylase) (Fig. 10–4) as a coenzyme. The degradation of fatty acids to two carbon compounds (Fig. 13–4) does *not* require the presence of thiamine pyrophosphate. In animals on a low-thiamine, high-fat diet the supply of two carbon fragments from carbohydrate is limited owing to the small quantity of cocarboxylase in the tissues, but this deficiency is compensated by the generation of these metabolic intermediates in adequate amounts from fat.

A compound of cocarboxylase and lipoic acid, lipothiamide (LTPP), acts as a coenzyme for the oxidative decarboxylation of pyruvic acid and α-ketoglutaric acid by certain bacteria, *e.g., E.coli.* In an enzyme system obtained from this organism the following series of reactions has been demonstrated:

pyruvate (α-ketoglutarate) + LTPP + DPN →
acetyl LTPP (succinyl LTPP) + CO_2 + $DPNH_2$

acetyl LTPP (succinyl LTPP) + *Co A* → acetyl *Co A* (succinyl *Co A*) + LTPP

2. *Coenzyme A.* Lipmann and co-workers discovered that a coenzyme is necessary for the acetylation of sulfanilamide. Subsequent studies demonstrated that the same substance is required for the metabolic formation of acetylcholine from choline and for the condensation of oxalacetic acid with the two carbon fragments from fat or carbohydrate metabolism to produce citric acid (Fig. 13–4). Since in each case acetic acid or an acetyl group seemed to be involved, the coenzyme was named coenzyme *A* (*Co A*), a "coenzyme for acetylation." Chemical investigations re-

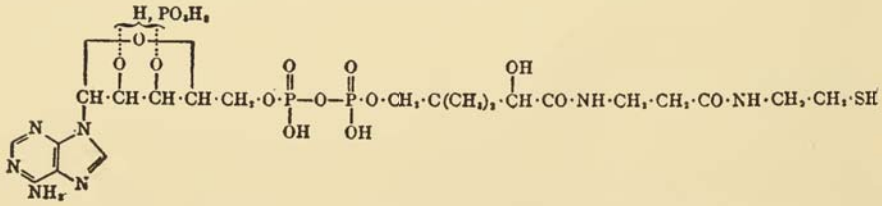


Fig. 10–5. Structure of coenzyme *A* suggested by Baddiley and Thain. It is possible that this formula will require some revision as fuller information becomes available.

vealed that *Co A* was a derivative of pantothenic acid, thus providing an insight into the metabolic functions of this B vitamin. The *Co A* molecule also appears to contain adenine, ribose, β-thioethylamine, and two or three phosphate radicals. Although the exact chemical formula is not yet known, a suggested structure is given in Fig. 10–5. The substance seems to function by accepting acetyl groups from one metabolite

and then donating them to another; in other words, it serves as an acetyl carrier. This is illustrated in the following scheme:

$$\text{acetyl } X + Co\ A \xrightarrow{\text{apoenzyme 1}} X + \text{acetyl } Co\ A$$

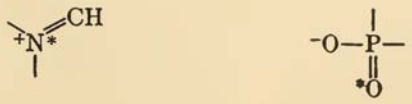
$$\text{acetyl } Co\ A + Y \xrightarrow{\text{apoenzyme 2}} \text{acetyl } Y + Co\ A$$

The apoenzymes 1 and 2 are specific for X and Y; for example, different apoenzymes are required for the formation of citric acid than for acetylation of choline.

3. *Pyridino Coenzymes.* Several coenzymes have been found necessary for the numerous dehydrogenation reactions which constantly occur in all living cells. Warburg and co-workers demonstrated the need for one such substance for the enzymatic dehydrogenation of glucose-6-phosphate to phosphogluconic acid. The coenzyme was isolated in pure form and shown to contain three molecules of phosphoric acid, two of pentose, one of adenine, and one of nicotinamide (later identified as the pellagra-curing vitamin). This substance was called "coenzyme II," but now is preferably designated as *triphosphopyridine nucleotide* (TPN, Fig. 10–6). It has been shown to be a component, for example, of the dehydrogenases that act on glucose-6-phosphate and on isocitrate, and for the enzyme system that converts malate to pyruvate and carbon dioxide (reaction 15, Fig. 13–4).

Another coenzyme in this group is called cozymase, coenzyme I, or preferably *diphosphopyridine nucleotide* (DPN). It has exactly the same chemical structure as TPN except that it contains only two phosphate groups, as is indicated by the name. The extra phosphate group in TPN is the one on the second carbon of the ribose residue in the adenylic acid half of the molecule. Among dehydrogenases which require DPN are those involved in the oxidation of D-glyceraldehyde-3-phosphate, lactate, ethanol, malate, L-α-glycerophosphate, and glucose.

DPN and TPN are called "pyridino" coenzymes because of the pyridine ring in the nicotinamide component. It is also the pyridine ring which undergoes chemical reaction when the coenzymes function in oxidation-reduction reactions. The exact nature of this important change, which is the same for both DPN and TPN, may be understood by studying the structural formulas given in Fig. 10–6. In the oxidized form the pyridine nitrogen has a valence of *five* and exists as the basic ion of a quaternary ammonium salt. This positive charge is neutralized by one of the negatively (acidic) charged phosphate groups of the molecule. In Fig. 10–6 these groupings are starred and appear as follows:



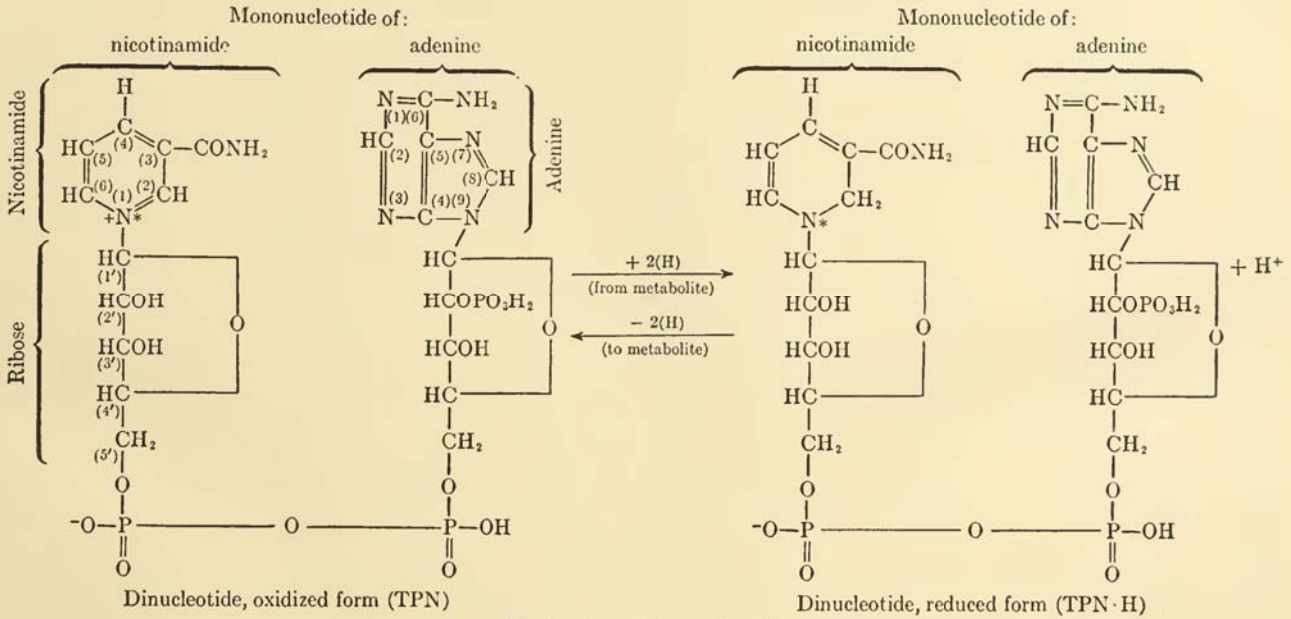
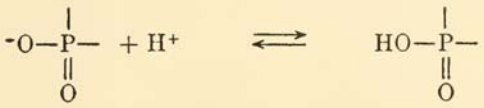


Fig. 10-6. Chemical formula of triphosphopyridine nucleotide (TPN). Both oxidized and reduced forms are shown, and the main components of the complex molecule are indicated. The parts affected by oxidation and reduction are starred (*).

When the oxidized coenzyme is reduced, the nitrogen changes to a valence of *three,* the double bond between the nitrogen and the adjacent carbon atom is reduced, and a hydrogen ion is formed. These changes may be represented as follows:

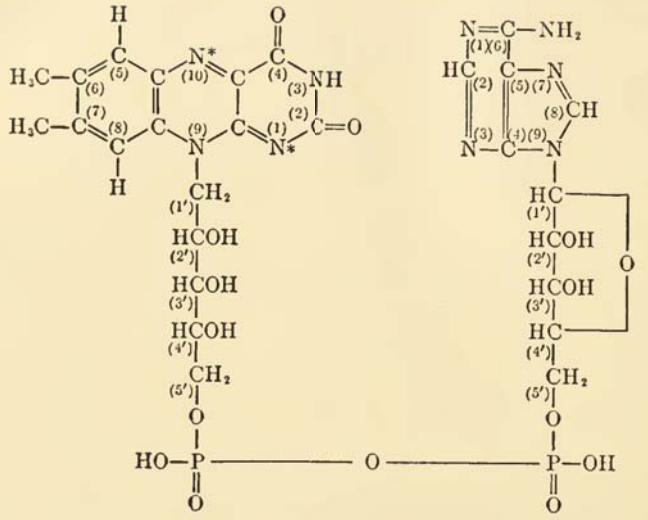
The trivalent nitrogen is much less basic and no longer neutralizes a phosphate group. Consequently the hydrogen ion formed might add to this group:



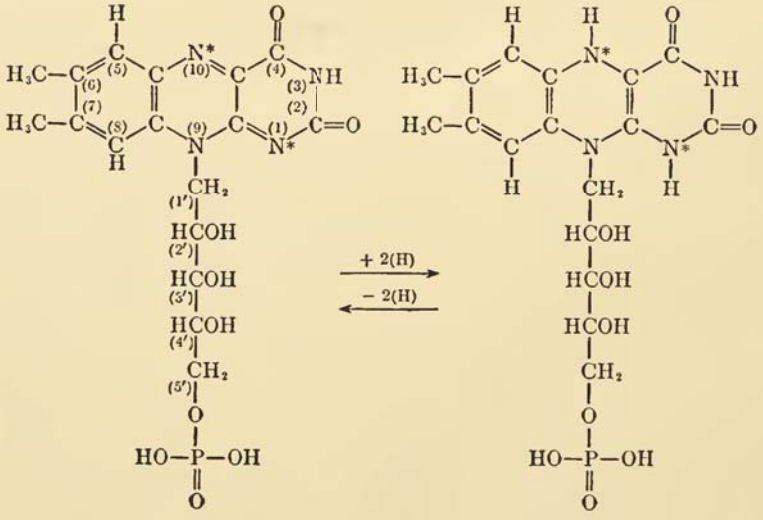
However, the phosphate group is strongly acidic and in the physiological pH range is almost completely dissociated (*i.e.*, the equilibrium point of the above reaction lies far to the left). What actually happens is that the newly formed hydrogen ion is picked up by the buffer systems of the cell and is used later in the reaction of reduced cytochrome *c* with oxygen (see below).

It should be remembered in this connection that an atom of hydrogen consists of a *proton* (hydrogen ion, H^+) and an *electron.* The electron corresponding to the proton set free in the reduction of DPN or TPN becomes attached to the coenzyme, neutralizing the positive charge on the nitrogen atom. The reduced coenzyme thus actually carries one *hydrogen atom* and one *electron,* the proton of the second hydrogen being carried in the cell buffers.

4. *Riboflavin Coenzymes.* These substances are also coenzymes of oxidation-reduction reactions. There are two of these: the so-called riboflavin mononucleotide (FMN), which is more accurately named riboflavin-5′-phosphate, and riboflavin adenine dinucleotide (FAD). The abbreviations start with "F" because riboflavin is often called simply "flavin." The formulas of these compounds are given in Fig. 10–7. Since the union between the isoalloxazine and ribitol (alcohol corresponding to ribose) residues is not glycosidic, neither substance strictly speaking is a nucleotide, but the above names are in common use and are likely to be retained. These coenzymes act by taking up and giving off two hydrogens. In each case the hydrogen atoms are attached to positions 1 and 10 in the flavin part of the molecule (Fig. 10–7). FMN is a coenzyme for TPN-cytochrome reductase and L-amino acid oxidase. FAD is required by xanthine oxidase and glycine oxidase.



Riboflavin adenine dinucleotide (FAD, oxidized form)



Oxidized form Reduced form

Riboflavin mononucleotide (FMN)

Fig. 10-7. Riboflavin coenzymes. The atoms which acquire hydrogens on reduction are starred.

5. *Iron Porphyrin Compounds.* A number of enzymes such as catalase and peroxidase have iron porphyrin compounds as the prosthetic group. The cytochromes, a group of pigments present in a large number of organisms and tissues, are also of this type, since they consist of a char-

acteristic protein and a heme compound. There are at least three cytochromes, designated as *a*, *b*, and *c*. The heme portion is more firmly attached to the protein in these pigments than the corresponding functional groups of the pyridino- or flavo-proteins. The cytochromes are concerned with oxidation-reduction reactions, and their concentration in aerobic organisms bears a direct relationship in many instances to the respiratory activities of the cell. The best characterized of these respiratory pigments is cytochrome *c*. It contains 0.43 per cent of iron and is believed to have a molecular weight of 13,000. The most probable formula for the heme component of cytochrome *c*, according to the evi-

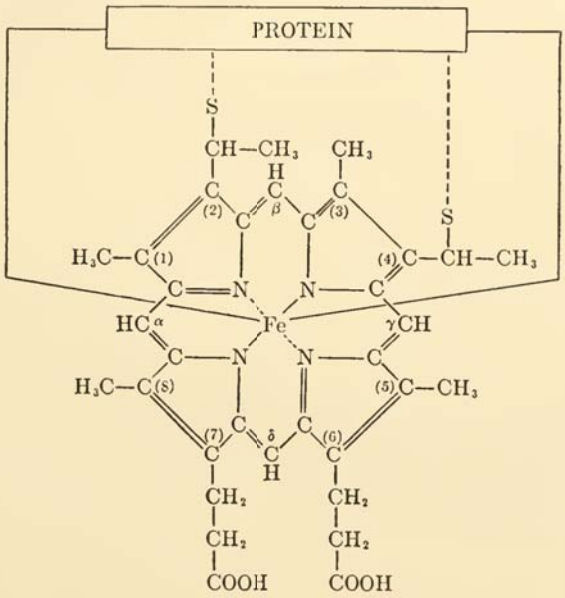


Fig. 10–8. Cytochrome *c*. The heme component (prosthetic group) is shown and also its attachment to the protein part of the molecule by two sulfur linkages and the iron atom.

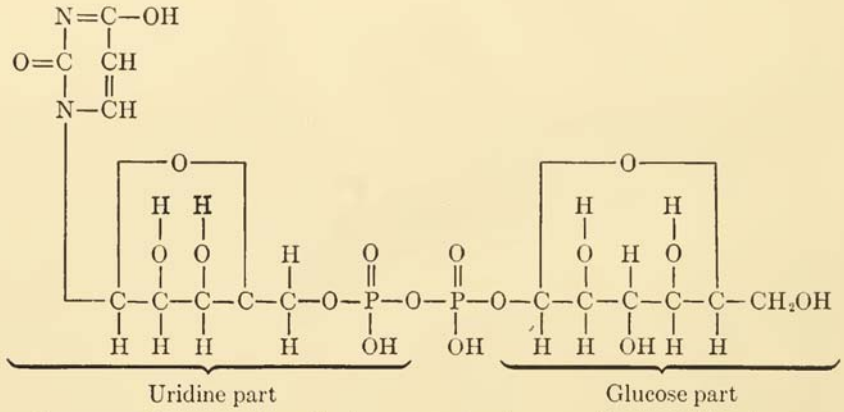
dence available at present, is shown in Fig. 10–8. Cytochrome *c* (abbreviated Cyt. *c*) functions as an electron carrier in cellular oxidation-reduction reactions by virtue of its iron atom which alternately changes its valence from 2 to 3:

$$\text{Cyt. } c\ (Fe^{+++}) + e \rightleftarrows \text{Cyt. } c\ (Fe^{++})$$

6. *Pyridoxal Phosphate.* Enzymes which catalyze the decarboxylation of histidine, tyrosine, lysine, and glutamic acid to form carbon dioxide and the corresponding primary amine require pyridoxal phosphate as a coenzyme. This coenzyme is also a necessary cofactor for transaminase

(Table 10–1, class G.1.a.). Its chemical formula has been given on p. 243.

7. *Other Coenzymes.* Three definite chemical substances are known to serve as coenzymes for the interconversion of several organic phosphates during carbohydrate metabolism. Glucose-1,6-diphosphate is a coenzyme for phosphoglucomutase, which catalyzes the migration of a phosphate group between the 1 and 6 positions of glucose. Glyceric acid-2,3-diphosphate acts in an entirely analogous manner in catalyzing the migration of phosphate groups between the 2 and 3 positions of glyceric acid. A coenzyme necessary for the enzymatic conversion of galactose-1-phosphate to glucose-1-phosphate (reaction II, Fig. 13–1) has been purified by Caputto and co-workers. The suggested formula is given below:



Coenzyme for conversion of galactose-1-phosphate to glucose-1-phosphate

A number of other compounds or their derivatives are suspected to be coenzymes on the basis of their chemical properties or their gross metabolic effects. The tripeptide glutathione (GSH, p. 130) can be oxidized to form a double molecule, the parts of which are held together by a disulfide (—S—S—) linkage. Specific pyridinoproteins have been studied which catalyze this reaction. Although the role of glutathione in oxidation-reduction reactions is not fully understood, it is known to be a cofactor in the glyoxalase reaction (Table 10–1, class D.1.a.) and to be a functional part of glyceraldehyde phosphate dehydrogenase. Ascorbic acid is also capable of undergoing alternate oxidation and reduction, but the mechanism of its metabolic function has not been explained. Biotin has been implicated in certain carbon dioxide-fixation reactions, *e.g.*, the condensation of carbon dioxide and pyruvate to form oxalacetate (reaction 14, Fig. 13–4), but to date no enzyme has been purified which has been proven

to require a biotin containing coenzyme. Vitamins containing para-aminobenzoic acid, *e.g.*, folic acid and the "citrovorum factor," seem to be concerned with the transfer of formyl or formaldehydo groups in the organism (Chap. 9). The family of B_{12} vitamins has an effect on the metabolism of methyl groups (Chap. 9) and on the synthesis of desoxyribonucleotides.

Role of enzymes in tissue oxidation

It has already been noted in the discussion of coenzymes and in Table 10–1 (section F) that a large group of enzymes is concerned with oxidation-reduction reactions. There are three general groups of enzymes in this class, the *oxidases*, *peroxidases*, and *dehydrogenases*. In the 1920's there were two concepts concerned with the oxidation of substrates in the organism. The advocates of the Warburg school contended that substances were oxidized because of *activation of oxygen* by iron. In model experiments with iron-containing charcoal and enzyme preparations, it was shown that the oxidation of substrate was accompanied by a reduction of iron from the ferric to the ferrous state.

However, Wieland and co-workers demonstrated that an organic substance in the reduced form could be oxidized in the presence of palladium black. Palladium is known to have a strong affinity for hydrogen, and the process was termed "dehydrogenation." The idea was advanced that biological oxidations occurred more as a result of *activation of hydrogen* than of oxygen. This view was greatly advanced by the work of Thunberg who demonstrated that the removal of hydrogen from succinate, for example, could be accomplished in the absence of oxygen by methylene blue and a specific enzyme. Methylene blue is a dye which is readily reduced and is thereby decolorized:

$$\underset{\text{Succinic acid}}{HOOC{-}CH_2CH_2{-}COOH} + \underset{\substack{\text{Methylene}\\\text{blue}}}{MB} \xrightarrow{\text{enzyme}} \underset{\text{Fumaric acid}}{HOOC{-}CH{=}CH{-}COOH} + \underset{\substack{\text{Leuco}\\\text{methylene}\\\text{blue}\\\text{(colorless)}}}{MB\cdot H_2}$$

It will be seen later that both principles apply to oxidation processes in living organisms.

Oxidases are enzymes which lead to oxidation of a substrate by molecular oxygen. Thus cytochrome *c* is converted from the ferrous to the

ferric state by molecular oxygen under the influence of *cytochrome oxidase* (Table 10–1, class F.II.a.). *Tyrosinase* is another example of an oxidase.

Peroxidases lead to the oxidation of substrates by hydrogen peroxide. *Catalase* also activates hydrogen peroxide and decomposes it to water and oxygen in the absence of added substrates. However, in the presence of certain oxidizable substrates, catalase can act as a peroxidase. To illustrate:

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2 \qquad (H_2O_2 \text{ decomposition})$$

$$H_2O_2 + CH_3CH_2OH \xrightarrow[\text{or peroxidase}]{\text{catalase}} 2H_2O + CH_3CHO \qquad (\text{peroxidation})$$

Dehydrogenases result in a removal of hydrogen ions and an equal number of electrons from an organic molecule. Examples of various dehydrogenases have already been encountered in the discussion of pyridino and flavin coenzymes. The nature of the coenzymes of certain other important dehydrogenases, *e.g.*, succinic dehydrogenase (Table 10–1, class F.I.3.), has not been completely elucidated to date. When the biocatalyst is concerned with the oxido-reduction of a compound from which electrons, but not hydrogens, are removed, the enzyme is called a *reductase, e.g.*, cytochrome reductase (Table 10–1, class F.I.2.a.).

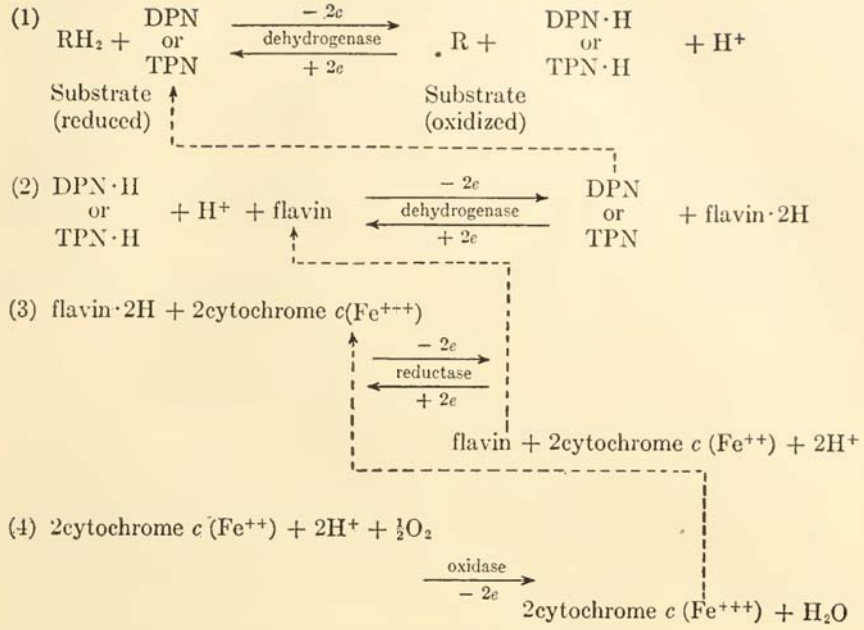
The basic mechanism of all oxidation-reduction reactions involves the transfer of electrons, and the enzymes or coenzymes concerned can be considered as carriers of electrons. To illustrate schematically:

(1) $HOOC{-}CH_2{-}CH_2{-}COOH$
Succinic acid $\underset{\text{dehydrogenase}}{\rightleftarrows}$ $HOOC{-}CH{=}CH{-}COOH + 2H^+ + 2e$
Fumaric acid

(2) 2Cytochrome *c* (Fe^{+++}) + $2e$ $\underset{\text{reductase}}{\rightleftarrows}$ 2Cytochrome *c* (Fe^{++})

(3) Oxidase
(a) 2Cytochrome *c* (Fe^{++}) $\rightleftharpoons$ 2Cytochrome *c* (Fe^{+++}) + $2e$
(b) $2H^+ + 2e + \frac{1}{2}O_2 \longrightarrow H_2O$

If one considers the oxido-reduction enzymes in relation to each other, a certain pattern emerges. For example, a dehydrogenase containing TPN or DPN can extract hydrogen and electrons from a given substrate, reduced TPN or DPN can react with flavo-protein, and this then can react with cytochrome *c*, which can be oxidized by oxygen in the presence of cytochrome oxidase. This relationship can be summarized in the following series of equations:



(1) + (2) + (3) + (4) $RH_2 + \frac{1}{2}O_2 \xrightarrow{-2e} H_2O + R$

Substrate (reduced) — Substrate (oxidized)

It will be observed that the *net* result of the oxidation of the substrate to product is the removal of two hydrogen atoms [two hydrogen ions plus two electrons, since H (atom) = H^+ (ion) + e (electron)] from the substrate, which combine with oxygen to form water. It should also be noted that the coenzyme that becomes reduced in one reaction is reoxidized in the next and thus is ready for the first reaction again; this is indicated by the broken arrows in the scheme.

In subsequent chapters (13, 16), it will be explained that the degradation of foods is a stepwise process which proceeds through a series of intermediary compounds before the end products are excreted by the organism. The above scheme suggests that this situation also applies to oxidation-reduction processes. It should be realized, furthermore, that a certain increment of energy becomes available at each of the steps of the oxidation-reduction chain and that this energy can be transferred to functions useful to the organism by appropriate, stepwise mechanisms, such as the formation of high energy phosphate linkages (Chap. 16).

Although some tissues contain all the electron transferring systems indicated in the scheme, it should be remembered that not all of the steps are necessary for every process. For example, the oxidation-reduc-

tion reactions of anaerobic glycolysis (Chap. 13) proceed through DPN-linked enzymes only.

REVIEW QUESTIONS ON ENZYMES

1. Explain the terms (1) apoenzyme, (2) coenzyme, (3) zymogen, (4) activator, (5) carrier.
2. How general is the occurrence of enzymes in nature? Distinguish between endo- and exo-enzymes.
3. (1) Name five of the enzymes that have been obtained in the crystalline state. (2) What has proved to be the chemical nature of all crystallized enzymes?
4. (1) What is the essential nature of enzyme action? (2) What is the effect of an enzyme on the chemical equilibrium of a reaction?
5. (1) List the factors that influence the rate of enzyme action and discuss each briefly. (2) What is meant by specificity of enzymes?
6. What is probably the mechanism of enzyme action?
7. Define the following: (1) dehydrogenase, (2) catalase, (3) oxidase, (4) peroxidase.
8. What is the chemical nature of the cytochromes, and to what substance previously studied are they therefore related?
9. Explain wherein the structure of (1) glutathione, (2) DPN, (3) riboflavin phosphate, and (4) cytochrome *c* could make it possible for these compounds to function in tissue oxidation.

REFERENCES AND SUGGESTED READINGS

Baldwin, E., *Dynamic Aspects of Biochemistry*, 2nd ed., Cambridge University Press, Cambridge, 1952.

Baddiley, J. and Thain, E. M., "Coenzyme A. Part III. Synthesis of Pantothenic Acid-2′:4′ Phosphate and Further Structural Considerations," *J. Chem. Soc.*, 3421 (1951).

Caputto, R., Leloir, L. F., Trucco, R. E., Cardini, C. E., and Paladini, A. C., *J. Biol. Chem.*, **179**, 497 (1949).

Haldane, J. B. S., *Enzymes*, Longmans, Green and Company, New York, 1930.

Lardy, H. A. (editor) *Respiratory Enzymes*, Burgess Publishing Company, Minneapolis, 1949.

Lardy, H. A., "Vitamins and Carbohydrate Metabolism," *J. Chem. Ed.*, **25**, 262 (1948).

Lipmann, F., Kaplan, N. O., Novelli, G. D., Tuttle, L. C., and Guirard, B. M., "Coenzyme for Acetylation, a Pantothenic Acid Derivative," *J. Biol. Chem.*, **167**, 869 (1947).

McElroy, W. D. and Glass, B., *Phosphorus Metabolism*, Vol. I, The Johns Hopkins Press, Baltimore, 1951.

Northrop, J. H., Kunitz, M., and Herriot, R. M., *Crystalline Enzymes*, Columbia University Press, New York, 1948.

Peters, R. A. and Thompson, R. H. S., "Pyruvic Acid as an Intermediary Metabolite in the Brain Tissue of Avitaminous and Normal Pigeons," *Biochem. J.*, **28**, 916 (1934).

Sumner, J. B. and Myrbäck, K., *The Enzymes*, Academic Press Inc., New York, 1951.

Thunberg, T., "Action of Animal Tissues on Methylene Blue," *Skand. Arch. Physiol.*, **35**, 163 (1917).

Velick, S. F. and Ronzoni, E., "The Amino Acid Composition of Aldolase and D-Glyceraldehyde Phosphate Dehydrogenase," *J. Biol. Chem.*, **173**, 627 (1948).

Warburg, O., *Die Katalytischen Wirkungen der lebendigen Substanz*, Julius Springer, Berlin, 1928.

Warburg, O. and Christian, W., "Activation of the Robison Hexosemonophosphoric Acid Ester in the Red Blood Cells and the Method for Preparation of Activating Enzyme Solutions," *Biochem. Z.*, **242**, 206 (1931).

West, E. S. and Todd, W. R., *Textbook of Biochemistry*, Macmillan Company, New York, 1951.

Wieland, H., "Über den Mechanismus der Oxydationsvorgänge," *Ergebnisse Physiol.*, **20**, 477 (1922).

Chapter 11

HORMONES

The hormones have been defined by Houssay as "specific chemical substances produced by an organ or tissue which, after being discharged into the circulating fluids, may reach all parts of the organism and in small amounts markedly influence the functions of other organs or systems without themselves contributing important quantities of matter or energy." Thus they resemble the vitamins very closely, differing only by being produced in the body rather than having to be supplied ready-made in the food. The hormones are produced by specialized organs called the glands of internal secretion, or *endocrine glands*, such as the pancreas, thyroid, ovaries, and others. Hormone manufacture and secretion is the physiological function of these glands, and the effects which follow their removal or alteration are merely the result of too little or too much hormone production. Often it is possible to overcome the effect of glandular lack by supplying the necessary hormone from an outside source (for example, insulin). The various hormones were discovered by showing that the effects of removing certain endocrine glands could be counteracted with extracts of the same glands from other individuals.

Chemical types

The known hormones of higher animals are sometimes grouped roughly into three chemical types. Those of the pancreas and pituitary, plus a few others, are *proteins* or *peptides*. The sex hormones and adrenal cortex hormones are *steroids*. The third group is made up of adrenalin and thyroxine, which are classified together as phenolic compounds, although they are otherwise quite dissimilar. Most of the plant and insect hormones do not fit into any of these classes. Some of the former are considered briefly in Chap. 15.

Control of hormone production

The various glands of internal secretion and the hormones they produce make up a closely interrelated system, which is delicately balanced and responsive to many influences. The functioning of this system helps

the organism to adjust its metabolic activities so as to cope with changes in the outside environment and to maintain a stable internal condition. The system operates for the most part automatically. Thus, for example, an increase in the blood glucose level stimulates the pancreas to secrete more insulin, which promotes utilization of the sugar and hence brings the concentration down again. The pituitary produces, among others, hormones which stimulate growth and activity of the thyroid, ovaries, testes, and adrenal cortex. The characteristic hormones of these glands depress pituitary function. In general, the rate of hormone production is controlled either by other hormones, by various other chemical substances in the body, or to a lesser degree by nervous stimulation originating in the external environment.

Hormone metabolism and function

The smooth operation of various bodily processes often involves the concerted action of a whole series of hormones. For example, carbohydrate metabolism cannot proceed normally without the help of hormones from the pancreas, pituitary, thyroid, and adrenal cortex. Sexual reproduction in mammals depends on the hormones of the ovaries, testes, pituitary, adrenal cortex, and, to some extent, the thyroid.

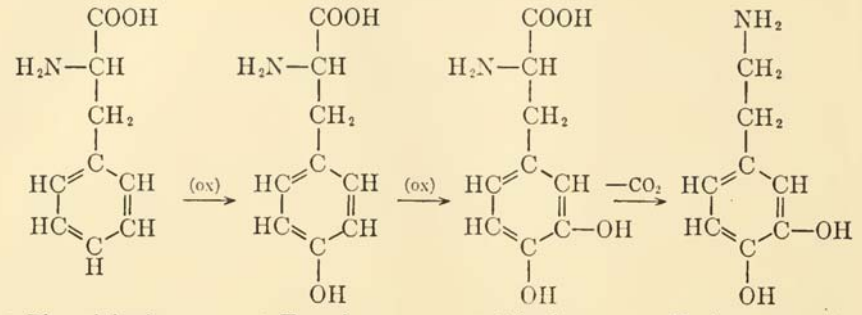
Just how the observed effects are brought about is, in most cases, not known. Presumably, since they are active in very small amounts, the hormones must act through certain enzyme systems (*e.g.*, effect of epinephrine on phosphorylase, p. 289). Likewise the metabolic fate of the hormones, that is, what becomes of them after being secreted into the blood, is still largely unknown, although some of the steroid hormones have been found recently to be converted into modified products and excreted in the urine.

With this general introduction, attention will now be directed to the individual endocrine organs and the hormones they produce.

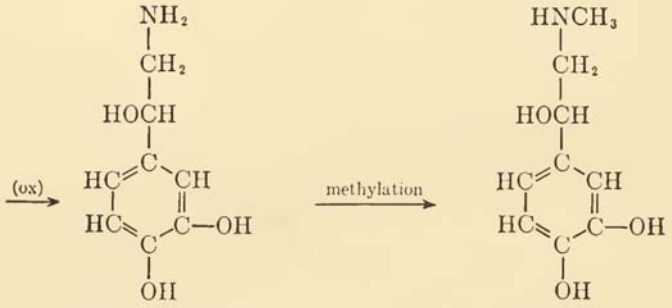
NONPROTEIN HORMONES

Hormones of the adrenal medulla

Epinephrine (adrenalin) and *norepinephrine* (noradrenalin or arterenol) are the hormones produced by the *adrenal medulla,* the inner part of a small endocrine gland located just above each kidney. About five to six times as much of the former substance is normally formed by the adrenal as of the latter. These two hormones are almost certainly synthesized from phenylalanine in the body. The biosynthetic pathway used is not entirely known, but is probably somewhat as follows:



L-Phenylalanine — L-Tyrosine — L-Dihydroxyphenylalanine — Hydroxytyramine



l-Norepinephrine — *l*-Epinephrine

It has been demonstrated that slices of the adrenal medulla convert hydroxytyramine *in vitro* into a substance with the biological properties of epinephrine and that norepinephrine is methylated *in vivo* according to the reaction shown. Furthermore, Gurin and Delluva injected into rats D L-phenylalanine labeled with C^{14} (radioactive) in the —COOH group and (or) in the alpha position (carbon atom next to the —COOH). They showed that radioactive epinephrine with all of its C^{14} located in the terminal carbon of the side chain was formed. This is good evidence that epinephrine is actually made from phenylalanine by the living animal. There is no direct proof that dihydroxyphenylalanine takes part in the biosynthesis, but some such dihydroxy substance must obviously be involved at some stage of the process.

Both hormones contain an asymmetric carbon atom and are therefore optically active. The levorotatory or *l*-forms are the naturally occurring isomers and are about fifteen times more effective than the corresponding *d*-forms. Both isomers of each hormone have been prepared by chemical synthesis, and the pure *l*-forms have been isolated from the adrenals of various animals.

Injection of epinephrine is followed by a rapid rise of blood pressure

due to increased heart rate and contraction of the arteriols (small arteries). The blood pressure falls again rather quickly unless additional doses are given. The hormone also brings about contraction of the iris, relaxation of bronchial muscles, increased salivary secretion, and other effects. These responses, in general, are the same as those caused by stimulation of the sympathetic nerves going to the same tissues or organs. In fact, it appears that epinephrine, or norepinephrine, is necessary for transmission of nerve impulses in the sympathetic (or "adrenergic") nerve system. Some sympathetic nerve cells produce epinephrine, and others, norepinephrine. According to Tainter and Luduena, these hormones probably pass through the nerve trunks to the endings, are released when the nerve is stimulated, and act on the particular tissue concerned to produce the effect finally seen.

Epinephrine also causes a rise in the amounts of glucose and lactic acid in the blood stream and increases the basal metabolic rate (p. 424). Blood sugar is derived from glycogen by the following reactions (see Fig. 13–1):

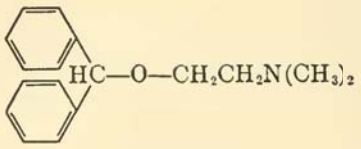
$$\text{glycogen} + H_3PO_4 \xrightarrow{\text{(phosphorylase)}} \text{glucose-1-phosphate} \xrightarrow{\text{(phosphogluco-mutase)}}$$

$$\text{glucose-6-phosphate} \xrightarrow{\text{(phosphatase)}} \text{glucose} + H_3PO_4$$

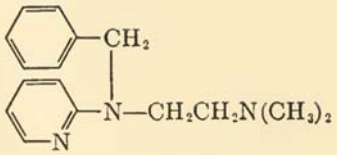
It is the first of these steps which is stimulated by epinephrine. Norepinephrine has only about one-eighth the effect of epinephrine in raising the blood sugar level.

The secretion of the adrenal medulla is under nervous control and is increased in times of stress or intense emotion (suffocation, rage, fear, etc.). The net result is a general mobilization of the resources of the individual to meet the crisis. Aside from its direct natural functions, epinephrine has also found a number of medical applications, particularly as a vasoconstrictor (blood vessel constrictor), and for relief of the bronchial spasms of asthma and hay fever. Injected together with a local anesthetic such as Novocaine, it contracts small blood vessels and reduces blood flow through the area affected. It thus permits a longer and more intense response from a given dose of the anesthetic. It also has antihistamine action and usually gives relief in a variety of allergic conditions thought to be due to the liberation of a histamine-like substance.

Histamine is the amine formed by decarboxylation of histidine (p. 321). Even small doses of it can produce symptoms of allergy. A number of drugs intended to destroy or counteract histamine have been developed. They are called *antihistamines*, and their effect is called an antihistamine action. Benadryl and Pyribenzamine are two widely used antihistamine drugs:



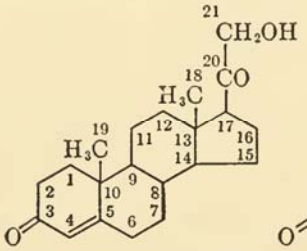
Benadryl



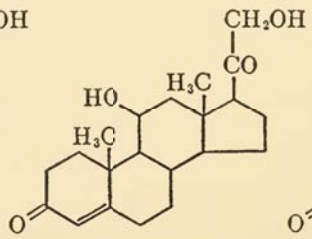
Pyribenzamine

Hormones of the adrenal cortex

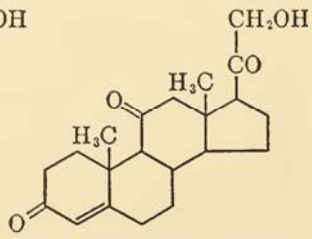
The outer part, or *cortex,* of the adrenal gland produces a series of hormones which are essential for life. In this respect the cortex differs from the medulla, for the latter can be removed from animals without causing death. However, in 1930 Hartman and Brownell found that adrenalectomized animals [1] could be kept alive if injected at regular intervals with material extracted from the adrenals of other animals of the same or different species. The life-maintaining principle was contained in the nonsaponifiable part of the extract from the cortex and was eventually found to consist of a group of closely related steroids. At least 28 individual steroids have been separated from such extracts as pure crystalline substances, of which six possess marked adrenal cortical activity.[2] The chemical structures of these six compounds are as follows (see p. 94 for diagram and numbering of the steroid ring system):



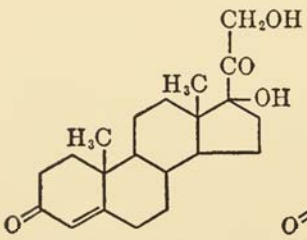
11-Desoxycorticosterone, $C_{21}H_{30}O_3$



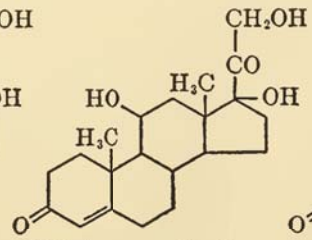
Corticosterone, $C_{21}H_{30}O_4$



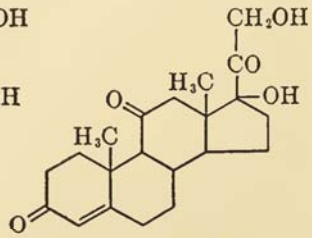
11-Dehydrocorticosterone, $C_{21}H_{28}O_4$



17-Hydroxy-11-desoxy-corticosterone, $C_{21}H_{30}O_4$



17-Hydroxycorticosterone, $C_{21}H_{30}O_5$



Cortisone, $C_{21}H_{28}O_5$ (17-Hydroxy-11-dehydro-corticosterone)

[1] Animals with both adrenals completely removed.

[2] In addition, there is a noncrystalline residue which still contains one-fourth to one-half the biological activity of the original extracts.

Note that the six substances differ chiefly in the presence or absence of oxygen on carbons 11 and 17. They were first obtained as pure chemical substances during the period 1935 to 1939.

That the normal adrenal cortex has a number of functions has been revealed by study of adrenalectomized animals and of human victims of Addison's disease, a fatal illness caused by insufficient secretion of adrenal hormones. In such cases there is a marked decrease in the ability of the organism to work and to withstand stresses of any sort (for example, fasting or exposure to cold). Sodium and chloride ions are excreted in the urine in such excessive amounts that bodily supplies are depleted, whereas excretion of potassium and urea are subnormal. Glycogen disappears from the liver, great muscular weakness develops, and growth ceases.

The cortical hormones listed above differ in their ability to counteract these symptoms. 11-Desoxycorticosterone is the most active member of the group for regulating sodium, potassium, and chloride metabolism, and for maintaining the life of adrenalectomized animals. Cortisone, on the other hand, is relatively inactive in these respects, but it is the most potent member of the group for increasing the ability to work and resist stress and for stimulating glycogen formation. The other four hormones have effects similar to one or the other of these two, or both.

Addison's disease was for a time (and to some extent still is) treated with cortical extracts following the work of Hartman and Brownell in 1930. This treatment prolongs the life of the patients, but it is prohibitively expensive and only partly successful. The use of 11-desoxycorticosterone, after it became available about 1940, resulted in a major gain in life expectancy, but even this hormone did not fully replace the missing adrenal secretion. Most patients so treated lack normal vigor, and about half die within seven years. The additional injection of cortisone may quite possibly make up the deficiency, but sufficient amounts of cortisone for clinical use have been produced only recently, and some years will be needed to decide this question definitely. As might be expected from the nature of their disturbed electrolyte metabolism, Addison's disease patients are greatly benefited by diets low in potassium and high in common salt.

The opposite situation—oversecretion of adrenal cortical hormones—is also a serious clinical condition. This may result from tumor growth of the pituitary gland, which causes an increased secretion of a pituitary hormone (adrenocorticotropic hormone, or ACTH) that stimulates the adrenal cortex. In this condition (Cushing's syndrome) blood levels of sodium are high and those of potassium low, that is, just opposite from the situation in Addison's disease.

Tumor growth on the adrenal itself leads to secretion of male sex hormones. If the patient happens to be an adult female, this results in sex inversion (virilism), which manifests itself by deepening of the voice,

growth of a beard, atrophy of the breasts, cessation of menstruation, and development of a masculine-type musculature.

In the last few years several of the most common and distressing human diseases of previously unknown origin have been found to respond to treatment with adrenal hormones. The outstanding example is *rheumatoid arthritis,* a painful crippling disease characterized by swelling and inflammation of the joints. Arthritic patients had sometimes been observed to show improvement under stress (late pregnancy, starvation, major surgery, etc.). Since it was known that adrenal function also is increased by stress, Hensch and Kendall reasoned that some of the adrenal hormones might be of value in arthritis. Their report describing the beneficial effect of cortisone and ACTH appeared in April 1949 and was quickly followed by confirmatory findings elsewhere.

Great efforts have been made by chemical and pharmaceutical firms to produce these substances in adequate amounts. Cortisone has been obtained synthetically by an involved process starting with cholic acid from cattle bile (p. 96). Recently, certain plant steroids have been found which can be used as starting materials and greatly simplify the synthetic process. As a result, prices have been reduced and supplies increased, although larger amounts are still needed. ACTH, on the other hand, has not been synthesized and can only be obtained from animal pituitary glands. This is a limited and costly source. Investigations of the structure suggest that only part of the ACTH protein is needed for the biological effect and that the activity may, in fact, reside in a rather small peptide portion of the molecule. If the exact structure of this peptide can be established, there will be a possibility of producing it synthetically in any desired amount.

Sex hormones

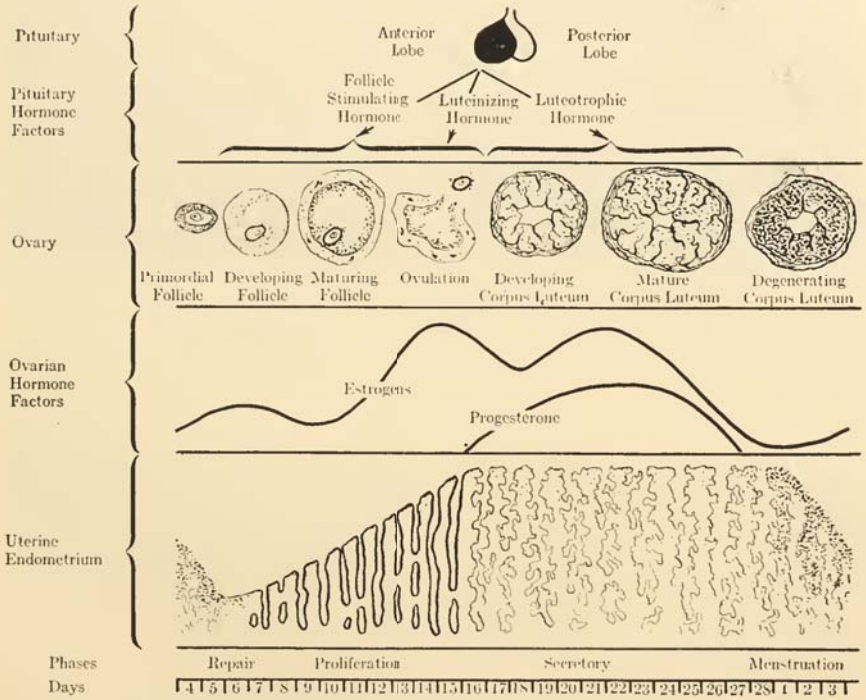
This term is applied to hormones secreted by the gonads (ovaries and testes), although several other glands are equally essential for reproduction. Whether or not the adrenals normally produce sex hormones is uncertain, although the effect from abnormal production is well known (see above).

Functions. The periodic sexual cycles of female mammals are controlled largely by the ovarian hormones, *estradiol* and *progesterone.* These substances are made in the ovary as a result of stimulation by two *gonadotropic hormones* from the pituitary, namely the *follicle-stimulating,* or *follicle-ripening* (FSH), and *luteinizing* (LH) hormones. Figure 11–1 shows how these substances function during a normal human menstrual cycle.

On the first day of the cycle (first day of menstruation) secretion of FSH begins and causes one of the primitive, undeveloped egg sacs, or

primary follicles, in the cortex of the ovary to start growing. After about 13 to 15 days the mature, or *Graafian, follicle* bursts, releasing an egg cell or ovum (ovulation). The bursting of the Graafian follicle is caused by LH. The egg finds its way into one of the Fallopian tubes and thence to the uterus. The developing follicle, and to a lesser extent the entire ovary, secrete estradiol in increasing amounts after about the fourth day. Estradiol causes the endometrial cells in the mucosa of the uterus to start dividing so that by the time of ovulation the mucosa is actively growing. A similar effect is produced on the cells lining the vagina. Any substance which produces these effects is called an *estrogenic hormone,* or an *estrogen.* In female animals, estrogens also produce sexual heat or *estrus;* hence, the name.

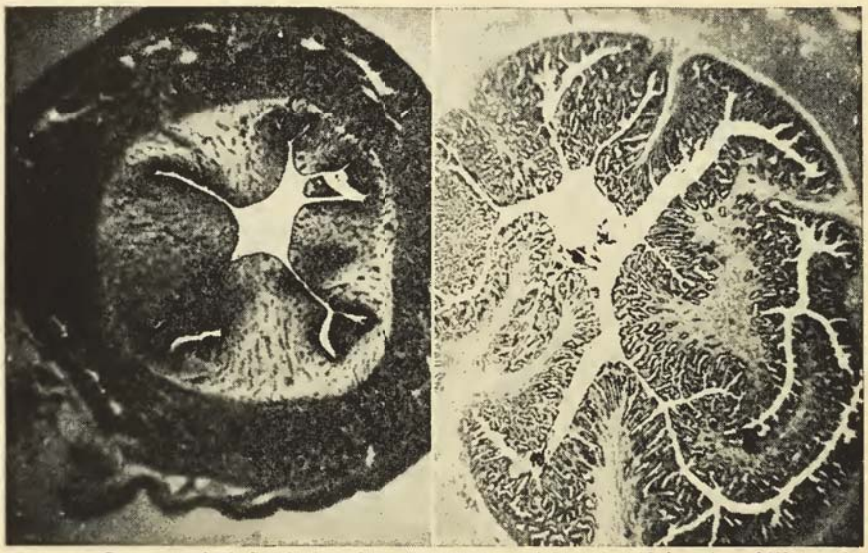
After discharging its ovum the remainder of the follicle changes into another structure called the *corpus luteum.* The corpus luteum develops into a mature state under the influence of LH, which starts to be secreted



Courtesy of J. C. Stucki and the Department of Zoology, University of Wisconsin.

Fig. 11–1. Hormonal control of the menstrual cycle. This is a simplified diagram which does not take into account interactions between the three pituitary hormones nor the action of the ovarian hormones on the pituitary gland. The curves indicate approximate variations in the urinary excretion of ovarian hormones during the cycle. For further explanation see text.

by the pituitary gland about the twelfth to fourteenth day of the cycle (see Fig. 11–1), and is maintained in that condition partly by LH and partly by the luteotropic hormone. The corpus luteum, in turn, produces progesterone, which has a further profound effect on the lining of the uterus, causing the lining and especially the tiny glands present in it to develop enormously (Fig. 11–2) and to secrete a nutritive fluid. In



Courtesy of W. G. Black and the Department of Genetics, University of Wisconsin.

Fig. 11–2. Effect of progesterone on the uterus. Spayed rabbits were given 10 daily injections of 1 mg. each of progesterone in oil, and killed 24 hours after the last injection. Left, untreated control; right, treated with progesterone.

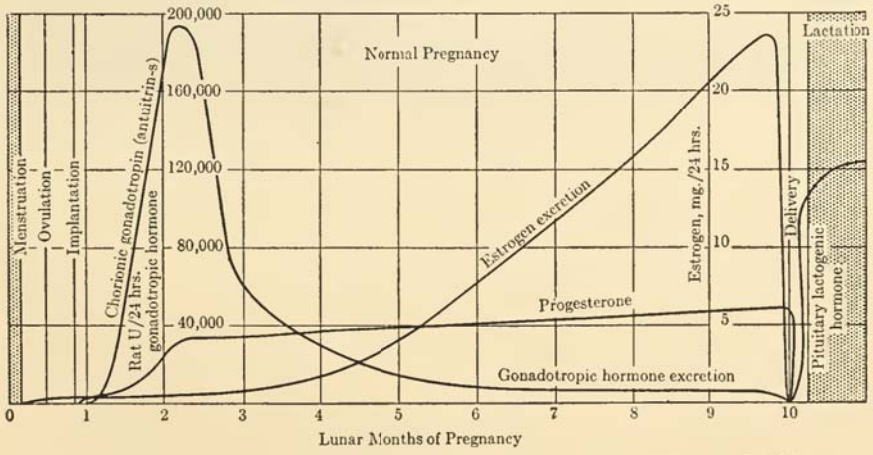
this state the uterus is prepared to receive the ovum, in case fertilization has occurred during its passage through the Fallopian tube. If fertilization does not occur, the ovum dies a few hours after ovulation, and in a few days the corpus luteum stops functioning and degenerates. Secretion of progesterone consequently diminishes, and as a result much of the lining of the uterus sloughs off, and in primates it is discharged as the menstrual flow.

In case the ovum has been fertilized, it becomes embedded in the specially prepared uterine lining and begins to grow. The corpus luteum continues to secrete progesterone and thus to maintain the uterus in a condition favorable for the developing fetus. Any deficiency or interruption in the supply of progesterone results in death of the embryo and consequent miscarriage. This is one of the reasons why certain women suffer repeated miscarriages and are unable to bear a living child. This

difficulty has been completely overcome in many cases by the clinical use of progesterone.

After about the third month of pregnancy the placenta also begins to function as an endocrine organ and partially takes over the production of progesterone. It also secretes hormones with FSH and LH action, and probably others. The urinary excretion of various hormones during pregnancy is shown in Fig. 11–3. Toward the later months estradiol is again produced in increasing amounts. This renders the muscles of the uterus more responsive to the *oxytocic hormone* of the pituitary (a "contracting" hormone, see p. 303) and eventually brings about the onset of labor. Delivery is facilitated by still another hormone, *relaxin*. This substance has been studied mainly in animals, where it appears to be produced by the placenta under the influence of estradiol and progesterone. As the name implies, relaxin loosens the pelvic ligaments and thus facilitates birth. Chemically it appears to be a peptide.

The final hormone cooperating in the reproductive process in the female is the *lactogenic hormone*, which is produced immediately following delivery (Fig. 11–3) and stimulates milk production. It is probable that the luteotropic hormone is, in fact, the same substance as the lactogenic hormone.



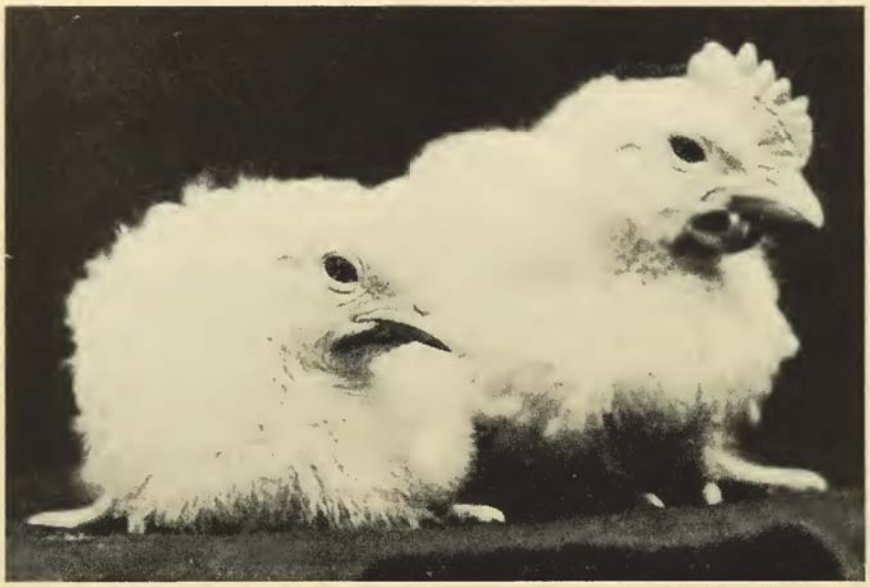
Reproduced by courtesy of Parke, Davis & Company's *Therapeutic Notes*.

Fig. 11–3. Hormone production during pregnancy.

In addition to the roles described above, the female sex hormones exert a profound influence on the development of the secondary sex characteristics such as body shape, body hair distribution, and voice tone, and on the growth of the reproductive organs.

The male sex hormones, *testosterone* and *androsterone*, control the development of the male genital organs, the production of spermatozoa,

and the appearance of male characteristics peculiar to the species. In man these include a typical distribution of body hair, deep voice, masculine shape of body and muscular development, and growth of the beard. Substances producing these effects are called *androgens*, or *androgenic hormones*. A typical effect is shown by androgens on the growth of the comb and wattles of the chick (Fig. 11–4). This response has been

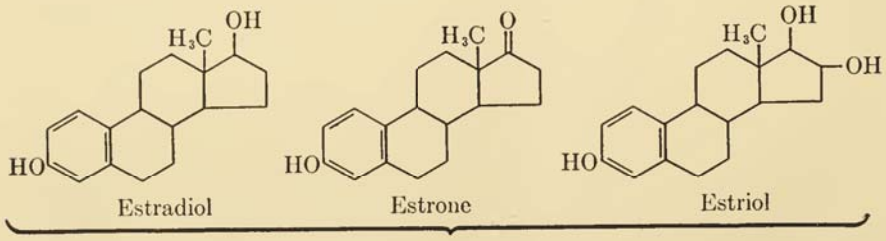


Courtesy of University of Wisconsin, Department of Poultry Husbandry.

Fig. 11–4. Effect of androgen on growth of comb and wattles of immature cockerels. Both birds were 19 days old when photographed, but the one on the right had been treated with 15 mg. of testosterone propionate 9 days previously.

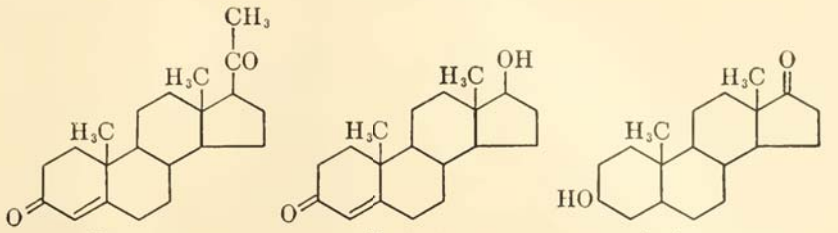
made the basis for a quantitative assay method.

Chemical Nature. The chemical formulas of the more important sex hormones obtained from natural sources are given below:



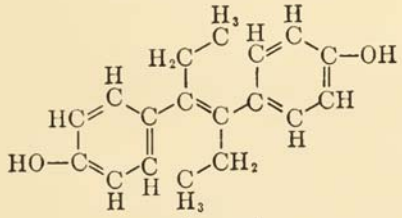
Estradiol Estrone Estriol

Estrogenic hormones

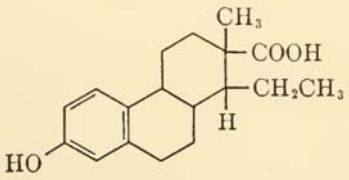


Androgenic hormones

Estradiol, progesterone, and testosterone appear to be the true, primary hormones, since they are considerably more active, weight for weight, than the others. The chemical structures of all the steroid hormones appear to be remarkably similar for substances having such widely different biological properties (compare p. 290). The structures of the estrogens and androgens can be modified considerably, however, without marked loss of potency. For example, *stilbestrol* and *doisynolic acid* are about as effective as estrone, and the former, in fact, is even more active than estrone when given orally (Table 11–1). On the other hand,



Stilbestrol



Doisynolic acid

almost any change in the progesterone molecule produces an inactive product.

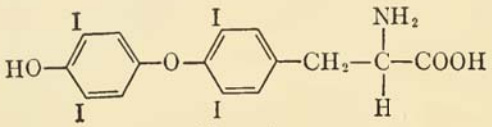
Thyroid hormone

The thyroid gland is a small mass of specialized tissue—about 30 g. in the human adult—located in the neck near the larynx. It produces a hormone which greatly stimulates metabolic activity, particularly the oxidative, energy-yielding processes. Iodine has long been recognized as related to thyroid function (for example, in goiter), but it was not until 1919 that the isolation of an active substance, *thyroxine*, from thyroid tissue was reported by Kendall. A related substance, *diiodotyrosine* or iodogorgoic acid, has also been isolated from thyroid, and, in fact, accounts for about two-thirds of the total iodine content of the gland.

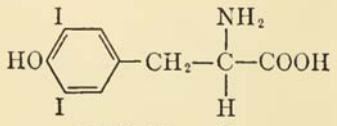
Table 11–1

Estrogenic potency of various substances

Substance	*Effective dose for rats* *Injected* (μg.)	*Oral* (μg.)
Natural estrogenic hormones		
Estradiol	0.08–0.13	50
Estrone	0.7–0.8	50–60
Estriol	10	10
Synthetic products		
Stilbestrol	0.3–0.4	0.7–1.0
Hexestrol	0.2	
Doisynolic acid	0.8–0.9	



L-Thyroxine



L-Diiodotyrosine

Thyroxine contains no less than 65.4 per cent of iodine by weight. It was obtained from thyroid glands only after drastic alkaline hydrolysis, which, incidentally, converted the natural L-form into the corresponding DL-mixture. Thyroxine and diiodotyrosine are not present in the living gland in the free state but are contained in a protein, *thyroglobulin.* This substance has a high molecular weight, estimated at 700,000, and is consequently nondiffusible. It contains a rather constant total amount of tyrosine, diiodotyrosine, and thyroxine residues amounting to 3–4 per cent, but the relative proportions of the three vary with the iodine intake. Iodine ingested with food is quickly absorbed by the thyroid and is used to convert more of the tyrosine of thyroglobulin into diiodotyrosine and thyroxine residues. Thus the gland acts both as a factory and storehouse for bound thyroid hormone.

When the hormone is needed in other parts of the body, some of the thyroglobulin is broken down by proteolytic enzymes so that either free thyroxine or small, water-soluble, diffusible peptides containing thyroxine as one of the component amino acids are liberated and carried away by the blood stream. While circulating in the blood the thyroxine is bound rather loosely to one of the plasma proteins. Just what happens when these substances reach the tissues ultimately affected is not known, but free thyroxine apparently is not involved. Nevertheless, pure L-thyroxine, either natural or synthetic, does produce the effects of whole thyroid when administered to animals or to human patients. The term "thyroid hormone" applies to any substance capable of causing the characteristic physiological effects and thus includes both free thyroxine and the various bound forms of it existing in the animal body. Diiodotyrosine has no appreciable thyroid hormone activity.

The mechanism of thyroid hormone synthesis in the body is not known

with certainty, but very likely involves addition of iodine to tyrosine followed by self-condensation of the diiodotyrosine produced to form thyroxine. Similar reactions, at any rate, take place quite readily outside the body. Thus, when proteins such as casein or egg albumin are treated with iodine for several hours in the presence of a mild alkali (sodium carbonate), iodine becomes incorporated into the protein molecule and thyroxine is formed. The amount of hormone formed is closely correlated with the tyrosine content of the protein used; gelatin, which does not contain tyrosine, fails to yield thyroxine under this treatment. Iodinated casein has been used extensively as a source of thyroid hormone, for example, to feed dairy cows. Milk production is thereby increased, but higher feed costs plus possible injury to the animals make the practice of doubtful economic value.

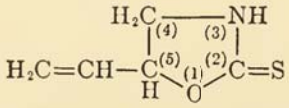
Thyroid Disorders. Common goiter is an enlargement of the thyroid resulting from low iodine intake. Where the iodine supply is not greatly deficient, the enlarged gland is able to maintain normal function. The only serious result is pressure on surrounding organs; for example, pressure on the windpipe may be sufficient to cause difficulty in breathing. The condition can usually be corrected by supplying adequate iodine (see p. 192), or in extreme cases by surgically removing a part of the gland.

A more serious condition arises whenever the thyroid is damaged in any way (for example, by an infection) so that it can no longer produce an adequate supply of hormone. This may occur at any time from fetal life onward. In adults the condition (called *myxedema*) is characterized by a general slowing down of metabolic activities, slow heart rate, low blood pressure, easy fatigue, increased blood cholesterol levels, diminished urinary excretion of 17-ketosteroids, and decreased amounts of "plasma-bound iodine." This plasma-bound iodine, which represents the amount of circulating thyroid hormone, may drop from the normal range of 4–6 to below 2 μg. per 100 ml. of blood plasma. Replacement therapy with thyroxine or whole thyroid substance (dried, ground animal thyroids) is effective but must be continued throughout life.

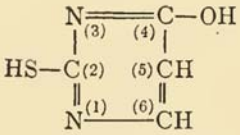
Thyroid deficiency in early life has all the above effects and, in addition, retards both mental and physical growth. The affected individuals often survive to adulthood, but are dwarfs and idiots. This condition is called *cretinism*, and the sufferers from it, *cretins*. Treatment with thyroid hormone is effective, but only if started before normal development has been stunted.

Overactivity of the thyroid (Grave's disease) is likewise most injurious to health. Excessive production of the hormone leads to high basal metabolism, excess energy production, restlessness, tremor of extremities, excessive flushing and perspiration, loss of weight, low blood cholesterol, high excretion of 17-ketosteroids, and frequently exopthalmos (protruding eyeballs). Psychic disturbances often accompany Grave's disease and may be a factor in causing it. Treatment is designed, of course, to

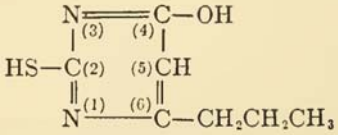
counteract the excessive hormone production. This has been accomplished by partial or complete removal of the gland, or, more recently, by the use of antithyroid drugs and chemicals. Many common foods including spinach, cabbage, turnips, walnuts, lima beans, peas, carrots, grapes, grapefruit, and others have distinct goitrogenic (goiter-producing) or antithyroid effects. The responsible substance was obtained in pure form from yellow turnips and was shown to be *l-5-vinyl-2-thiooxazolidone*. Various synthetic drugs such as 2-thiouracil and 6-propyl-2-thiouracil also have marked antithyroid action. These substances do not prevent absorption of iodine by the thyroid, but interfere with the incorporation of it into the thyroid hormone. Some of them, *e.g.*, propylthiouracil, have proved to be very valuable in the treatment of Grave's disease.



l-5-Vinyl-2-thiooxazolidone



2-Thiouracil



6-Propyl-2-thiouracil

Radioactive iodine compounds containing the I^{131} isotope have also found application in the diagnosis and treatment of excessive thyroid activity. A small test dose of the radioactive iodine, for example, in the form of potassium iodide, is given the patient, and the accumulation of the iodine in the thyroid followed with a Geiger counter placed near the throat. If an abnormally high fraction of the test dose enters the gland, excessive thyroid activity is indicated. In this case, larger doses of I^{131} are given, and as the iodine becomes lodged in the gland, the radiations emitted by it kill a part of the thyroid tissue. Surrounding tissues are essentially unaffected, and the extent of thyroid destruction can easily be controlled by regulating the dosage.

PROTEIN AND PEPTIDE HORMONES

Hormones of the pancreas

The pancreas is a pale pink organ about ten inches long in an adult person, which produces a group of digestive enzymes and discharges them into the small intestine. It also has the function of producing at least one hormone, *insulin*, which is secreted directly into the blood stream.

The name insulin comes from the Latin *insula,* meaning *island,* and refers to the fact that the hormone is formed by small groups of specialized cells called the "islets of Langerhans." There are about 250,000 to 2,500,000 of these islets in man. Lack of insulin causes *diabetes mellitus,* a fatal human disease characterized by excessive urinary excretion and by the presence of large amounts of glucose in the urine. After this fact was established around 1890, many efforts were made to prepare active extracts from animal pancreas glands. They were without success because the hormone was destroyed by the proteolytic enzymes present. The first really effective extracts were obtained in 1922 by Banting, Best, Collip, and MacLeod through the use of acidified alcohol, which inactivated the enzymes. Because of the great demand for insulin for the treatment of diabetes, commercial production was soon undertaken, and many workers studied methods of purifying the crude extracts. Pure crystalline insulin was finally isolated by Abel and co-workers in 1926.

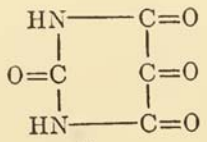
Chemical Nature. Insulin is a water- and alcohol-soluble protein, having its isoelectric point at pH 5.3. The molecular weight has been estimated at 36,000 to 48,000, but it is now known that these values represent aggregations of smaller units. The true molecular weight is generally considered to be 12,000.[1] Insulin has a sulfur content of 3.1 per cent, which is much higher than that of most other proteins. In spite of much searching no organic prosthetic group or structure other than the usual amino acids has ever been found as a component of insulin. Crystalline insulin contains about 0.3 per cent of zinc, but this is of doubtful significance as essentially zinc-free amorphous preparations have equal biological activity. As a result of a brilliant series of researches carried out during the last few years, chiefly by Sanger, the structure of insulin is known in greater detail than is that of any other protein (p. 132).

Physiological Function. The appetite and thirst of the untreated diabetic are enormous, but in spite of the great quantities of food and drink consumed, the body weight becomes progressively less. Blood glucose levels rise so much above the renal threshold that large amounts are excreted in the urine (p. 327). Bodily glycogen stores are depleted. Urinary excretion of nonprotein nitrogen compounds is increased as a result of increased conversion of deaminated amino acids into carbohydrate. Excessive oxidation of fat occurs, and ketosis develops (p. 338). These symptoms have usually been interpreted as being due to decreased utilization of carbohydrates by the body, but some authorities believe that the disease is more a result of *increased formation* of carbohydrates (from fat and protein) than of *decreased utilization.* The over-produc-

[1] However, Fredericq and Neurath have recently obtained evidence that the actual value may be only 6,000. Larger apparent molecular weights result from reversible aggregation in solution, the extent of which depends on the pH, concentration, temperature, and kind and amount of inorganic ions present.

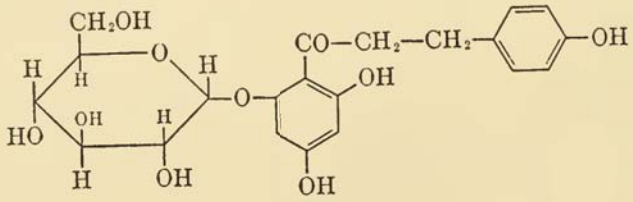
tion theory is supported by the observation that removal of the liver from either diabetic or normal animals is followed by an equally rapid decline of blood glucose levels in both. Under these conditions at least, the diabetic animal utilizes carbohydrate as well as the normal one.

All the symptoms of the disease are relieved by injection of insulin. The hormone is not effective by mouth because it is destroyed by digestive enzymes, as mentioned above. Excessive doses are sometimes administered accidentally to diabetic patients. In such cases, as might be expected, the blood sugar level falls sharply and may go so low as to result in coma and, if not treated, death. This condition, *insulin shock,* is counteracted quickly and completely by injection of glucose. Symptoms of insulin deficiency can be produced in animals by feeding *alloxan,* and it has been suggested, but not proved, that alloxan may be in some way connected with the development of diabetes. When present in the body, its effect is to destroy the islet cells of the pancreas.



Alloxan

A condition closely resembling diabetes mellitus can be produced in experimental animals by feeding the drug, *phlorhizin,* a glucoside present in the root and bark of apple, pear, and plum trees. It has the following chemical structure:



Phlorhizin

The blood sugar level in phlorhizin diabetes, however, is *lower* than normal. The drug produces the symptoms of diabetes by preventing the resorption of glucose by the tubules of the kidney, thus, in effect, lowering the renal threshold and causing urinary excretion of sugar even while the blood sugar level is in or below the normal range.

The mechanism by which insulin acts is not definitely known. It has been claimed by Cori and co-workers that insulin counteracts the inhibition of hexokinase by hormones of the pituitary and adrenal glands. However, the experimental results have been interpreted differently by other workers, and the status of this suggestion, therefore, is still in doubt.

Hyperglycemic Factor of the Pancreas. Certain commercial preparations of both amorphous and crystalline insulin have been found to contain an impurity which, surprisingly enough, causes an *increase* in blood sugar levels. This substance, the *hyperglycemic factor*, is present in the pancreas and also in the stomach lining. It appears to be a protein and to act by causing breakdown of glycogen (glycogenolysis). Its physiological role has not been clarified.

Lipocaic. Animals which have been rendered diabetic by removal of the pancreas have been observed to accumulate great amounts of fat in the liver. Since the development of such fatty livers can be prevented by the feeding of raw pancreatic tissue, but not by insulin, the existence of another hormone in the pancreas was postulated. This substance, which was named *lipocaic*, is probably not a hormone, since the effect can be produced by pancreatic juice (*i.e.*, the *external* secretion of the pancreas) and can also be duplicated by choline or methionine. Prevention of fatty livers by raw pancreas is apparently due to the presence of proteolytic enzymes which make methionine more readily available from food proteins.

Hormones of the posterior pituitary

The *pituitary* or *hypophysis*, a small endocrine organ located in the center of the head, is the master gland of the whole hormone system of higher animals. Its special importance is due to the large number of hormones it produces and to the fact that several of these have the particular function of stimulating other glands to secrete their characteristic hormones. Thus the pituitary directly or indirectly influences a great number of bodily processes. The gland consists of anterior and posterior lobes and a small center section. Extracts of the posterior lobe have three well-defined effects on the animal, namely, those of raising the blood pressure (pressor effect), stimulating the contraction of uterine muscle and, to a smaller extent, of smooth muscles generally (oxytocic effect), and suppressing urinary secretion (antidiuretic effect).

The antidiuretic effect is caused by the same substance that produces the pressor effect (vasopressin). Lack of it causes *diabetus insipidus*, a disease in which the volume of urine excreted is enormously increased, although sugar is not present.

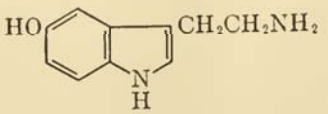
Oxytocin. The substance responsible for the oxytocic effect, *oxytocin*, has been very highly purified, and the best preparations of it seem to be substantially one substance. It is a white, amorphous, water-soluble powder with the properties of a basic polypeptide. According to Pierce and du Vigneaud it yields on hydrolysis one molecule each of leucine, isoleucine, tyrosine, proline, glutamic acid, aspartic acid, glycine, and cystine, and three molecules of ammonia. The molecular weight is close to 1,000. Thus it is an eight-membered peptide. The sequence of the

amino acid residues has not been determined. One physiological role of oxytocin seems to be to expedite labor by increasing uterine contractions. Partially purified preparations have found clinical application for this purpose and for control of hemorrhage after delivery. The hormone is ineffective orally. Oxytocin also plays a role in milk secretion in that it stimulates contraction of the smooth muscles in the walls of the milk ducts.

Vasopressin. The pressor hormone of the posterior lobe has been variously called *vasopressin, pressin,* or *pitressin.* Like oxytocin it is a basic octapeptide, which has a molecular weight of about 1,000. It has been obtained as a white, amorphous, water-soluble powder. On hydrolysis it gives the same products as does oxytocin, except that arginine and phenylalanine are present, but leucine and isoleucine are not. When injected into animals vasopressin causes a pronounced but temporary rise in blood pressure. Presumably it must function normally to help regulate blood pressure, along with thyroxine, epinephrine, and other substances.

Abnormally high blood pressure (hypertension) is a common human disease and a major cause of death. Attempts to learn its cause have uncovered several other substances of natural occurrence which greatly influence blood pressure. One of these is *renin,* a protein secreted into the blood stream by the kidney. Renin is a proteolytic enzyme. It acts on a particular protein, one of the globulins in the blood plasma, which therefore is called *renin substrate.* Renin itself does not have pressor activity, but the product of its action on the substrate, a peptide called *angiotonin* or *hypertensin,* is highly active. The elevated pressure is caused by contraction of small arteries combined with increased heart action. The effect from a single dose lasts only a few minutes because angiotonin is quickly destroyed in normal individuals by another enzyme, *angiotonase.* Pathological hypertension could conceivably be produced by overproduction of vasopressin or renin, or by lack of angiotonase, but these possibilities have not been proved correct, and no really satisfactory treatment for the disease has yet been found.

Still another pressor substance normally present in the animal body is *serotonin.* This substance was isolated from blood serum in the form of a crystalline product which proved to be a mixed sulfate of serotonin and creatinine. The effective component, serotonin itself, is 5-hydroxy tryptamine:



Serotonin

Hormones of the anterior pituitary

The anterior lobe produces six well-recognized hormones, which have been named according to their biological effects. All six have been extensively purified and found to be proteins of relatively low molecular weight. The functions of several have been considered briefly in previous sections of this chapter. Additional properties are listed in Table 11–2.

One of the most interesting and important of the group is the adrenocorticotropic hormone (ACTH, p. 291). The active protein can be hydrolyzed with pepsin or hydrochloric acid until about half of the peptide bonds have been broken without diminishing the activity. This indicates that the effective substance most probably is a peptide composed of far fewer amino acids than the ACTH protein. This peptide has not been isolated in pure form, but partly purified products have been obtained which are reported to have molecular weights in the range 1000–2000 and to be much more active than ACTH protein on a weight basis.

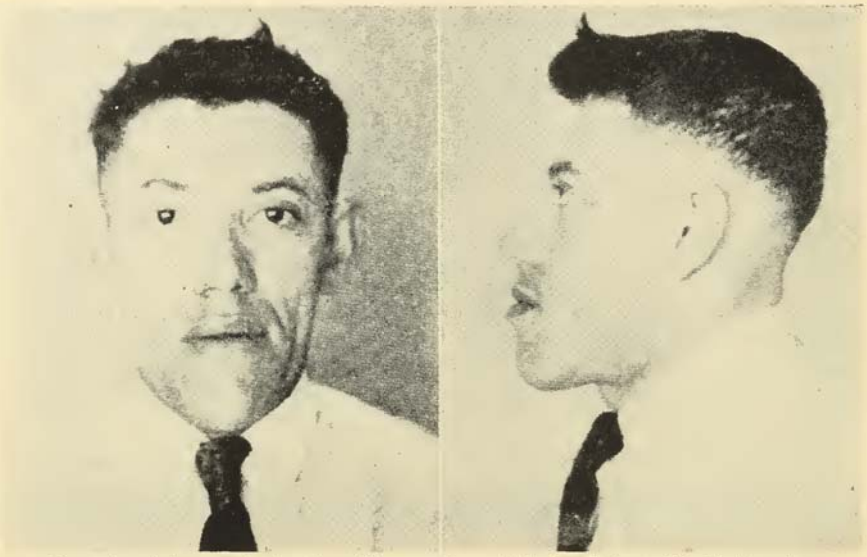
The growth hormone (GH) has the power of stimulating growth, both of the skeleton and soft tissues of the animal body. Normally the long bones are "closed off" at the ends and stop developing shortly after the attainment of sexual maturity. This cessation of bone elongation is probably caused by the sex hormones produced at that time. However, in some cases this does not occur, and the continued production of excess GH leads to gigantism. Heights of eight and nine feet have occurred in human beings. If extra secretion of GH occurs after full maturity, some parts of the body are still able to grow and others are not. This results in distorted growth, which causes a gorilla-like appearance. This condition is called *acromegaly* (Fig. 11–5). Conversely, a deficiency of GH causes one type of dwarfism. Such dwarfs have normal intelligence but are physically small, delicately formed, and doll-like. The diabetogenic activity of the pituitary (p. 325) probably is also the result of GH, or of a substance closely associated with it.

The lactogenic hormone of the anterior pituitary, sometimes called prolactin, is another very interesting substance, not only because it stimulates milk production by mammals, but also because it seems to influence mental attitudes. Its effects have been described by R. G. Hoskins as follows:

"In addition to its effect on milk production, the anterior-lobe product *prolactin* has a striking influence on animal behavior. It induces broodiness in the fowl and modifies the nesting behavior in certain fish (Riddle). Its influence on the instinctual behavior of rats has been studied by Wiesner and Sheard and by Riddle.

"The method of procedure is to place young female rats in cages with materials for nest building. They are then tested as to the strength of their maternal urge by

being offered new-born baby rats for adoption. In most instances the females remain indifferent to the intruders. But if, to these nonchalant misses, a few doses of prolactin are administered, not only are their mammary glands stimulated but a remarkable change in their behavior takes place. They will now eagerly adopt as many babies as may be offered, build elaborate nests for them, and eagerly mother them.



Reproduced by permission from Turner, *General Endocrinology*, W. B. Saunders Company.

Fig. 11-5. Acromegaly.

The yearning seems to be universal. Their maternal reactions are not confined to infants of their own kind but are extended to baby mice, baby rabbits, or even helpless squabs. For a normal, vigorous rat to do other than promptly make a feast of a proffered squab is proof positive that something fundamental has happened to her instincts. What part prolactin may play in the determination of human instincts and emotions is as yet unknown, but the stimulus to imagination is tempting."

Gastrointestinal hormones

The secretion of digestive juices and the movements of the stomach and intestines incidental to the digestion of food are partly controlled and regulated by several *gastrointestinal hormones*. These processes are also influenced markedly by nervous stimulation, and it has been difficult to sort out the two types of effect. At present four gastrointestinal hormones have been quite definitely proved to exist, and a number of others are suspected. None of them are definitely known to be essential for life or to cause any disease if produced in abnormally large or small amounts.

Before considering these substances in detail, a distinction should be

Table 11–2

Properties of anterior pituitary hormones

Property	*Luteinizing hormone* (LH)*	*Follicle stimulating hormone* (FSH)	*Lactogenic hormone*	*Adrenocorticotropic hormone* (ACTH)	*Thyrotropic hormone*	*Growth hormone* (GH)
Organ or function stimulated	ovary, testis, secretion of androgens	ovarian follicles, spermatogenesis	milk production	adrenal cortex	thyroid	skeletal development
Isolated in pure state †	yes	no	yes	yes	no	yes
Molecular weight	40,000	?	22,000–26,500	20,000	(est. 10,000)	39,300–44,250
Solubility:						
Water	very sol.	very sol.	insol.	very sol.	very sol.	insol.
Other solvents	sol. 40% alcohol	sol. 70% alcohol	sol. absolute alcohol	sol. 60–70% alcohol or acetone	sol. 40% acetone	sol. phenol
Isoelectric pH	4.6	4.8	5.7	4.7	?	6.85
Analysis:						
Nitrogen, %	14.2	?	15.8	15.6	12–13	15.6
Sulfur, %		?	1.8	2.3	1.0	1.3
Phosphorus, %			none	none	none	none
Carbohydrate, %	10.3	ca. 10	none	none	ca. 6	none
Approx. amount to produce biol. response (in μg.)	5–10	5–20	30	20–200	10–30	20–100

* From sheep.

† That is, satisfying physical-chemical tests for purity.

made between them and "secretogogues." The latter are chemical substances in the food, or derived from food during digestion, which directly or indirectly stimulate the secretion of digestive juices. They are not classified as hormones because they are not produced by the body.

Gastrin. This hormone is produced by the mucosa of the lower, or pyloric, end of the stomach and to a lesser extent by the mucosa of the duodenum (that portion of the small intestine immediately beyond the stomach). It is secreted into the blood stream (though not directly) as a result of stimulation by secretogogues and has the effect of increasing the flow of gastric juice. The juice so formed is high in hydrochloric acid but low in pepsin.

Gastrin can be extracted from suitable mucosa and has been purified considerably, although not completely. It is destroyed by proteolytic enzymes or by boiling in one-tenth normal sodium hydroxide solution and is precipitated by trichloracetic acid. It is probably a peptide or low molecular weight protein. Histamine in very small doses has the same effect as gastrin, and it may be that histamine actually is the hormone. This has not been proved, however, mainly because the effective level of histamine is too low to be detected in the blood stream by the analytical methods at present available. An observable gastric response is produced in human beings by the injection of only 0.004 μg. of histamine per kilogram body weight per minute.

Secretin. This substance stimulates the secretion of water by the pancreas and of bile by the liver. The increased flow of pancreatic juice is relatively poor in enzyme content.[1] Secretin exists in an inactive form (prosecretin) in the mucosa of the upper small intestine, or duodenum, and is released by the action of dilute hydrochloric acid from the stomach (pH ca. 4.6).

Two crystalline secretin preparations have been isolated, both in the form of salts with picrolonic acid. One appears to be a peptide, while the other is a compound of low molecular weight. The peptide, however, can be extensively hydrolyzed by aminopolypeptidase without loss of secretin activity. It is suggested that the two products may be related in much the same way as thyroxine and thyroglobulin. The exact formula of secretin is not known.

Cholecystokinin. The name of this hormone (literally "gall bladder mover") indicates its physiological function which is to stimulate contraction and emptying of the gall bladder. Like secretin, it is produced in the mucosa of the duodenum. It is secreted indirectly into the blood stream whenever fat, fatty acids, peptone, or dilute hydrochloric acid enter the intestine. It has been only partially purified, and its chemical

[1] Another gastrointestinal hormone, the existence of which is very probable but not conclusively proved, stimulates the secretion of *enzymes* by the pancreas. Purified preparations of this substance, *pancreozymin*, have no effect on the *volume* of pancreatic secretion.

constitution is therefore not known. However, it tends to follow secretin in extraction and purification procedures and may be a peptide.

Enterogastrone. This hormone is also secreted by the duodenal mucosa, but it has the effect of inhibiting the movements of the stomach, as well as the stomach's secretion of hydrochloric acid. Secretion of enterogastrin into the blood stream is brought about by the presence in the small intestine of fatty acids, especially oleic acid, soaps, neutral fat, or relatively concentrated solutions of sucrose, glucose, or lactose. The hormone has not been isolated in pure form, but the best preparations contain amino acids and have the properties of peptides. If sufficiently pure preparations to avoid undesirable side effects were available, enterogastrone would be of value for the treatment of gastric ulcers in human patients.

REVIEW QUESTIONS ON HORMONES

1. Distinguish between hormones and vitamins.
2. Name two hormones which are derived from amino acids in the animal body, and outline the process by which this conversion takes place.
3. Define the terms: vasoconstrictor, secretogogue, androgen, estrogen, endocrine organ, gastrin, oxytocin.
4. Point out the similarities and differences between the types of diabetes caused by alloxan, phlorhizin, pancreatic deficiency, and posterior pituitary deficiency.
5. Give examples to illustrate three different ways in which the quantity of hormones secreted by various glands is controlled in the body.
6. Make a list of hormones known to participate in the process of reproduction in mammals. Indicate briefly the function of each.
7. Outline the mechanisms by which the blood calcium level is controlled, explaining the influence of each factor. What are the consequences of abnormal blood calcium levels?
8. To which chemical classes do the majority of hormones belong? Give examples.
9. List diseases caused by abnormal hormone production in animals, and name the hormone associated with each.
10. Why is the pituitary sometimes called the "master gland" of the animal or human body?

REFERENCES AND SUGGESTED READINGS

Abel, J. J., Geiling, E. M. K., Rouiller, C. A., Bell, F. K., and Wintersteiner, O., "Crystalline Insulin," *J. Pharmacol.*, **31**, 65 (1927).

Banting, F. G., Best, C. H., Collip, J. B., and MacLeod, J. J. R., "Preparation of Pancreatic Extracts Containing Insulin," *Trans. Roy. Soc. Canada,* **16**, Sect. V, 1 (1922).

Colowick, S. P., Cori, G. T., and Slein, M. W., "The Effect of Adrenal Cortex and Anterior Pituitary Extracts and Insulin on the Hexokinase Reaction," *J. Biol. Chem.*, **168**, 583 (1947).

Cori, C. F., "Enzymatic Reactions in Carbohydrate Metabolism," *The Harvey Lectures* (1945–46), p. 253.

Fieser, L. F. and Fieser, M., *Natural Products Related to Phenanthrene,* Reinhold Publishing Corporation, New York, 1949.

Fredericq, E. and Neurath, H., "The Minimum Molecular Weight of Insulin, *J. Am. Chem. Soc.,* **72**, 2684 (1950).

Gurin, S. and Delluva, A. M., "The Biological Synthesis of Radioactive Adrenalin from Phenylalanine," *J. Biol. Chem.,* **170**, 545 (1947).

Harris, R. S., Marrian, G. F., and Thimann, K. V., *Vitamins and Hormones,* vols. 1–10, Academic Press, Inc., New York, 1943–1952.

Hartman, F. A. and Brownell, K. A., "The Hormone of the Adrenal Cortex," *Science,* **72**, 76 (1930).

Hensch, P. S., Kendall, E. C., Slocumb, C. H., and Polley, H. F., "The Effect of a Hormone of the Adrenal Cortex and of Pituitary Adrenocorticotrophic Hormone on Rheumatoid Arthritis," *Proc. Staff Meet., Mayo Clinic,* **24**, 181 (1949).

Hoskins, R. G., *Endocrinology,* W. W. Norton and Company, New York, 1941.

Houssay, B. A., Chapter 13 on Hormones, in *Currents in Biochemical Research,* ed. by D. E. Green, Interscience Publishers, Inc., New York, 1946.

Kendall, E. C., "Isolation of the Iodine Compound Which Occurs in the Thyroid," *J. Biol. Chem.,* **39**, 125 (1919).

Lesh, J. B., Fisher, J. D., Bunding, I. M., Kocsis, J. J., Walaszek, L. J., White, W. F., and Hays, E. E., "Studies on Pituitary Adrenocorticotropin," *Science,* **112**, 43 (1950).

Pierce, J. G. and du Vigneaud, V., "Studies on High Potency Oxytocic Material from Beef Posterior Pituitary Lobes," *J. Biol. Chem.,* **186**, 77 (1950).

Pincus, G., editor, *Recent Progress in Hormone Research,* vols. 1–7, Academic Press, Inc., New York, 1947–1952.

Pincus, G. and Thimann, K. V., *The Hormones,* vols. I and II, Academic Press, Inc., New York, 1948.

Roche, J. and Michel, R., "Natural and Artificial Iodo-Proteins," *Advances in Protein Chemistry,* **6**, 253 (1951).

Sanger, F., "The Arrangement of Amino Acids in Proteins," *Advances in Protein Chemistry,* **7**, 1 (1952).

Soskin, S. and Levine, R., *Carbohydrate Metabolism,* University of Chicago Press, 1946.

Stephens, G. A., *Hormones and Vitamins,* Geo. Newnes, Ltd., London, 1947.

Tainter, M. L. and Luduena, F. P., "Sympathetic Hormonal Transmission," *Recent Progress in Hormone Research,* **5**, 3 (1950).

Turner, C. D., *General Endocrinology,* W. B. Saunders Company, Philadelphia, 1948.

Chapter 12

DIGESTION

by G. W. E. PLAUT

ASSISTANT PROFESSOR, INSTITUTE FOR ENZYME RESEARCH,
UNIVERSITY OF WISCONSIN

Most foods have to be converted to the proper physical and chemical state before they can be utilized by the body. Digestion is the series of mechanical and chemical processes which accomplishes this result.

SALIVARY DIGESTION

Composition of saliva

Food particles are reduced to smaller size by the mechanical action of the teeth. While in the mouth they are moistened and mixed with saliva, the secretion of the submaxillary, sublingual, and the parotid glands. The sublingual glands secrete a thick fluid which is rich in the glycoprotein, mucin. When mucin is hydrolyzed it yields, in addition to protein, sulfuric acid, acetic acid, glucuronic acid, and glucosamine. Mucin serves to lubricate the food for its subsequent passage through the esophagus to the stomach. A thin watery fluid, low in organic matter (serous secretion), is produced by the parotid gland. The submaxillary gland contributes a mixture of the two types of secretion.

The saliva contains inorganic constituents found in blood. A small amount of thiocyanate is also present. Some organic compounds characteristic of blood such as uric acid, urea, and creatinine are also present. An α-amylase called ptyalin is present in saliva. It catalyzes the hydrolysis of starch and glycogen to maltose and polysaccharides of lower molecular weight. The action of the amylase on starch persists on the way from the mouth to the stomach. The activity stops in the stomach when the acidity becomes too unfavorable. This enzyme has been crystallized from human saliva. It is inactivated upon dialysis against distilled water, but the activity can be restored by the addition of chloride ions. Human saliva has a neutral reaction (about pH 6–8).

Secretion of saliva under natural conditions

The secretion of saliva appears to be mainly controlled by the nervous system. There are two general modes of nervous stimulation of salivary flow. (1) The presence of materials in the mouth leads to the secretion of saliva. There is a remarkably purposeful variation in the composition of saliva depending on the nature and mechanical state of the material present in the mouth. Thus a watery secretion is produced in the presence of dry powder, whereas acid leads to the secretion of a fluid high in mucin, which would tend to neutralize the acid. (2) Stimulation of other organs of sense, aside from that of taste, also leads to salivation. We are all familiar with the experience of our mouths watering when we smell or see a particularly tasty food. It is obvious that one must have had the experience of tasting the particular food at one time and that the stimulation due to smell or sight must have been acquired then. Such an acquired stimulation is known as a conditioned reflex.

When the body is exposed to a situation of water loss, the salivary secretion is depressed. As a result the mouth becomes dry, and the person experiences the sensation of thirst. A normal human adult secretes 1–1.5 l. of saliva per day.

After the food has been prepared in the mouth for further digestion, it is swallowed and passes through the esophagus to the stomach.

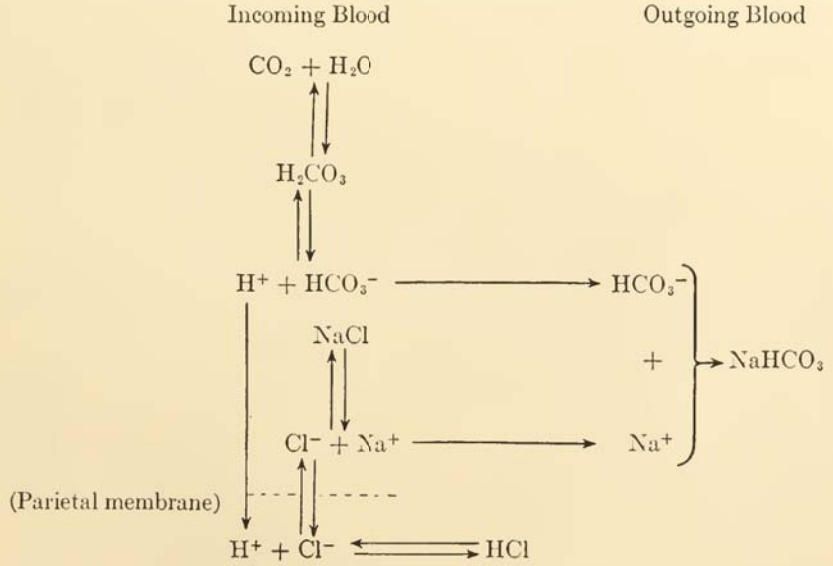
GASTRIC DIGESTION

In the stomach the foods are mixed with the gastric juice. The gastric movement renders the food creamy and semifluid in consistency. This mass is then known as the *chyme*. It passes out of the stomach through the pyloric opening into the duodenum.

Gastric juice is secreted by three main types of cells. The secretion of *mucous cells* is high in mucin, the *parietal cells* contribute hydrochloric acid, and the *zymogenic cells* supply the zymogen, pepsinogen. In contrast to other fluids of the body, gastric juice has a very acid reaction, *e.g.*, pH 1.5–1.9, in the case of man. It has been estimated that the secretion of parietal cells is 0.16*N* hydrochloric acid. If only hydrochloric acid were secreted in the stomach, the pH would be around 1; however, the other secretions contain substances such as proteins and sodium bicarbonate which partially neutralize the hydrochloric acid. The unneutralized portion of the acid can be determined by titration with alkali and is known as the *free acid* of the gastric juice (0.05–0.1*N* HCl), while the sum of the neutralized and the free acid is called the *total* acid. When food is mixed with the gastric juice still more of the free acid is neutralized, and the acidity of the chyme is pH 3–5, depending on the nature of the food.

The hydrochloric acid of gastric juice is made from blood which has an approximately neutral reaction. The hydrogen ion of hydrochloric acid comes from carbonic acid and the chloride ion originates from sodium chloride. These ions are selectively absorbed from the blood by the parietal cells, and the hydrochloric acid thus formed is secreted into the stomach. The loss of hydrogen ions from the blood is evidenced by an increase in the alkalinity of the blood which has passed through the gastric mucosa of the stomach during a period of active hydrochloric acid secretion. The precise *mechanism* by which hydrogen and chloride ions are concentrated by parietal cells to form an acid from the almost neutral blood has not been completely elucidated. It should be realized that a great deal of energy is required to raise the hydrogen ion concentration from $4 \times 10^{-8} M$ (pH 7.4) in blood to $0.16 M$ (pH 0–1), the hydrogen ion concentration of parietal secretion. This transformation constitutes an approximate 4,000,000 fold increase in the concentration of H^+.

The relationship of some components of the blood to the secretion of hydrochloric acid by the parietal cells is pictured in the following scheme.



The high acidity of gastric juice has a bactericidal effect on the microorganisms ingested with the food and is ideal for the action of some of the digestive enzymes present in this fluid. Most of the enzymes of gastric juice work best at pH 2–4.

The principal proteolytic enzyme of gastric juice is pepsin. It attacks proteins and reduces them to smaller fragments such as proteoses, pep-

tones, and some amino acids. The enzyme is secreted by the zymogenic cells in an inactive form, *pepsinogen*. Pepsinogen is converted to pepsin by hydrogen ions and pepsin (see p. 273). Rennin is present in particularly high concentrations in the stomachs of young mammals. This enzyme is involved in the curdling of milk. Rennin catalyzes the conversion of casein to soluble paracasein. Paracasein combines with calcium ion to form insoluble calcium paracaseinate. Rennin obtained from calf stomach is used commercially to curdle milk in cheese making. Pepsin can also curdle milk, but accomplishes this result by a process different from that of rennin.

The flow of gastric juice is regulated by several factors. Nervous impulses due to various stimuli, such as food in the mouth, or even the odor or sight of food, cause secretion. Fear or worry have been shown to suppress secretion. Mechanical pressure inside the stomach has a slight effect, but the presence of certain foods (*e.g.*, meat extract, peptone) in the stomach causes a tremendous increase in gastric flow. This effect of food seems to be independent of the nervous system, and some evidence has been obtained to show that a substance may be present in gastric mucosa which reacts with a food component to form a hormone, *gastrin* (p. 308). This substance is liberated into the blood and causes gastric secretion. The injection of histamine, a compound present in gastric mucosa as well as in other body tissues, causes the secretion of a gastric juice which is high in hydrochloric acid but low in pepsin, in contrast to the normal composition. The composition of chyme leaving the stomach for the duodenum has an effect on gastric digestion. Thus when fat or acid are placed into the duodenum, gastric secretion and motility are inhibited. A material extracted by Ivy from intestinal mucosa, when injected into the blood stream, produces the same type of depression of gastric activity. It is a hormone called *enterogastrone* (p. 309). *Urogastrone,* isolated from urine, has a similar effect.

Various chemicals have a profound effect on the secretion and composition of gastric juice. Ethyl alcohol leads to a secretion high in hydrochloric acid and mucin and low in pepsin; liver, meat, and vegetable extracts are powerful stimulants of normal gastric juice secretion, while acid depresses secretion. The concentration of hydrochloric acid is chronically lowered or raised in certain pathological conditions. The hydrochloric acid is completely absent (achlorhydria), *e.g.*, in pernicious anemia, and is produced in excessive amounts (hyperacidity) in most cases of duodenal ulcers.

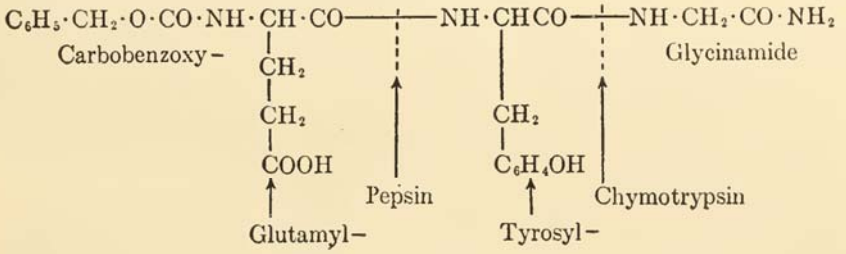
Bland diets, for example, milk, are used in treating ulcer patients to prevent excessive gastric secretion. The high buffering capacity of such diets helps to neutralize the free acid of the gastric secretion.

INTESTINAL DIGESTION

When the chyme enters the duodenum it is mixed with the secretions of the pancreas and bile.

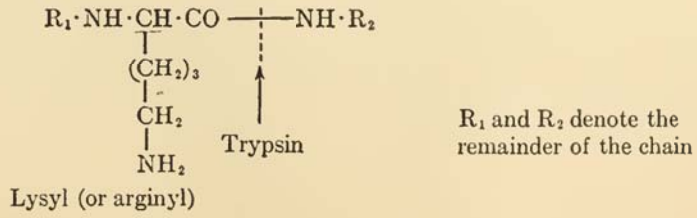
Pancreatic secretion

Pancreatic juice has an alkaline reaction, pH 7.1–8.2, and contains a variety of very active enzymes which can attack carbohydrates, proteins, and fats. Trypsin and chymotrypsin hydrolyze proteins and polypeptides to smaller polypeptides and amino acids. The action of the proteolytic enzymes depends in part on the sequence of amino acids in the protein or peptide which they attack. Peptides that are the products of one of the proteinases can therefore be hydrolyzed further by another proteinase of different specificity. In model experiments with the synthetic peptide, carbobenzoxy-L-glutamyl-L-tyrosyl-glycinamide, it was found that pepsin split the amino linkage of tyrosine, and chymotrypsin split the carboxyl linkage of tyrosine. In the following formulation the site of cleavage is indicated by dotted lines:

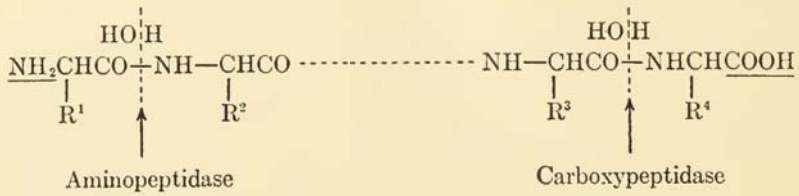


From experiments with other synthetic peptides, it was found that phenylalanine can be substituted for tyrosine. From studies on native proteins, *e.g.*, insulin, it appears that pepsin splits linkages other than those involving the amino group of tyrosine and phenylalanine. For example, the Leu. Val. bond is readily hydrolyzed, and the Ala. Leu. bond to a considerable degree. Pepsin appears to have a much wider range of specificity than chymotrypsin or trypsin.

Trypsin splits peptides at the carboxyl linkage of either lysine or arginine. Thus

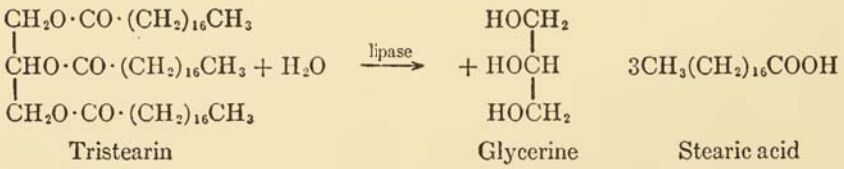


The proteinases are therefore complementary in action and can break down the large protein molecules to smaller and smaller units. Carboxypeptidases attack polypeptides at the carboxyl end, liberating the terminal amino acid of the chain. In contrast, the aminopeptidases (mainly secreted in the intestines) attack the peptide linkage at the free amino end of the chain. To illustrate:



Neither trypsin, chymotrypsin, nor carboxypeptidase are secreted as the active enzyme by the pancreas, but rather as an inactive precursor, called a zymogen. Trypsinogen, the precursor of trypsin, is converted to the latter by the action of enterokinase (an enzyme present in intestinal juice) or by trypsin itself. Chymotrypsinogen goes to chymotrypsin in the presence of trypsin. Trypsin is also instrumental in the conversion of procarboxypeptidase to the active enzyme. Trypsin, trypsinogen, chymotrypsin, chymotrypsinogen, and carboxypeptidase have been isolated in crystalline form.

The pancreatic juice also contains lipases, enzymes that catalyze the hydrolysis of fat, and amylases, enzymes that catalyze the hydrolysis of



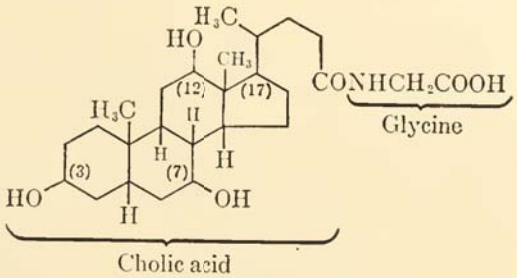
starch to lower molecular weight polysaccharides (dextrins) and maltose. Pancreatic amylase has been crystallized and appears to be identical in chemical, physical, and enzymatic properties with ptyalin.

The flow of pancreatic juice is regulated in part by the nervous system; however, it has also been found that the injection into the blood stream of an extract of duodenal mucosa results in copious secretion of pancreatic juice. This extract is a hormone called *secretin*. Another hormone from duodenal mucosa, *pancreozymin*, has no effect on the volume of pancreatic secretion, but it does effect an increase in the trypsin, amylase, and lipase content of the juice (p. 308). About 600 ml. of pancreatic juice are secreted daily by an adult man.

Bile

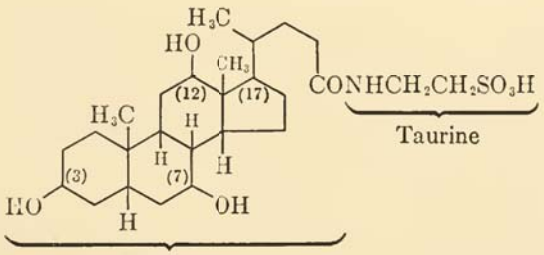
Bile is continuously produced by liver cells. It is collected by a series of ducts from these sources and stored in the gall bladder (some animals, *e.g.*, the rat, do not have a gall bladder, and consequently the bile is also stored in the liver cells). The pH of liver bile is about 8–8.6, while that of the gall bladder is around 7. The main components of bile are bile salts, bile pigments, cholesterol, and lecithin.

An inspection of the formulas of the principal bile acids reveals that



Glycocholic acid

they are related to the sterols (p. 95). The prefix glyco or tauro is



Taurocholic acid

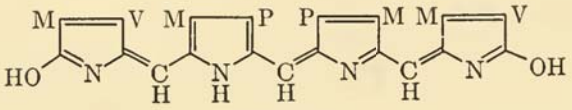
used to show that the sterol portion of the bile acid (cholic acid in the above structures) is combined by a peptide linkage with glycine or taurine, respectively. Glyco and taurodesoxycholic acids have been isolated (the hydroxyl group in carbon 7 of cholic acid is replaced by a hydrogen in desoxycholic acid) from bile. Similar conjugates of chenodesoxycholic acid (hydroxyl of cholic acid in position 12 replaced by hydrogen) and lithocholic acid (hydroxyls of cholic acid in carbons 7 and 12 replaced by hydrogens) have been demonstrated to be present in bile. The sterol portion of the bile acids have a great affinity for

nonpolar substances, *e.g.*, fat, while the carboxyl and hydroxyl groups of the molecule have a great affinity for polar solvents, such as water. Bile acids, therefore, have the properties of a detergent, and their mode of action is akin to that of soaps.

As a result of these chemical properties the bile salts have the ability to increase the water solubility of lipides such as fats and cholesterol, and vitamins A, D, E, and K. The increased water solubility of these otherwise practically water-insoluble materials facilitates their passage through the intestinal wall into other body fluids. The speed of hydrolysis of fats to fatty acids and glycerol in the presence of lipase is increased in the presence of bile salts.

Once the bile salts have been secreted they are reabsorbed in the intestines and transported via the bloodstream to the liver, where they are used over again. However, it has been shown with isotopically labeled compounds that the sterol portion of the bile salts can be formed new from administered cholesterol.

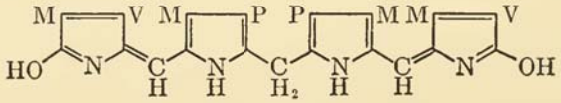
The principal pigments of bile are bilirubin and biliverdin, which are



M = CH_3—
V = CH_2=CH—
P = —CH_2CH_2COOH

Biliverdin

products of degradation of heme. The color of feces is mainly due to



Bilirubin

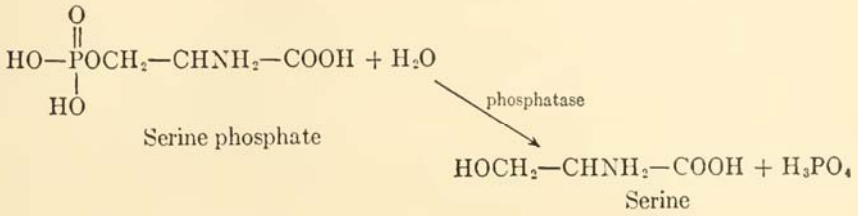
the products of bacterial reduction of bile pigments, *e.g.*, stercobilin and stercobilinogen.

The discharge of bile into the duodenum is regulated in part by the nervous system and by secretin. Another substance, *cholecystokinin*, has been implicated in the contraction of the gall bladder. The presence of the bile salts themselves in the duodenum exerts a powerful stimulation on the flow of bile.

Intestinal secretion

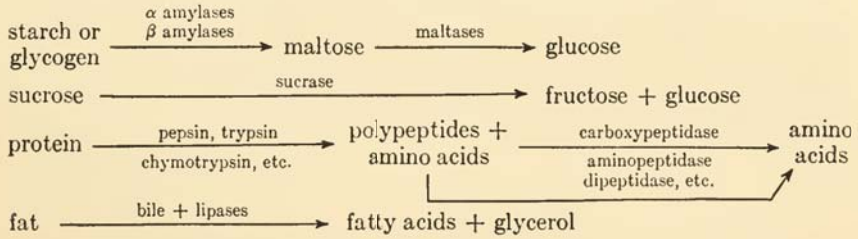
The intestinal juice is secreted by a large number of glands in the mucosa. This fluid has a reaction of pH 7–8.5. It contains a large number of enzymes; among them are enterokinase (converts trypsinogen to trypsin), peptidases (hydrolyze peptides to free amino acids), nucleases

(hydrolyze nucleic acids to polynucleotides and nucleotides), nucleotidases (hydrolyze nucleotides to the corresponding nucleoside and phosphate, *e.g.*, adenylic acid + $H_2O \rightarrow$ adenosine + phosphoric acid), nucleosidases (split nucleosides into purine or pyrimidines and pentose, e.g., adenosine + $H_2O \rightarrow$ adenine + ribose), phosphatases (hydrolyze phosphate esters into the corresponding alcohol and phosphoric acid, *e.g.*,



sucrase (hydrolyzes sucrose to glucose and fructose), maltase (hydrolyzes maltose to two molecules of glucose), and lactase (lactose + $H_2O \rightarrow$ glucose + galactose). Mucin is secreted by epithelial cells of the small and large intestines; it lubricates the movement of material through this portion of the intestinal tract. The flow of intestinal juice is markedly stimulated by the application of mechanical pressure to the intestinal wall; therefore, the mere physical presence of food will stimulate secretion.

Schematic representation of enzymatic degradation of major foodstuffs



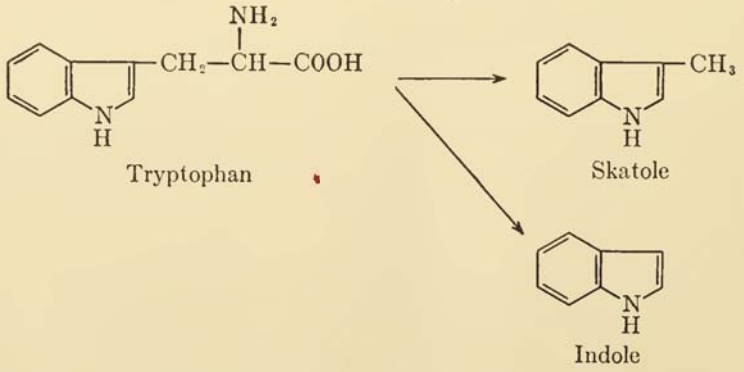
ABSORPTION FROM THE GASTRO-INTESTINAL TRACT

Fairly large quantities of ethanol, methanol, and water are absorbed in the stomach, and hydrocyanic acid is rapidly taken up at this site in fatal amounts. The mucosa of the small intestines, however, is the most important location for the absorption of foodstuffs. The intestinal wall is covered with a large number of microscopic, protruding processes known as *villi*. Each of the villi contains a small blood vessel and a lymph vessel (lacteal). The villi are the principal absorbing units of the small intestines. The materials that can be absorbed are transported across the membranes which separate the intestinal content from the blood and

lymph vessels. These breakdown products of foods enter the general circulation from the smaller vessels and are taken up by the various organs of the body. Amino acids and monosaccharides pass into the capillaries and from there into the portal blood. Some authorities claim that small quantities are transported into the lymph. Over 90 per cent of the fatty acids of fat absorbed in the intestines of the rat have been demonstrated to be transferred to intestinal lymph. It is doubtful if the fatty acids of fats can be transported directly into the portal blood.

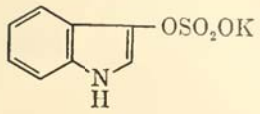
Calcium and iron are absorbed mainly from the upper part of the small intestines. The intestinal content has a profound effect on the extent of calcium absorption. Soluble calcium salts such as the gluconate, lactate, and chloride are readily available, but the phosphate is not. Cereals reduce calcium absorption since they contain phytic acid (inositol hexaphosphoric acid), which binds calcium; spinach has a similar effect because of its oxalic acid content. As has been mentioned previously, vitamin D enhances calcium absorption (p. 211). Iron absorption is notoriously inefficient. For example, if a normal child is fed 5 mg. of iron, only about 12 per cent of the iron is absorbed. However, the greater the need of the body for iron, the greater is the increase in the uptake of this element. It has been shown that in cases of iron-deficiency anemia, absorption increases many times over normal. Most of the other inorganic salts and the bulk of the water are absorbed in the colon.

As a result of water removal in the colon, the materials there acquire a semisolid consistency. A large number of bacteria act in the colon on materials which have been passed there from the small intestines or which are secreted by the walls of the colon. It has been estimated that 10–90 per cent of the weight of the feces is derived from bacteria. The characteristic odor of feces is due mainly to indole and skatole. These are products of degradation, presumably of tryptophan. Part of the



Tryptophan Skatole Indole

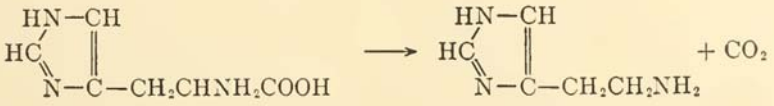
indole and skatole are reabsorbed into the blood and are mainly converted into the sulfate esters (indoxyl sulfate and skatoxyl sulfate)



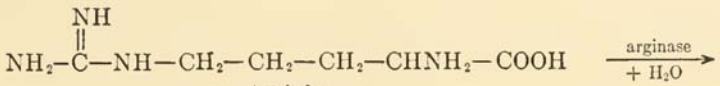
Indican
(potassium indoxyl sulfate)

in the liver and are excreted in this form in the urine. Tyramine, histamine, putrescine, and cadaverine occur in feces; they are probably the products of bacterial decarboxylation of the amino acids tyrosine, histidine, ornithine, and lysine, respectively:

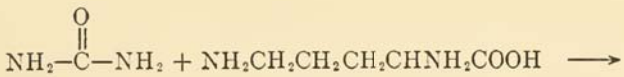
Tyrosine Tyramine



Histidine Histamine

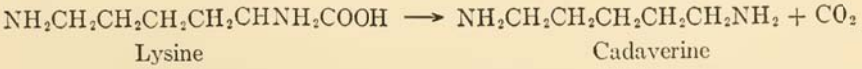


Arginine



Urea Ornithine

Putrescine



Lysine Cadaverine

Hydrogen sulfide and methane are among the gaseous products of putrefaction in the colon. A typical analysis of the intestinal gases of swine gave 25 per cent methane, 50 per cent carbon dioxide, and 25 per cent hydrogen. In herbiverous animals large quantities of gas are produced in the paunch, in addition to those in the intestines. The decomposition of foods by bacteria, leading to gas formation in the paunch, may account for as much as 25 per cent of the energy loss from the food during the digestive process.

The nutritional significance of the intestinal bacterial flora has been

thoroughly appreciated only in the last few years. It was observed in earlier nutritional experiments that some rats receiving a diet deficient in B complex vitamins would recover spontaneously from the deficiency without supplementation of the diet with the missing vitamins. Although receiving a B vitamin deficient diet, other rats which were prevented from eating their feces (coprophagy is a common practice in the animal world) required much more supplementation with B vitamins than those consuming feces. When rats are fed a purified diet to which all the vitamins except folic acid have been added, they will develop normally; however, when succinylsulfathiazole (which is not absorbed from the intestines) is added to this ration, typical symptoms of folic acid deficiency develop, and the amount of this vitamin in the cecal content and various tissues decreases. It is thought that the sulfa drugs under these conditions depress the bacterial synthesis of folic acid in the intestines. In contrast to the depression of vitamin production by an antibacterial agent described above, it has been found more recently that the addition of certain antibiotics, *e.g.*, penicillin, aureomycin, terramycin, and streptomycin, to the ration will increase the rate of growth of animals usually 10–20 per cent, even under farm conditions. The quantity fed is small (2–5 mg. per pound of feed). It is thought that the antibiotics inhibit the growth of those organisms which assimilate large quantities of certain vitamins present in the intestinal tract and which therefore reduce the supply for absorption by the animal.

REVIEW QUESTIONS ON DIGESTION

1. Discuss the digestion of (1) proteins, (2) starch, (3) fat.
2. Define (1) conditioned reflex, (2) chyme, (3) secretin.
3. Which reactions are catalyzed by the following enzymes: (1) pepsin, (2) rennin, (3) ptyalin, (4) lipase, (5) carboxypeptidase, (6) amino peptidase, (7) nucleotidases?
4. Persons with obstructed bile ducts hemorrhage easily, even when their diets contain large quantities of vitamin K. What may be the reason for this condition?

REFERENCES AND SUGGESTED READINGS

Best, C. H. and Taylor, N. B., *Physiological Basis of Medical Practice,* Williams and Wilkins Company, Baltimore, 1950.

Elvehjem, C. A., "Nutritional Significance of the Intestinal Flora," *Federation Proceedings,* **7**, 410 (1948).

Grossman, M. I., "Gastrointestinal Hormones," *Physiol. Rev.,* **30**, 33 (1950).

Hawk, P. B., Oser, B. L., and Summerson, W. H., *Practical Physiological Chemistry,* 12th ed., The Blakiston Company, Philadelphia, 1947.

Northrop, J. H., Kunitz, M., and Herriot, R. M., *Crystalline Enzymes,* Columbia University Press, New York, 1948.

Chapter 13

ANIMAL METABOLISM

METABOLISM OF CARBOHYDRATES

The chief function of carbohydrate in the animal body is to provide energy in a form which the animal can use. Like all fuels, it must be burned, or oxidized, for the energy to be released. The end result of the burning process is the conversion of the sugar into carbon dioxide and water, which is the reverse of photosynthesis:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 683\,\text{Cal.}$$

When carried out by living organisms, this process is called *respiration*. This term is often used in a broader sense to include all metabolic processes by which gaseous oxygen is used to oxidize organic matter chiefly to carbon dioxide and water. This type of metabolism is most pronounced in animals, but is also carried out by plants and by a few microorganisms.

If the carbohydrate is burned directly in a flame, all the energy is released in the form of heat. Some heat is produced also in the animal body, but much of the energy released is stored up in the form of certain chemical by-products, particularly adenosine triphosphate (ATP), which can later be used for muscle contraction or other useful purposes. Furthermore, the energy is released in small portions and at temperatures low enough so the living tissues are not injured. Direct burning of carbohydrate material (*e.g.*, wood, paper) of course never takes place except at a temperature fatally high to all living things; yet the same net result is accomplished rapidly and continuously in all living animals. Obviously, nature must have devised some very special and effective method of "low-temperature, biological burning."

After a very great deal of painstaking research, many of the details of this complicated process have now been discovered. In brief, what happens is that the carbohydrate undergoes a long series of chemical changes, each altering it slightly, so that it is gradually converted into the final end products, carbon dioxide and water. Each chemical reaction involved in the process is catalyzed by a particular enzyme, without which the reaction will not proceed fast enough to be of any use to the organism. Many of these essential enzymes in turn require the presence

of coenzymes and (or) activators in order to function properly (Chap. 10). This whole sequence of linked chemical changes forms a pathway over which each molecule of carbohydrate passes, as need for energy arises.

All of these changes, and any others which carbohydrates undergo in body tissues, are referred to collectively as *intermediary carbohydrate metabolism.* Although the major part of carbohydrate metabolism has to do with breakdown into simpler substances, the process is in large part reversible, and certain carbohydrates (*e.g.,* glycogen, lactose) are formed in the body from other carbohydrates or from intermediate break-down products. The "building-up" aspects of metabolism are called *anabolism;* break-down processes are termed *catabolism.*

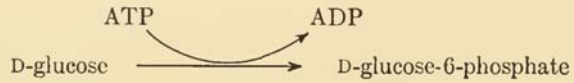
Interconversion of digested carbohydrates

Formation of Glycogen. Carbohydrate metabolism starts when the products of carbohydrate digestion pass through the intestinal wall and enter the blood stream. These products, from a normal diet, are D-glucose, D-fructose, and D-galactose. If mannose is eaten, it can also be metabolized. All four sugars are interconvertible in the animal body and give rise to glycogen by means of the metabolic reactions shown in Fig. 13–1.

At first, each sugar combines with a phosphate radical taken from ATP (reaction I, Fig. 13–1).[1] This is an irreversible[2] reaction catalyzed by *hexokinase* and Mg^{++} ions. It may be represented by the usual type of equation. For example,

$$\text{D-glucose} + \text{ATP} \xrightarrow[(Mg^{++})]{\text{(hexokinase)}} \text{D-glucose-6-phosphate} + \text{ADP}$$

or more concisely by the scheme used in Fig. 13–1:



The ATP here serves as the biological equivalent of a match used to light a fire. ATP is a concentrated storehouse of chemical energy. When one of its three phosphate radicals is transferred to the sugar, some of the energy is transferred too. This activates the sugar so that

[1] Well established chemical reactions occurring in the animal body have been numbered for easy reference throughout the chapter.

[2] Although this reaction, as such, is irreversible, the glucose-6-phosphate can easily be *hydrolyzed* back to free glucose (see p. 325).

it can begin to undergo "biological burning," just as a match heats a piece of paper to its kindling point so that it will burn.

The other reactions shown in Fig. 13–1 are reversible, equilibrium reactions (note double arrows). Such reactions go either in one direction or the other, depending on the relative amounts of the various reacting substances present. Thus after a meal, when a large amount of sugar comes into the blood stream, a considerable part is converted into glycogen, but when the sugar phosphates are consumed during exercise, the glycogen is broken down again.

Glycogen may also be formed from a variety of other substances which are involved in the further metabolism of carbohydrates (see below). Consequently, the amount of glycogen present in the body at a given time reflects a balance between the intake of all glycogen-forming food materials and the metabolic consumption of carbohydrate as energy sources.

The amount of glycogen which can be stored, however, is limited. In a normal human adult, the top level of glycogen is seldom over 6 per cent in the liver and 0.7 per cent in the muscles. These percentages correspond to a total quantity of about 110 g. in the liver and 250 g. in the muscles. Consumption of additional amounts of food above those needed to maintain this amount of glycogen in the body leads, as is well-known, to the formation of fat.

Blood Sugar Level. There is also a close interrelationship between glycogen, blood sugar, and the action of several *hormones*. The only sugar which is present in appreciable amounts in the general blood circulation is D-glucose, which for this reason is often called *blood sugar*. The blood glucose supply is furnished partially by direct absorption from the intestine, but mainly by hydrolysis of D-glucose-6-phosphate coming from glycogen: [1]

$$H_2O + \text{D-glucose-6-phosphate} \xrightarrow{\text{(phosphatase)}} \text{D-glucose} + H_3PO_4$$

The hormone *adrenalin* acts to increase the amount of glucose in the blood-stream (p. 289). Adrenalin is secreted by the adrenal gland in response to intense emotions such as rage or fear. It is usually assumed that this secretion represents a physiological preparation for intense muscular activity to cope with the situation which aroused the emotion.

Two other hormones, *insulin* from the islets of Langerhans in the pancreas and the *diabetogenic hormone* from the anterior pituitary gland, also affect the blood sugar level. It is claimed that the latter hormone is

[1] Blood sugar directly absorbed from the intestine may also be formed by hydrolysis of a glucose phosphate, since phosphorylation probably occurs during absorption.

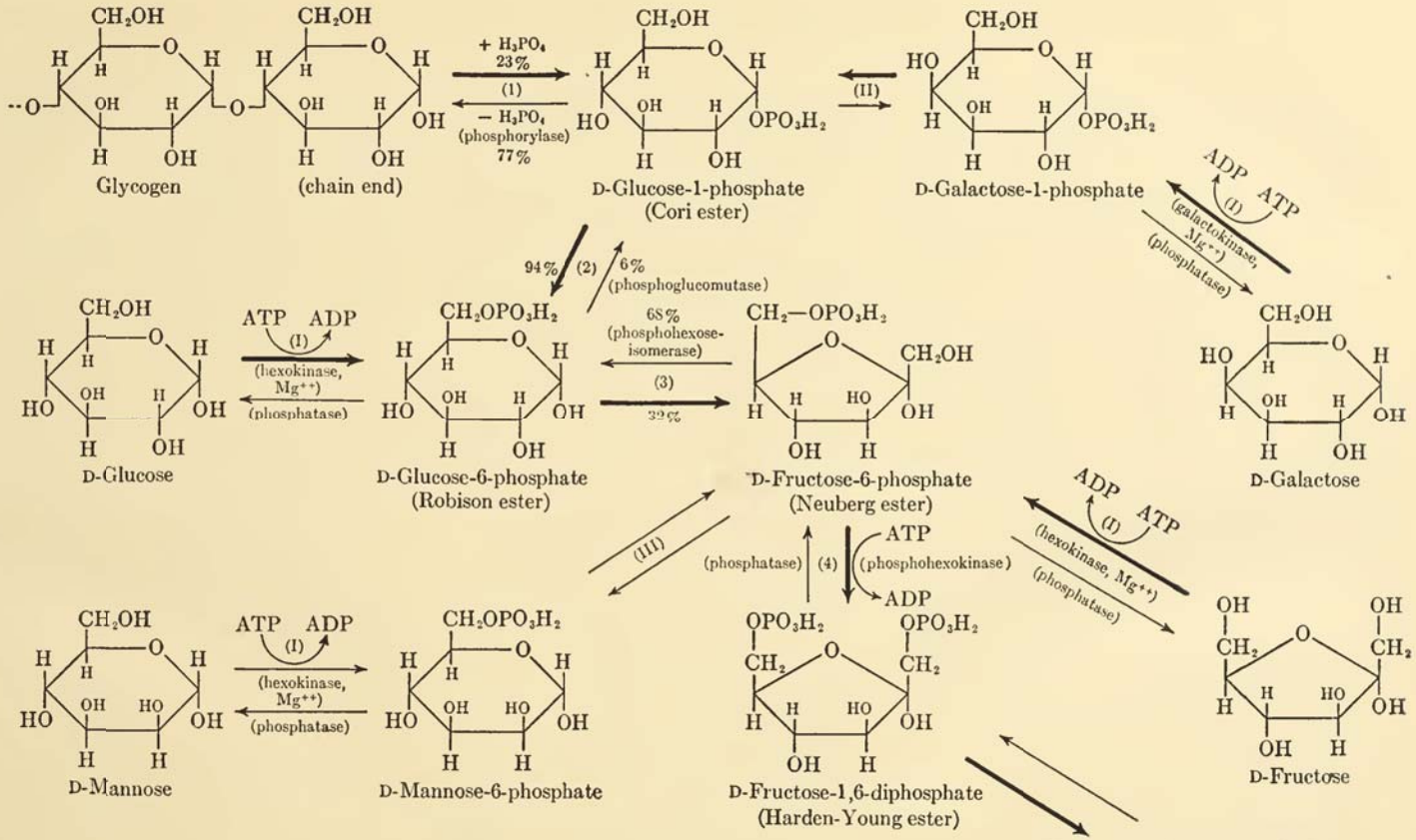


Fig. 13–1. Metabolic interconversion of food carbohydrates, and the first four reactions of glycolysis. Equilibrium reactions are indicated by double arrows, and percentage figures placed by the arrows show the point of equilibrium under physiological conditions. Enzymes catalyzing each reaction are given in parentheses. Reactions of the main metabolic pathway are numbered with algebraic numerals and are shown with heavy arrows; those leading into this pathway, with Roman numerals.

a powerful inhibitor of hexokinase, whereas insulin counteracts this inhibition. The effect of the diabetogenic hormone therefore is to raise the blood sugar level by preventing the phosphorylation essential for the utilization of blood glucose. Insulin has the opposite effect. The disease, diabetes, may be caused either by too much diabetogenic hormone or too little insulin.

The normal blood sugar level in man varies between 0.07 and 0.10 per cent (70 to 100 mg. per 100 ml. of blood) during fasting, but rises to 0.12–0.15 per cent after a meal. Some of the controlling influences which operate to maintain this level have been listed above and are presented diagrammatically in Fig. 13–2. Another factor which sets an upper limit to the blood sugar concentration is excretion in the urine. Normally

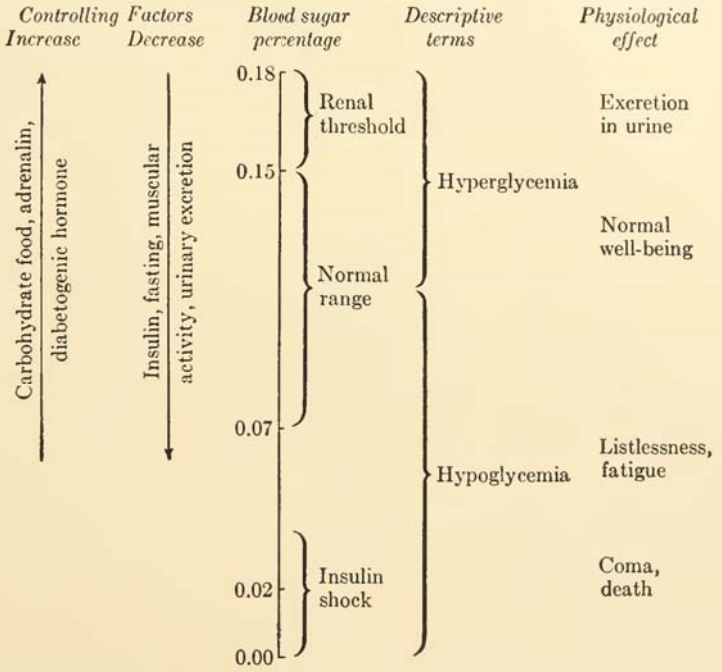


Fig. 13–2. Blood sugar level and its control.

only traces are excreted (an average of only 142 mg. in the urine of a normal man during 24 hours), but whenever the blood sugar level rises to a certain point, called the *renal threshold,* urinary excretion occurs. Thus in cases of diabetes the urine usually contains 3–5 per cent of glucose (about 50–100 g. excreted per day). The renal threshold varies with the individual, but ordinarily it is about 0.15–0.18 per cent: Levels of blood sugar much below 0.07 per cent lead to unconsciousness and

death, and even values only slightly below the normal range result in feelings of listlessness and fatigue.

Glycolysis. The catabolism of carbohydrate in the animal body may be divided for purposes of study into two main phases, the *anaerobic* and the *aerobic.* The anaerobic phase, which is called *glycolysis,* precedes the aerobic part and consists in the conversion of glycogen into pyruvic acid and (or) lactic acid. The metabolic reactions which make up glycolysis are shown in Figs. 13–1 and 13–3.[1] The whole process is often called the Embden-Meyerhof scheme.

Starting with glucose, two moles of phosphoric acid or inorganic phosphate, are converted into "organic phosphate" (reaction 8, Fig. 13–3),[2] and two moles of ATP are used up, being converted into ADP (reactions I and 4, Fig. 13–1). However, four moles of ATP are again formed from ADP (reactions 9 and 12) so there is a net gain of two moles of ATP for each mole of glucose used. In reaction 7, hydrogen is removed from glyceraldehyde-3-phosphate and is held in the form of a reduced coenzyme, $DPN \cdot H_2$ (p. 332). Four atoms of hydrogen are thus produced per mole of glucose. The net result of glycolysis, under conditions of mild exercise, can be summarized by the equation:

$$C_6H_{12}O_6 + 2H_3PO_4 + 2ADP + 2DPN \rightarrow 2CH_3COCOOH + 2ATP + 2H_2O + 2DPN \cdot H_2$$

During mild exercise, the hydrogen of the $DPN \cdot H_2$ is converted into water by combining with oxygen *via* the cytochrome system (p. 332). However, when exercise is very violent, oxygen cannot be carried by the blood stream to the muscles quickly enough to reoxidize the $DPN \cdot H_2$ as fast as it is formed. When this situation occurs, pyruvic acid is reduced to lactic acid (reaction 13, Fig. 13–3) so that lactic acid becomes the end product of anaerobic glycolysis. This process gives the organism an extra burst of energy for a short time, but the muscles soon become loaded with lactic acid, and exhaustion results. During rest, about four-fifths of the lactic acid is converted back into glycogen, and the remainder is oxidized to carbon dioxide and water.

Lactic acid formation (reaction 13) therefore is essentially an offshoot from the main line of carbohydrate metabolism. The main pathway leads to pyruvic acid, ATP, and $DPN \cdot H_2$, as given in the equation above. These products are disposed of during the aerobic phase of carbohydrate metabolism.

It should be noted that each carbon atom of the pyruvic acid comes

[1] The glyceraldehyde and glyceric acid phosphates, appearing in these charts, are frequently called phospho-glyceraldehydes and phospho-glyceric acids, respectively.

[2] Only one molecule of H_3PO_4 is shown in Fig. 13–3, but two C–3 fragments are formed from each C–6 unit (reaction 5) so that the products shown subsequently (reactions 7–13) represent only one-half of the molecules coming from one mole of glucose.

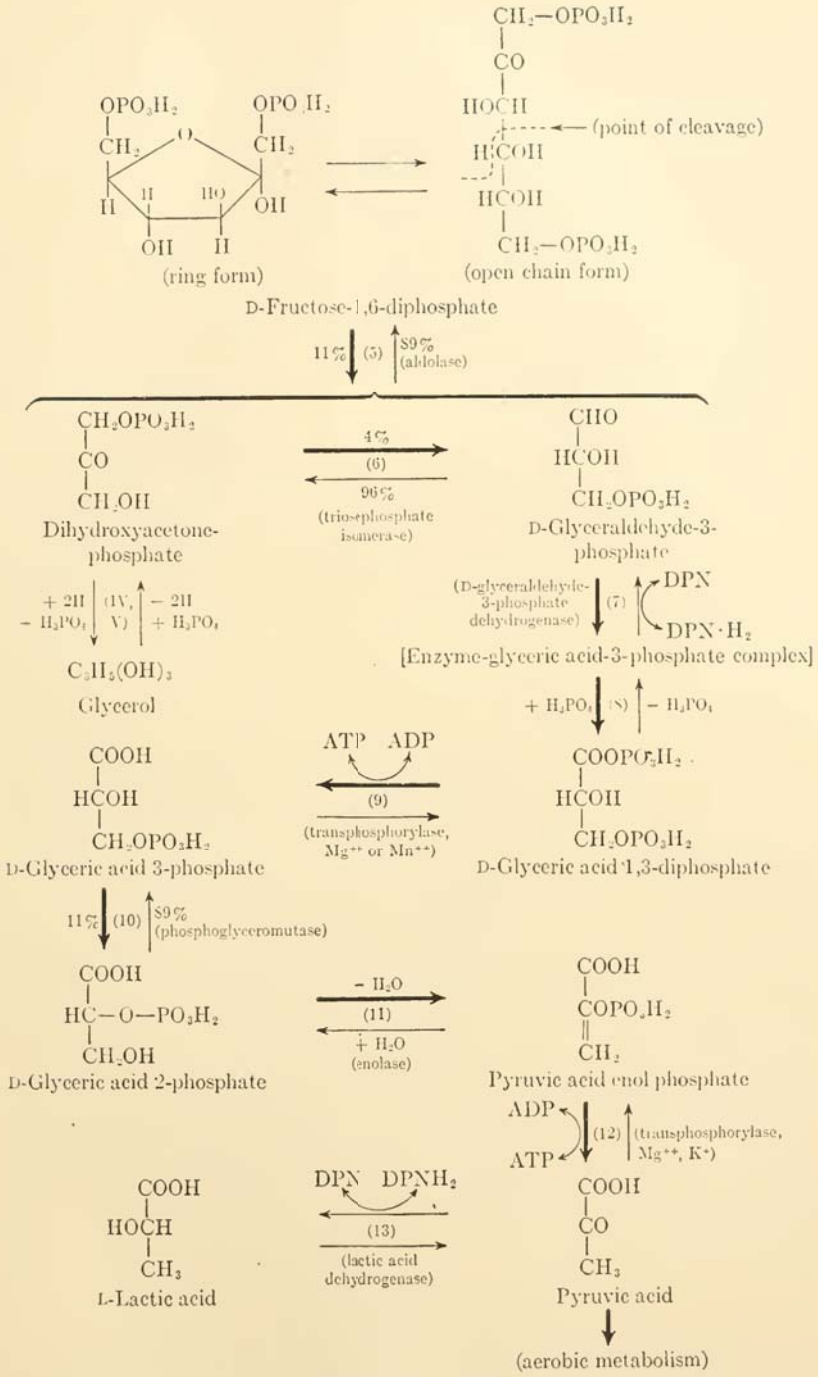
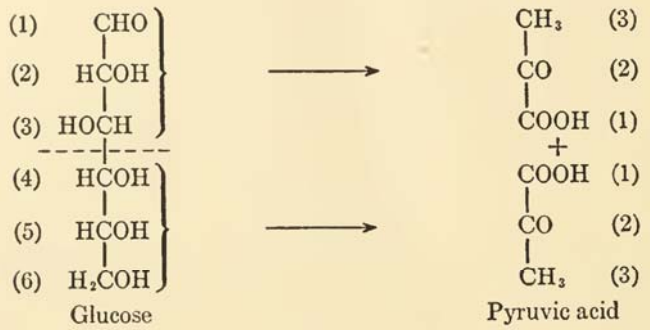


Fig. 13–3. Reactions of glycolysis (*continued*). Labeling as in Fig. 13–1.

from a definite part of the original glucose molecule (see Fig. 13–3, especially reactions 5 and 6). This may be pictured as follows:



The carbon atoms for the methyl groups of the two pyruvic acid molecules (carbon 3 of the pyruvic acid) come from carbons 1 and 6 of the glucose, those for the CO groups from 2 and 5, and those for the COOH groups from 3 and 4. The correctness of these relationships has been well established by studies with compounds containing isotopic carbon atoms in known positions.

Oxidation of pyruvic acid

The Citric Acid Cycle. Pyruvic acid is metabolized by the reactions shown in Fig. 13–4.[1] Although oxygen does not appear in this scheme, the process is an aerobic one because the hydrogen atoms produced at several points are constantly being combined with oxygen by the cytochrome system. The outstanding feature of the process is its cyclic nature. Oxalacetic acid and acetic acid combine to form citric acid, which then goes back to oxalacetic again (reactions 18–25). This is called the *citric acid cycle*.[2] The main sequence of reactions, normally followed by the bulk of the pyruvic acid metabolized, is indicated in Fig. 13–4 by heavy arrows. Reverse reactions are shown with light arrows, and various associated processes by broken lines.

The result of the operation of the citric acid cycle is that the original molecule of pyruvic acid is completely broken down into carbon dioxide and hydrogen, which later becomes water (see below). This may be seen by reading clockwise around the cycle and noting what is added or subtracted in each step. Starting with pyruvic acid, four moles of water are added (reactions 16, 19, 22, 24) and one removed (reaction

[1] In this figure, and throughout this chapter, the two-carbon substance arising from the metabolism of pyruvic acid and from fats is shown for simplicity as free acetic acid. It is almost certain, however, that this intermediate is actually an acetyl group (CH_3CO—), which is taken up by a coenzyme (*Co A*, p. 274) as fast as it is formed and later transferred to some other substance (*e.g.*, oxalacetic acid).

[2] Also called the tricarboxylic acid cycle, or Krebs cycle.

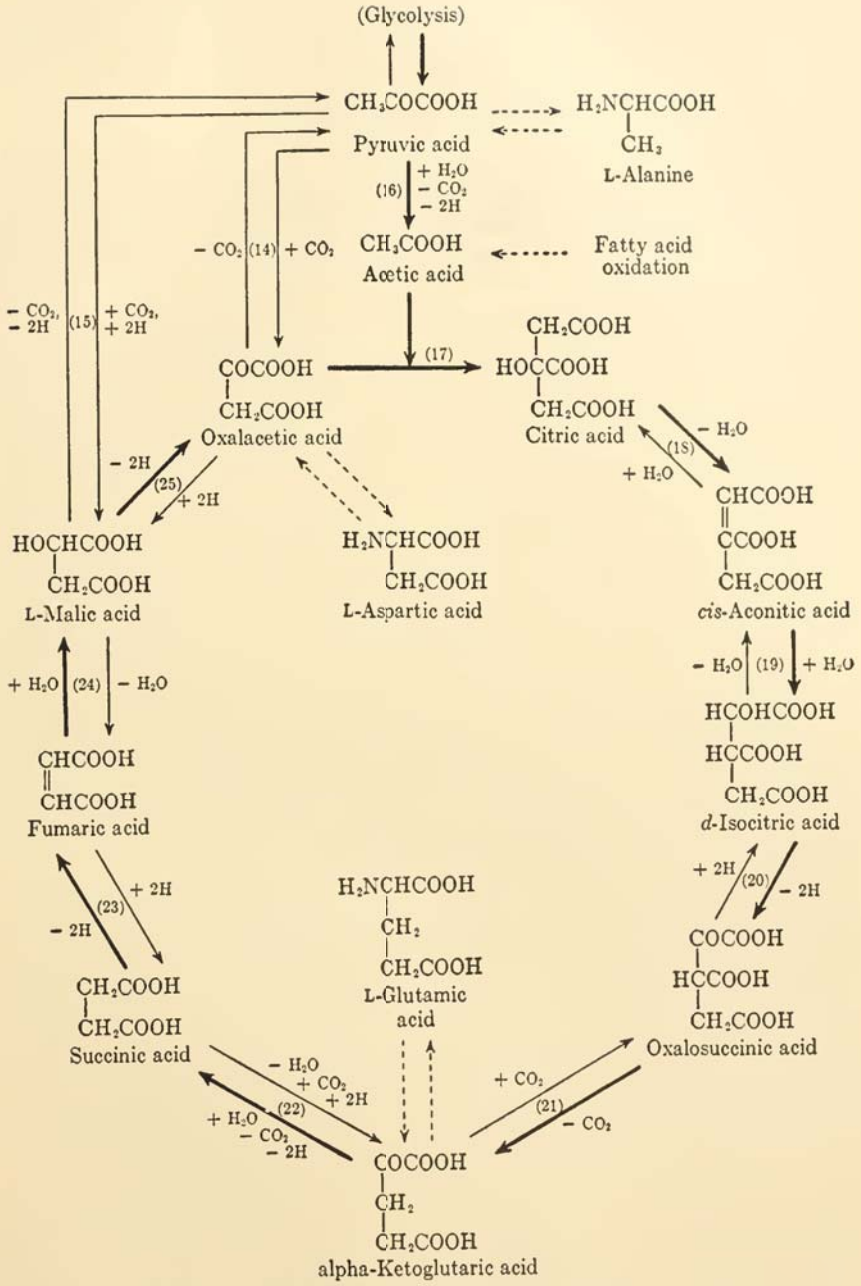


Fig. 13–4. The citric acid cycle and related metabolic reactions.

18). Ten atoms of hydrogen (two each in reactions 16, 20, 22, 23, 25) and three moles of carbon dioxide (reactions 16, 21, 22) are also removed. The net result, therefore, is:

$$CH_3COCOOH + 3H_2O \rightarrow 10(H) + 3CO_2$$

Since at the end of the cycle another molecule of oxalacetic acid is formed, more pyruvic acid can at once be catabolized. The citric acid cycle may be regarded as a sort of machine for metabolizing pyruvic or acetic acids, or any other substance which can be converted into one of the compounds involved in the cycle (*e.g.*, glutamic acid, p. 343).

Oxalacetic acid occupies a position of special importance, since it is the substance with which the incoming stream of acetic acid molecules must combine in order to set the cycle in operation. Although oxalacetic acid is regenerated at each "turn of the wheel," it is obvious that at least a small amount must be present *before* the cycle can start at all. In other words, there must be some source of oxalacetic acid other than that regenerated by the cycle itself. This other source is pyruvic acid, which can combine with carbon dioxide to give oxalacetic acid directly (reaction 14) or with carbon dioxide and hydrogen (from TPN · H_2) to form malic acid, which then goes to oxalacetic (reactions 15 and 25). It is probable that the latter pathway is quantitatively the more important in animal tissues.

Cytochrome System. The only oxidative processes shown in Fig. 13–4 are indirect ones consisting of the addition of water and removal of hydrogen. Thus succinic acid, for example, is converted into oxalacetic acid, which contains one more oxygen atom. This indirect method of oxidation is a very common biochemical process.

The hydrogen so produced is never present in the free state in the tissues. It forms reduced coenzymes (*e.g.*, DPN · H_2) and from them is passed through the *cytochrome system* to combine with the oxygen brought to the muscles by the blood stream. It is important to note that, of the two metabolic end products—carbon dioxide and water—only the latter comes from a direct union with the inhaled oxygen. The carbon of the original sugar is never oxidized directly to carbon dioxide. Likewise, the bulk of the energy derived from the metabolism of fats and carbohydrates comes from the oxidation of hydrogen (p. 422).

The most important coenzymes which receive hydrogen from metabolites and transfer it to cytochrome are the pyridine nucleotides, DPN and TPN, and the flavin nucleotides, FAD and FMN (p. 277). In most cases the hydrogen from the metabolite first passes to one of the pyridine coenzymes, which is thereby converted into the reduced form, DPN · H_2[1]

[1] These abbreviations are used merely for convenience. In reality, one of the two extra hydrogens is ionized:

$$DPN \cdot H_2 \rightleftarrows (DPN \cdot H)^- + H^+$$

or $TPN \cdot H_2$ (see Chap. 10 for exact formulas). Next, the hydrogen is most probably transferred to one of the flavin nucleotides. This may be represented, for example, as follows:

$$TPN \cdot H_2 + FMN \xrightarrow{(26)} TPN + FMN \cdot H_2$$

Note that the pyridine nucleotide is returned to its original condition, ready to take up more hydrogen. The reduced flavin coenzyme then hands its hydrogen to cytochrome *c* (Cyt. *c*):

$$FMN \cdot H_2 + 2\text{Cyt. } c\ Fe^{+++} \xrightarrow{(27)} FMN + 2\text{Cyt. } c\ Fe^{++} + 2H^+$$

The final reaction is the reoxidation of the reduced cytochrome *c* by molecular oxygen (from oxyhemoglobin) under the influence of cytochrome oxidase, with the formation of water:

$$2\text{Cyt. } c\ Fe^{++} + 2H^+ + \tfrac{1}{2}O_2 \xrightarrow[\text{oxidase}]{(28)\ \text{cytochrome}} 2\text{Cyt. } c\ Fe^{+++} + H_2O$$

The transport of hydrogen through the cytochrome system may be represented by the scheme shown in Fig. 13–5. The light curved arrows are used to indicate the alternate reduction and reoxidation of the hydro-

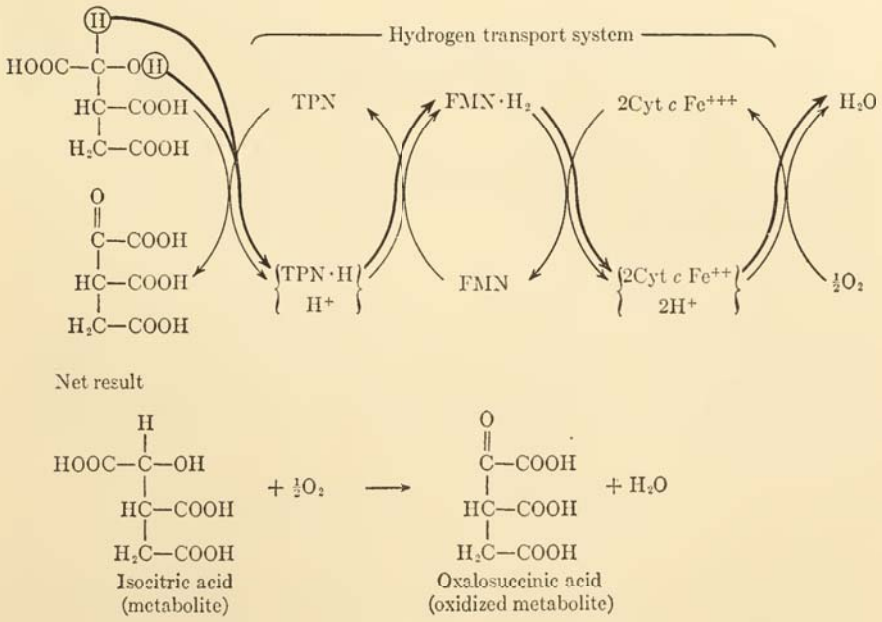


Fig. 13–5. Transport of hydrogen through the cytochrome system. Alternate oxidation and reoxidation of the hydrogen carriers is indicated by light curved arrows. Heavy arrows show path of hydrogen from metabolite to oxygen.

gen carriers, TPN, FMN, and cytochrome *c*. Note that the two hydrogen atoms from one molecule of the metabolite are passed from one coenzyme to another before they are finally combined with oxygen (heavy arrows). This seems like an unwieldy and roundabout method of bringing hydrogen and oxygen together. Apparently, the purpose of this procedure is to release energy in small steps (p. 420) rather than in a sudden burst, which probably would injure living tissues. Through the action of the cytochrome system all the hydrogen released from pyruvic acid by the reactions of the citric acid cycle is converted into water.

Cytochrome *c* is an iron-containing protein (p. 279). The enzyme, cytochrome oxidase, is poisoned by cyanide. The importance of the cytochrome system to higher animals is apparent from the fact that cyanide inhibits the respiration of animal tissue preparations to the extent of 80 per cent or more.

Summary of carbohydrate metabolism

The conversation of food carbohydrates into glycogen and their oxidation to carbon dioxide and water have been considered above under the headings, glycolysis, citric acid cycle, and cytochrome system. It must be emphasized that these phases of carbohydrate metabolism are not in any way separate from each other but operate continuously and simultaneously in the living animal. In order to gain a clearer over-all picture, the result of these processes as applied to a molecule of glucose may be summarized as follows:

$$C_6H_{12}O_6 \longrightarrow 2CH_3COCOOH + 4(H)$$

$$2CH_3COCOOH + 6H_2O \longrightarrow 20(H) + 6CO_2$$

$$24(H) + 6O_2 \longrightarrow 12H_2O$$

$$\textit{Sum}: \quad C_6H_{12}O_6 + 6O_2 \longrightarrow 6H_2O + 6CO_2$$

At various stages of the process, energy is stored up by conversion of ADP into ATP, and some energy is released as heat. These aspects will be considered in Chap. 16.

METABOLISM OF LIPIDES

The fat which is poured into the blood stream by way of the lymph system, following a fatty meal, can be used by the animal organism in four different ways. These are storage, excretion, oxidation, or conversion into essential lipides.

Fat storage

The main purpose of fat metabolism is to provide energy by oxidation of the fat. However, before this occurs a large part of the fat eaten is temporarily deposited in the fatty tissues of the body. This deposit provides a reserve of energy for the organism far greater than that in the form of glycogen for not only is a much greater quantity of fat deposited, but it has an energy value of 9 Calories per gram as compared to only 4 for carbohydrate. A certain amount of stored fat is also desirable as a protective covering for certain organs, especially the kidney.

Dynamic State of Stored Fat. Until rather recently it was supposed that stored fat was more or less inert metabolically—excess food laid away and left undisturbed until needed. This viewpoint was entirely changed by the experiments of Schoenheimer, who fed animals fatty acids containing deuterium, an isotope of hydrogen, in place of some of the hydrogen atoms ordinarily present. He found that after four days about half of the deuterium was present in the stored fats and that much of the isotope had been shifted to several other fatty acids besides the one fed. Also, when water containing deuterium was injected into mice, much of the isotopic hydrogen quickly appeared in the body fats. He concluded that the stored fat was normally in a constant state of flux, even in adult animals having a substantially constant weight and total fat content. About one-half of the body fat is synthesized and one-half broken down each week.

Nature of Stored Fat. In general, each animal species tends to lay down a type of depot fat characteristic of the species, but the nature of this fat is also greatly influenced by the kind of food eaten. This is true because the animal possesses only a limited ability to transform one fatty acid into another.

With the aid of isotopic tracers, chiefly deuterium, it has been demonstrated that the animal can shorten or lengthen the chain of saturated fatty acids. Thus stearic acid, for example, can be converted into palmitic and myristic acids, and palmitic can be changed back into stearic again. Animal tissues also contain enzymes which can change saturated acids into certain unsaturated ones, for example, stearic into oleic acid. This process, however, is limited to the introduction of one double bond at the 9,10-position. Desaturation at the α,β-position also probably occurs during beta oxidation (p. 336).

That the animal cannot synthesize more highly unsaturated fatty acids such as linoleic or linolenic is shown by the fact that these are essential components of the diet (p. 79).

The tissues of animals are able to bring about a saturation of α,β-unsaturated acids. However, if the food fats are more highly unsaturated

than the body fats normally are, the latter will become more unsaturated also. This is a matter of considerable economic importance in the feeding of hogs, where a very soft fat is undesirable in the pork. Whenever the body fats are produced by the feeding of carbohydrates, a type of fat characteristic of the animal results. Hogs fed on soybean or peanut meals are "finished" on corn for this reason.

Fat transport

The blood stream serves as the vehicle for carrying fats to various organs of the body. The blood normally contains simple fat (triglycerides) only for a few hours after a meal. These absorbed glycerides are carried in the form of tiny fat droplets called *chylomicrons* to the fat-storage tissues (*e.g.*, under the skin). Later, the fat to be oxidized is carried to the liver, apparently in the form of phospholipides.

A number of conditions are known which bring about a greatly increased amount of fat in the liver. For example, interruption of the normal flow of pancreatic juice in dogs was found experimentally to cause an accumulation of over 300 g. of fat in the liver, whereas the liver of a normal dog of similar size contains only 10–15 g. At the same time, the blood phospholipide level fell from 60 to about 30 mg. per 100 ml. This "fatty liver" condition is prevented or corrected by feeding choline, which presumably acts by way of forming more phospholipide and thus promoting the transport of fat away from the liver. Methionine also shows *lipotropic* action (*i.e.*, prevents accumulation of fat in the liver), probably because it can be used in the metabolic synthesis of choline (p. 345).

Metabolic oxidation of fat

Whether or not the fat is stored, eventually it becomes oxidized to carbon dioxide and water with the liberation of energy. This oxidative catabolism of fat is an aerobic process, which is started chiefly in the liver and finished in the muscles and kidneys.

The glycerol part of the fat is most probably dehydrogenated and phosphorylated to form D-glyceraldehyde-3-phosphate (Fig. 13–3), which may then be metabolized by the carbohydrate pathways already discussed. Thus it may either be converted into glycogen or oxidized to carbon dioxide and water.

Beta Oxidation. The fatty acids cannot enter the sugar metabolism pathway so simply because of their widely different chemical nature. It now appears quite certain that these long chain acids are chiefly broken down according to *Knoop's theory of beta oxidation.* Briefly, Knoop's theory states that two carbon pieces, which appear to be molecules of acetic acid or some closely related substance, are broken off from the

—COOH end of the fatty acid. These are then further oxidized to carbon dioxide and water. The exact details of how the two-carbon piece is broken off have not been completely worked out, but are probably somewhat as follows:

$$\text{----}CH_2CH_2CH_2CH_2COOH \xrightarrow[(29)]{-2(H)} \text{----}CH_2CH_2CH{=}CHCOOH$$

Carboxyl end of saturated fatty acid molecule — Corresponding α,β-unsaturated acid

$$\xrightarrow[-2(H)]{+H_2O}_{(30)} \text{----}CH_2{-}CH_2{-}\overset{\overset{O}{\|}}{C}{-}CH_2COOH \xrightarrow[(31)]{+H_2O}$$

Corresponding β-keto acid

$$\text{----}CH_2CH_2COOH + CH_3COOH$$

Saturated fatty acid with two less carbon atoms — Acetic acid

Note that it is the *beta* carbon atom (second from the —COOH group) which is oxidized. This process is then thought to occur over and over until the original fatty acid molecule has been broken down entirely to acetic acid and hydrogen. For example, stearic acid, containing eighteen carbon atoms, is split in 8 places to yield 9 molecules of acetic acid as follows:

$$CH_3(CH_2)_{16}COOH + 16H_2O \rightarrow 9CH_3COOH + 32(H)$$

Block and Rittenberg have estimated the normal acetic acid production in rats to be about 1 g. per 100 g. of body weight per day. The exact amount will of course be influenced by the proportion of fat in the ration. Thus acetic acid, and possibly also acetoacetic acid, represent the end products of fat catabolism in the liver. These products are transported by the blood to the muscles and kidneys, where the oxidation is completed.

Other Theories of Fat Oxidation. There is evidence that the methyl group of fatty acids (the "omega" carbon atom at the opposite end of the chain from the —COOH) may be oxidized to a second carboxyl group. This oxidation would produce a dibasic acid which could then undergo β-oxidation from each end. The "*omega oxidation*" probably occurs to a minor extent, and only with fatty acids of intermediate chain length (about 8–12 carbon atoms). That it can occur, however, has been shown by feeding dogs triglycerides of such fatty acids as undecanoic (saturated C-11 acid). The urine of these dogs was found to contain dibasic acids of 11, 9, and 7 carbon atoms. Omega oxidation is probably not a major pathway of normal fat catabolism.

A third type of fat catabolism is *multiple alternate oxidation.* According to this idea the fatty acid is oxidized at the β-carbon and at each

alternate carbon beyond the β-position toward the methyl end of the chain. This results in a polyketo acid of the type, $\ldots COCH_2COCH_2$-$COCH_2COCH_2COOH$, which breaks down all at once to form acetic (or acetoacetic) acid. It is still uncertain whether this type of oxidation occurs extensively during fat metabolism in animals.

Oxidation of Acetic Acid. The two-carbon fragment produced during fat catabolism, as described above, can be further metabolized in a variety of ways. Probably the bulk of it, under normal circumstances, is completely oxidized to carbon dioxide and water. This oxidation occurs chiefly in the muscles and kidneys, the main energy-using organs. The acetic acid condenses with oxalacetic acid to form citric acid (reaction 17, Fig. 13–4), which is then further metabolized by the reactions of the citric acid cycle. This condensation, therefore, is a connecting link between fat and carbohydrate metabolism. Following the reactions from acetic acid back to oxalacetic acid (Fig. 13–4), it may be seen that 8(H) and $2CO_2$ are removed, while $2H_2O$ have been added. This means that the acetic acid has been completely broken down:

$$CH_3COOH + 2H_2O \rightarrow 8(H) + 2CO_2$$

Since stearic acid was shown above to form nine acetic acid molecules, the complete catabolism of this acid can now be represented as follows:

$$CH_3(CH_2)_{16}COOH + 34H_2O \rightarrow 104(H) + 18CO_2$$

The hydrogen atoms, of course, are united with coenzymes as fast as they are produced and are immediately transferred through the cytochrome system to oxygen, thereby being converted into water.

Ketosis. One of the most important features of fat metabolism is the fact that *fat is not oxidized efficiently to carbon dioxide and water unless carbohydrate is also being oxidized at the same time.* The reason probably is that the supply of oxalacetic acid, which is formed from pyruvic acid and carbon dioxide (reactions 15 and 25, Fig. 13–4), is low when carbohydrates, and hence pyruvic acid, are not being metabolized. The essential relationships involved may be illustrated by an hypothetical case. Suppose only one molecule of oxalacetic acid is present in a cell which needs to oxidize three molecules of acetic acid. *Three* separate "turns" of the citric acid cycle, one after another, will be required to complete the job. However, if two molecules of pyruvic acid are also present, they can be converted into two extra molecules of oxalacetic acid, and hence all of the acetic acid—as well as the pyruvic—can be metabolized at *one* turn of the cycle. At any rate, when carbohydrates are not being metabolized, the acetic acid coming from fats is not oxidized as fast as it is produced. Instead it piles up and is recombined into acetoacetic acid:

$$CH_3COOH + CH_3COOH \xrightarrow{(32)} CH_3COCH_2COOH + H_2O$$

From this, in turn, are formed acetone and β-hydroxybutyric acid:

$$\underset{\beta\text{-Hydroxy butyric acid}}{CH_3CHOHCH_2COOH} \underset{-2(H)}{\overset{+2(H)}{\underset{(34)}{\rightleftharpoons}}} \underset{\text{Acetoacetic acid}}{CH_3COCH_2COOH} \xrightarrow[(33)]{-CO_2} \underset{\text{Acetone}}{CH_3COCH_3}$$

These three substances collectively are called "ketone bodies." When fat, but not carbohydrate, is metabolized, the ketone bodies accumulate in the blood and are excreted in the urine. This condition is called *ketosis*. Since two of the ketone bodies are acids, ketosis also involves a condition of *acidosis*, which if not relieved, leads to coma and death.

Ketosis may be caused by eating a diet high in fat and low in carbohydrate. For most people a diet having over 75 per cent of the calories in the form of fat and less than 15–20 per cent as carbohydrate is ketogenic (*i.e.*, produces ketosis). However, Eskimos, for example, can tolerate even higher amounts of fat. Ketosis may also develop during starvation or after long-continued vomiting, because in such cases the main food material being metabolized is the stored fat. Diabetics are very apt to develop ketosis because of their lowered ability to metabolize sugars. The excretion of ketone bodies in cases of ketosis in human beings often amounts to 15–20 g. per day and has been reported in extreme cases to be more than 100 g. per day.

The exact manner in which acetoacetic acid is formed in ketosis has been the subject of much dispute. Formerly, it was thought to arise only from the four carbon atoms at the methyl end of fatty acid molecules, *i.e.*, the last to be degraded by normal β-oxidation. However, it was later found that enzymatic oxidation of caprylic acid (the saturated C-8 acid) gave rise to *two* moles of acetoacetate. The β-oxidation theory, of course, could account for only one mole from one mole of the fatty acid. It was found further that if the caprylic acid were labeled with C^{13}, a heavy isotope of carbon, in the —COOH group, the C^{13} appeared in both the —COOH *and* CO groups of the acetoacetic acid. These experimental findings showed that, at least in this case, the caprylic acid was first oxidized to C_2 fragments, which then recombined as indicated in reaction 32 above.

Other metabolic reactions of acetic acid

Fat Synthesis. It is a matter of common observation that consumption of excess food leads to fatness. Fat can be synthesized in the animal body from either carbohydrates or proteins, although the carbohydrates are the only important source.

The production of fat from carbohydrate is a process of reduction and requires energy. Part of the sugar must be oxidized in order that the rest may be converted into fat. Although the mechanism of the conversion is not positively known, the sugar is probably broken down

in the usual way to pyruvic acid, which in turn forms acetic acid or a related C_2 substance. The long chain fatty acids are then most probably produced by uniting a number of these C_2 units. This plan accounts for the fact that nearly all the natural fatty acids contain an even number of carbon atoms.

Furthermore, it has been shown by the isotope tracer technique that acetic acid does form fatty acids in the animal body. Acetic acid labeled with deuterium in the methyl group and C^{13} in the —COOH was given to mice and rats, and the body fats examined after a few days. Both deuterium and C^{13} were present in the fatty acids, and in the same amounts relative to each other as in the acetic acid fed. The isotopes were present in all parts of the fatty acid molecules. Other animals were fed deuterium oxide ("heavy water") in place of ordinary water, and the body fats were found to have taken up the deuterium. These facts are all consistent with the idea that the fatty acids are synthesized by condensation of C_2 fragments, followed by reduction with hydrogen derived from water in the body tissues.

Thiamine is required for fat synthesis, possibly because it is a part of cocarboxylase which is required for the oxidative decarboxylation of pyruvic acid to form acetic acid (reaction 16, Fig. 13–4). Recently it has been found that another vitamin, namely biotin, is involved in the synthesis of oleic acid, particularly in microorganisms.

Steroid Synthesis. Acetic acid has also been found to serve as a metabolic precursor of cholesterol in the animal body. At least half, and probably more, of the carbon and hydrogen atoms in cholesterol are derived from this source. Several other substances such as ethyl alcohol, leucine, and butyric acid can also take part in cholesterol synthesis, but probably only because they are first converted into acetic acid.

Other important animal steroids are known to be formed, in turn, from cholesterol. Thus the transformation of cholesterol into cholic acid and pregnanediol has been demonstrated with isotopic compounds.

Acetylation of Amines. When amines, *e.g.*, sulfanilamide, not normally present in the body are given to animals, they often are converted at least partially into acetyl derivatives, which are excreted. This represents a bodily mechanism for throwing off foreign and possibly toxic materials. As a rule, the acetylated products are less toxic than the original amines.

Acetylation also occurs in the case of normal tissue constituents (*e.g.*, amino acids, choline) and is, in fact, a very common metabolic reaction. It has been amply demonstrated that the acetyl groups come from acetic acid. Acetylcholine, produced by acetylation of choline, is an essential substance for nerve functioning.

The participation of acetic acid in the metabolic production of porphyrins and uric acid is discussed under protein metabolism (p. 351).

In general, it must be concluded that acetic acid, or some closely related C_2 substance, is a very active material metabolically and enters into many of the catabolic and anabolic activities of the living animal cell.

METABOLISM OF PROTEINS

Synthesis and interconversion of amino acids in animal tissues

Essential Amino Acids. The metabolism of proteins in the animal body is largely a matter of the transformations of amino acids. At least 20 of these "building blocks" are present in animal proteins and must therefore be supplied to the animal, either directly from food proteins or indirectly by synthesis from other food constituents. Those acids which cannot be synthesized by the animal at measurable rates are called *nutritionally essential amino acids*. Those which can be synthesized, but at rates which are sometimes inadequate (*e.g.*, during rapid growth) may be called *semiessential*. Presumably all other amino acids present in body proteins must be capable of being synthesized in adequate amounts (and fast enough) to meet all requirements. Of course, these "nonessential" amino acids also may be present in the food, and, in fact, the main supply normally comes from this source.

Lists of essential and semiessential amino acids are given in Table 13–1 for several species. These lists are based mainly on studies of the growth of young animals and on nitrogen balance studies with adults. The latter method, which is the one used for experiments with human beings, involves a comparison of the total intake and output of nitrogen when only certain amino acids are given the subjects. If the lack of a particular amino acid results in a negative nitrogen balance (output larger than intake), this is evidence that the acid is needed and cannot be synthesized in the body. For the best nutrition the diet should supply not only the essential and semiessential amino acids, but a good selection of the nonessential ones as well. Although the latter can be produced in the tissues from other materials (see below), it is probably more efficient to consume them ready-made in the form in which they are needed.

It will be noted in Table 13–1 that some of the essential amino acids can be replaced by certain closely related substances, namely, the D-isomers, or the alpha-keto or alpha-hydroxy analogs. This situation probably results from the fact that these amino acids can enter into the process of transamination (see below). In these cases it is the carbon chain of the amino acid which is the essential feature, and not the alpha-amino group. It has also been shown that α-amino adipic acid can replace lysine for the rat, that is, it acts as a physiological precursor of this essential amino acid.

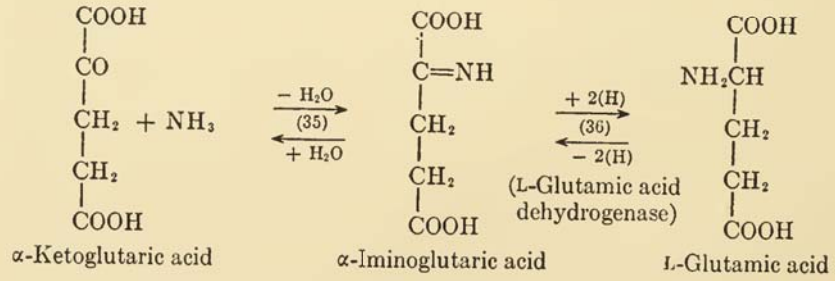
Table 13–1

Nutritionally essential and semiessential amino acids

Classification	*Man*	*Rat*	*Chick*
Essential amino acids (no synthesis)	isoleucine leucine lysine methionine * phenylalanine * threonine tryptophan valine	histidine *† isoleucine *† leucine† lysine methionine *† phenylalanine *† threonine tryptophan *† valine †	arginine histidine isoleucine leucine * lysine methionine * phenylalanine * threonine tryptophan valine
Semiessential amino acids (synthesis sometimes inadequate)	arginine * histidine	arginine cystine glutamic acid proline tyrosine	cystine glutamic acid glycine proline

* Can be replaced by D-isomer.
† Can be replaced by corresponding alpha-keto or alpha-hydroxy acid.

Amination. The process of oxidative deamination, which all the amino acids undergo (p. 351), is reversible in the case of glutamic acid. This reversibility makes possible the synthesis of glutamic acid from α-keto-glutaric acid and ammonia, as follows:



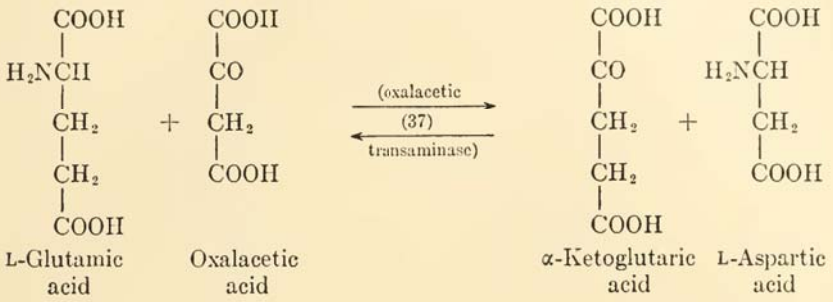
This process takes place mainly in the liver and kidneys.

The necessary hydrogen is obtained from the reduced forms of either DPN or TPN, which of course are always available in the body as a result of fat and carbohydrate metabolism. Since the α-ketoglutaric acid is produced from carbohydrates, this process constitutes a link between the metabolism of proteins and sugars.

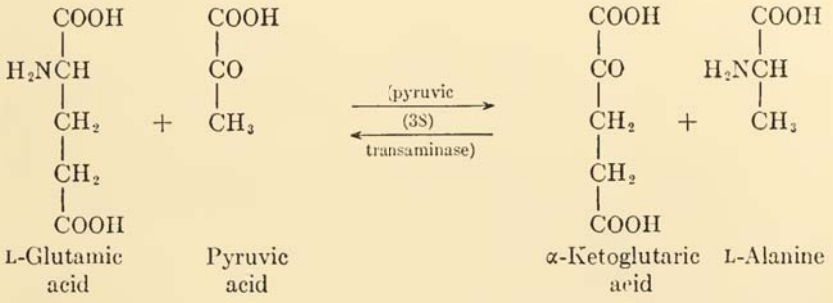
The ammonia must be provided from some dietary source, which normally comes from the deamination of other amino acids. This means that the above process, which may be called *amination,* does not result in a net increase in the total supply of amino acids. Its value lies, rather,

in the fact that, in conjunction with transamination, it enables the body to convert one amino acid into another.

Transamination. Two enzymes have been found in animal tissues which catalyze the transfer of an amino group from an amino acid to a keto acid. Each of these *transamination* reactions requires glutamic acid as the amino group donor or α-ketoglutaric acid as the acceptor, and pyridoxal phosphate as a coenzyme:



L-Glutamic acid — Oxalacetic acid — α-Ketoglutaric acid — L-Aspartic acid



L-Glutamic acid — Pyruvic acid — α-Ketoglutaric acid — L-Alanine

The presence of these highly active transaminases in nearly all animal tissues suggests that transamination is a major metabolic reaction. Two additional amino acids, aspartic acid and alanine, are thus obtained from sugar metabolism intermediates. There are indications that other amino acids can take part in transamination also, but the importance of the reaction in these cases is doubtful as far as normal metabolism is concerned.

Transmethylation. Many of the organic substances present in the tissues of higher animals contain methyl groups attached to nitrogen or to sulfur (examples: creatine, choline, methionine). Other substances (for example, pyridine), not normally present, when fed to animals are converted into methylated derivatives and are excreted in that form. A clearer understanding of this process of *methylation* was obtained by du Vigneaud from a study of methionine in relation to rat growth. He found that this essential amino acid could be replaced by choline plus homocysteine and proved, by using deuterium as a tracer, that methionine

was formed in the animal by the transfer of methyl groups from choline (reaction 39, Fig. 13–6).

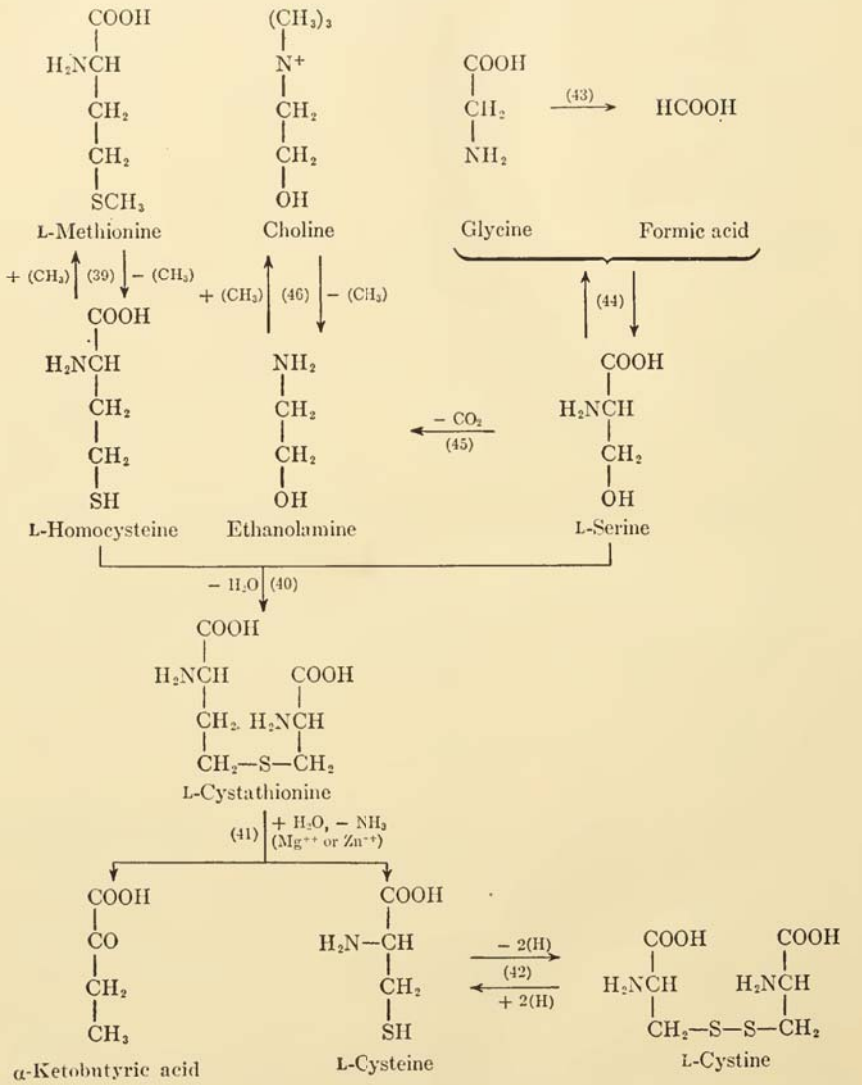


Fig. 13–6. Metabolic interrelationships of glycine, serine, methionine, and cystine, and some methylation reactions in animal tissues.

Without choline in the diet, homocysteine was unable to replace methionine for rat growth. It was concluded that the animal was unable to synthesize methyl groups needed for certain methylation reactions, but could transfer them, by the process of *transmethylation,* from other methylated substances, such as choline. Such substances are called *methyl donors* and are said to contain *labile methyl groups.* An adequate

source of labile methyl groups is one of the essential components of a complete diet for higher animals. However, this requirement can be met indirectly if the diet contains certain vitamins (see below).

Methionine itself is also a methyl donor and has been shown to provide methyl groups for the formation of both choline and creatine (p. 348). Choline is produced in the animal body by the addition of methyl groups from methionine to ethanolamine (reaction 46, Fig. 13–6), which in turn is derived from serine (reaction 45). Five other substances have now been found which can serve as methyl donors in biological systems. Two of them, *betaine* [$(CH_3)_3N^+CH_2COO^-$] and dimethyl-propiothetin [$(CH_3)_2S^+CH_2CH_2COO^-$] occur in nature and probably take part in methylation reactions in living cells.

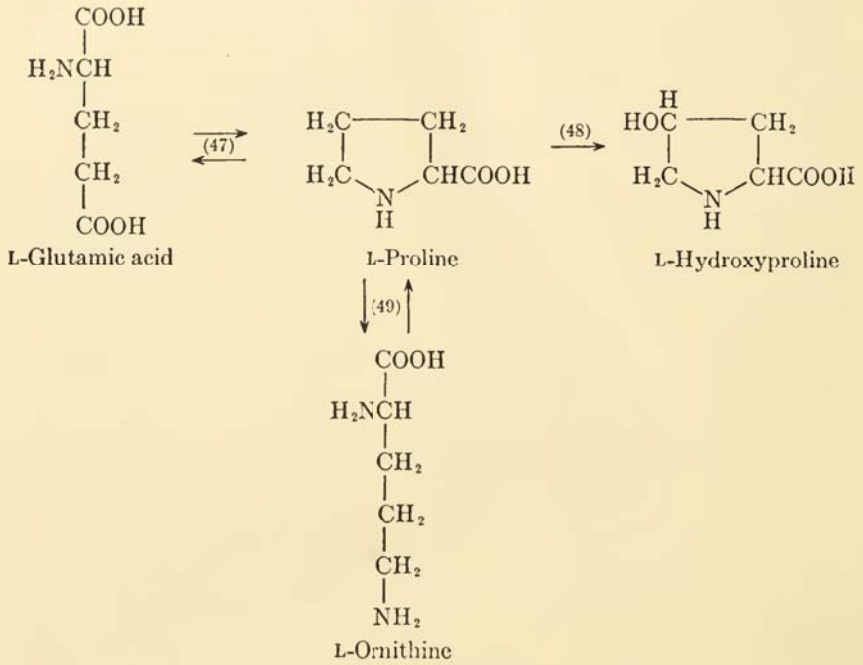
Recent studies have proved that when adequate supplies of folic acid and vitamin B_{12} are present in the diet, rats can synthesize labile methyl groups from glycine, serine, acetone, or formic acid and hence do not require a methyl donor for growth. This was established by isotopic tracer studies which showed that carbon atoms from these substances appeared in the methyl groups of choline and thymine. Also, when rats were fed a diet containing all the known vitamins including folic acid and vitamin B_{12}, but without any methyl donor, and with homocysteine as the only sulfur-containing amino acid, good growth occurred. Presumably the rats converted the homocysteine into methionine under these conditions.

Other Metabolic Interconversions of Amino Acids. As a result of recent investigations, based almost entirely on the use of isotopic tracers, several other metabolic relationships among amino acids have been discovered. For example, L-cystine is synthesized in the animal body from L-serine and L-methionine. The intermediate steps, which involve homocysteine and cystathionine, are shown in Fig. 13–6. In some still obscure manner the cleavage of cystathionine (reaction 41) results in the formation of α-ketobutyric acid as the other product besides cysteine. Note that only the sulfur of the cystine is derived from methionine and that the rest of the molecule comes from serine.

These reactions provide a reasonable explanation for the fact that cystine is not a nutritionally essential amino acid and that it has a "sparing action" for methionine. That is, when the diet contains plenty of cystine, no methionine has to be diverted to cystine synthesis so that less methionine is needed. Another substance which can give rise to cystine in the body of the rat is L-lanthionine (p. 117).

Another metabolic relationship, which is now well established, is the formation of serine from glycine. The most probable route of this synthesis is shown in Fig. 13–6, reactions 43 and 44. One molecule of glycine is changed into formic acid, which then combines with a second molecule of glycine to form serine. The reverse conversion of serine into glycine also occurs readily in the animal body.

Higher animals also are able to convert glutamic acid into proline, hydroxyproline, and ornithine, as follows:



L-Glutamic acid — L-Proline — L-Hydroxyproline

L-Ornithine

The ornithine produced is readily converted into two additional amino acids, citrulline and arginine (see urea formation, p. 352). Although arginine can thus be synthesized in the animal, the rate of production is often too slow to meet bodily needs. It has been shown, for example, that the growth of rats fed a ration lacking arginine is greatly stimulated by adding this amino acid.

Tyrosine is another amino acid which is synthesized in the animal body, the precursor being phenylalanine:

L-Phenylalanine — L-Tyrosine

Accordingly, it has been found that tyrosine has a sparing action for phenylalanine, just as cystine has for methionine.

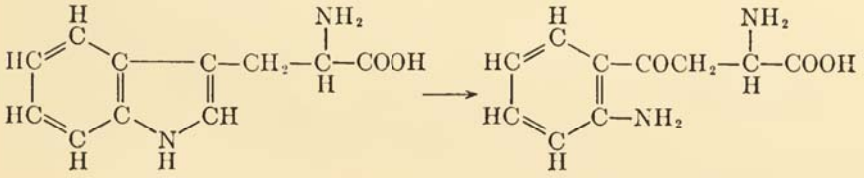
Utilization of amino acids

The main use for amino acids in the animal body is the synthesis of tissue proteins. Such synthesis is not only necessary for young, growing animals, but it is also essential for full-grown adults, because tissue proteins are continually being broken down and resynthesized. Borsook gives 10 days as the half-life (period in which one-half of a substance is decomposed) of the proteins in the internal organs of man and 158 days for those in other tissues (mainly the muscles).

The amount of dietary protein needed to supply the normal requirements of human beings depends on age (stage of growth) and on the amino acid composition of the proteins consumed. Assuming good quality food proteins, satisfactory allowances per kilogram of body weight are: men, 1 g.; women, 1–1.8 g.; children, 1.5–3 g.; infants, 3.5 g.

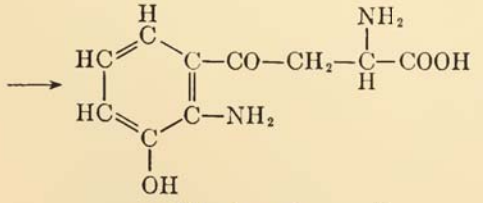
Conversion of amino acids into other metabolites

In addition to protein synthesis, amino acids are used as raw materials for the synthesis of a series of essential substances by animal tissues. The formation of *nicotinic acid* from tryptophan (p. 237) probably follows the pathway indicated below, although some details remain unproved:

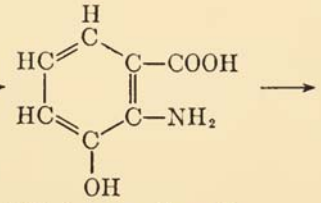


L-Tryptophan

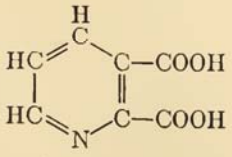
Kynurenine



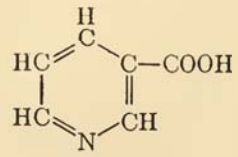
3-Hydroxy kynurenine



3-Hydroxy anthranilic acid

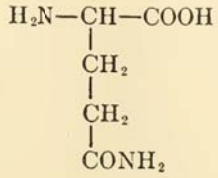


Quinolinic acid



Nicotinic acid

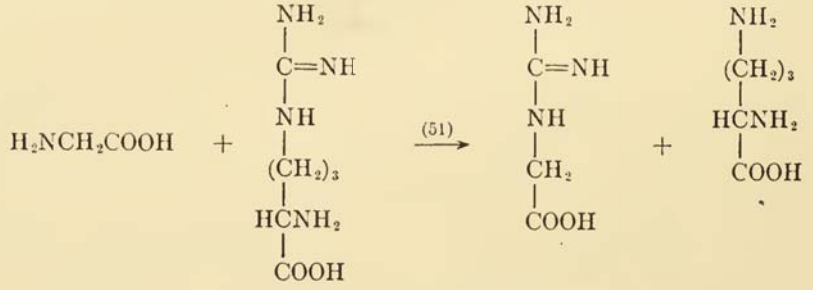
It has been established that nicotinic acid can be formed from tryptophan in the actual tissues of the animal body, although the same conversion can also be brought about by intestinal microorganisms.



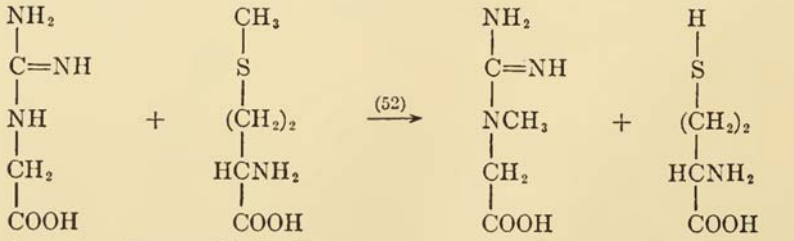
L-Glutamine

Glutamic acid is extensively converted into *glutamine* in animal tissues. In fact, a large part of the glutamic acid in the body, both free and combined, probably exists in the form of glutamine.

Creatine and *creatinine* are produced from three amino acids, glycine, arginine, and methionine. The guanidine group of arginine first combines with glycine to form *guanidoacetic acid*, which then is methylated by methionine:



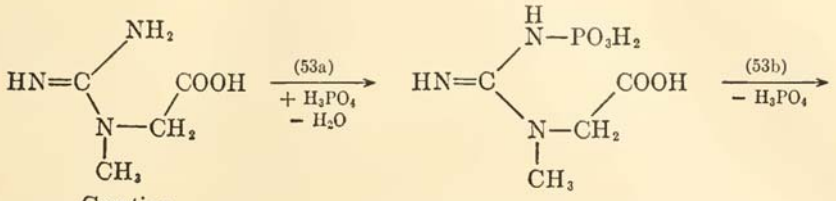
Glycine L-Arginine Guanidoacetic acid Ornithine



Guanidoacetic acid L-Methionine Creatine L-Homocysteine

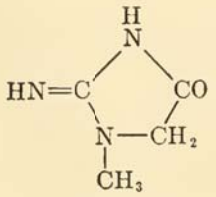
The formation of ornithine in the first reaction has not been established, but would certainly be expected. Choline can also furnish the methyl group for the second reaction, but only indirectly by first transferring it to methionine. The creatine so formed is converted into the

anhydride, creatinine, through the intermediary formation of creatine phosphate.



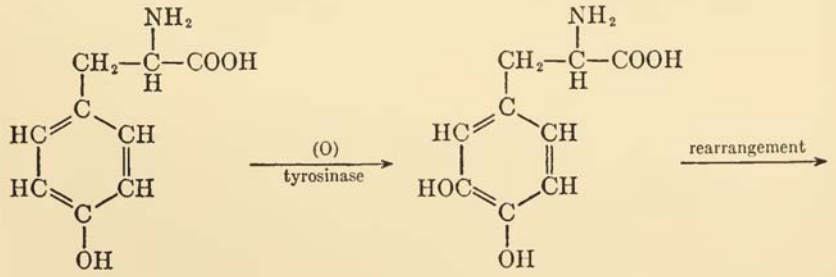
Creatine

Creatine phosphate



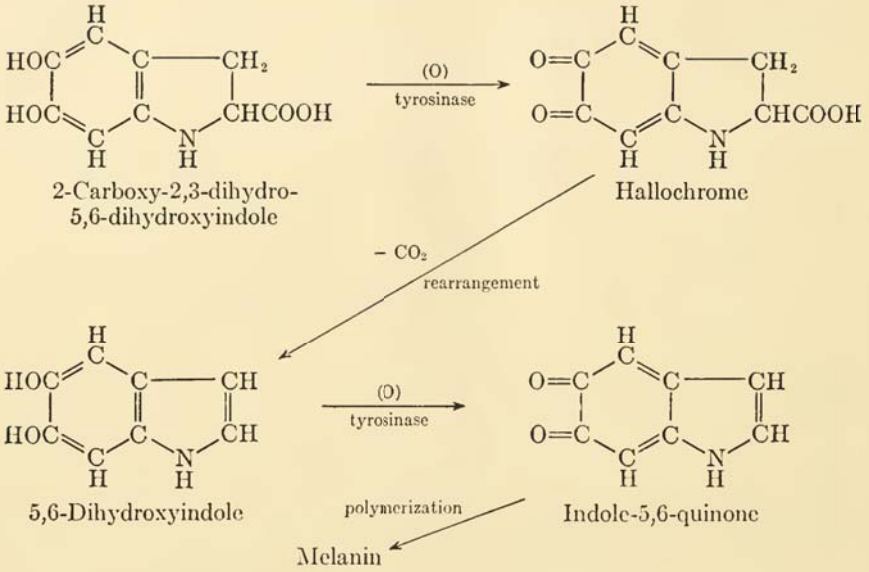
Creatinine

Tyrosine, itself produced from phenylalanine, is the starting point for the biosynthesis of *melanin*, the dark-colored pigment of the hair and skin, and for the hormones, *adrenalin*, *noradrenalin*, and *thyroxine* (see Chap. 11). Melanin is produced by the enzymatic oxidation of tyrosine to an unstable intermediate product which polymerizes (reacts with itself) to form the high molecular weight pigment. According to Mason the most probable course of the process is as follows:

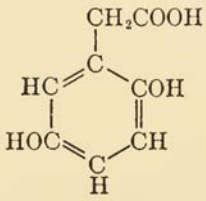


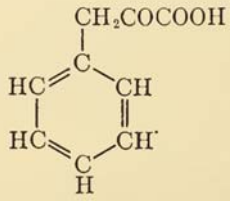
L-Tyrosine

Dihydroxyphenyl alanine, or "Dopa"



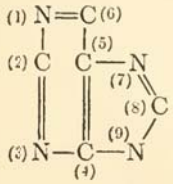
Abnormal metabolism of tyrosine and phenylalanine is exhibited by some people. In the condition known as alcaptonuria, tyrosine is converted into *homogentisic acid*. This substance is excreted in the urine


Homogentisic acid


Phenylpyruvic acid

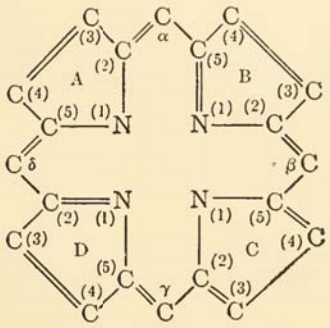
and causes it to turn black on standing exposed to air. In another condition, phenylketonuria, large amounts of *phenylpyruvic acid* are formed from phenylalanine and are excreted in the urine. This apparently results from an inability to convert phenylalanine to tyrosine. Both of these conditions represent hereditary abnormalities of amino acid metabolism.

Certain amino acids, particularly glycine, have also been demonstrated to be among the building blocks used by the animal in the biosynthesis of purines and porphyrins. The various carbon and nitrogen atoms of the purine skeleton come from the following sources: carbons 4 and 5,



Purine skeleton

and nitrogen 7 from glycine; carbons 2 and 8 from acetic acid; carbon 6 from carbon dioxide; and nitrogens 1, 3, and 9 from ammonia. In the porphyrin ring system,



Porphyrin ring system[1]

carbons *A*-2, *B*-2, *C*-2, *D*-2, α, β, γ, and δ come from CH_2 of glycine, while *A*-4, *B*-4, *C*-4, and *D*-4 are derived from the CH_3 of acetic acid. Also the four nitrogen atoms come from the NH_2 group of glycine. The mechanism by which these components are put together to form purine and porphyrin compounds in the animal body is still unknown.

Breakdown of amino acids in the animal body

Deamination. In the average American diet more protein is consumed than is needed for synthesis of tissue proteins and the other essential substances derived from amino acids. Consequently, the body receives an excess of amino acids which must be disposed of in some manner. Direct excretion would be wasteful and, in fact, occurs to only a small extent (in the urine). Most of the excess amino acids seem to be broken down by the process of *oxidative deamination.* Two steps are involved; first, dehydrogenation of the amino acid forms an *imino acid:*

[1] The four individual pyrrole rings are sometimes designated as I, II, III, and IV, respectively, rather than by *A*, *B*, *C*, and *D* (p. 137).

$$\underset{\text{Amino acid}}{H_2N-\underset{\displaystyle R}{\underset{|}{CH}}-COOH} \xrightarrow[(54)]{\text{amino acid oxidase}} \underset{\text{Corresponding imino acid}}{HN=\underset{\displaystyle R}{\underset{|}{C}}-COOH} + 2(H)$$

which in the second step is hydrolyzed to the corresponding keto acid and ammonia:

$$HN=\underset{\displaystyle R}{\underset{|}{C}}-COOH + H_2O \xrightarrow{(55)} O=\underset{\displaystyle R}{\underset{|}{C}}-COOH + NH_3$$

A number of enzymes present in animal tissues catalyze the dehydrogenation of various amino acids (see Chap. 10). The hydrogen split off in the first step is transferred directly to an acceptor, which differs according to the enzyme involved, but frequently is oxygen. Assuming that oxygen is the acceptor, the net result of oxidative deamination may be summarized by the following equation:

$$H_2N-\underset{\displaystyle R}{\underset{|}{CH}}-COOH + O_2 \xrightarrow{+H_2O} O=\underset{\displaystyle R}{\underset{|}{C}}-COOH + NH_3 + H_2O_2$$

The hydrogen peroxide is decomposed by catalase, or used to oxidize other metabolites. The other products are further metabolized as described below.

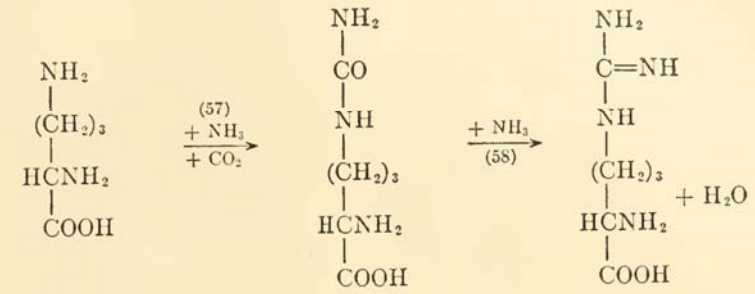
Formation of Urea. Ammonia is a toxic substance that cannot be tolerated by animal tissues in large amounts and therefore must be eliminated as fast as it is formed. For most higher animals it is combined with carbon dioxide to form the waste product, *urea*. A summary equation indicating the net result of this combination is as follows:

$$2NH_3 + CO_2 \rightarrow \underset{\text{Urea}}{H_2N-CO-NH_2} + H_2O$$

However, such a direct union does not occur in the body. Urea is formed instead by a cyclic process involving several intermediate substances. Almost certainly, the immediate source of the urea is *arginine,* which is broken down into *urea* and *ornithine* by the enzyme, *arginase:*

$$\underset{\text{L-Arginine}}{\begin{array}{l} NH_2 \\ | \\ C=NH \\ | \\ NH \\ | \\ (CH_2)_3 \\ | \\ HC-NH_2 \\ | \\ COOH \end{array}} + H_2O \xrightarrow[(56)]{\text{arginase}} \underset{\text{L-Ornithine}}{\begin{array}{l} NH_2 \\ | \\ (CH_2)_3 \\ | \\ HC-NH_2 \\ | \\ COOH \end{array}} + \underset{\text{Urea}}{\begin{array}{l} NH_2 \\ | \\ CO \\ | \\ NH_2 \end{array}}$$

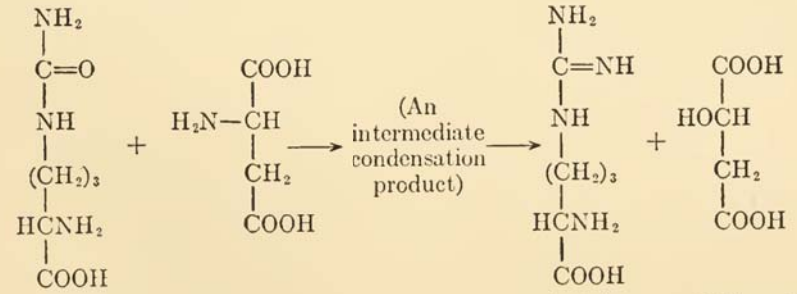
Arginase is present in the livers of those species which excrete urea as the main end product of nitrogen metabolism, but is absent from others such as birds, reptiles, and insects which excrete uric acid instead of urea. Evidently there must be some way by which ammonia is incorporated into arginine in the body, in preparation for urea formation. A possible method, suggested in 1932 by Krebs and Henseleit, is known as the *ornithine cycle:*



L-Ornithine L-Citrulline L-Arginine

Splitting of the arginine which was formed would then give the end product, urea, plus another molecule of ornithine and start the cycle over again.

Most investigators still regard this scheme as essentially correct, although later work has shown that aspartic and glutamic acids are also involved in the process and also that an energy source (probably ATP) is necessary to drive the reactions in the direction indicated. According to Ratner and Pappas the conversion of citrulline to arginine (reaction 58) probably does not occur simply by addition of ammonia, as shown in the above equation, but rather by interaction with aspartic acid:



L-Citrulline L-Aspartic acid L-Arginine L-Malic acid

This indicates that the nitrogen of certain amino acids may be converted into urea in the body without ever having existed as free ammonia.

Another modification of the Krebs-Henseleit cycle was proposed by Cohen and Grisolia. They presented evidence that the carbon dioxide

needed for reaction 57 does not react directly with ornithine, but first combines with glutamic acid to form an intermediate substance, which then transfers its carbon dioxide to ornithine. Glutamine probably is not involved in urea formation; but it does serve as the source of urinary ammonia (*i.e.*, ammonium salts) in mammals.

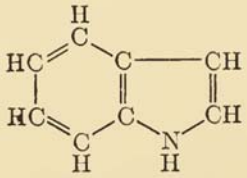
Metabolism of deaminated amino acid residues

The products resulting from deamination of amino acids (usually α-keto acids) are in most cases utilized as a source of energy by the animal body, that is, oxidized to carbon dioxide and water. The metabolic pathways by which this oxidation occurs are not fully known, but are certainly different for each amino acid.

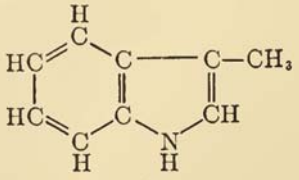
The keto acids from the deamination of alanine, aspartic acid, and glutamic acid are normal intermediates in carbohydrate metabolism and can be either broken down to carbon dioxide and water or built up into glycogen or glucose by the reactions already considered (Figs. 13–1, 13–3, and 13–4). In diabetic dogs several other amino acids also are convertible, wholly or in part, into glucose. Included in this group are glycine, serine, arginine, and proline.

Three of the amino acids, phenylalanine, tyrosine, and leucine, produce acetoacetic acid when fed to diabetic dogs. This substance, it will be recalled, is one of the compounds resulting from oxidation of fatty acids in the animal body. These amino acids therefore appear to be oxidized, after being deaminated, in the manner of fatty acids. Amino acids which give rise to acetoacetic acid in the body are said to be *ketogenic* (form ketone bodies), whereas those convertible into carbohydrate are *anti-ketogenic*.

In some cases ring structures of the more complex amino acids are nonutilizable. Tryptophan, for example, is partly degraded into indole and skatole, which are excreted in the feces.



Indole



Skatole

REVIEW QUESTIONS ON ANIMAL METABOLISM

1. Define metabolism, catabolism, anabolism.
2. What is the main purpose of carbohydrate metabolism in the animal body? In which respects does this process resemble actual burning of carbohydrate in a flame?

3. Summarize the main factors operating to control the blood sugar level.

4. Is lactic acid one of the intermediate substances formed on the main pathway of normal carbohydrate metabolism? Under which conditions is it formed in considerable amounts? Why is it formed? What becomes of it?

5. Show by balanced equations the net result of glycolysis, starting with glucose; of the combined operation of the citric acid cycle and the cytochrome system, starting with pyruvic acid.

6. List four phosphorylated and six nonphosphorylated intermediate substances involved in carbohydrate metabolism.

7. Summarize the evidence as to whether stored fat is metabolically active or inert.

8. What is meant by "beta" oxidation of fatty acids; by "omega" oxidation? Which is most important in animal tissues?

9. List eight substances which may be more or less directly produced from acetic acid in animal tissues.

10. Discuss the relationship between the ability of animal tissues to carry out synthetic reactions and the need for amino acids or proteins in an animal's food.

11. Which metabolic reactions serve to link together the metabolism of carbohydrates and proteins; of carbohydrates and fats?

12. What is transmethylation? Name the substances now known to serve as methyl group donors, and list several substances produced in the body as a result of methylation.

13. Name 5 substances produced in the animal body wholly or partially from glycine.

14. Which amino acids are ketogenic; antiketogenic?

REFERENCES AND SUGGESTED READINGS

Baldwin, E., *Dynamic Aspects of Biochemistry,* 2nd ed., Cambridge University Press, Cambridge, 1952.

Bloch, K., "The Metabolism of Acetic Acid in Animal Tissues," *Physiol. Rev.,* **27**, 574 (1947).

Bloch, K. and Rittenberg, D., "An Estimation of Acetic Acid Formation in the Rat," *J. Biol. Chem.,* **159**, 45 (1945).

Bloor, W. R., *Biochemistry of the Fatty Acids,* Reinhold Publishing Corporation, New York, 1943.

Borsook, H., "Protein Turnover and Incorporation of Labeled Amino Acids into Tissue Proteins in Vivo and in Vitro," *Physiol. Rev.,* **30**, 206 (1950).

Breusch, F. L., "The Biochemistry of Fatty Acid Catabolism," *Advances in Enzymology,* **8**, 343 (1948).

Chaikoff, I. L. and Entenman, C., "Anti-Fatty Liver Factor of the Pancreas," *Advances in Enzymology,* **8**, 171 (1948).

Cohen, P. P. and Grisolia, S., "The Intermediate Role of Carbamyl-L-Glutamic Acid in Citrulline Synthesis," *J. Biol. Chem.,* **174**, 389 (1948).

Duel, H. J. and Morehouse, M. G., "The Interrelation of Carbohydrate and Fat Metabolism," *Advances in Carbohydrate Chemistry,* **2**, 119 (1946).

du Vigneaud, V., Ressler, C., and Rachele, J. R., "The Biological Synthesis of 'Labile Methyl Groups,'" *Science,* **112**, 267 (1950).

Gortner, R. A. Jr. and Gortner, W. A., *Outlines of Biochemistry,* 3rd ed., John Wiley and Sons, Inc., New York, 1949, Chapters 19, 20, 26, and 32.

Greenberg, D. M. (editor) *Amino Acids and Proteins,* Charles C. Thomas, Publisher, Springfield, 1951, Chapters X, XIII.

Kleinzeller, A., "Synthesis of Lipids," *Advances in Enzymology*, **8**, 299 (1948).
Knoop, F., "Catabolism of Aromatic Fatty Acids in the Animal Body," *Beitr. Chem. Physiol. Path.*, **6**, 150 (1905).
Krebs, H. A. and Henseleit, K., "Urea Formation in the Animal Body," *Z. Physiol. Chem.*, **210**, 33 (1932).
Lardy, H. E. (editor) *Respiratory Enzymes*, Burgess Publishing Company, revised ed., Minneapolis, 1949.
Martins, C. and Lynen, F., "Problem des Citronensäure cyklus," *Advances in Enzymology*, **10**, 167 (1950).
Mason, H. S., "The Chemistry of Melanin," *J. Biol. Chem.*, **172**, 83 (1948).
Medes, G., "Fat Metabolism," *Annual Rev. Biochem.*, **19**, 215 (1950).
Ratner, S. and Racker, E., "Carbohydrate Metabolism," *Annual Rev. Biochem.*, **19**, 187 (1950).
Ratner, S. and Pappas, A., "Biosynthesis of Urea," *J. Biol. Chem.*, **179**, 1183, 1199 (1949).
Schoenheimer, R., *The Dynamic State of Body Constituents*, Harvard University Press, Cambridge, 1942.
Swanson, P. P. and Clark, H. E., "Metabolism of Proteins and Amino Acids," *Annual Rev. Biochem.*, **19**, 235 (1950).

Chapter 14

METABOLISM OF MICROORGANISMS

Interrelations of microorganisms, animals and plants

Microorganisms form an integral and indispensable part of a living world. Some consideration of their chemical activities is not only desirable but essential if an over-all view of biochemistry is to be obtained. Chlorophyll-containing plants are the "factories" in which organic matter is made and energy is stored. Many of the organic compounds produced in nature contain nitrogen, and in the fixation of atmospheric nitrogen, bacteria, such as rhizobia, are undoubtedly the most important agents. The building of organic matter is balanced by its destruction; here again microorganisms seem to play the leading role. Dead plant and animal materials are converted by microorganisms into simple compounds such as carbon dioxide, ammonia and nitrates, which are used again by plants. Although animals contribute to the breakdown of organic matter, in the over-all effect microorganisms undoubtedly are the principal agents. The balance between construction and destruction of organic matter is often spoken of in connection with single elements and designated as the carbon cycle and the nitrogen cycle in nature.

The intimate association of microorganisms and animals is of course obvious from the fact that animals act as hosts to vast numbers of bacteria in the intestinal tract. The importance of bacteria to the host is conspicuous in the case of ruminants, where they probably are indispensable. They break down cellulose and other resistant plant materials to compounds that can be utilized by the animal, synthesizing all the B vitamins needed by the ruminant. Even nonruminants appear to derive a large part of their supply of certain vitamins, *e.g.*, biotin, from the synthetic action of bacteria.

On the debit side of the association account is the production of disease by infectious microorganisms in animals and plants. The practical problem then is to promote the development of useful microorganisms and to retard the growth of harmful types.

From a scientific viewpoint, the study of the metabolism of microorganisms has been a most fruitful effort. A first insight into intermediary metabolism came from a study of yeast. This has been extended to animals and bacteria, and from these studies a diversified but also a unified pattern of metabolism is emerging.

Growth requirements

Energy and Carbon. Because of the thousands of species of microorganisms, it is much more difficult to state their growth requirements than it is those of higher animals, where only a few species have to be considered. Perhaps the only general statement one can make is that all require some source of energy. A few species of microorganisms can, like plants, use light as a source of energy, but the vast majority obtain their energy from chemical elements or compounds. Merely listing a few examples shows the diversity of sources: elemental H, C, and S, simple compounds such as H_2S and NH_3, carbon compounds ranging from carbon dioxide and methane through the carbohydrates, lipides, and proteins to such resistant materials as lignin and paraffin.

Stephenson cites an example from the work of den Dooren de Jong to illustrate the amazing synthetic powers of microorganisms. The bacterium, *Pseudomonas putida,* can meet all its carbon requirements from 77 different carbon compounds out of 200 tested. The utilizable compounds included 6 carbohydrates, 10 alcohols, 13 fatty acids, 17 amino acids, 9 amides, and 7 amines. It would probably be a safe statement to make that there is no form of combined carbon in the world that cannot be utilized by some microorganism.

Nitrogen. Since all living cells contain protein, some form of nitrogen must be supplied. In some cases atmospheric nitrogen is utilized (*e.g.*, *Azotobacter vinelandii*); in others, inorganic nitrogen, such as nitrates and ammonia, is adequate (*e.g.*, yeasts, molds, and autotrophic bacteria); but in many others only amino acids can meet the needs of the cell. Lactic acid bacteria are conspicuous examples of cells that require preformed amino acids for growth. One of these, *Leuconostoc mesenteroides,* requires 17 amino acids, many more than are required by higher animals.

The requirement for certain amino acids may depend upon the absence of a vitamin. For example, some strains of propionic acid bacteria require riboflavin if ammonium sulfate is the source of nitrogen, but if amino acids are supplied, no riboflavin is needed. Another example is *Lactobacillus arabinosus,* which grows without tryptophan if vitamin B_6 is present, and without B_6 if tryptophan is present. In both cases it synthesizes the compound that is omitted. Hence there is not an absolute requirement for either compound, but the cell cannot make both compounds simultaneously.

The interesting phenomenon of imbalance among amino acids exists in microorganisms, perhaps more markedly than with higher animals. Thus a certain strain of *Escherichia coli* will grow in the absence of tyrosine, but not in its presence unless phenylalanine is also present. In

this instance tyrosine and phenylalanine are antagonistic to one another. There are many other examples of imbalance between amino acids.

Growth of some bacteria requires, or is increased by, purines and pyrimidines in the culture medium (*e.g., Staphylococcus aureus* and *Clostridium tetani*). Some bacteria use uric acid and other purines as their sole source of carbon and nitrogen (*e.g., Clostridium acidiurici*).

Growth Factors (Vitamins, etc.). The growth factor requirements of microorganisms vary over a wide range of compounds. For example, *E.coli* can grow in a medium containing no B vitamins, whereas *Lactobacillus casei* requires at least seven of these vitamins and, in addition, some growth factors of undetermined nature. In some cases only a part of the vitamin molecule is required preformed in the medium; *e.g.*, the β-alanine part of pantothenic acid by yeast and the pantoyl part of it by *Acetobacter suboxydans*. In other cases, a combined form of the vitamin is required; *e.g.*, nicotinamide riboside by *Hemophilus parainfluenzae* and pantetheine (pantothenic acid-β-aminoethanethiol) by *Lactobacillus bulgaricus*. An example of a progressively more complex series of compounds, and bacteria requiring them, follows:

Bacteria	*Compounds required*
Clostridium acetobutylicum	*p*-aminobenzoic acid
Streptococcus fecalis	pteroic acid
Lactobacillus casei	pteroyl glutamic acid
Lactobacillus citrovorum	formyltetrahydropteroylglutamic acid

The probable explanation of this series is that the last compound in the series is either the one that functions in metabolism or is nearer to it than the earlier members. The bacteria that need only the simpler compounds probably synthesize the complex compound from the simpler ones. For example, *Cl. acetobutylicum* can perform all the syntheses between *p*-aminobenzoic acid and formyltetrahydropteroylglutamic acid, and *L. citrovorum* cannot perform some, if any, of them.

Another such progressively complex series starts with pantothenic acid, proceeds to pantetheine, and ends with coenzyme *A*. A third series can be formed from pyrimidine (or thiazole), thiamine, and lipothiamide. The existence of these series of compounds suggests that the compound actually functioning in the metabolism of the cell is the complex compound and not the simple one.

The fat-soluble vitamins (A, D, E, and K), so essential for animals, are not required by microorganisms. Many microorganisms synthesize K and carotene and ergosterol, the precursors of A and D. Ascorbic acid stimulates the growth of some bacteria, but it seems to act as a reduction-oxidation compound rather than as a vitamin. On the other hand, many other compounds not required in the diet of animals serve as growth factors for microorganisms. Examples of such compounds, in addition to those already named, and the associated microorganisms

follow: hemin (*Hemophilus influenzae*); putrescine, $NH_2(CH_2)_4NH_2$ (*H. parainfluenzae*); α-lipoic acid, also called thioctic acid, p. 254 (*Streptococcus lactis* and *Tetrahymena geleii*); coprogen, an organic-iron compound (*Philobolus kleinii*).

Inorganic Elements. The requirement of K, Mg, Mn, Fe, S, and P for the growth of microorganisms is well established, and numerous reports indicating the need for Ca, Cu, Zn, Mo, and Co have appeared. The reason for the uncertainty regarding the need for some of these elements is the small amount that is required and the difficulty of removing traces of these elements from the medium. An example of the difficulties that exist is illustrated by a study of the vitamin B_{12} requirements of bacteria. This vitamin is synthesized by many bacteria, and since it contains cobalt, this element must have been present in the medium. While the vitamin can be shown to have been formed, the presence of cobalt in the medium is not easily demonstrated. The quantity of cobalt required for the synthesis of all the vitamin needed by the bacteria is of the order of 0.4 mμg Co per liter [1] of medium—a quantity that is not easily detected.

On the other hand, microorganisms that synthesize large quantities (*e.g.*, 2–3 μg per milliliter) of vitamin B_{12} must be supplied with cobalt salts if maximal yields of vitamin B_{12} are to be obtained.

In practical work the needs of microorganisms for all the inorganic elements are met by adding phosphate or sulfate salts of potassium, magnesium, manganese, and iron to the medium. These salts usually carry enough impurities to meet the needs, if any, of the microorganism for other elements.

Growth efficiency

Cells differ greatly as to the efficiency with which they convert nutrients into cell material. The comparison is best made on the basis of dry matter of food converted into dry matter of cells, in order to eliminate the effect of varying moisture contents on the calculations. If the figures are expressed on a percentage basis, the efficiency of the cell is obtained. The efficiency varies under different conditions, but if those giving optimal values are taken, the conversion of nutrients into cell dry matter is about as follows:

Group	*Example*	*Per cent*
Aerobic bacteria	*Azotobacter vinelandii*	14
Anaerobic bacteria	*Clostridium acetobutylicum*	1
Yeast	*Saccharomyces cerevisiae*, aerobic growth	50
Yeast	*Saccharomyces cerevisiae*, anaerobic growth	5
Molds	*Penicillium chrysogenum*, in penicillin production	40
Molds	*Aspergillus niger*, in citric acid production	5

[1] One milligram is equivalent to 1,000 micrograms (μg) and one microgram is equivalent to 1,000 millimicrograms (mμg).

Young of some common higher animals:

Cattle	4
Swine	7
Chicken	10
Fish	10

Yeast when grown under aerobic conditions is probably the most efficient of the microbial cells; molds come next and, from the meager data available, bacteria are third. Animals, even the most efficient, are far below aerobic microorganisms in their ability to convert food into living cells.

Slow-growing animals are less efficient than animals that attain maturity in a short time. This is to be expected since cells that grow slowly use up a larger proportion of the food for maintenance than rapidly growing cells.

Perhaps the most noteworthy figures are those comparing aerobic and anaerobic growth. For example, the same species of yeast gives about 10 times more weight of cells under aerobic than under anaerobic conditions. Producers of baker's yeast understand this fact and blow enormous quantities of air through the medium to obtain high yields of yeast. Under anaerobic conditions the nutrients, *e.g.*, glucose, are converted mainly into ethyl alcohol and carbon dioxide instead of into yeast cells. It is impossible to have high yields of cells and alcohol in the same fermentation. The situation is analogous to that with cattle; the farmer obtains a high production of either beef or milk, but not both from the same animal.

The effect of aerobic conditions in stimulating the growth of cells is seen also by comparing the two bacteria, *A. vinelandii* and *Cl. acetobutylicum*. The first is an aerobe and gives high yields of cells. The second, an anaerobe, gives a low yield of cells but large amounts of products such as acetone, ethyl alcohol, and butyl alcohol.

Molds are aerobic microorganisms. If allowed to grow under favorable conditions, *e.g.*, penicillin fermentation, they are about as efficient as yeast in converting nutrients into cells. In citric fermentation, growth is deliberately restricted in order to promote the yield of citric acid.

Metabolic rate

Microorganisms transform matter at a much faster rate than do animals. It takes about 100 days for an adult human being to consume his own weight of food. Cattle accomplish this in 40 days and swine do it in 20 days. Yeast cells take about 30 minutes to metabolize their weight of nutrients. The mold *A. niger* converts its own weight of glucose to gluconic acid in about 2 minutes, and a urea-splitting bacterium transforms its weight of urea to ammonia in a few seconds. One reason why

some microorganisms work so fast is that they derive very little energy from the chemical changes that they bring about. Hence, of necessity, they must work over a large amount of matter in order to meet their energy needs.

Comparison of microorganisms with respect to end products

The term fermentation may be defined as the chemical process by which organic compounds are converted into new compounds by microorganisms or by enzymes obtained from microorganisms. With modifying words it is used in a broad sense to designate products formed, materials utilized, or agents involved. When dealing with products formed, we have such phrases as alcohol fermentation, citric acid fermentation, penicillin fermentation, tea fermentation, etc. To feature the materials utilized, such terms as glucose-, xylose-, cellulose-fermentation are used. In designating the microbiological agent such expressions as yeast fermentation, bacterial fermentation, and mold fermentation are employed. In this chapter the word fermentation is used in all three of these ways. No distinction is made among processes that are anaerobic and produce gas, *e.g.*, alcoholic fermentation, those that are anaerobic and produce no gas, *e.g.*, lactic acid fermentation, and those that are aerobic and produce gas (carbon dioxide), *e.g.*, citric acid and penicillin fermentations.

In a restricted sense the term fermentation is used to denote an anaerobic type of metabolism. Associated with this usage is the term respiration, brought over from animal metabolism to denote what in effect is complete oxidation of the substrate to carbon dioxide and water. Attempts to separate microbial metabolism into fermentation and respiration processes seem highly artificial, since the yeast cell, for example, may operate on either an aerobic system or an anaerobic system, and at times even on both systems simultaneously.

In Table 14–1 are listed the products that are characteristic of bacteria, yeasts, and molds. The percentage given for the glucose or fructose converted into the corresponding product is maximal, or nearly so. It is believed that figures showing such a performance of a microorganism are more meaningful than data obtained under conditions that do not permit the cells to function at or near their optimal capacity.

An inspection of this table shows that there are about a half dozen products that are common to all three groups. Carbon dioxide is the compound that is not only common to all three, but is also produced in large amounts by many members of each group. It is probably a universal product of cell metabolism. In certain cases a product is more generally found in one or two of the groups rather than in the

others. Lactic acid, for example, is produced by many bacteria, but only a few molds make it, and yeasts produce it only in traces or under special conditions.

Products that are limited to bacteria are hydrogen, the lower fatty acids, butylene glycol, butyl alcohol, acetone, and isopropyl alcohol. Several of these products are closely related, as will be shown later.

Di- and tri-basic acids are characteristic of molds. Succinic acid is the only one of this type common to all three groups. Although it has been found in yeast fermentations, it appears to originate from oxidation and decarboxylation of glutamic acid rather than from glucose.

If a fourth column to include animals were tabulated, it would be found to be mostly negative, except for carbon dioxide. Lactic acid, acetic acid, and acetone are found at times in the urine, but they are not normal end products of animal metabolism.

The high yields obtained with some microorganisms have made possible a number of industrial fermentations. The mechanism of the formation of these products will be discussed later.

Table 14–1

Characteristic end products of carbohydrate metabolism formed by bacteria, yeasts, and molds *

Compound	*Bacteria*	*Yeasts*	*Molds*
Carbon dioxide	*Cl. acetobutylicum*, 55	*S. cerevisiae*, 45	*P. chrysogenum*, 70
Lactic acid	*L. delbrückii*, 90	*S. cerevisiae*, trace	*R. oryzae*, 75
Ethyl alcohol	*T. mobile*, 45	*S. cerevisiae*, 45	*F. avenaceum*, 40
Acetic acid	*L. gayonii*, 10	*S. cerevisiae*, 1–3	*A. niger*, trace
Glycerol	*L. lycopersici*, 20	*S. cerevisiae*, 1–40	*Aspergillus sp.*, 10
Mannitol	*L. gayonii*, 65	None	*Aspergillus sp.*, 35
Gluconic acid	*A. suboxydans*, 95	None	*A. niger*, 95
Propionic acid	*P. pentosaceum*, 60	None	?
Formic acid	*E. typhi*, 10	None	None
Hydrogen	*E. coli*, 0.5	None	None
Succinic acid	*E. coli*, 12	Doubtful	*Rhizopus, sp.*, small
Acetoin	*A. aerogenes*, 0.5	Trace	None
Butylene glycol	*A. aerogenes*, 45	?	None
Butyric acid	*Cl. saccharobutyricum*, 40	None	None
Butyl alcohol	*Cl. acetobutylicum*, 20	None	None
Acetone	*Cl. acetobutylicum*, 7	None	None
Isopropyl alcohol	*Cl. butylicum*, 6	None	None
Oxalic acid	None	None	*A. niger*, 80
Fumaric acid	None	None	*R. nigricans*, 60
Citric acid	None	None	*A. niger*, 90

* The microorganisms named are typical of the best producers of the compounds. The figures denote the maximum percentage of product, based on glucose or fructose fermented, that has been found in the literature. The yield of product, usually less, will vary with other substrates, microorganisms, and fermentation conditions.

AEROBIC METABOLISM OF CARBOHYDRATES

The conventional and most convenient method of classifying the varied types of metabolism performed by microorganisms is on the basis of utilization of oxygen. If oxygen is used, the metabolism is called aerobic; and if not, it is designated as anaerobic. This is an arbitrary classification, as many organisms have both an aerobic and an anaerobic system and operate on one or the other as circumstances require. Examples of such microorganisms are *E. coli* and ordinary baker's yeast, *S. cerevisiae*. Bacteria that can grow either in the presence or absence of air are termed facultative aerobes or facultative anaerobes depending upon which condition appears more favorable.

By bacteria

In general, aerobic bacteria oxidize sugars to carbon dioxide and water, but there are certain bacteria that are exceptions to this rule. *Acetobacter suboxydans*, for example, oxidizes the carbons along the chain of a polyhydroxy substance like glucose, but cannot cut the chain into shorter pieces. From glucose it forms gluconic acid and 5-ketogluconic acid, and from sorbitol it makes sorbose. The oxidizability of a compound is very specific and depends upon the structure of the molecule. For example, sorbitol and mannitol are oxidized to the corresponding keto-sugars, sorbose and fructose, respectively; but ducitol, the alcohol corresponding to galactose, is not attacked. From a study of these and other sugar alcohols, Bertrand concluded that two alcohol groups adjacent to the primary alcohol must be *cis* to one another for oxidation to take place. Dulcitol and xylitol do not have such a structure and are not oxidized. However, as more polyhydric alcohols and bacteria have been studied, it has been found that the requirements are both less specific and more complex than is expressed by Bertrand's rule.

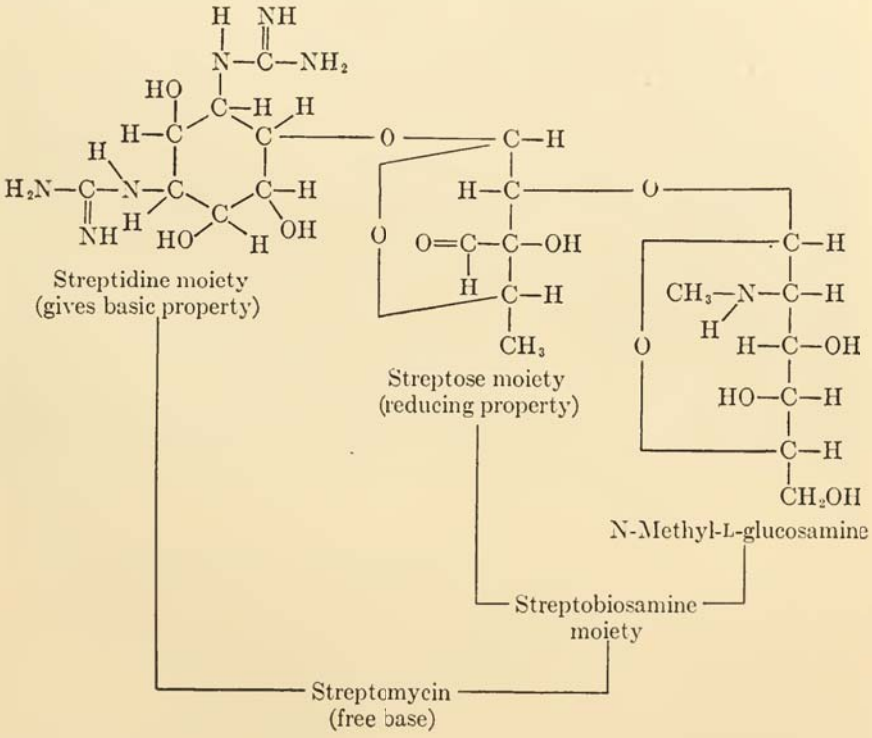
Aerobic bacteria are very important in the production of vinegar, antibiotics, and enzymes; in the retting of flax; and in the disposal of sewage by the activated sludge process. Antibiotics are of special interest and recent development. An antibiotic is generally defined as an organic compound produced by microorganisms which in small concentrations inhibits or kills other microorganisms, usually pathogenic in character. The definition is admittedly arbitrary, as it excludes inhibitory compounds produced by higher plants, such as quinine, and purely synthetic compounds, such as sulfa drugs. The term as thus defined, however, is useful and convenient for practical purposes.

The number of antibiotics reported in the literature runs into several hundreds. Most of these are poorly characterized chemically, but approximately 60 have been obtained sufficiently pure to permit determina-

tion of their elementary composition. Some of these antibiotics are well-characterized structurally, but most of them are poorly defined chemical compounds. One reason why so little is known regarding the chemical nature of many antibiotics is the fact that they are too toxic for clinical use. Toxicity removes one of the strong incentives for determining their structure. This is unfortunate, because a knowledge of the structure of a toxic substance is perhaps as important as an understanding of the make-up of the less toxic substance. There is a fine opportunity for qualified chemists in this field.

The best known antibiotic in actual use is of course penicillin, but since it is a mold product it will be discussed in the section on molds. The other antibiotics are produced either by bacteria or streptomycetes. The latter are classified as bacteria, although they have mold-like characteristics, for example, growth in long thread-like filaments. Each of these antibiotics will be discussed separately.

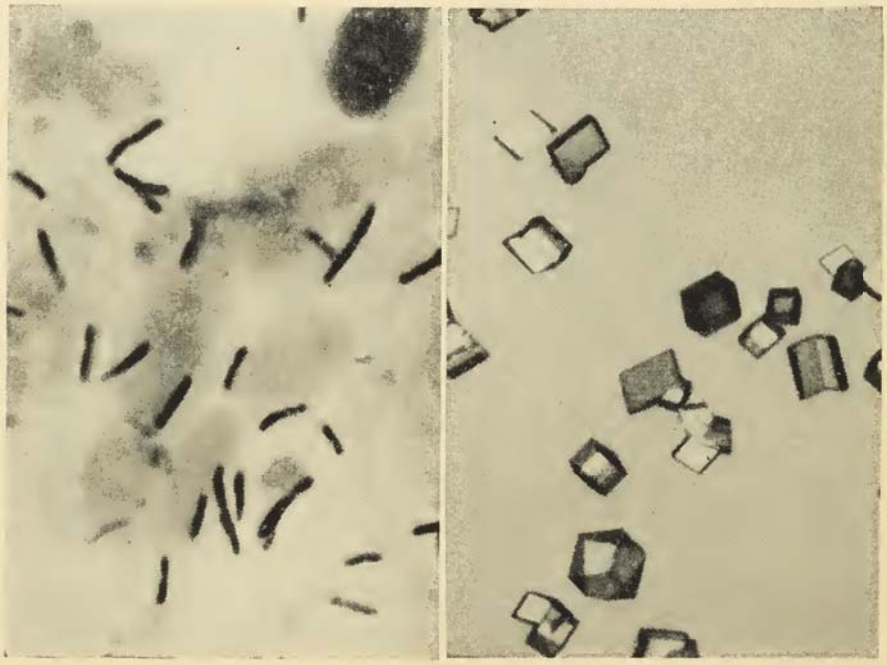
Streptomycin. This antibiotic was discovered by Waksman and asso-



ciates in 1944 and is produced by the microorganism *Streptomyces griseus*. Like most microorganisms, *S. griseus* produces several other antibiotics, namely, mannosidostreptomycin, a combination of mannose and streptomycin; actidione, an antifungal compound ($C_{15}H_{23}NO_4$); and grisein ($C_{40}H_{61}N_{10}O_{20}SFe$). By selection, strains of *S. griseus* have been obtained

which give mainly streptomycin, and in high yields—about 2–3 g. per liter of medium. This antibiotic is produced by many companies in the United States and foreign countries. In 1952 the production in the United States was 440,000 lb., and the market value at the plants was about $50,000,000.

Its use is attended with some drawbacks. The tubercle and other bacteria become resistant to streptomycin. It also has toxic effects on the eighth cranial nerve which may lead to deafness on prolonged use. However, in spite of much effort, no other antibiotic has been found equal to streptomycin in the treatment of tuberculosis. See Fig. 14–1.



Courtesy of Abbott Laboratories.

(a)

Courtesy of The Squibb Institute for Medical Research.

(b)

Fig. 14–1. Tubercle bacillus and streptomycin. (a) Photomicrograph of infected lung tissue of mouse. The rod-like particles are *Mycobacterium tuberculosis,* the microorganism that causes tuberculosis. Other dark areas show accompanying cellular lung tissue. (b) Crystals of streptomycin trichloride, the most useful antibiotic in the treatment of tuberculosis.

Streptomycin, as can be seen from the structural formula, is a complex organic base consisting of three parts: streptidine, streptose, and a methyl derivative of glucosamine. The streptidine base contains two guanidino groups (compare arginine, p. 120) which give streptomycin its salt-forming characteristics. Commercial preparations of streptomycin are usually the trihydrochloride or trihydrosulfate. Streptidine is clearly a deriva-

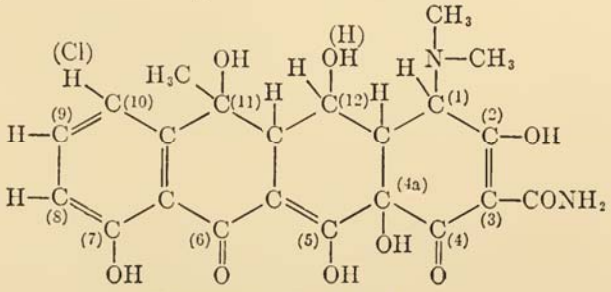
tive of inositol, a vitamin which is found in many plant and animal materials.

Streptose is an unusual type of sugar. It has an aldehyde group attached along the carbon chain to give a branching structure. The aldehyde group can be reduced chemically to give the corresponding alcohol. This derivative was thought for a time to be superior to streptomycin clinically, but later work indicated that it had the same disadvantages as streptomycin.

It has been suggested that streptomycin interferes with nucleic acid metabolism. Since it is a basic substance, it may react with the acidic groups of nucleic acid and form complexes that are not metabolized. According to Umbreit, the mode of action of streptomycin is through interference with pyruvic acid metabolism. This is a complex phenomenon involving many enzymes, coenzymes, and chemical changes. If streptomycin interferes with the pyruvate metabolism of microorganisms, the question naturally arises as to why it does not affect the same metabolism in the animal? The explanation usually given is that streptomycin does not penetrate the animal cell but remains in the extracellular fluid. The bacteria infecting the animal cannot prevent the entrance of streptomycin into their cells, hence their metabolism becomes deranged and they die.

Bacteria rapidly acquire resistance to streptomycin, and in such cells Umbreit has found that the oxalacetate-pyruvate relation has disappeared. The bacteria have apparently been able to develop a new metabolic pathway with which streptomycin does not interfere. A still more puzzling phenomenon is the development of dependent bacteria, that is, bacteria that will not grow unless streptomycin is added to the medium. No satisfactory explanation has as yet been found for the development of dependent strains. A possible explanation that has been advanced is that the dependent strain produces so much of some metabolite that it kills itself when no streptomycin is present to counteract the effect of this metabolite. Some support for this theory is found in the increased production of the metabolite para-aminobenzoic acid by certain bacteria when sulfanilamide is added to the medium.

Aureomycin and Terramycin. These two antibiotics are very similar



Terramycin, $C_{22}H_{24}N_2O_9$

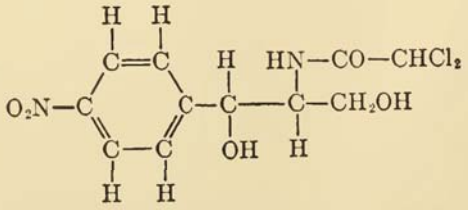
biologically and chemically. The structural formula for terramycin was worked out first and is given above. Aureomycin, $C_{22}H_{23}N_2O_8Cl$, has the same basic structure as terramycin. Both are derivatives of naphthacene and have many groups in common. Aureomycin contains a chlorine atom and one less hydroxyl group than terramycin, but the chlorine and hydroxyl group are not interchangeable. They occupy different places in the molecule, as indicated by the Cl and H in parentheses in the structural formula. Chlorine replaces a hydrogen at position 10, and a hydrogen takes the place of the hydroxyl at 12 in the terramycin formula. There seems also to be some doubt as to the location of the dimethylamino group in aureomycin; it may be interchanged with the hydroxyl group attached at 4a. Both compounds form yellow-colored salts and have many other similar physical and chemical properties. For further details the many papers on these antibiotics that have appeared recently should be consulted.

As would be expected from their close chemical relationship, they are much alike in their bacterial spectrum. Both act on gram-positive [1] and gram-negative bacteria, on organisms producing rickettsial diseases, *e.g.*, typhus fever, and are potent in certain virus infections, such as virus pneumonia. Neither antibiotic is effective against the tubercle bacillus, bacteria of the proteus and pseudomonas types, or fungi. Aureomycin and terramycin are given by mouth in clinical treatments.

Aureomycin and terramycin are produced commercially by fermentation. The microorganism producing aureomycin is called *Streptomyces aureofaciens* because of the golden yellow appearance of the colonies of the microorganism on agar plates and also because the antibiotic is yellow. The terramycin organism is named *Streptomyces rimosus* because of the cracked appearance of the colonies on agar plates.

Since each antibiotic is produced by a single company, no official figures are available as to the yearly production. Judging from the widespread use of these antibiotics, their production must be several hundred thousand pounds per year and their market value must run into millions of dollars.

Chloromycetin (Chloramphenicol). Chloromycetin is the name com-



Chloromycetin

[1] Bacteria that take the gram-stain are called gram-positive and those that do not are said to be gram-negative. Consult a book on bacteriology for the reagents (*e.g.*, gentian violet, etc.) used in making the stain and the method of performing it.