monly used for this antibiotic, but it is also known by the more chemical name chloramphenicol. The compound contains chlorine and is produced by a streptomyces, hence it is quite apparent how the word Chloromycetin came to be devised. The microorganism producing Chloromycetin is called *Streptomyces venezuelae,* an obviously poor name since it denotes geographical origin and not an inherent characteristic. The microorganism was obtained from a sample of soil from Venezuela. It has also been found in soils from Illinois and Japan and is probably widely distributed in nature.

It is produced industrially both by fermentation and by synthesis. To date it is the only commercial antibiotic that is produced synthetically as well as by fermentation.

The most distinctive feature about the chemical structure of Chloromycetin is the nitro group. Few organic compounds in nature contain a nitro group. It also contains chlorine, which, though not common, occurs in aureomycin and a number of mold products, *e.g.,* erdin. The presence of an amide linkage relates it to peptides and explains its hydrolysis by enzymes found in cells of *Proteus vulgaris.* Chloromycetin contains no acidic or basic groups, hence it does not form salts. It is a neutral compound that crystallizes as colorless needles or elongated plates.

Chloromycetin is relatively inactive against gram-positive bacteria, but is very potent against the gram-negative bacteria associated with intestinal diseases such as typhoid fever and dysentery. It is active against the same rickettsial and viral diseases as aureomycin and terramycin.

Chloromycetin is relatively stable to acids and alkali, is rapidly absorbed from the gastro-intestinal tract, and hence is usually given by mouth. Liver and kidney tissues reduce the $-NO_2$ group to an $-NH_2$ group. About 90 per cent of the administered dose is excreted as an inactive compound and 10 per cent as unchanged Chloromycetin in 24 hours.

Terramycin, aureomycin, and Chloromycetin are alike in bacterial spectrum and appear to be similar in their mode of action; they interfere strongly with protein synthesis but are much less effective in stopping nucleic acid synthesis. A more specific reaction in protein metabolism has been observed for Chloromycetin. It acts as an antagonist against phenylalanine, but the antagonism is noncompetitive. Only low concentrations of Chloromycetin can be overcome by addition of phenylalanine. At higher concentrations its effect cannot be reversed by adding more phenylalanine. This makes Chloromycetin a particularly effective antimetabolite since the inhibited organism cannot counteract the Chloromycetin by producing more phenylalanine.

Bacitracin, Polymyxin, and Tyrothricin. These three antibiotics are all polypeptides and are produced respectively by *Bacillus licheniformis,*

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Bacillus polymyxa, and *Bacillus brevis*. The amino acid content of bacitracin is given in Table 5–4 and presents no unusual features. There are several polymyxins, A, B, C, D, and E, and each one contains large amounts (more than 50 per cent) of the unusual amino acid, L-α,γ-diaminobutyric acid. A second distinctive feature is the presence in the molecule of a nine-carbon fatty acid, probably 6-methyloctanoic acid.

Tyrothricin is not a homogeneous substance but consists mainly of gramicidin, a neutral cyclic polypeptide. On hydrolysis gramicidin gives five amino acids and ethanolamine, $NH_2CH_2CH_2OH$.

Bacitracin resembles penicillin in being most active against gram-positive bacteria. It causes kidney damage (evidenced by albumin in the urine). Because of this toxic effect, its use is limited to combating local infections.

The polymyxins are very potent against gram-negative bacteria, including the very resistant *Proteus* and *Pseudomonas* bacteria. Unfortunately, the polymyxins cause more or less kidney damage, so their use will probably be limited to refractory infections that do not respond to other treatments.

Tyrothricin is the oldest commercial antibiotic, but probably the least used of the commercial products. It acts on gram-positive bacteria but is not suitable for injection or oral administration. It is used only for topical purposes, that is, where it can be brought into direct contact with the infecting organism, *e.g.*, surface abscesses.

Because of the millions of gallons of media that must be used for the production of antibiotics, the fermentation is done in deep tanks of 5–15,000 gallon capacity. Sterile air is forced through the medium at the rate of about one-half volume of air per volume of medium per minute. The medium is also stirred vigorously to increase aeration. Deep tank fermentation was first developed for the production of penicillin and later applied to the production of other antibiotics and vitamins.

Vitamin B_{12} is produced simultaneously with streptomycin, aureomycin, and terramycin. Hence producers of these antibiotics obtain a second valuable product in the same fermentation. Vitamin B_{12} is also produced commercially by a mixed aerobacter-proteus type of fermentation. The vitamin is formed by many different kinds of bacteria and molds. Yeasts produce little, if any, of it.

By yeast

Baker's yeast can grow under both aerobic and anaerobic conditions. If an abundance of air and a low concentration of sugar (*e.g.*, 0.5 per cent) are supplied, the end products of metabolism are mainly carbon dioxide and yeast (50 per cent of the weight of sugar is obtained as dry weight of yeast); there is practically no alcohol. See Fig. 14–2.

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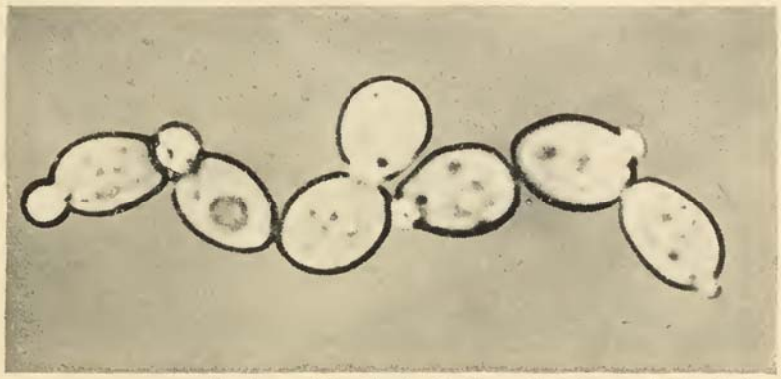
If the sugar content of the medium is raised to 5 per cent, the yield of yeast is markedly reduced, and much alcohol is formed. In other words, an anaerobic type of metabolism comes to the front, although an abundance of air is present. The explanation for this is that ordinary yeast



Courtesy of Dr. Charles N. Frey, Fleischmann Laboratories.

Fig. 14–2. Budding yeast cells.

has a weak aerobic enzyme system and a strong anaerobic system. If more sugar is present than can be metabolized aerobically, the anaerobic system begins to operate.

By molds

Molds cannot grow in the absence of air; carbon dioxide and water are the usual products of metabolism. However, many species convert a large percentage of the sugar in the medium into other carbon products. Examples of products that make up more than 40 per cent by weight of the sugar consumed and the molds producing them are given in Table 14–1. The highest yields of compounds in the table are for gluconic acid. Actually over 100 per cent has been obtained, if allowance is made for glucose going to mycelium. This yield is possible since one oxygen is added per mole of glucose, which amounts to 196 g. of gluconic acid from 180 g. of glucose, or 109 per cent.

Citric Acid. This acid has been obtained many times in yields of 80–90 per cent, but the usual yields, without allowing for glucose going to mycelium, are around 70 per cent. Very special conditions have to be maintained to keep the mold growth low. Such conditions are low concentrations of metals, particularly manganese, high concentrations of sugar, and low pH in the medium. An explanation for the effect of manganese is that this metal serves as a cofactor for some enzyme system that functions in the breakdown and oxidation of citric acid. If there is a deficiency of manganese, the enzyme cannot operate, and then citric

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acid accumulates. Associated with citric acid production is sparse spore formation. The mycelium presents a beaded or braided appearance, and this appearance supports the idea that accumulation of citric acid is an abnormal type of metabolism.

One of the theoretical problems connected with citric acid production is how to harmonize the high yield with the conventional system of intermediary metabolism that operates in yeast and animals (p. 331). This system would require three 2-carbon pieces, or one and one-half moles of glucose per mole of citric acid. On a percentage basis only 71 per cent of citric acid could be obtained. Many theories have been proposed to account for higher yields. The current and best explanation is the uptake of carbon dioxide by pyruvic acid to form oxalacetic acid (Wood-Werkman reaction) and condensation of this acid with acetic acid to form citric acid:

$$CO_2 + CH_3 \cdot CO \cdot COOH \rightarrow HOOC \cdot CH_2 \cdot CO \cdot COOH$$

$$HOOC \cdot CH_2 \cdot CO \cdot COOH + CH_3 \cdot COOH \rightarrow HOOC \cdot CH_2 \cdot \underset{\displaystyle {| \atop CH_2 \cdot COOH}}{C(OH)} \cdot COOH$$

Uptake of isotopic carbon dioxide has been shown to take place, but whether this is adequate to account for the high yields is still not certain. The mechanism of citric acid formation is under active investigation in both animal and mold studies, and many of the questions now unanswered should be cleared up in the near future.

Penicillin. Besides the major products mentioned in Table 14–1, molds produce hundreds of other compounds in amounts from a fraction of a per cent to 10 per cent. The best known of these products is penicillin. Approximately 20 tons of penicillin are produced every month in the United States alone. Yields of 1 g. of penicillin per liter of medium are usual, and about 70 per cent of the penicillin in the broth is recovered as finished product. A typical medium is: lactose, 3 per cent; corn steep solids (the concentrate of the water extract obtained in the industrial manufacture of starch, gluten, and other corn products), 3 per cent; calcium carbonate, 0.5 per cent; sodium sulfate, 0.1 per cent; phenylacetic acid, 0.3 per cent. The medium is sterilized, inoculated with a high-yielding strain of *Penicillium chrysogenum,* and aerated and stirred vigorously during the fermentation period, 3–4 days. The penicillin is extracted from the acidified broth with amyl acetate, transferred to a buffer, purified, and finally crystallized as the sodium, potassium, or procaine salt. Since penicillin is an acid, many different salts can be made, but the above three are those in commercial use. More than a dozen companies are producing penicillin in this country, and the market value of the yearly product has been more than 100 million dollars for several years. See Fig. 14–3.

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Courtesy of Abbott Laboratories.

(b)

Courtesy of Myron P. Backus.

(a)

Courtesy of Abbott Laboratories.

(c)

Fig. 14–3. Production of penicillin. (a) Colony of the high-yielding penicillin mold, *Penicillium chrysogenum*, Wis. Q176. This culture was used industrially for many years to produce penicillin. (b) Fermentation tanks of 6000 gal. capacity used in the submerged production of penicillin. (c) Crystals of the sodium salt of penicillin.

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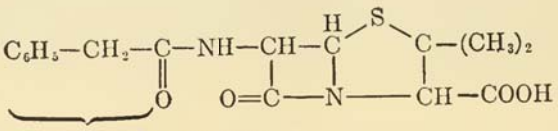
The yield of penicillin has been increased about a thousandfold over that obtained in the beginning of its production. The high yield has been attained largely by selection of better cultures. The best of these have been obtained by treating the mold spores with x-ray, ultraviolet light, or N-mustard gas to give high-yielding mutants.

Other factors in obtaining high yields have been the use of better media and better methods of aeration and agitation of the media. The improvement in penicillin yields is strikingly similar to the development in wheat-raising. Penicillin production might be called factory farming, for the principles operating are the same as in wheat production.

Molds produce at least a half dozen different types of penicillin in the same medium. These differ only in the R group part of the molemule. Today, only one type of penicillin is wanted in commerce, that is the benzyl or G penicillin, which has the formula



Penicillin G

If a suitable precursor, *e.g.*, phenylacetic acid, is added to the medium, the mold obligingly responds by incorporating this compound into the molecule. Other R groups are: in F penicillin, pentenyl ($CH_3 \cdot CH_2 \cdot CH = CH \cdot CH_2—$); in K penicillin, heptyl ($CH_3 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2—$). More than 20 penicillins have been obtained by addition of the appropriate precursors.

Penicillin acts on gram-positive bacteria, and in exceedingly low concentrations. For example, 0.03 units per milliliter will inhibit the growth of the assay organism *Micrococcus pyogenes* var.aureus (formerly called *Staphylococcus aureus*). Since a unit of penicillin is 0.6 μg., 0.03 unit is less than 0.02 μg. per milliliter or 2 mg. per 100 l. of medium. A clinical dose of 100,000 units is only 60 mg. Unfortunately, strains of microorganisms that are resistant to penicillin are beginning to appear. These resistant strains probably come from patients who have been recently treated with penicillin.

The most obvious effect of penicillin on the microbial cell is that although the cell grows larger, it does not divide. This shows that formation of cell constituents, *e.g.*, proteins and nucleic acids, continues for some time after the penicillin enters the cell. Eventually the enlarged cell bursts.

Interference with absorption of amino acids, protein synthesis, nucleic acid synthesis, and phosphorylation reactions have all been attributed to penicillin. It is difficult to determine which of these are primary effects and which are secondary manifestations. Metabolism is a series of events, and interference at one place will show up in all subsequent

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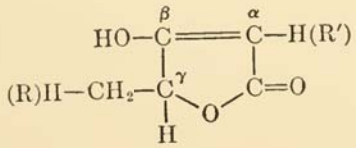
places and eventually reflect back to the original point of interference. It would probably be more correct to think of metabolism as a cycle rather than a chain of events.

Penicillin is specifically and irreversibly bound by gram-positive bacteria. For example, 0.49 units of penicillin per gram of dry weight are bound by cells of *Bacillus cereus*. Extracts of the cells also bind the penicillin, and it should be possible to identify the substance in the extract that possesses binding power. It may be that the reaction between penicillin and this cell constitutent is the primary reaction and other effects are secondary.

A well-defined effect of penicillin on nucleic acid metabolism is reported by Park. This is the accumulation of uridine-5′-pyrophosphate complexes in cells of *Staphylococcus aureus* that have been treated with penicillin. In addition to uracil, ribose, and phosphoric acid, one of these complexes contains an N-acetyl amino sugar. A second complex contains L-alanine in addition to the other four components, and a third has attached to it a peptide made up of DL-alanine, L-lysine, and D-glutamic acid. Probably the accumulation of these complexes is a secondary effect caused by the blocking of some reaction that utilizes the uracil compounds.

From the discussion given, it is evident that the specific effect of penicillin is still undetermined. However, since many able investigators are attacking the problem, distinct progress toward its solution may be expected.

Tetronic Acids. The production of a series of compounds closely related in structure is a characteristic feature of mold metabolism. Besides the penicillins, another such series is the tetronic acids.



1. γ-Methyl tetronic acid, *Penicillium charlesii*.
2. Carolinic acid, *P. charlesii:*
   $R' = CO(CH_2)_2COOH$ (succinyl group).
3. Carolic acid ($+H_2O$), *P. charlesii:*
   $R' = CO(CH_2)_2CH_2OH$ (γ-hydroxybutyryl).
4. Terrestric acid ($+H_2O$), *P. terrestre:*
   $R' = CO(CH_2)_2CHOH \cdot C_2H_5$
   (an ethyl derivative of the R′ group in carolic acid).
5. Dehydrocarolic acid ($+H_2O$), *Penicillium cinerascens:*
   CH_3- of carolic acid is replaced by $CH_2=$
6. Carlic acid, *P. charlesii:*
   $R = HOOC$; $R' = CO(CH_2)_2CH_2OH$ (γ-hydroxybutyryl)
7. Carlosic acid, *P. charlesii:*
   $R = HOOC$; $R' = CO \cdot CH_2CH_2CH_3$ (butyryl).

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Formulas with ($+H_2O$) mean that their peculiar structure is present only in water. These compounds crystallize as anhydrides. γ-Methyl tetronic acid may be regarded as the parent substance, and the others, as substitution products. Carolinic acid has a succinyl group in place of the hydrogen on the α-carbon. Carolic acid has a carbinol group instead of a carboxyl group in the side chain. In carlosic acid the end group in the R′ side chain is methyl. These three compounds clearly represent different degrees of oxidation. In carlosic acid and carlic acid there are also carboxyl groups replacing the hydrogen at R in the formula. All of these compounds are produced in only small amounts, in the order of 1 to 2 per cent of the sugar fermented. It is of special interest that five of the compounds are produced by the same mold. These must be interrelated in the metabolism of *P. charlesii*.

Many other such series of compounds have been described by Raistrick as characteristic of mold metabolism, *e.g.*, a citric, an anthraquinone, and a tropolone series. Over 200 mold products have been isolated in more than a quarter century of research work by Raistrick and co-workers. From this wealth of material many interesting features of mold metabolism have been discovered. For more information see his review paper listed in the references at the end of this chapter.

Another noteworthy aspect of mold metabolism is the formation of organic chlorine compounds. Examples of such compounds are erdin ($C_{16}H_{10}O_7Cl_2$), and geodin ($C_{17}H_{12}O_7Cl_2$). These two compounds are produced by the same mold, *Aspergillus terreus*, and are closely related in structure.

Spoilage of such commercial products as wood, paper, leather, hay, grain, bread, etc., constitutes a debit side of mold activities. An inhibitor of mold growth, propionic acid, is widely used in the bread industry. For use on nonfood materials, there are a number of mold inhibitors, *e.g.*, pentachlorphenol, C_6Cl_5OH.

ANAEROBIC METABOLISM OF CARBOHYDRATES

By bacteria

The anerobic metabolism of bacteria is probably more diversified than the aerobic and probably results in a larger number of products. (See Table 14–1.) The principal types of anaerobic fermentations can be classified by their major end products, as follows:

1. The homolactic type of fermentation (*e.g.*, by *S. lactis*) accounts for more than 90 per cent of the glucose as lactic acid. Thus

$$C_6H_{12}O_6 \rightarrow 2CH_3 \cdot CHOH \cdot COOH$$

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2. The heterolactic fermentation (*e.g.*, by *L. pentoaceticus*) turns about half of the glucose into lactic acid and converts the other half mainly into carbon dioxide and ethyl alcohol. The equation is

$$C_6H_{12}O_6 \rightarrow CH_3 \cdot CHOH \cdot COOH + CO_2 + CH_3 \cdot CH_2OH$$

Sometimes considerable amounts of acetic acid and small quantities of glycerol are formed.

These two types of lactic acid fermentation, homolactic and heterolactic, are important in the industrial production of lactic acid and in the making of cheese, sauerkraut, pickles, and silage.

3. The propionic fermentation (*e.g.*, by *P. pentosaceum*) gives propionic acid, acetic acid, succinic acid, and carbon dioxide as major products, but under certain conditions considerable amounts of lactic acid are formed. The propionic fermentation may be regarded as superimposed upon a homolactic fermentation, but it does not seem probable that lactic acid is an intermediate in the production of propionic acid. In this and the following fermentations the reactions are too complicated to be readily expressed by simple equations.

4. The colon-aerogenes-typhoid bacteria, not only produce all of the compounds formed by the mixed lactics except glycerol, but in addition make formic acid, hydrogen, and butylene glycol. This is a very heterogeneous group of organisms, and the proportion of the products to one another varies greatly with the species of bacteria. Perhaps the most distinctive products are: formic acid by *Eberthella typhi*, acids and hydrogen by *Escherichia coli*, and acetoin and butylene glycol by *Aerobacter aerogenes*.

5. The butyric acid fermentation (*e.g.*, by *Cl. acetobutylicum*) is characterized by the almost complete absence of lactic acid and the appearance of acetic acid, butyric acid, carbon dioxide, hydrogen, butyl alcohol, ethyl alcohol, and acetone. Isopropyl alcohol may replace acetone wholly or in part in certain butyric fermentations. Some bacteria in this group do not form the last four compounds, collectively called solvents, while others produce them in large amounts.

6. The naturally occurring methane fermentation (*e.g.*, by *Methanobacterium omelianskii*) involves a unique type of metabolism. The two extremes of oxidized and reduced carbon products, carbon dioxide and methane, meet here. This apparent contradiction did not appear so strange when it was discovered that methane arose, at least partially, by reduction of carbon dioxide with hydrogen. Methane (also called marsh gas) occurs extensively in coal mines, stagnant waters, sewage, certain types of plants, and in the intestinal tract of animals.

7. Fairly well-characterized polysaccharides have been obtained from more than 60 species of bacteria. Some of these give unusual products

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on hydrolysis such as glucuronic acid, D-arabinose, and inositol. Some of the most notable polysaccharides, their important characteristics, and the bacteria producing them are as follows:

(a) *Cellulose.* This is true cellulose, identical in chemical and physical properties with that found in higher plants. It is produced by *Acetobacter xylinum* and other members of this genus.

(b) Polysaccharides with marked physiological and chemical properties are produced by many pneumococci. The polysaccharide produced by Type III consists of alternate glucose and glucuronic acid units bound together through oxygen by a β-linkage from carbon 4 of the glucose to carbon 1 of the glucuronic acid and a second β-linkage between carbon 3 of the glucuronic acid and carbon 1 of the glucose. There appear to be over 600 units each of glucose and glucuronic acid in the polysaccharide chain. The polysaccharide has marked antigenic properties; when injected into a rabbit, it evokes production of antibodies and immunity against infection with Type III pneumococcus.

(c) *Dextrans.* Many bacteria produce dextrans, but *L. mesenteroides* is the best known dextran-producer. One reason for the current interest in this bacterium is that it produces a dextran that is now being manufactured as a substitute for blood plasma. *L. mesenteroides* is found as a contaminant in sugar factories, and the dextran produced interferes seriously with manufacturing operations. A 10 per cent sucrose solution is fermented in about 24 hours and gives a yield of 25–35 per cent dextran, based on sucrose used. The dextran comes from the glucose part of the sucrose molecule, but glucose itself does not give any dextran, although the microorganism grows well on this sugar. On a glucose medium the microorganism behaves as a heterolactic. The dextran appears to be formed from sucrose according to the following equation:

$$n(C_{12}H_{22}O_{11}) \rightarrow (C_6H_{10}O_5)n + n(C_6H_{12}O_6)$$

Sucrose Dextran Fructose

Some of the glucose and most of the fructose are fermented to lactic acid, acetic acid, ethyl alcohol, carbon dioxide, and mannitol. Potent enzyme preparations which bring about the rapid formation of dextran and fructose from sucrose have been obtained from the culture solution.

The dextran has a branching structure with apparently α-1,6-linkages in the main chain and α-1,4-linkages at the branching points (pp. 50 and 60). The molecular weights of dextrans from different strains of *L. mesenteroides* are enormous, *e.g.,* 25 to 80 millions. These dextrans are too large for use directly as blood plasma substitutes. They are partly degraded by controlled acid hydrolysis and fractionated to give products of suitable size, *e.g.,* molecular weights of about 75,000. Only about 10 per cent of the original dextran is obtained as material suitable for clinical use. An extensive search is now in progress for microorganisms that will pro-

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duce more suitable polysaccharides than those obtained from *L. mesenteroides*.

(d) *Levans.* Polysaccharides of this type are produced from sucrose by several microorganisms, *e.g.*, *Bacillus subtilis*. Yields of levan up to 30 per cent, based on the sucrose used, have been obtained. Enzyme preparations give approximately the same yields, and the reaction seems to follow the same equation as for dextrans, but the polysaccharide and free sugar are reversed. Thus:

$$n(C_{12}H_{22}O_{11}) \rightarrow (C_6H_{10}O_5)_n + n(C_6H_{12}O_6)$$

Sucrose Levan Glucose

By yeast

In the absence of air, ethyl alcohol and carbon dioxide account for about 90 per cent of the sugar fermented, as indicated by the following equation:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

Small amounts of acetic acid and glycerol are also produced. If the medium is kept alkaline, pH about 8.5, large quantities of acetic acid and glycerol are formed. The metabolism of the yeast is shifted so that a minor product, glycerol, becomes a major product. The equation for the fermentation may be represented as

$$2C_6H_{12}O_6 + H_2O \rightarrow 2CO_2 + CH_3COOH + CH_3CH_2OH + 2CH_2OH \cdot CHOH \cdot CH_2OH$$

This theoretical distribution of products is not realized, since more alcohol and less glycerol are usually formed.

Glycerol production may also be increased by adding sulfites to the medium. This fixes the intermediate acetaldehyde as $CH_3CHOH \cdot O \cdot SO_2Na$ and prevents its reduction to ethyl alcohol. A corresponding amount of another intermediate, dihydroxyacetone phosphate, is converted to glycerol. The sulfite process for production of glycerol was used by the Germans in World War I. It is still under consideration, but to date has not been operated successfully. The fermentation equation may be written as

$$C_6H_{12}O_6 \rightarrow CH_2OH \cdot CHOH \cdot CH_2OH + CH_3CHO + CO_2$$

Only about one-half of this yield of glycerol is obtained in practical operations because some of the acetaldehyde escapes fixation and instead goes to ethyl alcohol.

Under anaerobic conditions the yield of yeast (dry weight) is around 5 per cent of the sugar fermented, about one-tenth as much as is produced under aerobic conditions.

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SYSTEMS OF INTERMEDIARY METABOLISM

Aerobic metabolism

Microorganisms that use oxygen have a glycolysis and oxidizing system such as animals possess and metabolize glucose via pyruvic acid and the citric acid cycle to carbon dioxide and water. However, another route, by which glycolysis is side-stepped and possibly also the citric acid cycle, appears to function in yeast and bacteria, and to some extent in liver. This route has been known for a long time but has not received much attention until recently. It has been called "the hexose monophosphate shunt," but this is a poor name since the route appears to be more than a detour around glycolysis. It is a direct oxidation of glucose in which a number of entirely new compounds appear as intermediates in the following sequence:

$$\text{Glucose} \xrightarrow{(1)} \text{glucose-6-PO}_4 \xrightarrow{(2)} \text{gluconic acid-6-PO}_4$$

$$\xrightarrow{(3)} \text{2-ketogluconic acid-6-PO}_4 \xrightarrow[-\,CO_2]{(4)} \text{ribulose-5-PO}_4$$

$$\xrightarrow{(5)} \left\{ \begin{array}{l} C_2 \text{ compound } (\times 2) \xrightarrow{(6)} \text{tetrose} \\ \text{triose-3-PO}_4 \longrightarrow \end{array} \right\} \xrightarrow{(7)} \text{sedoheptulose-7-PO}_4$$

$$+ \text{triose-3-PO}_4 \xrightarrow{(8)} \left\{ \begin{array}{l} \text{glucose-6-PO}_4 \xrightarrow{(9)} \text{recycled} \\ \text{tetrose-4-PO}_4 \xrightarrow{(10)} \text{? triose-3-PO}_4 + CO_2 \end{array} \right.$$

The nature of the C_2 compound in step 5 is still unknown; it does not appear to be a glycolic aldehyde. The ketopentose, ribulose-5-PO_4, is in equilibrium with the aldopentose, ribose-5-PO_4, but the predominant form seems to be the ketose. The occurrence of a C_7 sugar, sedoheptulose, in the metabolism of a C_6 sugar is an unexpected and noteworthy phenomenon. The disposition of the tetrose-4-PO_4 (step 10) is still uncertain, but it appears to go to a triose-phosphate and a one carbon compound, which may be carbon dioxide. All details of the direct oxidation pathway have not yet been worked out, but the main outlines of the route are evident.

Since some cells are equipped with both the glycolysis-citric acid cycle mechanism and the direct oxidation system, the question naturally arises as to the relative importance of the two systems. Investigators are cautious about expressing an opinion, because sufficient data are not yet available for answering this question. However, judging from the

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number of papers that are appearing, some information on this problem should soon be forthcoming.

A second type of direct oxidation that does not involve phosphorylation operates in the metabolism of glucose by certain aerobic bacteria, *e.g., Pseudomonas aeruginosa*. Oxidation of glucose leads to gluconic acid, 2-ketogluconic acid, pyruvic acid, and the formation of large amounts of α-ketoglutaric acid. Yields of this keto acid up to 0.55 mole per mole of glucose have been obtained. This provides a convenient method for the preparation of α-ketoglutaric acid.

ANAEROBIC METABOLISM

Yeast

Hexose diphosphate and pyruvic acid, so prominent as intermediary products in animal metabolism, were first observed in yeast. The steps in glycolysis are the same as far as pyruvic acid in both animal and yeast metabolism. In yeast fermentation the whole process is anaerobic; the pyruvic acid is decarboxylated to acetaldehyde, and this is then reduced to ethyl alcohol. The hydrogen necessary for the last step comes from the dehydrogenation (oxidation) of phosphoglyceraldehyde to phosphoglyceric acid via DPN · H_2 as carrier. If this reduction is blocked, for example, by fixing the acetaldehyde with sulfite, the hydrogen is used to reduce dihydroxyacetone to glycerol. Glycerol production always occurs to a slight extent (3–5 per cent of the glucose), but with the main route of fermentation blocked, the yeast makes the side line a main route. The alternative pathway is a very neat and convenient device for continuing metabolism under adverse conditions.

If alcoholic fermentation is studied with labeled glucose, it is found that carbon 1 appears in the methyl group, 2 in the carbinol group of ethyl alcohol, and carbon 3 in the carbon dioxide. This accords with the Embden-Meyerhof scheme of intermediary metabolism. (See Chap. 13.)

Bacteria

The so-called "mixed" lactic fermentation shows some unexpected departures from the alcoholic fermentation of yeast. Carbon 1 of glucose appears in the carbon dioxide. Carbons 2 and 3 are found in the methyl and carbinol (or carboxyl) groups, respectively, of ethyl alcohol (or acetic acid). Carbon 4 comes out in the carboxyl group of the lactic acid. The two halves of the glucose molecule are metabolized differently. Various intermediary compounds have been found. The first series of

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compounds appears to be the same as in the oxidative pathway of aerobic organisms:

$$\text{Glucose} \longrightarrow \text{glucose-6-phosphate} \longrightarrow \text{gluconic acid-6-phosphate}$$

$$\longrightarrow \text{2-ketogluconic acid-6-phosphate} \longrightarrow \begin{cases} CO_2 \\ \text{ribulose-5-phosphate} \longrightarrow \end{cases}$$

$$\begin{cases} C_2 \text{ compound} \longrightarrow \text{ethyl alcohol or acetic acid} \\ \text{triose-3-phosphate} \longrightarrow \text{lactic acid } (+ H_3PO_4) \end{cases}$$

The fermentation of glucose becomes a pentose fermentation after the loss of carbon 1. The latter part of this formulation fits well for the fermentation of pentoses by the same bacteria. A main difference between the two is that ethyl alcohol is a major product in the fermentation of glucose; it is absent from that of pentose. On the other hand, acetic acid is usually a minor product from glucose and a major product from pentoses. The explanation for these differences probably lies in the accumulation of a reduced coenzyme, perhaps DPN · H_2, in the glucose fermentation. The accumulated coenzyme may then be used for the reduction of the C_2 intermediate to give ethyl alcohol. In the fermentation of the pentoses there is no such accumulation of reduced coenzyme.

The discussion just given applies to the aldohexoses (glucose, galactose, and mannose) but does not fit the fermentation of fructose. Fructose gives approximately 1 mole each of carbon dioxide, acetic acid, and lactic acid, no ethyl alcohol, and 2 moles of mannitol. Mannitol is evidently formed by reduction of other molecules of fructose and appears to be paired with acetic acid as a reduction product. The interrelations of the two may be seen from the following equations:

$$C_6H_{12}O_6 + H_2O \rightarrow CO_2 + CH_3COOH + CH_3CHOHCOOH + 4H$$

$$2C_6H_{12}O_6 + 4H \rightarrow 2C_6H_{14}O_6$$

Fructose Mannitol

Usually less than 2 moles of mannitol are obtained per 3 moles of fructose fermented.

The reasons for the differences between fermentation of glucose and fructose will probably appear when labeled fructose can be used and data are obtained regarding the intermediary compounds that are formed. To date, there are no such data.

The mechanisms of other fermentations have been studied extensively, but space in this chapter does not permit a detailed discussion of the various steps leading from substrate to product. Only a few short statements and overall equations will be given.

Propionic acid appears to be either a reduction product of lactic acid

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or, more probably, a decarboxylation product of succinic acid. The latter may be formed by uptake of carbon dioxide with pyruvic to form oxalacetic acid, and subsequent reduction of this to succinic acid.

$$CO_2 + CH_3 \cdot CO \cdot COOH \longrightarrow HOOC \cdot CH_2CO \cdot COOH \xrightarrow{+4(H)}$$

$$HOOC \cdot CH_2 \cdot CH_2COOH + H_2O$$

The uptake of carbon dioxide by propionic acid bacteria was first demonstrated by Wood and Werkman, and this mechanism is therefore designated as the Wood-Werkman reaction.

Succinic acid is also assumed to be formed by oxidative condensation of two molecules of acetic acid. The overall equation is

$$HOOC\,CH_3 + CH_3COOH \rightarrow HOOC\,CH_2CH_2COOH + 2H$$

The two hydrogens are not released as gas but are used for the reduction of other compounds.

Acetoin (acetylmethylcarbinol) is formed in both yeast and bacterial fermentations. In yeast it is formed by condensation of pyruvic acid and acetaldehyde, and in bacteria by condensation of two moles of pyruvic acid. α-Acetolactic acid is an intermediate in each case. Equations for the formation by yeast may be expressed as follows:

$$CH_3CO \cdot COOH + CH_3CHO \rightarrow CH_3 \cdot \underset{\displaystyle CO \cdot CH_3}{\underset{|}{C}}OH \cdot COOH$$

$$CH_3CO \cdot \underset{\displaystyle COOH}{\underset{|}{C}}OH \cdot CH_3 \rightarrow CH_3 \cdot CO \cdot CHOH \cdot CH_3 + CO_2$$

In the biosynthesis by bacteria the first equation involves both condensation and decarboxylation.

$$2CH_3CO \cdot COOH \rightarrow CH_3 \cdot \underset{\displaystyle CO \cdot CH_3}{\underset{|}{C}}OH \cdot COOH + CO_2$$

In the aerogenes fermentation, acetoin is reduced to give a major product, 2,3-butylene glycol.

$$CH_3 \cdot CO \cdot CHOH \cdot CH_3 + 2H \rightarrow CH_3 \cdot CHOH \cdot CHOH \cdot CH_3$$

In the colon-aerogenes fermentation, formic acid and hydrogen are conspicuous products. These come from the hydrolytic cleavage of pyruvic acid

$$CH_3CO \cdot COOH + H_2O \rightarrow CH_3COOH + HCOOH$$

and subsequent breakdown of formic acid by the enzyme, hydrogenlyase.

$$HCOOH \rightarrow H_2 + CO_2$$

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Butyric acid, butyl alcohol, and acetone all appear to arise from condensation of acetic acid by way of acetoacetic acid.

$$CH_3 \cdot COOH + CH_3 \cdot COOH \rightarrow CH_3 \cdot CO \cdot CH_2 \cdot COOH + H_2O$$
$$CH_3 \cdot CO \cdot CH_2 \cdot COOH + 4H \rightarrow CH_3 \cdot CH_2 \cdot CH_2 \cdot COOH + H_2O$$
$$CH_3 \cdot CH_2 \cdot CH_2 \cdot COOH + 4H \rightarrow CH_3 \cdot CH_2 \cdot CH_2 \cdot CH_2OH$$
$$CH_3 \cdot CO \cdot CH_2 \cdot COOH \rightarrow CH_3 \cdot CO \cdot CH_3 + CO_2$$

The hydrogen for these reductions probably comes from the oxidative steps in the glycolysis scheme that lead to pyruvic acid. There is also hydrogen available from the breakdown of pyruvic acid to acetyl phosphate and carbon dioxide.

$$CH_3 \cdot CO \cdot COOH + H_3PO_4 \rightarrow CH_3 \cdot CO \cdot O \cdot PO_3H_2 + CO_2 + H_2$$

Methane arises, in part at least, by reduction of carbon dioxide with hydrogen:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

A puzzling situation exists in respect to conversion of acetic acid to methane. Isotope studies show that the methyl carbon, represented by (*), is found in the methane, and the carboxyl carbon, indicated by (**), appears in the carbon dioxide; hence methane, in this case, does not arise by reduction of carbon dioxide by hydrogen. The direct

$$C^*H_3C^{**}OOH \rightarrow C^*H_4 + C^{**}O_2$$

decarboxylation as shown in the above equation is probably not the actual mechanism.

REVIEW QUESTIONS ON METABOLISM OF MICROORGANISMS

1. Name some present-day foods that are the result of fermentation processes. Name the class of microorganisms involved in each case.

2. Define the terms "baker's yeast," "press yeast," "active dry yeast." What is the scientific name for these types of yeast? Are any other species of yeast produced commercially? (Consult *Chemical Abstracts* for references to some of these questions.)

3. Give the major and minor products in alcoholic fermentation of glucose. How can the ratio of the products be varied?

4. Make a table of all the microorganisms listed in this chapter, the products formed by them from sugar, and, where given, the percentage of sugar recovered as product.

5. Compare the fermentation of glucose by yeast with the metabolism of glucose in the animal body. Give similarities and differences.

6. What determines whether yeast or bacteria will develop in a food that is not sterilized? For example, fruit juices undergo an alcoholic fermentation, whereas milk becomes sour. Cabbage when shredded and packed into vats undergoes a mixed lactic acid fermentation. (Consult a book on bacteriology.)

7. What products, if any, would you expect to be formed by *A. suboxydans* from (1) glycerol, (2) dulcitol, (3) sorbitol, (4) 2,3-butylene glycol, (5) glucose?

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8. Name the direct precursor of each of the following intermediates in glucose fermentation by yeast: (1) fructose 1,6-diphosphate, (2) 3-phosphoglyceric acid, (3) carbon dioxide, (4) fructose-6-phosphate.

9. What products would you expect from the fermentation of 5-ketogluconic acid by a lactic organism?

10. If carbons 1 and 2 in glucose are labeled, where would you expect them to appear in the products from (1) a yeast fermentation, (2) a heterolactic fermentation? Explain answer.

11. In the production of baker's yeast, sugar is added slowly to the medium during fermentation, while in production of alcohol by yeast it is all added at the beginning. Why the difference in procedure?

12. Write out structural formulas for all the assumed intermediates in the fermentation of glucose by heterolactic bacteria.

13. Compile a list of references to original papers dealing with the direct oxidation (hexose monophosphate shunt) of glucose by microbial and animal cells.

14. Name some of the chemical and physiological characteristics of bacterial polysaccharides.

15. Compile a table of all the commercial antibiotics, giving the name, formula, and microorganism producing each and two infectious organisms against which it acts.

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Chapter 15

PLANT METABOLISM

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PHOTOSYNTHESIS

Man's existence on this planet is directly dependent upon plants, for they furnish him nearly all his food, fuel, and fiber. About a billion tons of organic matter are oxidized on the earth each day. It is obvious that such oxidation would exhaust the world's supply of organic matter in a few years if there were no process to balance the oxidation. Photosynthesis provides such a balance by reducing carbon dioxide, and the energy for the reduction comes from sunlight. The earth intercepts only one-half billionth part of the energy dissipated by the sun, and by photosynthesis only a fraction of one per cent of the radiant energy reaching the earth is stored as chemical energy, but this is sufficient to maintain a balance in the carbon cycle of nature (Fig. 15–1). One is forced to the conclusion that, from the standpoint of man's welfare, no chemical reactions surpass the photosynthetic reactions in importance.

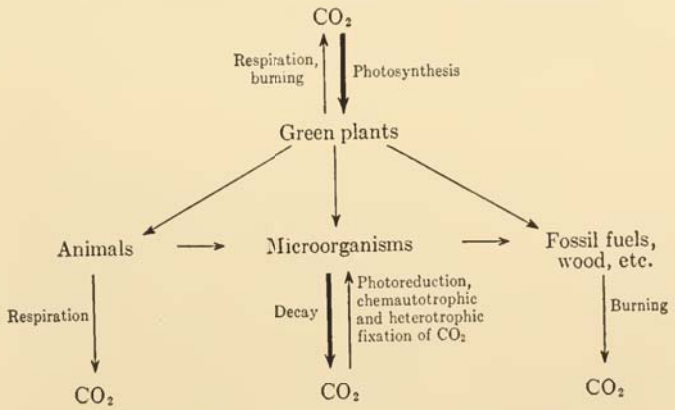

Fig. 15-1. Diagram of the carbon cycle in nature.

The chief reaction for fixing atmospheric carbon dioxide is photosynthesis, and carbon dioxide is released to the atmosphere mainly through

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the action of microorganisms. In addition to the reactions shown, carbon dioxide may be added to the cycle from volcanoes and hot springs, and removed from the cycle as carbonates.

In addition to reducing carbon dioxide, photosynthesis releases oxygen, and this oxygen is used by animals and plants for their energy-releasing respiratory processes. This oxidation destroys the substances produced in photosynthesis, but the energy made available in this way is essential for maintenance of animal and plant life, as is discussed in Chap. 16. Life is dynamic, having both its anabolic and catabolic phases, but only the plants are capable of the net synthesis essential for keeping the overall life processes in balance.

The simplest formulation of the photosynthetic reaction is:

$$n(CO_2) + 2n(H_2O) \xrightarrow[\text{radiant energy}]{\text{plant}} (CH_2O)_n + n(O_2) + n(H_2O) + n(119.6)\ \text{Cal.}$$

Carbon dioxide is reduced to an end product with the empirical formula of a carbohydrate $(CH_2O)_n$. The energy stored in this reduced compound is supplied the plant as radiant energy. *The oxygen evolved all originates from water,* so it is necessary to designate 2 molecules of water on the left side of the equation.

Photosynthesis can be described as a sensitized, photochemical, oxidation-reduction reaction. The sensitizer is *chlorophyll,* for it captures light and functions in the transformation of radiant into chemical energy. The reaction is in part a photochemical one, for light energy is required to drive it. It is an oxidation-reduction with *carbon dioxide* serving as the *oxidant* and water as the *reductant.* The carbon dioxide is reduced to the level of carbohydrate, that is, to substances containing as many oxygen atoms as carbons and twice this number of hydrogens. This description applies only to monosaccharides; polysaccharides have the same reduction level, but water has been removed from them.

Chlorophylls

The green pigment chlorophyll serves as the photosensitizer in the photosynthetic reactions. The first requirement in the utilization of light is that the light be absorbed, and chlorophyll accomplishes this. From the absorption spectrum for chlorophyll *a,* shown in Fig. 15–2, it is evident that the pigment absorbs strongly in the *blue* and *red* regions of the spectrum and transmits much of the green portion of white light.

Chlorophyll in the cells of higher plants is concentrated in *chloroplasts.* The chloroplasts usually are discs about 5 microns in diameter, and within them the chlorophyll is further concentrated in minute bodies called *grana.* Although lacking chloroplasts, the blue-green algae do contain grana, which are distributed throughout the cells.

The chlorophyll molecule apparently does not occur free in the cell but is bound to proteins and lipides. It is suggested that aqueous organic

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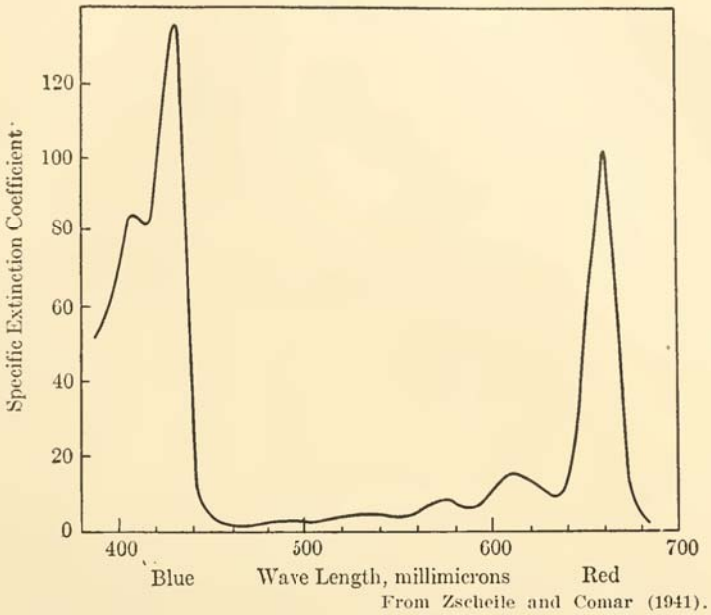
solvents, such as methanol plus water, effectively extract chlorophyll from plants because the water breaks the linkage of the protein, and the organic solvent, the linkage of the lipide to the chlorophyll molecule.



From Zscheile and Comar (1941).

Fig. 15-2. Extinction curve for chlorophyll *a* in ethyl ether.

The exact nature of the native chlorophyll complex is unknown, but apparently this lipoprotein has a molecular weight of 19,000 and contains two molecules of chlorophyll, about 40 per cent lipide and 8.5 per cent nitrogen.

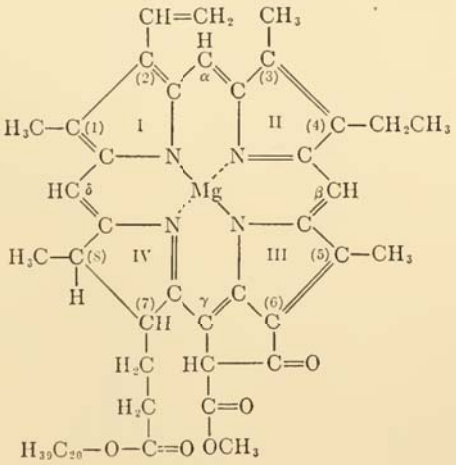


Fig. 15-3. Chlorophyll *a*.

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The green pigment from higher plants can be separated into chlorophyll *a* and chlorophyll *b* by repeated distribution between immiscible solvents or by adsorption chromatography. The first method was used by Willstätter in his classic investigations of the chemistry of the chlorophylls. Tswett's separation of plant pigments in 1906 introduced the useful tool of adsorption chromatography [1] to chemists. Chlorophylls other than *a* and *b* have been isolated from diatoms and algae, but their structures have not been determined in detail. In addition, bacteriochlorophyll has been isolated from purple sulfur bacteria and characterized. It differs from chlorophyll *a* (Fig. 15–3) only in having an acetyl group ($-COCH_3$) at position 2 and extra hydrogens at 3 and 4.

When seeds are germinated in the dark, the plants produced are termed etiolated plants, being devoid of chlorophyll. However, some protochlorophyll is present in etiolated plants, and when they are illuminated the protochlorophyll is rapidly converted to chlorophyll *a* by a process of reduction. It is suggested that chorophyll *b* then arises from chlorophyll *a*. Most plants have about 3 times as much chlorophyll *a* as *b*, and the sum of these chlorophylls usually constitutes 0.7 to 1.3 per cent of the dry weight of leaves.

In addition to chlorophylls, the chloroplasts contain other pigments that may function indirectly in photosynthesis. Some of the light that they absorb can be utilized, but there has been no demonstration that they can function photosynthetically in the complete absence of chlorophylls.

The determination of the structure of chlorophyll by Willstätter, Stoll, Conant, Hans Fischer, and their co-workers constitutes a brilliant contribution to organic chemistry. The empirical formula for chlorophyll *a* is $C_{55}H_{72}O_5N_4Mg$, and for chlorophyll *b* is $C_{55}H_{70}O_6N_4Mg$. The currently accepted structural formula for chlorophyll *a* is shown in Fig. 15–3. There remains some question whether the semi-isolated double bond [2] occurs in pyrrole ring II or is in ring III; if it were in ring III, the Mg would be bound between rings I and II rather than between I and III as shown.

There are a number of characteristics of the chlorophyll molecule which should be noted. First, much of the stability of the molecule can be attributed to the system of conjugated double bonds,[3] designated by the bold lines in the outline formula of Fig. 15–4. Second, the

[1] The procedure for adsorption chromatography is briefly as follows: a finely powdered adsorbent such as magnesium oxide is packed in a glass tube to form a column, and a solution of the mixture to be separated is poured on the top. When a suitable solvent is passed through the column, the individual components of the mixture move at different rates and separate into distinct bands.

[2] This is the double bond that is not a part of the conjugated system, shown between carbons 3 and 4 in Fig. 15–3.

[3] Alternate single and double bonds are said to be *conjugated*.

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molecule contains 4 pyrrole rings (Fig. 15–5) labeled I, II, III and IV. Third, the molecule contains an atom of Mg held by primary bonds to two nitrogen atoms of the pyrrole nuclei and by secondary bonds to the other nitrogen atoms. Fourth, the pyrrole rings are connected by

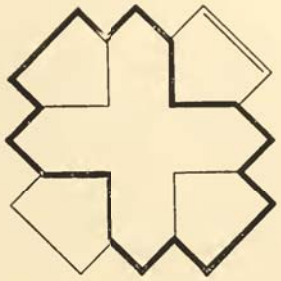

Fig. 15–4. System of conjugated double bonds in chlorophyll shown by bold lines.

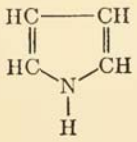

Fig. 15–5. Pyrrole.

methene bridges (—CH=). Fifth, pyrrole ring I carries a vinyl group (—CH=CH_2). Sixth, attached to pyrrole ring III is a homocylic ring bearing a carbonyl group and a carboxyl esterified with methanol. Seventh, pyrrole ring IV carries a propionic acid side chain esterified with phytol; phytol is a higher alcohol ($C_{20}H_{39}OH$) with a double bond between its α and β carbons. The phytyl group gives chlorophyll its solubility in fat solvents. Eighth, chlorophyll is optically active because of the presence of asymmetric carbon atoms. In chlorophyll *b* the —CH_3 of pyrrole ring II is replaced by —CHO. Protochlorophyll is like chlorophyll *a* but is dehydrogenated at carbon atoms 7 and 8.

Primary photochemical reaction

Photosynthesis consists, not of a single reaction, but of a sequence of reactions. Much interest attaches to the first reaction involving light, or the *primary photochemical reaction*. Photosynthesis was described as a sensitized, photochemical oxidation-reduction, and water was designated as the reductant. Water is not normally an effective reducing agent; somehow, energy must be added to it to make it effective. Much evidence suggests that the primary photochemical reaction is a *photolysis* of water, a reaction in which light energy is used for the splitting of water

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with the release of hydrogen to some hydrogen acceptor, which can function in subsequent reductions. This active hydrogen may be passed from one acceptor to another before it is finally used for the reduction of carbon dioxide.

Release of oxygen

The reaction releasing oxygen is dependent upon, but apparently not identical with, the primary photochemical reaction. If the photolysis of water is considered as an oxidation-reduction reaction, the hydrogen acceptor mentioned is reduced, and some other acceptor of the hydroxyl radical remaining is oxidized. This oxidized product releases O_2, and it may be assumed that an enzymatic mechanism is concerned in this release.

It has been implied throughout the discussion that all the oxygen formed in photosynthesis comes from water, but this fact has only been accepted in recent years. Early workers suggested that it came from carbon dioxide, or part from carbon dioxide and part from water. The work of van Niel with the photosynthetic bacteria suggested that, if bacterial photoreduction were analogous to the photosynthesis of higher plants, water should supply all the oxygen released in photosynthesis. This hypothesis was verified experimentally with the stable isotopic tracer O^{18}, when it was shown that the O^{18} level of photosynthetic O_2 corresponded exactly with the O^{18} level of H_2O^{18}, in which photosynthesizing algae were suspended.

Carbon dioxide

Normal air contains 0.03 per cent carbon dioxide, and upon this plants must depend for their source of carbon. Air is taken in through the stomatal openings on the leaf and is dissolved in the leaf sap. Carbonic anhydrase is an enzyme in the leaves which speeds the formation of carbonic acid from the carbon dioxide and water. There is no clear experimental answer to the question whether carbon dioxide is used in photosynthesis as the dissolved gas or as the carbonate and bicarbonate ions.

Evidently the carbon dioxide entering a leaf is bound in a loose complex on some large molecules, a binding which may be compared roughly to the binding of oxygen by hemoglobin. Many lines of evidence indicate that the carbon dioxide is utilized next in a carboxylation reaction. A general formulation of such a reaction is:

$$\mathrm{RCHO} + \mathrm{CO_2} \rightarrow \mathrm{RC(=O)OH}$$

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A carboxylation involves no reduction and requires relatively little energy. Much energy is needed for reduction of the carboxylated compound to the level of a carbohydrate, a reaction which can be pictured as:

$$\underset{\underset{\displaystyle O}{\|}}{RC}COOH + 2H \rightarrow RCHOHCOOH$$

$$RCHOHCOOH + 2H \rightarrow RCHOHCHO + H_2O$$

It is evident that 4 hydrogens are required to reduce one carbon dioxide molecule to the oxidation-reduction level of carbohydrate and that a molecule of water is produced.

Shortly after the turn of the twentieth century, it was realized that photosynthesis could be divided into distinct light and dark reactions, *i.e.*, reactions which do, or do not, require light in order to proceed. There is no evidence that the initial fixation of carbon dioxide, and its subsequent reduction, requires light directly. In fact, all evidence shows that *the reactions of carbon dioxide in photosynthesis are dark reactions.* Some of the most convincing experiments are based upon the use of specific inhibitors at varying light intensities. If an inhibitor does not decrease the rate of photosynthesis at low light intensities, but does at high intensities (where the dark reactions are the ones which limit the rate of the overall process), it is an inhibitor for a dark reaction. Cyanide, for example, acts in this fashion, and since it inhibits carboxylation, the conclusion is drawn that carboxylation does not require light.

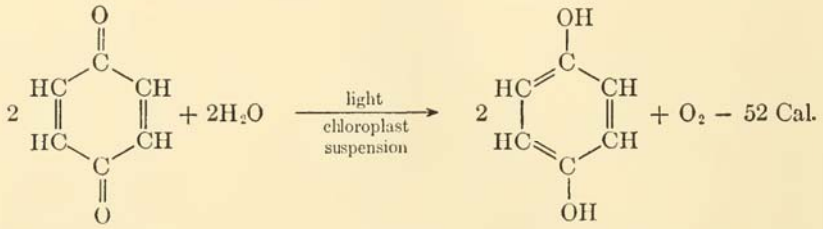
The rate of photosynthesis is dependent on the partial pressure of carbon dioxide, and though it is difficult to generalize for many plants at different light intensities, it may be said that raising the concentration of carbon dioxide from its usual level of 0.03 per cent to 0.1 per cent will usually double the rate of photosynthesis. Most plants are not inhibited by carbon dioxide up to 10 per cent, but their growth is reduced by concentrations greater than this.

Partial reactions

As pointed out, photosynthesis is a complex of reactions rather than a single reaction. The study of such an involved process is obviously much easier if it can be first separated into its individual steps. One of the most notable achievements of recent years has been the reconstruction of partial reactions in cell-free preparations; that is, certain ground-up plant tissues have been found to carry out parts of the photosynthetic process. This has permitted the study of individual reactions or reaction series in a system much simpler than that encountered in intact cells.

In 1937, Hill found that a suspension of chloroplasts plus leaf extract

would liberate small amounts of oxygen upon illumination. Substitution of potassium ferric oxalate solution for the leaf extract increased the rate of oxygen evolution, and later workers have further improved the system by substituting *p*-benzoquinone. The potassium ferric oxalate or *p*-benzoquinone serves in place of carbon dioxide, which is the oxidant in normal photosynthesis. The Hill reaction with *p*-benzoquinone is:



Benzoquinone Hydroquinone

Oxygen is liberated, the energy of light is used in the reduction of the quinone, and energy is stored in the hydroquinone formed. The Hill reaction should more properly be called the Hill reactions, for both photochemical and dark reactions apparently are involved. This isolated system again emphasizes the close relationship of the photochemical and oxygen-liberating reactions of photosynthesis.

Success in carrying out light-sensitive partial reactions concerned in the reduction of carbon dioxide without the use of intact cells was not achieved until 1951, when Vishniac and Ochoa reported that light energy could be used in the reductive carboxylation of pyruvic acid to malic acid (reaction 15, Fig. 13–4). Grana from spinach leaves used the energy captured from light for the reduction of TPN. The reduced TPN, in the presence of the proper enzymes, then caused the reductive carboxylation of pyruvic to malic acid. In a similar manner DPN, reduced in the light, could effect the reduction of pyruvic to lactic acid (reaction 13, Fig. 13–3). The overall reactions did not occur in the dark, but it should be noted that the reactions directly involving the carbon dioxide were dark reactions. Illuminated chloroplasts, or grana, which we already have seen can liberate O_2, also can transfer the hydrogen produced in the photolysis of water to oxidized DPN or TPN. The coenzymes in turn can reduce pyruvate to lactate or function in the reductive carboxylation of pyruvate to malate. These observations have been confirmed by Tolmach, and Arnon has reconstructed a system for the light-dependent reductive carboxylation of pyruvate to malate, in which all the parts (except TPN) were of plant origin.

It is justifiable to say that the Hill reaction represents a true partial reaction of photosynthesis, but there is some question whether or not this can be said about the reactions described by Vishniac and Ochoa.

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The important consideration is that the reactions involve the capture of radiant energy by chlorophyll and use of the energy in the reduction of coenzymes, which can serve in further reductions. These should be viewed as model reactions useful in the study of portions of the photosynthetic process rather than as proved partial reactions of photosynthesis as they actually take place in normal plants.

Storage of energy

The formulation of the photosynthetic reaction (p. 388) indicated that 119.6 Cal. were stored for each mole of carbon dioxide reduced to the level of carbohydrate; this is equivalent to 717.6 Cal. per mole of glucose formed. These values are in terms of change in free energy (ΔF, p. 414), when glucose, at molar concentration, is formed in aqueous solution at 25°C. from liquid water and carbon dioxide at a concentration of 0.03 per cent. That the value for ΔF differs from that given for glucose oxidation in Chap. 16 (683 Cal.) can be attributed to the difference in concentration of reactants and products in photosynthesis and in animal respiration.

The energy stored in photosynthetic products represents radiant energy which has been captured and converted to chemical energy. Light energy is absorbed or radiated in discrete units, or *quanta,* which are equal to $h\nu$, when h is Planck's constant (6.547×10^{-27} ergs per sec.), and ν is the frequency [frequency is calculated by dividing the wavelength of light (cm.) into the velocity of light, 3×10^{10} cm. per sec.]. The total amount of energy in 6.06×10^{23} quanta is called one *Einstein.* It is evident that the higher the frequency (shorter the wavelength), the greater is the energy of each quantum of light. Einstein's theory of photochemical equivalence indicates that a photochemical reaction is induced when one molecule absorbs one quantum of light of characteristic frequency. The question of the quantum efficiency of photosynthesis may be stated as follows: How many quanta are required for the reduction of one molecule of carbon dioxide to the level of carbohydrate?

In 1922 Warburg and Negelein determined the quantum efficiency of photosynthesis by the alga *Chlorella* in light of various frequencies. They found that *4 to 5 quanta were required for each molecule of carbon dioxide.* The value of 4 quanta was readily accepted, because it was attractive to think that one quantum was required to activate each of the 4 hydrogens necessary to reduce carbon dioxide to the carbohydrate level. Some 16 years later, Manning, Stauffer, Duggar, and Daniels employed a variety of methods but were unable to reproduce the high efficiency reported by Warburg and Negelein. Several other investigators likewise were unsuccessful and found that 8 to 12 quanta were required rather than 4. Recently Warburg has repeated his earlier experiments

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and has obtained essentially the same results as before. Warburg and Burk have consistently observed quantum efficiencies of about 4 with several modified techniques.

In red light with 43 Cal. per Einstein, a quantum efficiency of 4 represents almost 70 per cent efficiency [1] in storing the energy of the incident light. This is a higher efficiency than that of any known photochemical reaction which can be performed under controlled conditions. Even a quantum efficiency of 8 to 12 is good in terms of known photochemical reactions, and if photosynthesis actually has a quantum efficiency of 4, it is unique. It is impossible at present to make a categorical statement of the quantum efficiency of photosynthesis, for the matter remains highly controversial.

Intermediates and products of photosynthesis

One of the most interesting problems of photosynthesis is the determination of the organic compounds produced by the sequence of reactions. What substances are formed between the first reduction of carbon dioxide and the final appearance of the finished sugar molecule? There is an extensive literature on this subject, much of which has centered around the formaldehyde hypothesis. In 1870, Baeyer suggested formaldehyde as the first product of photosynthesis, and though there never has been substantial evidence to support it, the hypothesis has been perpetuated in textbooks of biochemistry and botany to the present.

Current interest centers around phosphoglyceric acid as the first demonstrable product of photosynthesis. Pioneering work from 1939 to 1942 with the short-lived radioisotope C^{11} resulted in the introduction of a number of new concepts and the discard of certain old ideas on photosynthetic intermediates. Since 1946, the use of the long-lived (5,568 ± 30 year half life) radioactive isotope C^{14} has led to real advances in our knowledge of intermediates. Although it remains incomplete, a general picture of the reaction sequence has now emerged. Calvin and coworkers have made particularly notable contributions. By skillful use of a combination of C^{14}, paper chromatography,[2] and "radioautography" [3]

[1] Calculation : $\frac{119.6}{4 \times 43} \times 100 = 69.5\%$.

[2] In paper chromatography a small drop of a mixture of compounds in solution is placed near one end of a long strip of filter paper and is dried. The end near the point of application is immersed in a suitable solvent. As this solvent moves by capillary action past the added mixture of compounds and on toward the other end of the paper, it carries with it the individual components of the mixture varying distances and thus achieves their separation.

[3] "Radioautography" is a procedure for locating radioactive substances separated by paper chromatography. After the chromatographic separation is completed, the paper is dried and allowed to "take its own picture" by placing it on a photographic film in the dark. Black areas on the developed film correspond to the location of radioactive substances on the paper strip.

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they have unraveled many of the complexities of the early photosynthetic reactions and have revealed the importance of phosphoglyceric acid as an intermediate.

Figure 15–6 gives reactions postulated by Calvin and co-workers as occurring between the initial fixation of carbon dioxide and its final reduction to sugar.[1] The initial reaction is the carboxylation of the hypothetical vinyl phosphate (1) to 2-*phosphoglyceric acid* (2), which is then converted to 3-*phosphoglyceric acid* (3). In extremely short periods (5 seconds) of photosynthesis by algal cells in the presence of $C^{14}O_2$, it has been shown that up to 85 per cent of the C^{14} fixed appears in 2- and 3-phosphoglyceric acids. By reversing the glycolytic scheme (see Figs. 13–1 and 13–3), 3-phosphoglyceric acid can be converted to sugar. It first is reduced to 3-phosphoglyceraldehyde (4), which is in equilibrium with dihydroxyacetone phosphate (5). Condensation of these compounds yields fructose-1,6-diphosphate (6), which in turn yields fructose-6-phosphate (7) and glucose-6-phosphate (8). Free fructose and glucose can be obtained from these by the removal of phosphate. The method by which plants form sucrose is unknown, and the sucrose phosphate shown is a hypothetical intermediate. However, all the glycolytic steps included in Fig. 15–6 have been demonstrated to occur in plants (preparations from pea seedlings).

The left part of the scheme represents a suggested method for regenerating the C_2 fragment, which must serve as the acceptor of carbon dioxide when phosphoglyceric acid is formed; this remains the least well-defined and most controversial portion of the scheme. In the first formulation shown, one molecule of 2-phosphoglyceric acid is used in sugar formation, while water is removed from the other molecule to yield 2-*phosphoenolpyruvic acid* (9). Addition of another molecule of carbon dioxide yields *enol-oxalacetic acid* (10). This is converted to an undefined C_4 acid, which splits to give 2 molecules of glyoxylic acid (11); upon reduction this would yield glycolic acid (12). Several C_2 compounds have been suggested between glycolic acid and the hypothetical vinyl phosphate (1).

An alternative method, postulated by Calvin and co-workers, for forming a triose to serve in the reversed glycolytic mechanism and for regenerating the C_2 fragments is shown in skeleton form in the lower portion of Fig. 15–6. A C_4 and a C_3 compound are condensed to form the C_7 sugar, *sedoheptulose*. This compound is split to yield a C_2 fragment which recycles and a C_5 keto-sugar, *ribulose*. The ribulose in turn is split to a triose and a C_2 compound, which also recycles. The complete cycle, involving 3 carboxylations and subsequent reductions, yields a molecule of triose. Most of the specific intermediates in this reaction sequence remain to be defined.

[1] In Fig. 15–6 "P" indicates either a phosphate group or a molecule of phosphoric acid.

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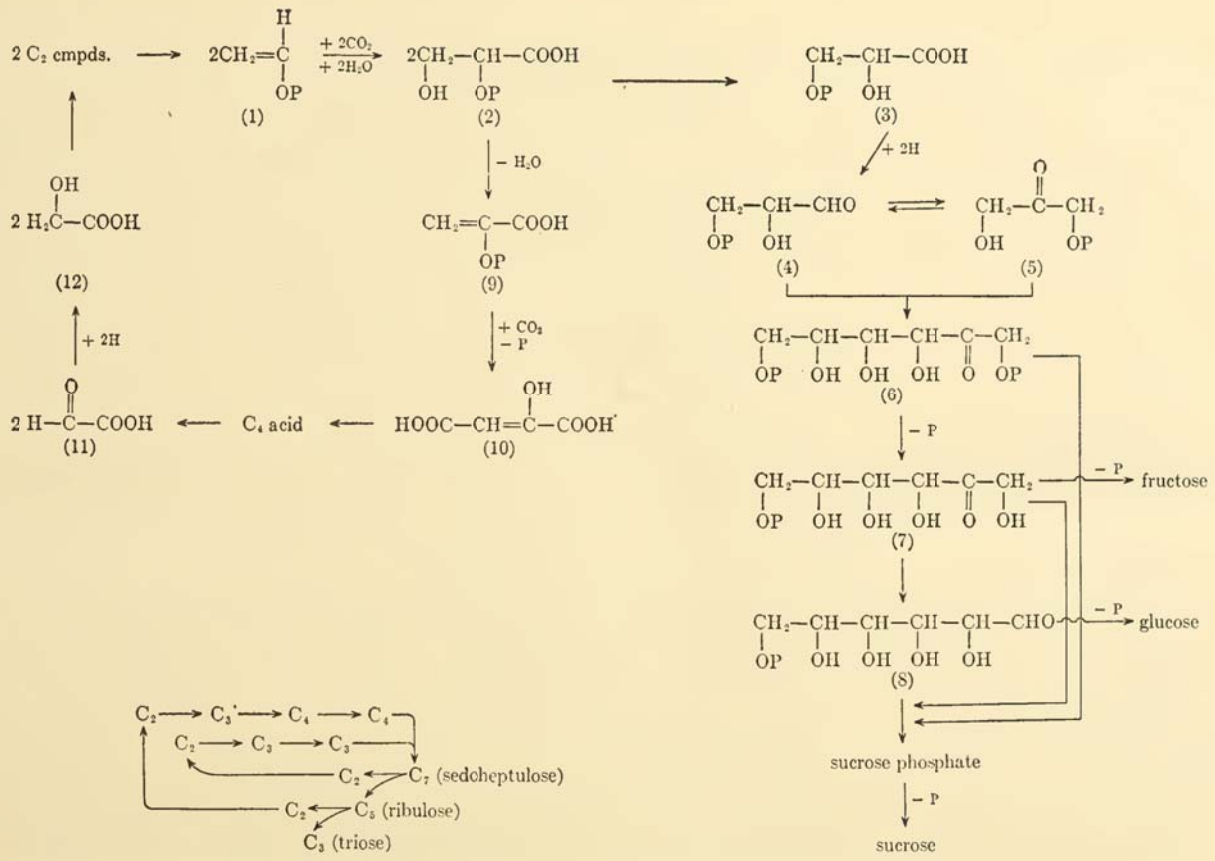

Fig. 15-6. Possible paths of carbon in photosynthesis, as suggested by Calvin and co-workers. See text for explanation.

Carbohydrates. Although information is incomplete on the early products of photosynthesis and their mode of formation, the total materials of the plant are the net final products of photosynthesis after modification by other phases of plant metabolism. The carbohydrates are quantitatively the most important group of compounds, and cellulose, starches, and sugars are particularly abundant.

Glucose and fructose commonly occur as free and combined sugars in plants, whereas mannose and galactose are found only in combined form. Among the disaccharides, sucrose is especially important. Maltose and cellobiose do not occur free in demonstrable concentrations but arise from the hydrolysis of starch and cellulose, respectively. Plants also may yield the disaccharides gentiobiose, trehalose, and melibiose, but they do not contain lactose. Raffinose, gentianose, and melizitose are the most common plant trisaccharides, and stachyose is a tetrasaccharide which has been isolated from lupine seeds.

Particular interest is attached to the method by which the plant synthesizes sucrose because the sugar is important to plants and man. It has not as yet been synthesized chemically. Sucrose can be formed by detached leaves when they are infiltrated with glucose, fructose, mannose, galactose, or glyceraldehyde; the synthesis will not take place in the absence of oxygen. The only controlled synthesis of sucrose by a cell-free preparation has been achieved with an enzyme system from the bacterium, *Pseudomonas saccharophila.* This organism produces a *sucrose phosphorylase* (more properly termed a *transglucosidase*), which by phosphorolysis of sucrose produces fructose plus glucose-1-phosphate. The equilibrium of the reaction is such that by a reversal of the reaction a synthesis of about 5 per cent of sucrose from glucose-1-phosphate plus fructose can be achieved. Though higher plants accumulate much sucrose, there has been no success to date in demonstrating their synthesis of sucrose by sucrose phosphorylase or by any other isolated enzyme system.

Even less is known concerning the synthesis of cellulose. Although bacterial preparations have been induced to form cellulose from a number of precursors, some as simple as C_2 compounds, there is no detailed information on the pathway of its formation.

Knowledge of starch synthesis is much more nearly complete. Peat, Bourne, and co-workers (see Bernfeld for discussion) have shown that potato tubers and other plant tissues contain a *starch phosphorylase* which by phosphorolysis converts starch to glucose-1-phosphate. The reversal of this reaction to yield starch from glucose-1-phosphate is readily demonstrable. The first preparations used, synthesized only amylose, the straight chain starch, but later preparations also yielded amylopectin, the branched chain starch. The enzyme which synthesizes amylose from glucose-1-phosphate is termed the *P-enzyme.* A separate enzyme, termed the *Q-enzyme,* is needed to form the linkages at the

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branch points in the amylopectin structure. The Q-enzyme, in conjunction with the P-enzyme, will form amylopectin:

$$\text{glucose-1-phosphate} \xrightleftharpoons{\text{P-enzyme}} \text{amylose} \xrightarrow{\text{Q-enzyme}} \text{amylopectin}$$

Lipides. Lipides are the chief storage materials in seeds. Much more energy is stored in a gram of lipide than in a gram of protein or carbohydrate (see p. 423). When oily seeds mature, their fat content builds up rapidly, chiefly at the expense of carbohydrate. Upon maturation, the fat increases in unsaturation, and the constituent fatty acids increase in average chain length. These changes in seeds are reversed when the seeds germinate. The fact that almost all the fatty acids have an even number of carbon atoms suggests that they originate from condensation of C_2 units.

NITROGENOUS COMPOUNDS AND THEIR METABOLISM

The animal requires an external supply of several amino acids (or their keto acid analogues) to survive, but the plant can synthesize its needed amino acids from inorganic sources of nitrogen, carbon dioxide, and water. Lack of an adequate supply of nitrogen in the soil, more frequently than the lack of any other element, limits the growth of plants. As a result, nitrogenous fertilizers, and crop rotations including nitrogen-fixing legumes, are of great importance in our agricultural economy.

Nitrogen nutrition

Nitrate has been used as a fertilizer for centuries. Because it can be employed in higher concentrations than ammonia, it often has been considered inherently superior to ammonia for the nutrition of plants. Actually, when ammonia is supplied in nontoxic concentrations, it normally supports a rate of growth in plants equal to or superior to that for nitrate. The conditions for optimum utilization of the two compounds differ, however; nitrate is used best at a pH below, and ammonia above, neutrality.

Urea and calcium cyanamide ($CaCN_2$) are widely used organic fertilizers which readily yield ammonia. Manures and composts serve as sources of nitrogen for plants, and it is generally suggested that their utilization by the plant is preceded by *ammonification* and *nitrification*.[1] In the process of ammonification, bacteria convert the nitrogen of proteins and other compounds to ammonia. This is followed by the action

[1] Nitrification is the process of converting ammonia to nitrites, or nitrites to nitrates, or both.

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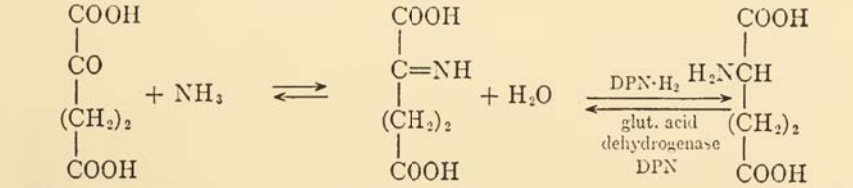
of the autotrophic [1] nitrifying bacteria which oxidize ammonia to nitrite (*Nitrosomonas* and *Nitrosococcus*) and nitrite to nitrate (*Nitrobacter*). Nitrification is an important process in the soil, but it is unreasonable to assume that plants can use only nitrate, the end product of the process, for their nutrition. We have already seen that they can use ammonia directly, and nitrite also is an excellent source of nitrogen for plants when it is present in subtoxic concentrations. Even complete ammonification may be unnecessary before the nitrogen of organic residues is used. In such material the nitrogen is present chiefly as protein, and upon hydrolysis this protein will yield peptides and free amino acids. For plants grown in the absence of bacteria (to avoid complications from bacterial action on the nitrogenous compounds) certain single amino acids can serve adequately as the sole source of nitrogen. Peptone, a mixture of peptides and free amino acids, also supports good growth. Evidently plants can use the products at all stages of protein breakdown: peptides, amino acids, ammonia, nitrites, and nitrates.

Synthesis of amino acids

A plant supplied nitrate or nitrite must reduce these compounds to the reduction level of ammonia for the synthesis of amino acids. Whether the reduction yields free ammonia or reduced nitrogen bound in a more complex form has not been established. The best defined amino acid synthesis from ammonia is the reductive amination of α-ketoglutaric acid to glutamic acid by way of α-iminoglutaric acid:



α-Ketoglutaric acid — α-Iminoglutaric acid — Glutamic acid

This reaction sequence has been accomplished with cell-free enzyme preparations from plants when reduced DPN has been added. Comparable reductive aminations may result in the formation of other amino acids, although they have not been demonstrated.

Glutamic acid is a key compound in the nitrogen metabolism of animals, plants, and microorganisms. It is probable that many amino acids in plants are formed by transamination from glutamic acid to various α-keto acids. By transamination the amino group of an amino acid is trans-

[1] Autotrophic bacteria are those microorganisms which can derive all their energy from the oxidation of a simple inorganic compound or element (*e.g.*, oxidation of S to $SO_4^=$) and are able to use carbon dioxide as their sole source of carbon.

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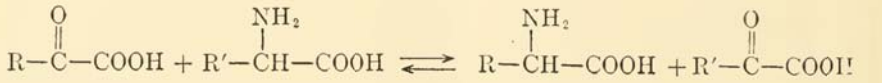
ferred to an α-keto acid, and a new α-amino acid and a new α-keto acid are formed (see Chap. 13, reactions 37 and 38):



Free ammonia does not function in the reaction. Transaminations from glutamic acid to pyruvic and oxalacetic acids to form alanine and aspartic acid, respectively, have been demonstrated in plants, and other transaminations occur but at considerably slower rates.

Metabolism of amides by seedlings

Germinating seeds may produce large quantities of the amides, *asparagine* and *glutamine*. Asparagine is the β-amide of aspartic acid ($HOOC—CHNH_2—CH_2—CONH_2$), and glutamine has a comparable structure ($HOOC—CHNH_2—(CH_2)_2—CONH_2$). When a high-protein seed such as a lupine seed germinates, there is a demand upon the protein as an energy yielding material for growth before the seedling establishes its synthetic capacity. When protein is broken down and respired, ammonia accumulates; high levels of ammonia would be toxic, but by forming amides the plant detoxifies it. As much as 85 per cent of the protein nitrogen disappearing in a germinating lupine seed may appear in the single compound, asparagine. Later in the growth of the plant the amide nitrogen is used in the resynthesis of protein, and when the plant matures, its amide content is very low.

Biological fixation of nitrogen

The nitrogen cycle in nature is pictured in Fig. 15–7, in a form which is simplified by omitting the marine cycle of nitrogen. Nitrogen is added to the cycle from the vast reservoir of atmospheric N_2 by chemical fixation,[1] by the symbiotic nitrogen fixation of leguminous plants, and by the nonsymbiotic nitrogen fixation of free-living bacteria and blue-green algae. Nitrogen is lost to the sea by leaching, erosion, and sewage disposal, and to the atmosphere by bacterial denitrification.[2] Man's installations for the chemical fixation of N_2 are impressive, but their contribution is minor compared to that of biological nitrogen fixation in maintaining the nitrogen cycle in balance. Chiefly because of improper

[1] "Fixation" is the conversion of gaseous nitrogen (N_2) into chemically combined forms such as ammonia, nitric acid, protein, etc.

[2] Denitrification is the release of free nitrogen (N_2) from nitrogenous compounds, or the opposite of fixation.

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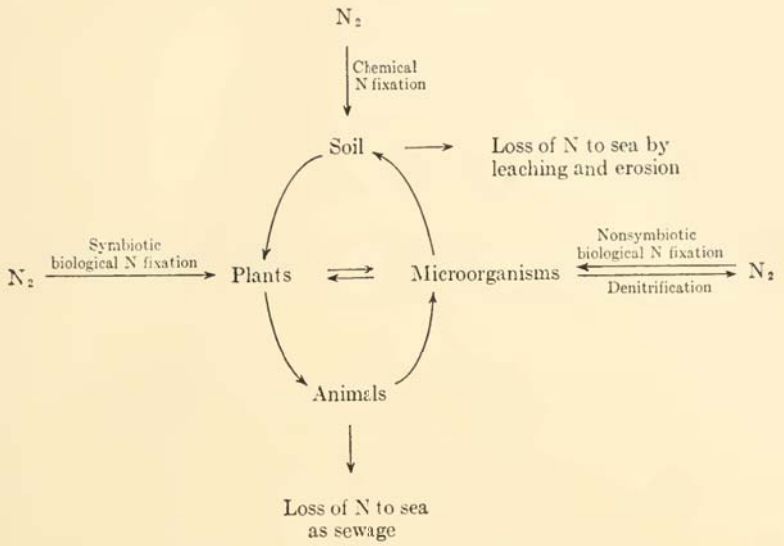

Fig. 15–7. The nitrogen cycle in nature.

land use and wasteful disposal of sewage, the land area of the earth yearly increases its nitrogen debt, despite nitrogen fixation by plants and bacteria.

Agents Capable of Fixing N_2. Leguminous plants such as clover, alfalfa, peas, and beans carry nodules on their roots. In these nodules, N_2 can be fixed into forms usable by the plant. The nodules are caused by the invasion of the roots by bacteria, the *rhizobia,* which cause the root tissue to grow at the site of invasion. Nodules have a characteristic, well-organized structure, and certain of the plant cells are packed with bacteria. It is interesting to note that a hemoglobin much like mammalian hemoglobin is present in nodules; this is the only reported occurrence of hemoglobin in the plant kingdom. Neither the leguminous plant alone nor the rhizobia alone can fix N_2, but in symbiotic association [1] they can. Symbiotic nitrogen fixation is quantitatively the most important means on the land surface of the earth for fixing N_2.

The nonsymbiotic nitrogen fixers, that is, those organisms capable of fixing N_2 by themselves, include the aerobic *Azotobacter,* the anaerobic *Clostridium* and *Desulfovibrio,* the photosynthetic bacteria *Rhodospirillum, Chromatium, Chlorobacterium,* and *Rhodomicrobium,* and representatives from 3 families of blue-green algae. It is very difficult to appraise the quantitative importance of the N_2 fixation by these organ-

[1] Symbiotic association, or *symbiosis,* is a relationship in which two different living organisms exist in close association with each other in such a manner that each derives benefit from the other's existence.

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isms. *Azotobacter* and *Clostridium* are important under normal soil conditions, and the blue-green algae function in maintaining the nitrogen supply under the wet conditions encountered in rice paddies. The other organisms probably function most extensively in aquatic habitats.

Mechanism of N_2 *Fixation.* Although the intermediates arising directly from the fixation of N_2 are unknown, these intermediates are quickly converted to ammonia before its incorporation into organic compounds. The reduction to ammonia logically should pass through hydroxylamine as an intermediate, but formation of amino acids from hydroxylamine *via* their oximes does not appear to be an important pathway of synthesis. Ammonia is the key compound in the sense that it is the compound which combines with carbon chains to yield organic nitrogenous substances in the plants or bacteria. The primary pathway of N_2 fixation involves conversion of N_2 to NH_3, through unknown intermediates, and the formation of glutamic acid by reductive amination of α-ketoglutaric acid. Glutamic acid in turn can form new amino acids by transferring its amino group by transamination.

The evidence that ammonia is the key intermediate in biological nitrogen fixation has been accumulated largely by investigating the fixation with the aid of the stable isotopic tracer, N^{15}. These studies, and tests with specific inhibitors of nitrogen fixation, indicate a unity in the mechanism of nitrogen fixation in all the diverse organisms investigated.

RESPIRATION OF PLANTS

Although plants are most notable for their photosynthetic capacities, they carry on respiration both in the light and in the dark. Their respiration per unit weight is less intense than that of most animals because many of the structural materials of the plant such as cellulose and lignin are metabolically inert. However, when activity is expressed per unit of nitrogen in the tissues, the respiratory activity of plants and animals is comparable.

The activity of cytochrome oxidase has been clearly demonstrated in plants, and cytochrome *c* has been isolated from plant materials. Plant cytochrome *c* and cytochrome oxidase are very similar to these constituents of the cytochrome system of animal origin. Other oxidases of importance in plants are ascorbic acid oxidase and tyrosinase (catecholase, dopa oxidase, and laccase are plant enzymes similar to tyrosinase). It is characteristic of the true oxidases that they use O_2, produce H_2O rather than H_2O_2, and have a heavy metal component. Both ascorbic acid oxidase and tyrosinase contain copper, and though cytochrome oxidase never has been purified, it apparently contains iron. A dominant position of the true oxidases characterizes plant respiration, in contrast to

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animal respiration where cytochrome oxidase is the only oxidase assuming an important role.

Peroxidases are widely distributed in plants, but their function is not well defined. They use H_2O_2 for the oxidation of a variety of phenols and aromatic amines. The peroxidase from horse radish has been crystallized and shown to be a hematin compound. Catalase, which converts hydrogen peroxide to water and O_2, and has peroxidatic activity, likewise is a hematin enzyme widely distributed in plants. Hemoglobin, cytochrome *c*, peroxidase, and catalase each has hematin as its prosthetic group.

Many organic acids are relatively abundant in plants, and they serve as substrates for their respective dehydrogenases. Malic, citric, and oxalic acids are quantitatively the most important plant acids. Among these, malic acid is a particularly active metabolite; citric acid is intermediate in activity; and oxalic acid is rather inert. Glycolic acid and lactic acid are rapidly oxidized in plants by glycolic and lactic acid dehydrogenases.

The formation, interconversion, and oxidation of organic acids in plants are competing processes which give rise to spectacular changes in the organic acid levels from day to day. For example, the succulent plants accumulate high concentrations of organic acids at night and convert them to starch during the day (isocitric acid, a rare acid, may constitute 18 per cent of the dry weight of the leaves of the succulent plant, *Bryophyllum calycinum*). In the dark, a large share of the malic acid of tobacco leaves is converted to citric acid.

Glycolysis in plants and animals yields pyruvic acid, which may be reduced to lactic acid or oxidized by aerobic processes. The oxidation of pyruvate *via* the Kreb's tricarboxylic acid cycle has been described for animal tissue in Chap. 13. Although information is much less complete for plants, there is good evidence that oxidation can occur by the same pathway outlined for animal tissue. Early work depended upon evidence that in the presence of inhibitors, such as malonate, intermediates in the tricarboxylic acid cycle accumulated. Now it has been possible to show that preparations of washed mitochondria [1] from mung bean and lupine seedlings can oxidize all the intermediates of the tricarboxylic acid cycle and can use some of the energy so obtained for converting ADP to ATP.

[1] Mitochondria are small, discrete particles existing within cells which can be separated from the rest of the cell contents by breaking the cell walls mechanically, suspending the mixture in a suitable liquid medium (*e.g.*, 0.4*M* sucrose), and centrifuging. The mitochondria settle out only after a certain centrifugal force has been reached. Larger particles settle first and are discarded, and smaller particles remain in suspension. Washing is accomplished by resuspending in fresh liquid and recentrifuging.

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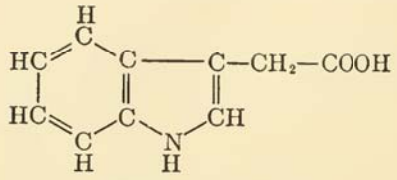
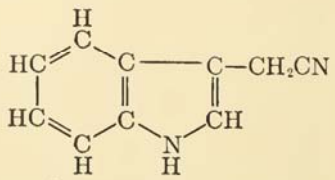
PLANT GROWTH SUBSTANCES

The growth of plants is regulated by a variety of chemical entities included under the broad heading, plant growth substances.[1] These compounds by being transported within the plant may effect changes at a distance from their point of formation. The most studied and versatile, naturally occurring plant growth substance is 3-*indoleacetic acid:*



3-Indoleacetic acid



3-Indoleacetonitrile

This compound, variously known as *β-indoleacetic acid* (IAA) and *hetero-auxin,* and the structurally similar 3-indoleacetonitrile, have been isolated directly from plants, as has traumatic acid ($HOOC—CH{=}CH—(CH_2)_8—COOH$), but evidence for most of the other growth substances reported to occur in plants lacks substantial chemical verification. Indoleacetic acid at a concentration of 0.01 mg. per liter will cause oat coleoptiles[2] to bend and will increase streaming of their protoplasm; 2.0 mg. per liter will inhibit the growth of oat roots by 50 per cent and will stimulate the respiration of oat seedlings; higher concentrations will inhibit both respiration and stem growth.

In addition to indoleacetic acid, many chemically synthesized substances will influence the growth of plants. For a compound to be effective it must as a rule contain an unsaturated ring and a side chain on the ring carrying a carboxyl group, or a group readily converted to a carboxyl; the carboxyl must be at least one carbon atom removed from the ring and must have a proper spatial relationship with the ring.

To the activity of growth substances are attributed such responses of plants as *phototropism* (bending toward light), *geotropism* (bending of roots toward and stems away from gravity), bending resulting from injury (traumatic acid is active), initiation of flowering, and *epinasty* (downward bending of leaves without wilting). Plant growth substances have been used in a large number of practical applications. They are employed to prevent premature dropping of blossoms and fruits. The

[1] "Plant growth substances" is a broad term covering the compounds often referred to as *plant hormones, phytohormones, growth regulating substances,* and *auxins.*

[2] The sheath around the first leaf sent out by the germinating oat seed.

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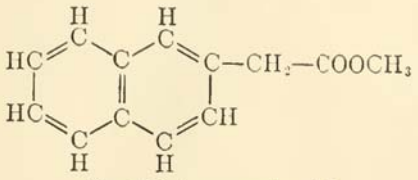
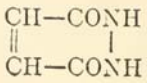
methyl ester of *naphthalene acetic acid* and *maleic hydrazide* will keep stored potatoes and carrots from sprouting.



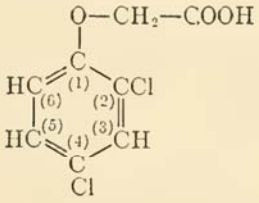
Naphthalene acetic acid, methyl ester



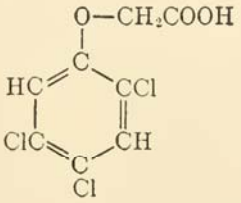
Maleic hydrazide

Growth substances will hasten the rooting of cuttings (Fig. 15–8) and will inhibit the development of buds. By application of growth substances to flowers, seedless fruits can be produced.

The most important use of plant growth substances is as *herbicides*, or *weed killers*, and this application has greatly modified current agricultural practice. General herbicides such as oils or acids have been employed for many years, but the plant growth substances have the great advantage of acting as *selective* herbicides. For example, much lower concentrations of 2,4-*dichlorophenoxyacetic acid* ("2,4-D") are required to



"2,4-D"
2,4-Dichlorophenoxyacetic acid



"2,4,5-T"
2,4,5-Trichlorophenoxyacetic acid

kill dicotyledonous plants (most broad-leafed plants such as beans and clover) than to kill monocotyledonous ones (cereals, other grasses, lilies, etc.). Thus broad-leafed weeds can be destroyed in a field of grain without damaging the grain. The closely related substance "2,4,5-T," especially in the form of an ester dissolved in an oily carrier such as kerosene, is very effective for killing weedy brush. Both compounds are very useful for the eradication of such weeds as poison ivy. The way in which selective herbicides act is still obscure.

MINERAL NUTRITION

The essential elements for the nutrition of plants include the macronutrient elements: C, H, O, N, P, K, S, Mg, Ca, and Fe; and the micronutrients: Cu, Zn, B, Mn, and Mo. As was pointed out in the discussion

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Courtesy of Dr. W. C. Cooper and the American Society of Plant Physiologists.

Fig. 15–8. Effect of indoleacetic acid on the rooting of lemon cuttings. Upper, untreated. Lower, treated for eight hours with a solution of 0.5 mg. of indoleacetic acid per ml. Photograph taken two and one-half weeks after treatment.

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Reproduced from *Hunger Signs in Crops*, a publication of the American Society of Agronomy and the National Fertilizer Association, Washington, D. C.

Fig. 15–9. Tobacco plants suffering from various mineral deficiencies—*B*, nitrogen; *C*, phosphorus; *D*, potassium; *E*, boron; *F*, calcium; *G*, magnesium. Reduction of growth has occurred in all cases. *A* is a normal plant.

of the general function of mineral elements in Chap. 8, animals require all of these elements except boron.

The cells of the root have a capacity for selective intake of ions. For example, from a solution high in sodium and low in potassium, the plant may absorb much more potassium than sodium. From soil the plant obtains its cations, such as Ca^{++}, by base exchange (that is, by a process by which the plant exchanges metabolic hydrogen ions for a cation *adsorbed* on a soil colloid). In contrast, the plant *absorbs* its anions, such as $SO_4^{=}$, from the soil solution around the roots.

Some effects of certain mineral deficiencies are illustrated in Figs. 15–9 and 15–10.

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Courtesy of Purdue University Agricultural Experiment Station. Reproduced from *Hunger Signs in Crops*, a publication of the American Society of Agronomy and the National Fertilizer Association, Washington, D. C.

Fig. 15–10. Oats in Crosby silt loam of low fertility. Pot 16 (NPK), treated with complete fertilizer, serves as check; plants healthy and vigorous. Pot 13 (PK), nitrogen starvation; plants spindling, yellowish green, slightly purplish stems. Pot 14 (NK), phosphorus starvation; plants dark green, stems weak, slightly purplish tinged. Pot 15 (NP), potassium deficiency; dark-green, weak plants, with oldest leaves brown and tip ends deadened.

REVIEW QUESTIONS ON PLANT METABOLISM

1. What is the prime source of most of the energy used by man?
2. Which process is of greatest importance in fixing carbon dioxide in the carbon cycle of nature; of greatest importance in releasing carbon dioxide?
3. How are photosynthesis and respiration interrelated?
4. What are the chemical differences between chlorophyll *a*, chlorophyll *b*, protochlorophyll, and bacteriochlorophyll?
5. What is the evidence that all oxygen released in photosynthesis arises from water?
6. Describe a light reaction and a dark reaction in photosynthesis. How can they be differentiated?
7. Why is a quantum efficiency of 4 attractive from a theoretical standpoint? Why is such a high efficiency questioned?
8. What is the first demonstrable organic intermediate in photosynthesis? Which products may it in turn yield?
9. How does the plant synthesize starch? What is the difference between amylose and amylopectin?
10. Do plants require any preformed amino acids?
11. Which are the most important sources of fixed nitrogen for maintaining the nitrogen cycle in balance?

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12. Which groups of organisms can fix N_2? What is the key intermediate in the fixation process?

13. What differences can you cite in the respiration of plants and animals? What similarities?

14. Why are plant growth substances often much superior to oils and acids as weed killers?

15. It is stated that growth substances are responsible for phototropism and geotropism. How can they cause such responses in plants?

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Chapter 16

BIOLOGICAL ENERGETICS

All living organisms must have a continuous supply of energy in a usable form. The study of energy sources, utilization, and quantitative requirements is called *biological energetics*. The great bulk of all food consumed goes to meet this need. In all cases the energy is derived from chemical reactions carried out by the living cell, whereby the foodstuffs are converted into products of lower energy content. The difference between the energy content of the foods eaten and waste products excreted represents approximately the energy which may be used (with greater or lesser efficiency) by the organism.

The chemical reactions on which living things depend for their energy supply are many and varied. Lower forms frequently live under anaerobic conditions, carrying out reactions which do not involve oxygen. Thus for example, glucose is converted into carbon dioxide and alcohol by yeast, or into lactic acid by lactic acid bacteria. Such conversions yield relatively little energy, and the yeast or bacteria accordingly are forced to metabolize large amounts of the foodstuff (here glucose). The higher animals and man, on the other hand, are aerobic organisms and oxidize their foodstuffs to the stage of carbon dioxide and water. Since this represents complete combustion, much larger amounts of energy are liberated, and less food per unit weight of living tissue is needed.

The energy used by living things appears partly in the form of heat, partly as muscular work, and partly in many other forms such as electrical, chemical, and light energy. It has become customary, however, to express all of these forms in terms of heat units, or calories. A calorie (cal.) is the amount of heat needed to raise the temperature of one gram of water one degree Centigrade, specifically from 14.5 to 15.5°C. The kilocalorie (Cal.) is one thousand times larger.[1] The energy difference between foods and waste products may be expressed quantitatively by means of these units. For example, the combustion of one mole (180 g.) of glucose gives 673,000 cal. The heat change accompanying a reaction is represented by the symbol, ΔH (Δ = change; H = heat). It is given

[1] The British Thermal Unit (BTU) is the amount of heat required to raise the temperature of one pound of water 1°F. One BTU equals 252 cal., or 1 Cal. equals very nearly 4 BTU. Another energy unit is the foot-pound, the amount of work done in lifting one pound through one foot. One calorie equals slightly more than three foot-pounds.

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a negative sign if heat is released when the reaction proceeds from left to right. For example, in the case above, ΔH is —673,000 cal.

The energy released from chemical processes which can be used for doing useful work is called the *free energy* change of the reaction and is represented by ΔF. For any particular process, ΔF may be either larger or smaller than ΔH. If ΔF happens to have a larger negative value than ΔH, more useful work can theoretically be obtained from the process concerned than corresponds to the amount of heat liberated. This rather surprising situation is caused by changes in *entropy*, which occur whenever chemical or physical transformations are carried out. The relation between the three quantities, for processes occurring at constant temperature and pressure—as is approximately true in biological systems—is given by the equation:

$$\Delta F = \Delta H - T\,\Delta S$$

where ΔS is the entropy change, and T the absolute temperature at which the process occurs. The entropy change, ΔS, is expressed in calories per degree. It is difficult to define exactly, but may be thought of as heat flowing into or out of the system from the surroundings while a process is going on. For a more complete discussion of entropy consult a textbook of physical chemistry or thermodynamics, such as those listed at the end of the chapter.

An illustration may help to make these abstract concepts more understandable. For the complete oxidation of glucose, ΔH is —673,000 cal. as stated above, whereas ΔF is about —683,000 cal. The exact ΔH and ΔF values depend on the state of the reactants and products, that is, whether solid, liquid, or gaseous, and at what concentration, temperature, pressure, etc. The value —683,000 cal. is based on approximately physiological concentrations, *viz.*, glucose 0.05*M*, carbon dioxide 0.1 atmosphere, oxygen 0.01 atmosphere, and on a temperature of 37°C. Useful work equivalent to 683,000 cal., therefore, could be obtained from burning one mole of glucose, if the machine or living tissue doing the work was able to operate with 100 per cent efficiency. Actual machines and tissues, of course, never reach this peak of perfection, but the efficiency which they do achieve may be determined by comparison with the free energy change of the process being used.

The ΔF value is also an indication of whether a particular chemical reaction can take place (if properly started) and to what extent it will proceed. Reactions which release free energy (ΔF negative) occur readily and are called *exergonic*. The opposite type, called *endergonic*, will not take place unless energy is supplied (ΔF positive). The change in free energy is the driving force of the reaction, and the larger the amount of free energy release, the more complete the reaction will be.

The completeness of equilibrium reactions is represented mathematically

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by the *equilibrium constant*, K. For a reaction of the type $A + B \rightleftarrows C + D$, K equals $[C] \times [D]$ divided by $[A] \times [B]$, where the brackets indicate molar concentrations at equilibrium (review the law of mass action in a textbook of general chemistry). Thus if K is one, the equilibrium point is reached when half of the starting materials have been converted into products, whereas if K is ten, the reaction is about 90 per cent complete.

The relation between ΔF and K is given by the equation,

$$-\Delta F = RT \ln K$$

where R is the gas constant (1.987 cal. per degree per mole), T the absolute temperature, and $\ln K$ the natural logarithm of K. Substituting numerical values and converting to ordinary logarithms, this equation becomes at body temperature (37°C):

$$-\Delta F = 1419 \log K$$

This equation enables one to calculate the equilibrium point of any reaction for which the free energy change is known, and vice versa. For example, if ΔF is zero, K is one, but if ΔF equals $-10{,}000$, K is 1.22×10^7. The latter value indicates an equilibrium point *very* far to the right.

Muscle contraction

The free energy available from oxidation of foodstuffs is not usable by animals and human beings in the form of heat. In other words, the body is not simply a heat engine,[1] although it is an engine or a machine in the sense that it converts energy from one form into others. Some heat of course is needed for warmth, but except under the most severe conditions of cold and exposure, more than enough heat for this purpose is always available. Heat production is primarily a consequence of the fact that the body, like other machines, operates at much less than 100 per cent efficiency. In fact, excess heat is an important waste product which must be eliminated to maintain health and vigor.

The question therefore arises as to just how the body does convert the chemical energy of foodstuffs into muscular work and other useful processes. A partial answer to this question has been obtained from studying the chemistry of muscle contraction. It was discovered around 1930 that muscle contains two substances which are readily hydrolyzed with the liberation of large amounts of energy. These compounds are adenosine triphosphate (ATP, p. 158) and creatine phosphate (CrP, p. 349). The latter was observed to decrease in amount as contraction occurred and to be completely used up if the muscle was stimulated to

[1] A heat engine is a machine which operates by virtue of temperature and pressure changes.

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exhaustion. Thus it appeared quite possible that the breakdown of CrP into free creatine and phosphoric acid might furnish the energy for contraction. However, it was demonstrated by Lohmann in 1934 that muscle contains no enzyme capable of catalyzing the direct hydrolysis of CrP, but that the substance is used up instead by reacting with adenosine diphosphate (ADP):

$$\mathrm{CrP} + \mathrm{ADP} \rightleftarrows \mathrm{Cr} + \mathrm{ATP}$$

The Lohmann reaction, as this is called, has a small ΔF and can therefore proceed readily in either direction, depending on changes in pH and concentrations of reactants. It obviously will go to the right and consume CrP only when ADP is available. ADP is present only in very small amounts in resting muscle, but can be formed from ATP by hydrolysis:

$$\mathrm{ATP} + \mathrm{H_2O} \xrightarrow{\text{ATP-ase}} \mathrm{ADP} + \mathrm{H_3PO_4}$$

This breakdown of ATP into ADP is catalyzed by adenosine triphosphatase (ATP-ase) and has a ΔF value of $-11{,}500$ cal. From the above considerations it is evident that this breakdown of ATP must take place *before* CrP can be used up by the Lohmann reaction. ATP, therefore, is most probably the *immediate* energy source for muscle contraction.

Just how the chemical energy in ATP is converted into the mechanical energy of contraction is not well understood, but it is known that ATP-ase is present in muscle fibers in large amounts. In fact, ATP-ase probably makes up a part of the fiber, being itself a long, thread-like protein, molecules of which are arranged lengthwise along the fiber. When ATP breakdown occurs, some of the side-chain groups in these or other protein molecules in the muscle probably become altered in such a way that they have an attraction for other groups in the same molecules. This would cause a puckering and shortening of the molecules so affected, and consequently a contraction of the whole fiber.

High energy phosphate bonds

The energy released when the terminal phosphate group of ATP is split off ($\Delta F = -11{,}500$ cal.) must have been contained in the particular valence bond which held this group to the rest of the molecule. Numerous other phosphate derivatives are also involved in metabolic reactions (see Chap. 13). All are of the type $(\mathrm{HO})_2\mathrm{P}(=\mathrm{O})\text{—X}$, where X may be either an oxygen or nitrogen atom, which is attached in turn to another phosphate radical or to some organic structure. These phosphorus compounds have been found to fall roughly into two main groups according

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to the amount of free energy liberated on hydrolysis of the P—X bond. A number of more important ones are collected in Table 16–1. For the low energy group, ΔF is about —2000 to —4000 calories per mole, whereas for the high energy compounds it amounts to around —11,000 to —15,000

Table 16–1

Free energies of hydrolysis of some phosphoric acid derivatives *

Compound	ΔF, cal.	pH	Temp., °C
Glucose-1-phosphate	—4,750	8.5	38
Glucose-6-phosphate	—3,000	8.5	38
Fructose-6-phosphate	—3,350	8.5	38
Glycerol-1-phosphate	—2,200	8.5	38
ATP (terminal group)	—11,500	7.5	20
Acetyl phosphate	—14,500	6.3	37
Pyruvic acid enol phosphate	—15,900	?	20
Creatine phosphate	—13,000	7.7	20
Arginine phosphate	—11,800	7.7	20

* Reproduced from Avison and Hawkins, "The Role of Phosphoric Esters in Biological Reactions," *Quart. Rev.*, **5**, 171 (1951) by permission of the authors and the Chemical Society (London).

calories. The latter substances are said to contain a *high energy phosphate* bond, which is written as "~P". *It is only the chemical energy of such bonds which can be transformed directly into useful work by living organisms, and so far as known, only ATP serves as the immediate source of such energy, both for muscular work and for all other purposes. The metabolic breakdown of foodstuffs, so far as energy requirements are concerned, is a matter of generating high energy phosphate bonds and of synthesizing ATP.*

Phosphagens

The presence in CrP of a high energy bond, taken together with the facts already presented, indicates that CrP serves as a ~P storehouse in muscle. When contractions begin, ATP starts to be used. It would be quickly exhausted except for the Lohmann reaction, which starts functioning as soon as some ADP is formed. ATP is thereby resynthesized at the expense of CrP, and the ATP level is kept up until most of the CrP is used, and the muscle becomes exhausted. This situation is reached, however, only during very severe work, because during moderate exercise the metabolism of glycogen soon starts, and ~P compounds are produced as fast as needed (see below). When muscular work stops, glycogen breakdown continues for a time, ATP is resynthesized, and the Lohmann reaction goes into reverse until the normal amount of CrP characteristic of resting muscle is restored.

This arrangement gives the muscle a much greater supply of quickly

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available energy than the ATP alone could provide, since considerably larger amounts of CrP are present (Table 16–2). In view of its metabolic role, CrP has been called a *phosphagen.* Another phosphagen, *arginine phosphate,* takes the place of CrP in the muscles of most invertebrates.

Table 16–2

Relation of muscular activity to concentration of various substances in muscle

Substance	*Muscle species and type*	*Concentration † when muscle is: Resting*	*Fatigued*	*In rigor*
Adenosine triphosphate	Average mammal, striated	5.0	2.5–4.5*	0.005*
Adenosine triphosphate	Average mammal, cardiac	1.5		trace or none
Adenosine diphosphate	Average mammal, striated	0.005*	0.5–2.5*	5
Creatine phosphate	Average mammal, striated	20	10–15	trace or none
Creatine phosphate	Average mammal, cardiac	2		trace or none
Creatine phosphate	Rat, smooth	1		trace or none
Arginine phosphate	Crab, striated	32	18	trace or none
Lactic acid	Average mammal, striated	1.7	45	68
Inorganic phosphate	Average mammal, striated	0.1*	10	30

* Values estimated from probable ATP-ADP-inorganic P ratios as calculated from energy relations of aerobic and anaerobic metabolism (see M. J. Johnson, Chap. XII, in *Respiratory Enzymes,* by Lardy: Burgess Publishing Company, Minneapolis, 1949).
† Millimoles per kilogram, fresh weight.

Still another phosphagen of unknown composition has been detected in certain lower organisms. The amounts of phosphagens in various tissues are shown in Table 16–2. By far the largest concentrations are present in those muscles which are capable of the greatest work output (striated muscle).

Generation of high energy phosphate bonds

Glycolysis. The reactions of glycolysis, which result in the formation of high energy bonds, are now known in detail. They have been presented in Chap. 13 (Figs. 13–1 and 13–3, reactions 9 and 12). In all, four ~P bonds are so generated for each hexose unit, that is, four molecules of ADP are converted to ATP. However, two molecules of ATP are used up along the way (reactions I and 4) so that the "net yield" to the organism is two moles of ATP per mole of glucose metabolized to lactic acid. Since each mole of ATP gives up 11,500 cal. when used for work (*i.e.,* hydrolyzed to ADP), this figure represents an energy yield from glycolysis of 23,000 cal. per mole of glucose. The free energy change

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for the conversion of glucose to lactic acid is not known with certainty, but is probably close to 40,000 cal. per mole. If this figure is correct, the efficiency of glycolysis is nearly 60 per cent.

It is perhaps not realistic to discuss the energy relationships of glycolysis in terms of the conversion of glucose to lactic acid, since this acid represents only an offshoot from the main pathway of carbohydrate metabolism and is not produced at all except during severe work (review p. 328). Even then, it is reconverted to pyruvic acid during rest. However, if carbohydrate breakdown is to be divided for purposes of study into anaerobic and aerobic phases, the anaerobic part must be treated as ending with lactic acid, even though discussion of the aerobic phase begins with pyruvic acid. Allowance for the energy released in converting lactic acid to pyruvic will be made below.

Aerobic Metabolism. Complete combustion of glucose to carbon dioxide and water releases about 683,000 cal. under physiological conditions. From the above figures it is obvious that only a small fraction of this total appears during anaerobic glycolysis. Approximately 94 per cent of the energy of the glucose remains to be released through the operation of the citric acid cycle. It is of great interest to discover what portion of this remaining energy becomes fixed in a biologically usable form (presumably ATP), and to learn just how the reactions of the citric acid cycle result in the formation of the necessary ~P bonds. During the oxidation of one molecule of pyruvic acid by one "turn" of the cycle, 10 atoms of hydrogen are released (2 in each of five steps, namely, reactions 16, 20, 22, 23, and 25, Fig. 13–4). The energy from the whole cycle is actually produced by the combination of these hydrogen atoms with the oxygen of the inhaled air, and ~P bonds are evidently formed at the same time. Before discussing this subject in greater detail, it seems desirable to consider briefly the nature of oxidation and the quantitative relations between oxidation and energy changes.

Oxidation is often defined as addition of oxygen or removal of hydrogen, but cases are also common in which oxidation occurs without either oxygen or hydrogen being directly involved. The most exact and general definition states that oxidation is a loss of electrons. For example, $Fe^{++} \rightarrow Fe^{+++} + e$, where e stands for an electron, the unit negative charge of electricity. The tendency of substances to give up electrons and become oxidized is expressed in terms of volts as an electrical potential, called the *oxidation-reduction* or *redox potential.* Strong oxidizing agents have positive potentials ranging up to about +2 volts, while reducing agents go down to about —1 volt, and even lower in a few cases. These relations provide a scale of oxidizing power, much as the pH scale measures active acidity. When the oxidized and reduced forms of an oxidizing agent are in equal concentrations, that is, when the oxidizing agent is half reduced, its redox potential is called by defini-

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tion the *normal potential*, E_0. If a substance of high E_0 reacts with one of lower E_0, the potential of the former (the oxidizing agent) drops as more and more of it becomes changed to its reduced form, and the potential of the latter (the reducing agent) rises as it is converted into its oxidized form. Finally, the two potentials become equal and no further reaction occurs. The free energy released depends on ΔE, the difference between the two E_0 values. This relationship is given by the following equation:

$$-\Delta F = n\mathrm{F}\,\Delta E$$

where n is the number of electrons involved in the reaction, and F is the Faraday.[1]

These principles may now be used to explore the possibilities of ~P generation during the biological oxidation of metabolites. As explained above, the energy released comes from hydrogen atoms split off at various stages. Each pair of hydrogens passes through a system of coenzymes or carriers before finally being united with oxygen (review cytochrome system, p. 333). A series of oxidation-reduction systems is therefore involved, each having its own characteristic E_0 value. The biological oxidation, for example, of lactic to pyruvic acid probably involves the various coenzymes and redox potentials shown in Fig. 16–1.

The two hydrogens are split off at a potential of —0.18 volt and, at first, combine with DPN:

$$\mathrm{CH_3CHOHCOOH} + \mathrm{DPN} \rightleftarrows \mathrm{CH_3COCOOH} + \mathrm{DPN \cdot H_2}$$

Since the ΔF of this reaction is +4600 cal., the equilibrium point lies far to the left, and the lactic acid will not be oxidized unless the $\mathrm{DPN \cdot H_2}$ is removed. If the amount of $\mathrm{DPN \cdot H_2}$ is in some manner kept very low, the equilibrium point is displaced to the right in accordance with the law of mass action. Of course, the hydrogens are, in fact, immediately transferred from the $\mathrm{DPN \cdot H_2}$ through the remaining steps shown in Fig. 16–1, and for each of these steps ΔF has a large negative value. This means that their equilibrium points lie far to the right, and the hydrogens are therefore pulled along until they unite with oxygen. They have then traveled over a potential span of 0.99 volt (—0.18 to +0.81), an interval which corresponds to a free energy change of —45,700 cal.[2] This energy, however, is not released in a single burst, but in three successive smaller portions, as shown in Fig. 16–1. One of these is near 10,000 cal., about the amount needed for a ~P bond, while the others are

[1] In any chemical process associated with electron transfer, a certain definite quantity of electricity is always needed to bring about the transformation of one gram equivalent weight of the reacting substance (examples, electrolysis of water, electroplating of metals). This quantity, the Faraday, is 96,500 coulombs, which is equivalent to 23,060 calories per volt.

[2] Calculation: $-\Delta F = n\mathrm{F}\,\Delta E = 2 \times 23{,}060 \times 0.99 = 45{,}700$.

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considerably larger. Therefore, approximately the right amount of energy is made available in about the right sized "packages" for the generation of three high energy bonds, when one mole of lactic acid is oxidized to pyruvic acid and water.

Overall reaction

$$\underset{\text{Lactic acid}}{CH_3CHOHCOOH} + \tfrac{1}{2}O_2 \rightleftharpoons \underset{\text{Pyruvic acid}}{CH_3COCOOH} + H_2O$$

Intermediate stages	*Corresponding redox potentials and energy changes* E_0 *volts*	ΔE *volts*	ΔF *cal.*
1. Lactic acid $\rightleftharpoons$ pyruvic acid + 2H	−0.18	−0.10	+4,600
2. DPN + 2H $\rightleftharpoons$ DPN H + H^+	−0.28	+0.22	−10,100
3. FAD + 2H $\rightleftharpoons$ FAD·H_2	−0.06	+0.32	−14,700
4. 2Cyt. *c* Fe^{+++} + 2H $\rightleftharpoons$ 2Cyt. *c* Fe^{++} + $2H^+$	+0.26	+0.55	−25,400
5. $\frac{1}{2}O_2$ + 2H $\rightleftharpoons$ H_2O	+0.81		

Fig. 16–1. Intermediate stages and energy relationships in the biological oxidation of lactic to pyruvic acid.

The five pairs of hydrogens that split out when pyruvic acid is completely oxidized by the citric acid cycle likewise pass through the hydrogen transport system. Each pair originates at a definite redox potential, which is given in Table 16–3. Calculations of the energy released as each pair becomes united with oxygen indicate that sufficient energy should be available to generate the number of ~P bonds shown in the last column. The total is 16 such bonds per mole of pyruvic acid. Since three more could have been formed in the conversion of lactic into pyruvic acid, the total for the aerobic phase of carbohydrate metabolism would be $2 \times (16 + 3)$ or 38 per mole of glucose. It must be emphasized that these figures are only estimates based on the information now available.

Table 16–3

Redox potentials at which hydrogen is released during oxidation of pyruvic acid via the citric acid cycle

Acid dehydrogenated	*Acid produced*	*Corresponding* E_0 *value *at* pH 7	*Number of* ~P *bonds possibly formed*
Pyruvic	Acetic	−0.63	4
Isocitric	Oxalosuccinic	−0.13	3
α-Ketoglutaric	Succinic	−0.60	4
Succinic	Fumaric	0.00	2
Malic	Oxalacetic	−0.10	3
		Total:	16

* Volts.

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Esterification of Inorganic Phosphate. Another line of evidence has been uncovered which bears on this question of the number of ~P bonds formed during the aerobic phase of carbohydrate metabolism. Ground-up preparations from tissues such as liver and kidney are able to take up molecular oxygen and use it to oxidize pyruvic acid, or any other acid involved in the citric acid cycle, to carbon dioxide and water. Inorganic phosphate is needed for this oxidation, and as the oxidation proceeds, some of the phosphorus becomes esterified, that is, united with organic substances. Lehninger has demonstrated with the aid of the isotope, P^{32}, that the newly formed organic phosphate has the properties of ATP. It is very probable that one molecule of ATP is produced for every atom of phosphorus esterified during the oxidation. The number of P atoms taken up for each atom of oxygen used is difficult to measure accurately because of side reactions which break down the new ~P bonds even as others are being formed. The best results, however, show values approaching those given in the last column of Table 16–3. This evidence, then, also tends to indicate that phosphorylations occur and ~P bonds are formed each time hydrogen atoms, from whatever source, are passed from one hydrogen carrier to the next. *In fact, this hydrogen transport system is almost certainly the chief energy transformer of aerobic organisms.*

Efficiency of Energy Metabolism. The above discussion deliberately goes somewhat beyond the bounds of present well-established knowledge in order to estimate the efficiency of energy metabolism in animals. If 38 ~P bonds are produced during the conversion of lactic acid to carbon dioxide and water and two more are produced during glycolysis, a total of 40 moles of ATP could be formed from the metabolism of one mole of glucose. If these figures are correct, the efficiency of the overall process would be

$$\frac{40 \times 11{,}500}{683{,}000} = 67\%$$

This is a very high value in comparison with other types of machines. The maximum efficiency of a steam engine, for example, is around 25 per cent and that of a diesel engine about 40 per cent. As a matter of fact, direct work measurements show that animals also have maximum efficiencies of about 30–40 per cent, but usually work at only about 15–20 per cent efficiency. This is not surprising since the above value of 67 per cent applies only to ATP *formation.* No information is available regarding the efficiency with which the chemical energy of ATP can be converted into muscular work by the animal.

Physiological Fuel Value of Foods. Until quite recently the study of energy metabolism in animals and man was conducted almost exclusively from the standpoint of the total heat produced by combustion of various foods and the total energy needs of metabolism under various conditions. Although this sort of knowledge does not provide as much

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insight into energy metabolism as the direct chemical approach described above, it nevertheless has important practical applications.

The heat of combustion of a food is determined by use of the *bomb calorimeter*. This is a heavy steel cylinder or "bomb" surrounded by water in an insulated container. The sample plus oxygen at high pressure is placed in the tightly closed cylinder and ignited by a spark. The amount of heat liberated as the sample burns is determined by noting the exact rise in the temperature of the water and making suitable corrections for heat taken up by the bomb itself. The results are expressed as calories liberated per mole or per gram of substance burned.

When pure chemicals are examined in this way, the heats of combustion are found to vary according to the composition of the sample. The figures given in Table 16–4 show that higher percentages of carbon and hydrogen are associated with higher heat values, whereas the opposite is true for oxygen. The reason for this, of course, is simply that substances like glucose with a high oxygen content are in effect already partly oxidized.

The fuel values of foods likewise depend on their elementary composition, but information of this sort is not usually available. Instead, foods are usually analyzed for their contents of carbohydrate, fat, protein, minerals, and moisture. These percentages constitute the *proximate composition*. The heat value of a given food can be calculated easily from its proximate composition, if the heat contributed by each of the major components is known. Minerals and moisture, of course, contribute nothing in this regard. Carbohydrates, proteins, and fats in the bomb calorimeter give about 4.1, 5.7, and 9.5 Cal. per gram, respectively (see Table 16–4). However, not all of this energy is available to the animal body partly because foods are not completely digested and absorbed and partly because they are not always oxidized completely in the body. Proteins in particular are oxidized to carbon dioxide, water, and free nitrogen in the calorimeter, but in the body the nitrogen is converted into excretory products (urea, creatine, etc.), which are themselves organic substances capable of being burned, and releasing additional heat.

Table 16–4

Relation of chemical composition to heat of combustion of various substances

Substance	*Composition*				*Heat of combustion*
	C%	H%	O%	N%	(Cal. per gram)
Glucose	40.0	6.7	53.3		3.73
Sucrose	42.1	6.4	51.5		3.96
Starch	44.4	6.2	49.4		4.22
Alanine	40.4	7.9	35.9	15.7	4.35
Casein *	53.1	7.0	22.5	15.8	5.85
Stearic acid	76.0	12.8	11.2		9.53
Animal fats (av.)	76.5	12.0	11.5		9.50

* Also contains 0.8% S and 0.8% P.

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When allowance is made for these facts, the average *physiological* fuel value of carbohydrates, proteins, and fats becomes, in round numbers, 4, 4, and 9 Cal. per gram, respectively. Note that these values are expressed in kilocalories and that they refer to the total energy obtained by the organism, both in the form of heat and in the form of ATP or other ~P compounds. However, no allowance for entropy changes is included, and as explained previously these changes are likely to provide additional energy. The entropy changes involved in the metabolism of most foodstuffs are not known.

The physiological fuel values of a list of common foods are given in the Appendix (Table A-1, p. 434). A study of this list emphasizes the tremendous effect of two components, namely fat and moisture, on the calorific value. Watery foods like fresh fruits and vegetables contribute very few calories, whereas concentrated, dried foods, especially those high in fat such as nuts, chocolate, vegetable oils, etc., have very high energy value. Sherman has pointed out that an ounce of olive oil is equal in fuel value to over three pounds of lettuce!

Energy requirements

Basal Metabolism. The most conspicuous use for food energy is in the performance of muscular work. However, even at rest, energy is required by a living animal or human being to keep various vital functions in operation. These include, not only such obvious processes as breathing, heart action, and blood circulation, but also the maintenance of a certain minimum muscle tension or tonus (even when lying down and completely "relaxed") and the normal operation of the organs of digestion, secretion, and excretion. The kidneys, for example, use energy to excrete waste products, and energy is needed to cause digested foods to pass through the intestinal wall. Even the synthesis and maintenance of the protein molecules which make up body tissues require energy, since the equilibrium point of the reaction

$$\text{protein} + \text{water} \rightleftarrows \text{amino acids}$$

lies far to the right, and energy must be supplied to shift it to the left.

The minimum rate of energy metabolism to provide for such functions is called the *basal metabolism,* and the energy thereby consumed is considered to be the minimum energy requirement. The basal metabolism is measured about 12 hours after eating so that no digestion of food is taking place, and with the subject lying down in a room of comfortable temperature. Under these conditions all the energy being used by the body appears directly in the form of heat since no external work of any sort is being done.

The basal metabolism can be measured by direct calorimetry, that is,

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by placing the subject in a large calorimeter and determining the heat output as described above for the bomb calorimeter. This actually has been done in many cases, but is so cumbersome and expensive that a shorter, indirect method has been devised. This method depends on the fact that heat output is closely related to oxygen consumed and carbon dioxide liberated by the subject. Thus for the oxidation of glucose,

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 678 \text{ Cal.}$$

it is apparent that six moles of oxygen are used for the oxidation of one mole of glucose and the production of 678 Cal.[1] Six moles of a gas occupy 6×22.4 or 134.4 l. at standard temperature and pressure. Therefore each liter of oxygen used in this reaction results in the production of $678 \div 134.4$ or 5.047 Cal. For fat oxidation the relations are somewhat different. Taking tristearin as an example,

$$2(C_{17}H_{35}COO)_3C_3H_5 + 163O_2 \rightarrow 114CO_2 + 110H_2O + 17{,}060 \text{ Cal.}$$

it is seen that in this case one liter of oxygen produces $17{,}060 \div (163 \times 22.4)$ or 4.67 Cal. Oxidation of average mixed food fats yields 4.69 Cal. and of mixed proteins, 4.82 Cal. per liter of oxygen consumed.

An indication of which type of foodstuff is being oxidized by the body at a given time is provided by the respiratory quotient (R.Q.), which is the ratio of carbon dioxide given off to oxygen used:

$$\text{R.Q.} = \frac{CO_2 \text{ given off}}{O_2 \text{ used}}$$

The amounts of the two gasses may be expressed in moles or in volumes (measured at the same temperature and pressure). It follows from the above equations that the R.Q. for oxidation of glucose is 1.00 and of tristearin 0.70 $(114 \div 163)$. In general, the R.Q. for average mixed carbohydrates is 1.00, for fats 0.71, and for proteins 0.81. It has been found that the R.Q. for both animal and human subjects under the conditions of the basal metabolism test is close to 0.82. For this R.Q. value each liter of oxygen consumed produces 4.825 Cal.[2] All that is necessary, therefore, to find the metabolic rate is to measure the liters of oxygen consumed per unit time and multiply by 4.825.

The basal metabolism is a fundamental characteristic of the living animal. Its magnitude depends on the body weight, body surface area, sex, age, and other factors. However, for healthy individuals of a given

[1] The current values of heat output per liter of oxygen are based on 678 Cal. as the heat of combustion of glucose, although 673 Cal. probably is more nearly correct.

[2] Calculated on the assumption that only fats and carbohydrates are being oxidized. The amount of protein oxidized, which can be estimated from the urinary nitrogen excretion, does not have to be considered since proteins also yield 4.82 Cal. per liter of oxygen.

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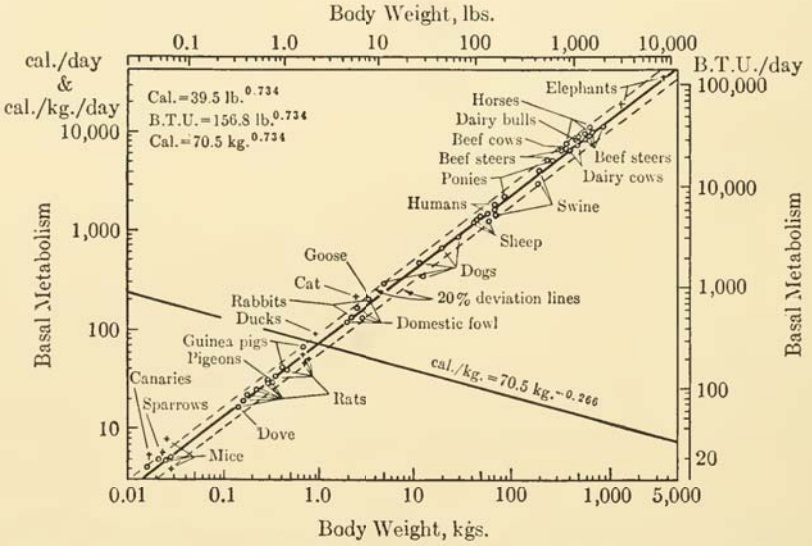
size and age there is a normal rate of basal metabolism, and any marked deviations therefrom indicate an abnormal, perhaps diseased, condition. Brody has emphasized the relation between the body size and basal metabolism of a large number of animal species. He found that the formula

$$\text{Cal. per day} = 70 \times (\text{body weight in kg.})^{0.73}$$

holds very well for a tremendous range of body sizes (Fig. 16–2). Note that the smallest animals have the highest basal metabolism per kilogram



From Brody, *Bioenergetics and Growth*, Reinhold Publishing Corporation.

Fig. 16–2. Relation of body weight to basal metabolism of mature animals of various species. The rising curve, showing basal metabolism per animal per day, is represented very well by the equation, Cal. per day = 70.5 × (body weight in kg.) $^{0.734}$. However, from the nature of the data the numerical values 0.734 and 70.5 are somewhat doubtful and probably should be rounded off to 0.73 and 70, respectively. The declining curve, showing metabolism per kg. of body weight per day, similarly corresponds to the equation, Cal. per kg. body weight = 70.5 × (body weight in kg.) $^{-0.266}$.

of body weight, although the largest animals, of course, have the highest total metabolism. More detailed formulas have been worked out for human beings, which take account of age, sex, height, and body surface area, as well as weight. For example, a 30 year old man who is 175 cm. (5 ft. 9 in.) tall and weighs 70 kg. (156 lb.) should have a basal metabolic rate (BMR) close to 1630 Cal. per day.[1]

Total Energy Requirements of Human Beings. The amount of food

[1] Calculated from Harris and Benedict's formula (see Sherman, *Chemistry of Food and Nutrition*, 7th ed., p. 164).

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needed above the basic metabolic level depends almost exclusively on muscular activity. Representative figures for various types of activity [1] are given in Table 16–5. An approximate idea of the total daily energy

Table 16–5

Total energy expenditure under different conditions of muscular activity *

Form of activity	*Calories per hour* Per 70 kg. (Av. Man)	Per kg.	Per lb.
Sleeping	65	0.93	0.43
Awake, lying still	77	1.10	0.50
Sitting at rest	100	1.43	0.65
Standing relaxed	105	1.50	0.69
Dressing and undressing	118	1.69	0.77
Typewriting rapidly	140	2.00	0.91
Walking, 2.6 mph.	200	2.86	1.30
Carpentry work	240	3.43	1.56
Walking, 3.75 mph.	300	4.28	1.95
Sawing wood	480	6.86	3.12
Swimming	500	7.14	3.25
Running, 5.3 mph.	570	8.14	3.70
Walking upstairs	1100	15.8	7.18

* Reproduced by permission from Sherman, *Chemistry of Food and Nutrition*, 7th ed., The Macmillan Company, New York, 1946, p. 189.

requirement might be obtained from these figures by estimating the time spent in various ways. However, more accurate estimates can be made by other methods. If, for example, an exact record of the total food intake of an individual is kept for a period of several months, and the body weight does not change appreciably during that time, the number of calories consumed per day must have been very nearly equal to the energy requirement. Another method is to measure oxygen consumption while the subject is engaged in various activities. Recommended allowances based on these and other types of measurements have been made by the Food and Nutrition Board of the National Research Council and are listed in Table 16–6. Note the relatively high allowances for growing children and the increased requirements caused by pregnancy and lactation and by heavy muscular work.

Obesity. The problem of obesity, or excessive fatness, is unfortunately a very common and serious one in many of the more prosperous, industrialized countries. The implications for health, longevity, and general well-being are too well known to require comment. However, the basic facts regarding control of body weight are apparently not well understood by many people.

[1] Note that the values in Table 16–5 are for *total* energy expenditures under various conditions, *i.e.*, they *include* the basal energy requirement.

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Table 16–6

Recommended daily calorie allowances *

	Calories
Men (154 lb., 70 kg.):	
Sedentary	2,400
Physically active	3,000
With heavy work	4,500
Women (123 lb., 56 kg.):	
Sedentary	2,000
Moderately active	2,400
Very active	3,000
Pregnancy (latter half)	2,400
Lactation	3,000
Children:	
Under 1 yr.	110/2.2 lb. (1 kg.)
1–3 yrs. (27 lb., 12 kg.)	1,200
4–6 yrs. (42 lb., 19 kg.)	1,600
7–9 yrs. (58 lb., 26 kg.)	2,000
10–12 yrs. (78 lb., 35 kg.)	2,500
Girls, 13–15 yrs. (108 lb.)	2,600
Girls, 16–20 yrs. (122 lb.)	2,400
Boys, 13–15 yrs. (108 lb.)	3,200
Boys, 16–20 yrs. (141 lb.)	3,800

* "Calorie allowances must be adjusted up or down to meet specific needs. The calorie values in the table are, therefore, not applicable to all individuals but, rather, represent group averages. The proper calorie allowance is that which over an extended period will maintain body weight or rate of growth at the level most conducive to well-being."

There is only one possible cause of obesity, namely, a greater average calorie intake over extended periods of time than is balanced by energy expenditures. No matter what the other circumstances may be, if the body weight is increasing (beyond the bounds of normal growth), the food intake *must* be excessive. Frequently this simple and obvious fact is either disbelieved or ignored. Perhaps one reason for this is that the relation between the calories eaten and the amount of work needed to use them up is not clearly appreciated. Some specific examples may help to emphasize this relationship. Suppose a young woman has a light snack, consisting of one ounce of shelled peanuts, in addition to regular meals sufficient for normal energy requirements. One ounce of peanuts has a physiological fuel value of about 180 Cal.[1] (Appendix, Table A-1), which is equivalent to slightly over 540,000 foot-pounds of work.[2] Assuming that this energy is converted into work with 20 per cent efficiency, a 56 kg. (123 lb.) woman would have to climb a vertical

[1] Most of the popular five-cent candy bars also contribute about this same number of calories.

[2] One Cal. equals slightly more than 3000 foot-pounds.

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distance of $(540{,}000 \times 0.20) \div 123$, or 878 feet in order to use it completely. If this very considerable exertion, or its equivalent in other work, is not made, the extra calories provided by the snack will inevitably go to form fat.

As another example, suppose a person wishes to exercise enough to remove one pound of fat. The fat tissues of the body contain about one-fifth moisture and four-fifths actual fat. One pound of such tissue, therefore, represents $454 \times 0.8 \times 9$, or 4080 Cal. According to Table 16–5, walking at the rate of 3.75 miles per hour involves a total energy expenditure of 300 Cal. per hour for a 70 kg. man. Therefore, the man would have to walk 13.6 hours $(4080 \div 300)$, or a distance of 51 miles (13.6×3.75) to "burn off" the pound of fat. Of course, considerably more weight might be lost in the form of perspiration, but this weight would be quickly regained through increased water intake. These examples show that it is very difficult, and usually impractical, to counteract excessive food intake by increased muscular activity.

Another source of confusion is the fact that some individuals gain weight on a food intake which would not be excessive for others. This may be due to differences in the efficiency with which the food is digested and absorbed, or to differences in basal energy requirements. Other things being equal, older people need fewer calories than younger ones, and a short stocky person probably has a lower basal metabolism than a tall gangling one.

The solution to the problem of overweight is very simple in theory, but, of course, in practice it is complicated by the appetite, which unfortunately is not an accurate guide to the energy requirements of the body. However, only a slight reduction of the calorie intake, if persistently continued, is capable of restoring normal weight. If as calculated above, one pound of fatty tissue corresponds to 4080 Cal., a reduction of only 100 Cal. a day would amount to the equivalent of 8 pounds a year. Adjustments of this magnitude can usually be made merely by intelligent food selection. The principles to be observed are to avoid fat insofar as possible and to increase the consumption of high protein foods such as fish, poultry, and lean meat. A high protein intake satisfies hunger with the consumption of relatively few calories. Bulky foods like fresh vegetables are also indicated. Severe dieting or use of drugs for reducing are at best undesirable and often actually dangerous to health. The most satisfactory procedure is a rearrangement of one's eating habits such that a gradual but steady weight reduction occurs.

REVIEW QUESTIONS ON BIOLOGICAL ENERGETICS

1. What is a calorie; a foot-pound? How many feet would a 150 lb. weight have to be raised to use up energy equivalent to 500 calories?
2. If the heat of combustion of ethyl alcohol is 326 Cal., how many calories would

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be provided by the alcohol in a glass of beer (assume 250 ml. per glass and 4 per cent alcohol in the beer)?

3. What is meant by a high energy phosphate bond? Name three substances that contain high energy and three that contain low energy phosphate bonds.

4. Which type of energy is used as the immediate source of energy for performing biological processes of various kinds? How does the animal body differ from a heat engine in its functioning?

5. List two reactions of glycolysis in which ~P bonds are formed. Which parts of the aerobic oxidation process in the body are probably mainly responsible for the liberation of energy and the formation of ~P bonds?

6. What is the Lohmann reaction? Explain the importance of this reaction for muscle contraction.

7. What is free energy, and how is it related to the heat of a chemical reaction? What is the relation between the free energy change of a reaction and the extent to which the reaction proceeds.

8. Define basal metabolism, and explain the general relationship between the basal metabolic rates of various animals.

9. What is there about the chemical nature of fats, proteins, and carbohydrates, and about the way they are metabolized in the body, which accounts for the difference in their physiological energy values?

10. Calculate the number of calories provided by a light lunch consisting of a glass of milk (250 ml.) and a sandwich made up of the following ingredients:

white bread	56.2 g.
lettuce	32.7 g.
butter	8.0 g.
oil dressing	2.1 g.
American cheese	50.7 g.

11. About how far could a person run by using the energy from the lunch itemized in Question 10?

12. What is the respiratory quotient? Is it possible to have an R.Q. greater than 1.0? Explain.

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Appendix

COMPOSITION AND ENERGY VALUE OF FOODS

The four tables making up this appendix are revisions of the tables contained in the authors' previous book, *Elements of Food Biochemistry*. The new material in these tables is taken largely from Watt and Merrill, *Composition of Foods*, U.S.D.A. Agricultural Handbook No. 8, 1950. A small number of new entries come from Sherman, *Chemistry of Food and Nutrition*, 7th ed., McCanse and Widdowson, *The Composition of Foods*, and Morrison, *Feeds and Feeding*, 21st ed. The tables include data for most of our raw food materials and for representative canned foods. By comparing these data an idea can be obtained of the differences in composition resulting from commercial canning operations. More comprehensive data on canned and cooked foods are contained in Watt and Merrill and in McCanse and Widdowson. No data on farm roughages have been included because of space limitations. Such data are found in Morrison.

In using these tables the student should bear in mind that the composition of any given sample that may be analyzed does not necessarily correspond to the figures recorded here. Some variations are found in the proximate composition of different samples, but greater variations occur in figures for the mineral and vitamin contents. Differences as great as 100 per cent may be encountered among a series of plant samples. These differences are due chiefly to soil and climate conditions, but they are also related to varieties and maturity of the plant. From the dietary viewpoint, such variations are not serious because variations in different foods cancel each other. The actual intake during a period of time will probably correspond closely to that calculated from the figures of the table.

Places marked with a question mark in Tables A-2 and A-3 indicate that no quantitative data have been as yet found. A fair idea of the probable amount present may be obtained by noting the figures given for a similar material. For example, the magnesium and other figures missing for buckwheat are probably in the same range as those given for another cereal, say, barley or rye. From a nutritional viewpoint, the important mineral elements are calcium, phosphorus, and iron, and these elements are given for all the foods in these tables. Values for sodium and chlorine are omitted as indicated by (a), where a considerable amount of sodium chloride is added in making the product, such as butter.

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Table A-1

Proximate composition of foods

(Edible portion)

Food	*Moisture* %	*Ash* %	*Protein* %	*Fat* %	*Carbohydrate* *Total** %	*Carbohydrate* *Available†* %	*Food energy* (Cal. per 100 g.)
Almonds	4.7	3.0	18.6	54.1	19.6	16.9	597
Apples, fresh	84.1	0.3	0.3	0.4	14.9	13.9	58
dried	23.0	1.4	1.4	1.0	73.2	69.3	277
Apricots, fresh	85.4	0.6	1.0	0.1	12.9	12.3	51
dried	24.0	3.5	5.2	0.4	66.9	63.7	262
canned, sirup	77.3	0.6	0.6	0.1	21.4	21.0	80
Artichokes	83.7	1.1	2.9	0.4	11.9	8.7	63
Asparagus, fresh	93.0	0.7	2.2	0.2	3.9	3.2	21
canned	93.6	1.3	1.9	0.3	2.9	2.4	18
Avocados	65.4	1.4	1.7	26.4	5.1	3.3	245
Bacon, medium fat	20.0	4.3	9.1	65.0	(1.1)	1.1	630
Bananas	74.8	0.8	1.2	0.2	23.0	22.4	88
Barley, grain	10.2	2.1	12.8	2.1	72.8	70.1	356
pearled	11.1	0.9	8.2	1.0	78.8	78.3	349
Beans, Lima, dried	12.6	3.8	20.7	1.3	61.6	57.3	333
fresh	66.5	1.7	7.5	0.8	23.5	22.0	128
navy, dried	10.5	3.9	22.0	1.5	62.1	58.2	338
string, fresh	88.9	0.8	2.4	0.2	7.7	6.3	35
baked, canned	70.0	2.0	5.8	3.0	19.2	18.3	125
green, canned	93.5	1.2	1.0	0.1	4.2	3.6	18
Beef, medium fat	60.0	0.9	17.5	22.0	0	0	273
chuck, medium fat	65.0	0.9	18.6	16.0	0	0	224
sirloin, medium fat	62.0	0.9	17.3	20.0	0	0	254
rib, medium fat	59.0	0.8	17.4	23.0	0	0	282
round, medium fat	69.0	1.0	19.5	11.0	0	0	182
corned, medium fat	54.2	5.0	15.8	25.0	0	0	293
Beets	87.6	1.1	1.6	0.1	9.6	8.7	42
Beet greens	90.4	1.7	2.0	0.3	5.6	4.2	27
Blackberries	84.8	0.5	1.2	1.0	12.5	8.3	57
Blueberries	83.4	0.3	0.6	0.6	15.1	13.9	61
Brains (all kinds)	78.8	1.4	10.4	8.6	0.8	0.8	125
Bran—*see* wheat							
Brazil nuts	5.3	3.4	14.4	65.9	11.0	8.9	646
Bread, rye	35.3	2.0	9.1	1.2	52.4	52.0	244
white, commercial	34.7	1.8	8.5	3.2	51.8	51.6	275
whole wheat	36.6	2.5	9.3	2.6	49.0	47.5	240
Broccoli	89.9	1.1	3.3	0.2	5.5	4.2	29
Brussels sprouts	84.9	1.3	4.4	0.5	8.9	7.6	47
Buckwheat, grain	12.0	1.9	10.3	2.3	73.5	62.8	356
Butter	15.5	2.5	0.6	81.0	0.4	0.4	716
Buttermilk (cult'd skim milk)	90.5	0.8	3.5	0.1	5.1	5.1	36
Butternuts	3.8	2.9	23.7	61.2	8.4	6.4	679
Cabbage	92.4	0.8	1.4	0.2	5.3	4.3	24
celery	95.4	0.7	1.2	0.3	2.4	1.9	14
Cantaloupe	94.0	0.6	0.6	0.2	4.6	4.0	20
Carrots	88.2	1.0	1.2	0.3	9.3	8.2	42
canned	92.2	0.9	0.5	0.4	6.1	5.5	28
Cashew nuts	3.6	2.7	18.5	48.2	27.0	25.7	578
Cauliflower	91.7	0.8	2.4	0.2	4.9	4.0	25
Celery	93.7	1.1	1.3	0.2	3.7	3.0	18
Chard, leaves	91.0	1.2	2.6	0.4	4.8	4.0	27
Cheese, cheddar, American	37.0	3.7	25.0	32.2	2.1	2.1	398
cottage, from skim milk	76.5	1.5	19.5	0.5	2.0	2.0	95
Swiss	39.0	3.8	27.5	28.0	1.7	1.7	370
Cherries	83.0	0.6	1.1	0.5	14.8	14.5	61

* Difference between 100 per cent and the sum of the percentages of water, ash, protein, and fat. Figures in parentheses were not obtained in this way.

† Carbohydrate exclusive of fiber.

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Table A-1 (Continued)

Proximate composition of foods

(Edible portion)

Food	Moisture %	Ash %	Protein %	Fat %	Carbohydrate Total* %	Carbohydrate Available† %	Food energy (Cal. per 100 g.)
Chicken, broilers	71.2	1.1	20.2	7.2	0	0	151
roasters	66.0	1.0	20.2	12.6	0	0	200
hens	55.9	1.1	18.0	25.0	0	0	302
Chocolate, bitter	2.3	3.2	5.5	52.9	29.2	26.6	501
sweetened	1.4	1.4	2.0	29.8	62.7	61.3	471
Clams	80.3	2.1	12.8	1.4	3.4	3.4	81
Cocoa, plain	3.9	5.0	8.0	23.8	48.9	44.3	293
Coconut, dried, shredded.	3.3	0.8	3.6	39.1	53.2	49.1	556
fresh	46.9	1.0	3.4	34.7	14.0	10.8	359
Collards	86.6	1.7	3.9	0.6	7.2	6.0	40
Corn, grain	11.0	1.3	10.0	4.3	73.4	71.3	372
popcorn, popped	4.0	1.6	12.7	5.0	76.7	74.5	386
sweet, fresh	73.9	0.7	3.7	1.2	20.5	19.7	92
canned	80.5	0.9	2.0	0.5	16.1	15.3	67
Corn flakes	3.6	2.9	8.1	0.4	85.0	84.4	385
Corn meal, whole	12.0	1.2	9.2	3.9	73.7	72.1	355
Cottonseed, whole	7.3	3.5	23.1	22.9	43.2	26.3	471
Cowpeas, fresh	65.9	1.4	9.4	0.6	22.7	22.7	130
dried, mature	10.6	3.5	22.9	1.4	61.6	57.4	342
Crab	80.0	1.7	16.1	1.6	0.6	0.6	86
Crackers, graham	5.5	2.2	8.0	10.0	74.3	73.5	393
soda	5.7	2.4	9.6	9.6	72.7	72.5	420
Cranberries	87.4	0.2	0.4	0.7	11.3	9.9	48
Cream, coffee	72.5	0.6	2.9	20.0	4.0	4.0	204
whipping	59.0	0.5	2.3	35.0	3.2	3.2	330
Cucumber	96.1	0.4	0.7	0.1	2.7	2.2	12
Currants, red	84.4	0.6	1.2	0.2	13.6	9.6	55
Dandelion greens	85.8	2.0	2.7	0.7	8.8	7.0	44
Dates	20.0	1.8	2.2	0.6	75.4	73.0	284
Doughnuts	18.7	1.0	6.6	21.0	52.7	52.5	425
Duck	54.3	1.0	16.0	28.6	0	0	321
Eel	71.6	1.0	18.6	9.1	0	0	162
Eggplant	92.7	0.5	1.1	0.2	5.5	4.6	24
Eggs, hen	74.0	1.0	12.8	11.5	0.7	0.7	162
white only	87.8	0.6	10.8	0	0.8	0.8	50
yolk only	49.4	1.7	16.3	31.9	0.7	0.7	361
duck	70.8	1.0	13.1	14.3	0.8	0.8	184
goose	70.4	1.1	13.9	13.3	1.3	1.3	180
Endive or escarole	93.3	0.9	1.6	0.2	4.0	3.2	20
Farina	10.5	0.4	10.9	0.8	77.4	77.0	370
Figs, dried	24.0	2.4	4.0	1.2	68.4	62.6	270
fresh	78.0	0.6	1.4	0.4	19.6	17.9	79
Fish, various (av.)	72.1	1.4	18.0	8.5	0	0	129
Flour, buckwheat, light.	12.0	0.9	6.4	1.2	79.5	79.0	348
rye, medium	11.0	1.1	11.4	1.7	74.8	73.8	350
wheat, whole	12.0	1.7	13.3	2.0	71.0	68.7	333
patent	12.0	0.4	10.5	1.0	76.1	75.8	364
Frog's legs	81.9	1.1	16.4	0.3	0	0	73
Gelatin, plain, dry	13.0	1.3	85.6	0.1	0	0	335
Goose	51.1	0.9	16.4	31.5	0	0	349
Gooseberries	88.9	0.4	0.8	0.2	9.7	7.8	39
Grapefruit	88.8	0.4	0.5	0.2	10.1	9.8	40
juice, canned, unsweetened	89.2	0.4	0.5	0.1	9.8	9.7	38
Grapes	81.9	0.4	1.4	1.4	14.9	14.4	70
Grape juice	81.0	0.4	0.4	0	18.2	18.2	67

* Difference between 100 per cent and the sum of the percentages of water, ash, protein, and fat.

† Carbohydrate exclusive of fiber.

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Table A-1 (Continued)

Proximate composition of foods

(Edible portion)

Food	Moisture %	Ash %	Protein %	Fat %	Carbohydrate Total* %	Carbohydrate Available† %	Food energy (Cal. per 100 g.)
Heart, beef	77.6	1.1	16.9	3.7	0.7	0.7	108
pork	76.8	1.1	16.9	4.8	0.4	0.4	117
Hominy, dry	12.0	0.4	8.7	0.8	78.1	77.7	362
Honey	20.0	0.2	0.3	0	79.5	79.5	294
Ice cream, plain	62.1	0.8	4.0	12.5	20.6	20.6	207
Jellies	34.5	0.3	0.2	0	65.0	65.0	252
Kale	86.6	1.7	3.9	0.6	7.2	6.0	40
Kidney, beef	74.9	1.1	15.0	8.1	0.9	0.9	141
pork	77.1	1.2	16.3	4.6	0.8	0.8	114
sheep	77.8	1.3	16.6	3.3	1.0	1.0	105
Kohlrabi	90.1	1.0	2.1	0.1	6.7	5.6	30
Lamb, medium fat	55.8	0.8	15.7	27.7	0	0	317
leg, medium fat	63.7	0.9	18.0	17.5	0	0	235
rib, medium fat	51.9	0.8	14.9	32.4	0	0	356
shoulder, medium fat	58.3	0.8	15.6	25.3	0	0	295
Lemons	89.3	0.5	0.9	0.6	8.7	7.8	32
Lentils, dried (entire seeds)	11.2	3.3	25.0	1.0	59.5	55.8	337
Lettuce	94.8	0.9	1.2	0.2	2.9	2.3	15
Limes	86.0	0.8	0.8	0.1	12.3	11.4	37
Liver, beef	69.7	1.4	19.7	3.2	6.0	6.0	136
calf	70.8	1.3	19.0	4.9	4.0	4.0	141
pork	72.3	1.5	19.7	4.8	1.7	1.7	134
Lobster	79.2	2.2	16.2	1.9	0.5	0.5	88
Loganberries	82.9	0.5	1.0	0.6	15.0	13.6	62
Macaroni, dry	8.6	0.7	12.8	1.4	76.5	76.1	377
Milk, fresh, whole	87.0	0.7	3.5	3.9	4.9	4.9	68
skim	90.5	0.8	3.5	0.1	5.1	5.1	36
canned, evaporated, unsweetened	73.7	1.5	7.0	7.9	9.9	9.9	138
condensed, sweetened	27.0	1.7	8.1	8.4	54.8	54.8	320
dried, whole	3.5	6.0	25.8	26.7	38.0	38.0	492
skim	3.5	7.9	35.6	1.0	52.0	52.0	362
goat, fresh	87.4	0.7	3.3	4.0	4.6	4.6	67
human, fresh	87.5	0.2	1.4	3.7	7.2	7.2	68
Molasses, cane, medium	25.4	3.2	2.1	0	69.3	69.3	286
Mulberries	82.8	0.8	1.2	0.6	14.6	12.6	69
Mushrooms	91.1	1.1	2.4	0.3	4.0	3.1	16
Mustard greens	92.2	1.2	2.3	0.3	4.0	3.2	22
Mutton—*see* lamb							
Nectarines	82.9	0.5	0.5	0.1	16.0	15.6	67
Oats, grain	9.8	4.0	12.0	4.6	69.6	58.6	368
Oatmeal or rolled oats	8.3	1.9	14.2	7.4	68.2	67.0	390
Okra	89.8	0.8	1.8	0.2	7.4	6.4	32
Oleomargarine	15.5	2.5	0.6	81.0	0.4	0.4	720
Olives, pickled, green	75.2	5.8	1.5	13.5	4.0	2.8	132
ripe, pickled	75.8	2.6	1.5	17.3	2.9	1.3	160
Onions, mature	87.5	0.6	1.4	0.2	10.3	9.5	45
green	87.6	0.6	1.0	0.2	10.6	8.8	45
Oranges	87.2	0.5	0.9	0.2	11.2	10.6	45
Orange juice, fresh or canned	87.5	0.4	0.8	0.2	11.1	11.0	44
Oysters, fresh, solids	80.5	2.0	9.8	2.1	5.6	5.6	84
Pancreas (all kinds)	63.3	1.3	15.8	19.2	0	0	236
Papayas	88.7	0.6	0.6	0.1	10.0	9.1	39
Parsley	83.9	2.4	3.7	1.0	9.0	7.2	50

* Difference between 100 per cent and the sum of the percentages of water, ash, protein, and fat.

† Carbohydrate exclusive of fiber.

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Table A-1 (Continued)

Proximate composition of foods
(Edible portion)

Food	Moisture %	Ash %	Protein %	Fat %	Carbohydrate Total* %	Carbohydrate Available† %	Food energy (Cal. per 100 g.)
Parsnips	78.6	1.2	1.5	0.5	18.2	16.0	78
Peaches, fresh	86.9	0.5	0.5	0.1	12.0	11.4	46
dried	24.0	3.0	3.0	0.6	69.4	65.9	265
canned (sirup packed)	80.9	0.4	0.4	0.1	18.2	17.8	68
Peanuts	2.6	2.7	26.9	44.2	23.6	21.2	559
Peanut butter	1.7	3.4	26.1	47.8	21.0	19.0	576
Pears	82.7	0.4	0.7	0.4	15.8	14.4	63
Peas, fresh	74.3	0.9	6.7	0.4	17.7	15.5	98
Peas, canned	82.3	1.0	3.4	0.4	12.9	11.5	68
dried, mature	11.6	3.0	23.8	1.4	60.2	54.8	339
Pecans	3.0	1.6	9.4	73.0	13.0	10.8	696
Peppers, green	92.4	0.5	1.2	0.2	5.7	4.3	25
Persimmons, native	64.4	0.9	0.8	0.4	33.5	32.0	141
Pineapple	85.3	0.4	0.4	0.2	13.7	13.3	52
canned (sirup packed)	78.0	0.4	0.4	0.1	21.1	20.8	78
juice, canned	86.2	0.4	0.3	0.1	13.0	12.9	49
Plums	85.7	0.5	0.7	0.2	12.9	12.4	50
Pork, fresh, medium fat.	42.0	0.6	11.9	45.0	0	0	457
fresh, ham, medium fat	53.0	0.8	15.2	31.0	0	0	344
loin, medium fat	58.0	0.9	16.4	25.0	0	0	296
cured, ham, medium fat	42.0	5.4	16.9	35.0	0.3	0.3	389
Potatoes	77.8	1.0	2.0	0.1	19.1	18.7	83
Potato chips	3.1	4.0	6.7	37.1	49.1	48.0	544
Prunes, dried	24.0	2.1	2.3	0.6	71.0	69.4	268
Pumpkin	90.5	0.8	1.2	0.2	7.3	6.0	31
Quinces	85.3	0.4	0.3	0.1	13.9	12.1	58
Rabbit	70.5	1.0	20.9	7.6	0	0	175
Radishes	93.6	1.0	1.2	0.1	4.2	3.5	20
Raisins	24.0	2.0	2.3	0.5	71.2	71.2	268
Raspberries, red	84.1	0.5	1.2	0.4	13.8	9.1	57
Rhubarb	94.9	0.7	0.5	0.1	3.8	3.1	16
Rice, brown	12.0	1.1	7.5	1.7	77.7	77.1	360
white	12.3	0.4	7.6	0.3	79.4	79.2	362
puffed	3.5	2.3	5.9	0.6	87.7	87.2	392
Rutabagas	89.1	0.8	1.1	0.1	8.9	7.6	38
Rye, grain	11.0	1.8	12.1	1.7	73.4	71.4	321
Salmon, Pacific, raw	63.4	1.0	17.4	16.5	0	0	223
canned, red	67.3	3.0	20.2	9.6	0	0	173
Sardines, canned in oil	57.4	4.7	25.7	11.0	1.2	1.2	214
Sauerkraut, canned	93.2	2.1	1.1	0.2	3.4	2.7	16
Scallops	80.3	1.4	14.8	0.1	3.4	3.4	78
Shrimp, canned	75.6	4.5	18.7	0.9	0.3	0.3	89
Sirup, table blends	25.0	0.6	0	0	74.0	74.0	286
Soybeans, whole	7.5	4.7	34.9	18.1	34.8	29.8	331
Soybean flour, low fat	11.0	5.5	44.7	1.1	37.7	35.4	228
Spinach	92.7	1.5	2.3	0.3	3.2	2.6	20
Squash, summer	95.0	0.4	0.6	0.1	3.9	3.4	16
winter	88.6	0.8	1.5	0.3	8.8	7.4	38
Strawberries	89.9	0.5	0.8	0.5	8.3	6.9	37
Sugar, granulated	0.5	0	0	0	99.5	99.5	385
Sweet potatoes	68.5	1.1	1.8	0.7	27.9	26.9	123
Tangerines	87.3	0.7	0.8	0.3	10.9	9.9	44
Tapioca	12.6	0.2	0.6	0.2	86.4	86.3	360
Tomatoes	94.1	0.6	1.0	0.3	4.0	3.4	20
canned	94.2	0.7	1.0	0.2	3.9	3.5	19
Tomato juice, canned	93.5	1.0	1.0	0.2	4.3	4.1	21

* Difference between 100 per cent and the sum of the percentages of water, ash, protein, and fat.

† Carbohydrate exclusive of fiber.

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Table A-1 (Continued)

Proximate composition of foods

(Edible portion)

Food	Moisture %	Ash %	Protein %	Fat %	Carbohydrate Total* %	Carbohydrate Available† %	Food energy (Cal. per 100 g.)
Tongue, beef	68.0	0.9	16.4	15.0	0.4	0.4	207
Tuna, canned, solids	60.0	2.7	29.0	8.2	0	0	198
Turkey	58.3	1.0	20.1	20.2	0	0	268
Turnips	90.9	0.7	1.1	0.2	7.1	6.0	32
Turnip tops	89.5	1.8	2.9	0.4	5.4	4.2	30
Veal, medium fat	68.0	1.0	19.1	12.0	0	0	190
Walnuts, English	3.3	1.7	15.0	64.4	15.6	13.5	654
Watermelons	92.1	0.3	0.5	0.2	6.9	6.3	28
Watercress	93.6	1.1	1.7	0.3	3.3	2.8	18
Wheat, grain	11.0	1.6	13.0	2.0	72.4	70.6	360
bran, crude	10.1	6.1	16.6	3.7	63.5	53.2	354
germ	11.0	4.3	25.2	10.0	49.5	47.0	361
puffed	3.8	3.6	10.8	1.6	80.2	78.5	355
shredded	5.6	1.7	10.1	2.5	80.1	77.8	360
Yams	72.6	1.0	2.1	0.2	24.1	23.3	107
Yeast, compressed	70.9	2.4	13.3	0.4	13.0	12.7	86
dried, brewer's	7.6	7.9	46.1	1.6	36.8	36.0	273

* Difference between 100 per cent and the sum of the percentages of water, ash, protein, and fat.

† Carbohydrate exclusive of fiber.

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Table A-2

Major mineral elements in the edible portion of foods

(Grams per 100 g.)

Food	*Calcium*	*Magnesium*	*Potassium*	*Sodium*	*Phosphorus*	*Chlorine*	*Sulfur*
Almonds	.254	.275	.756	.024	.475	.037	.164
Apples, fresh	.006	.006	.116	.015	.010	.004	.004
dried	.019	.029	.557	.072	.048	.019	.019
Apricots, fresh	.016	.012	.370	.021	.023	.004	.006
dried	.086	.062	1.924	.109	.119	.021	.031
canned in sirup	.010	.007	.256	.001	.015	.002	.001
Asparagus	.021	.015	.200	.008	.062	.047	.051
Bacon, medium fat	.013	?	?	?	.108	?	?
Bananas	.008	.024	.412	.023	.028	.163	.013
Barley, grain	.058	.126	.495	.070	.343	.139	.152
Beans, navy, dried	.163	.165	1.284	.189	.437	.007	.224
Lima, fresh	.063	.067	.606	.089	.158	.009	.068
dried	0.68	.181	1.899	.282	.381	.025	.156
string or green	.066	.032	.288	.012	.044	.045	.024
green, canned	.027	?	?	?	.019	?	?
baked, canned	.049	.037	.344	(a)	.113	(a)	.051
Beef, medium fat	.011	.032	.382	.066	.161	.056	.221
Beets	.027	.027	.235	.053	.043	.040	.017
Beet greens	.118	.097	.390	?	.045	?	.035
Brains	.011	.016	.269	.160	.338	.155	.130
Bread, white	.079	.034	.110	.517	.092	.602	.083
whole wheat	.096	?	?	?	.263	?	?
rye	.072	?	?	?	.147	?	?
Broccoli	.130	.024	.352	.030	.076	.076	.126
Brussels sprouts	.034	.015	.375	?	.078	?	.098
Buckwheat, grain	.090	?	.450	?	.310	?	?
Butter	.020	.002	.019	(a)	.016	(a)	.009
Cabbage	.046	.016	.217	.038	.031	.034	.074
celery	.043	.011	.400	.028	.041	.023	.013
Cantaloupe	.017	.016	.243	.048	.016	.048	.016
Carrots	.039	.020	.219	.050	.037	.035	.019
canned	.022	?	?	?	.024	?	?
Cashew nuts	.046	.267	?	?	.428	?	?
Cauliflower	.022	.023	.292	.048	.072	.038	.074
Celery	.050	.025	.320	.101	.040	.225	.021
Chard	.105	.053	.318	.086	.036	.039	.124
Cheese, cheddar	.725	.031	.116	.900	.495	.972	.214
cottage	.096	?	?	?	.189	?	?
Swiss	.925	?	?	?	.563	?	?
Cherries	.018	.012	.125	.015	.020	.004	.018
Chestnuts	.029	.048	.415	.037	.081	.010	.049
Chicken	.014	.047	.402	.054	.200	.034	.303
Chocolate	.098	.082	.400	.019	.446	.009	.114
Clams	.096	.090	.172	.603	.139	1.065	.219
Cocoa	.125	.192	.534	.060	.712	.050	.197
Coconut, fresh	.021	.040	.360	.040	.098	.120	.044
dried, sweetened	.043	.077	.693	.053	.191	.225	.076
Collards	.249	.017	?	?	.058	?	?
Corn, grain	.010	.142	.300	.036	.256	.041	.124
sweet, fresh	.009	.047	.278	.040	.120	.014	.037
sweet, canned	.004	?	?	?	.051	?	?
Cornflakes	.011	?	?	?	.058	?	?
Cottonseed, whole	.140	.320	1.110	.290	.070	?	.240
Cowpeas, dried	.077	.265	1.305	.036	.451	.019	.250
Crabs	.126	.117	.271	.366	.261	.570	.255
Crackers, graham	.020	?	?	.203	?	?	?
soda	.020	?	?	.096	?	?	?
Cranberries	.014	.005	.056	.002	.011	.004	.008
Cream	.097	.006	.112	.031	.077	.067	.033
Cucumbers	.010	.020	.170	.026	.021	.028	.011
Currants, fresh	.036	.031	.208	.015	.033	.010	.021
dried	.180	.155	1.040	.075	.220	.050	.105

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Table A-2 (Continued)

Major mineral elements in the edible portion of foods

(Grams per 100 g.)

Food	*Calcium*	*Magnesium*	*Potassium*	*Sodium*	*Phosphorus*	*Chlorine*	*Sulfur*
Dandelion greens	.187	.036	.461	.168	.070	.099	.170
Dates	.072	.065	.580	.040	.060	.253	.048
Duck	.015	?	?	?	.188	?	?
Eel	.018	.018	.241	.032	.202	.035	.133
Eggplant	.015	.015	.260	.026	.037	.063	.020
Eggs	.054	.009	.149	.111	.210	.100	.233
Egg white	.006	.011	.149	.175	.017	.131	.211
Egg yolk	.147	.013	.110	.078	.586	.067	.214
Endive	.079	.013	.381	.060	.056	.071	.032
Figs, fresh	.054	.020	.205	.043	.032	.037	.017
dried	.186	.068	.709	.151	.111	.126	.060
Fish, various (av.)	.024	.024	.375	.064	.225	.137	.199
Flour, patent	.016	.021	.137	.053	.087	.079	.155
whole wheat	.041	.122	.324	.160	.372	.177	.124
rye, medium	.027	?	?	?	.262	?	?
Frog	.018	.024	.308	.055	.147	.040	.163
Garlic	.006	.008	.130	.009	.090	.004	.318
Goose	.015	.031	.406	?	.188	?	.325
Gooseberries	.022	.009	.150	.010	.028	.009	.015
Grapefruit	.022	.007	.164	.006	.018	.007	.005
Grapefruit juice, canned	.008	?	?	?	.013	?	?
Grapes	.017	.004	.267	.011	.021	.002	.009
Heart	.009	.035	.329	.102	.203	.204	.151
Honey	.005	.004	.051	.006	.016	.015	.003
Horseradish	.169	.028	.550	.094	.059	.013	.234
Kale	.225	.055	.486	.050	.062	.120	.160
Kidney	.014	.019	.240	.238	.233	.376	.148
Kohlrabi	.046	.052	.370	.050	.050	.050	.039
Lamb (mutton)	.010	.033	.260	.070	.212	.069	.187
Leeks	.091	.037	.380	.036	.049	.110	.056
Lemons	.040	.006	.152	.009	.022	.006	.012
Lentils, dried	.059	.082	.662	.754	.423	.062	.123
Lettuce	.042	.015	.256	.028	.023	.085	.014
Liver	.009	.021	.255	.021	.333	.091	.258
Lobster	.061	.022	.258	?	.184	?	?
Macaroni	.022	.038	.054	.010	.165	.077	.119
Milk, cow, fresh	.118	.019	.129	.047	.093	.114	.031
evaporated	.243	.038	.258	.094	.195	.228	.067
powder	.949	.118	.955	.348	.728	1.029	.229
goat	.129	?	?	.026	.106	.163	?
human	.032	.005	.055	.011	.017	.058	.011
Molasses, medium	.290	?	?	?	.069	?	?
Mushrooms	.009	.012	.280	.013	.115	.026	.025
Mustard greens	.220	.016	.330	.020	.038	.090	.142
Oatmeal (rolled oats)	.053	.143	.365	.072	.405	.027	.207
Oats, grain	.094	.150	.450	.090	.318	.089	.187
Okra	.082	.038	?	?	.062	?	.014
Oleomargarine	.020	?	?	?	.016	?	?
Olives, pickled	.087	.012	.809	(a)	.017	(a)	.032
Onions	.032	.016	.200	.020	.044	.053	.065
Oranges	.033	.011	.177	.014	.023	.006	.011
Orange juice	.019	.014	.200	.006	.016	.008	.005
Orange juice, canned	.010	?	?	?	.018	?	?
Oysters	.094	.039	.204	.470	.143	?	.180
Parsley	.193	?	?	?	.084	?	?
Parsnips	.057	.038	.396	.010	.080	.038	.025
Peaches, fresh	.008	.015	.174	.012	.022	.006	.005
dried	.044	.087	1.009	.070	.126	.035	.029
canned in sirup	.005	.006	.151	.001	.014	.004	.001
Peanuts	.074	.169	.706	.052	.393	.040	.276
Pears	.013	.005	.110	.010	.016	.004	.010
Peas, green	.022	.035	.259	.024	.122	.049	.035

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Table A-2 (Continued)

Major mineral elements in the edible portion of foods

(Grams per 100 g.)

Food	*Calcium*	*Magnesium*	*Potassium*	*Sodium*	*Phosphorus*	*Chlorine*	*Sulfur*
canned	.025	.024	.201	(a)	.067	(a)	.044
mature	.057	.121	.943	.072	.388	.034	.178
Peppers, green	.011	.025	.270	.015	.025	.031	.030
Peppers, red	.035	.013	.120	.006	.042	.014	.030
Persimmons	.006	.005	.170	.013	.026	.019	.011
Pineapple	.016	.014	.230	.008	.011	.038	.003
canned in sirup	.029	.008	.057	.001	.007	.004	.003
Pineapple juice, canned	.015	?	?	?	.008	?	?
Plums	.017	.010	.212	.003	.020	.002	.004
Pork	.007	.027	.415	.081	.117	.040	.216
Potatoes	.011	.027	.498	.030	.056	.048	.033
Prunes, dried	.054	.032	.845	.101	.085	.004	.024
Pumpkins	.021	.021	.198	.011	.044	.025	.016
Rabbit	.018	.029	.415	.047	.244	.051	.184
Radishes	.037	.014	.166	.083	.031	.056	.038
Raisins	.078	.017	.796	.120	.129	.068	.043
Raspberries	.040	.018	.141	.007	.037	.010	.012
Rhubarb	.051	.015	.392	.010	.025	.070	.008
Rice, brown	.079	.141	.334	.068	.310	.066	.121
white	.024	.033	.046	.012	.136	.056	.114
puffed	.021	?	?	?	.116	?	?
Rutabagas	.055	.015	.210	.052	.041	.031	.069
Rye, grain	.075	.136	.477	.060	.376	.043	.152
Salmon, canned	.184	.030	.320	(a)	.292	(a)	.235
Sardines, canned	.386	.035	.433	(a)	.550	(a)	.283
Sauerkraut, canned	.036	?	?	(a)	.018	(a)	?
Shrimps, canned	.115	.105	.404	(a)	.263	(a)	.340
Soybeans, mature	.227	.287	1.693	.280	.586	.007	.269
Spaghetti—*see* macaroni							
Spinach	.081	.048	.416	.093	.055	.118	.027
Squash, winter	.019	.006	.161	.011	.028	.018	.029
summer	.015	.008	.150	.002	.015	?	?
Strawberries	.028	.019	.205	?	.027	?	.013
Sugar beets	.030	.041	.440	.130	.049	.180	.021
Sweet potatoes	.030	.035	.381	.031	.049	.022	.014
Tomatoes	.011	.016	.277	.013	.027	.048	.017
canned	.011	?	?	?	.027	?	?
Tomato juice, canned	.007	?	?	?	.015	?	?
Tuna, canned	.008	?	?	?	.224	?	?
Turkey	.023	.028	.367	.130	.320	.123	.234
Turnips	.040	.019	.193	.104	.034	.054	.048
Turnip greens	.259	.079	.300	.260	.050	.390	.051
Veal	.011	.030	.380	.086	.193	.073	.199
Venison	.010	.029	.336	.070	.249	.041	.211
Walnuts	.083	.132	.606	.013	.380	.030	.120
Watercress	.195	.010	.100	.031	.046	.059	.071
Watermelon	.007	.006	.071	.012	.012	.006	.005
Wheat, grain	.040	.163	.409	.060	.383	.088	.175
bran	.094	.420	1.252	.007	1.312	.042	.245
germ	.084	?	?	?	1.096	?	?
puffed	.046	?	?	?	.329	?	?
shredded	.047	?	?	?	.360	?	?
Yams	.041	.015	.290	.015	.042	.037	.013
Yeast, dried	.054	.145	2.230	.149	1.570	?	.015

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Table A-3

Trace elements in foods

(Fresh basis unless otherwise indicated)

Food	*Milligrams per 100 g. of edible portion*				*Micrograms per 100 g. of edible portion*
	Fe	Cu	Mn	Zn	Iodine
Almonds	4.4	1.2	1.2	1.9	?
Apples	.3	.1	.11	.07	6.6
Apricots, fresh	.5	.15	?	.04	?
dried	4.9	.32	.28	?	?
Artichokes	2.2	.32	.38	?	?
Asparagus	.9	.11	.19	.34	6.9
Avocados	.6	.21	.29	?	?
Bacon	.8	.41	.08	?	5.0
Bananas	.6	.21	1.1	.26	20.0
Barley, grain	8.9	1.2	1.6	2.3	9.1
pearled	1.3	.26	?	?	?
Beans, navy, dried	6.9	.98	1.9	3.1	4.8
kidney, dried	6.9	.92	1.6	5.2	1.8
Lima, dried	7.5	.86	1.1	?	?
Lima, fresh	2.3	.53	.6	1.5	?
string	1.1	.13	.37	.09	6.9
Beef, chuck	2.8	.1	?	?	?
heart	4.6	?	?	?	30.0
kidney	7.9	.11	1.0	2.4	9.0
liver	6.6	2.0	.32	3.5	14.0
"lean"	4.2	.05	.02	1.5	3.5
loin	2.6	.1	?	?	?
steak	2.5	.11	.02	?	9.1
sweetbreads	6.0	.08	.07	2.0	?
Beets	1.0	.12	.62	.65	3.3
Beet greens	3.2	.12	1.2	.02	8.0
Blackberries	.9	.15	.57	?	?
Blueberries	.8	.11	3.4	?	?
Brazil nuts	3.4	1.3	.94	?	?
Bread, rye	1.6	.28	1.3	?	9.0
white	.6	.25	.42	3.3	11.3
whole wheat	2.2	.33	3.2	?	11.0
Broccoli	1.3	.20	.26	?	15.0
Brussels sprouts	1.3	.11	.30	?	6.2
Butter	0	.04	.04	?	8.6
Buckwheat, grain	4.0	.9	3.3	?	?
Buttermilk	.1	.05	?	?	?
Butternuts	6.8	1.2	?	?	?
Cabbage	.5	.11	.21	.20	2.3
Calf's liver	10.6	6.3	.37	3.0	?
Cantaloupe	.4	.05	.05	.09	2.3
Carrots	.8	.12	.37	.35	4.4
Cauliflower	1.1	.27	.15	.22	1.6
Celery	.5	.12	.17	.21	12.3
Celery cabbage	.9	.06	.12	?	?
Chard	2.5	.11	.8	?	11.0
Cheese, hard	1.0	.09	.11	?	10.0
cottage	.3	?	.05	?	6.4
Cherries	.4	.13	.03	.15	.6
Chestnuts	2.2	.39	1.7	.19	?
Chicken	1.5	.54	?	.46	?
Chocolate	3.0	2.1	3.2	2.6	?
Citron	.8	.57	?	?	2.1
Clams	4.3	0	?	3.6	124.0
Cocoa	11.6	2.4	3.5	2.6	?
Coconut, dried	3.6	.62	?	?	?
fresh	2.0	.53	1.3	.84	1.8
Cod-liver oil	?	?	0	.9	860.0
Coffee, beans	5.4	1.3	?	.5	8.6
water extract	.46	?	?	?	4.0
Collards	1.6	?	2.0	?	1.0

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Table A-3 (Continued)

Trace elements in foods

(Fresh basis unless otherwise indicated)

Food	*Milligrams per 100 g. of edible portion*				*Micrograms per 100 g. of edible portion*
	Fe	Cu	Mn	Zn	Iodine
Corn, grain	3.0	.71	.7	2.2	12.0
germ	25.0	.91	3.6	9.4	?
meal, yellow	1.1	.19	.22	1.8	?
sweet	.5	.08	.31	?	3.3
Cornflakes	1.3	?	?	?	?
Cottonseed	14.0	5.0	1.2	?	?
Cowpeas	2.5	.17	1.5	?	5.7
Crab	2.0	1.3	0.3	2.5	30.2
Crackers, graham	1.9	?	?	?	?
soda	1.1	?	?	?	?
Cranberries	.6	.11	.38	?	3.3
Cream	.1	.15	?	?	5.7
Cucumber	.31	.13	.13	.12	.83
Currants, dried	3.3	.8	.31	?	?
fresh	.9	.13	?	.2	?
Dandelion greens	3.1	.17	.34	1.2	?
Dates	2.1	.23	2.6	.32	?
Duck	1.8	.46	.03	.34	?
Eggplant	.4	.09	.23	.28	.8
Eggs, hen	2.7	.17	.04	1.3	12.0
Egg white	.2	.04	?	.01	6.8
Egg yolk	7.2	.25	.11	3.8	16.0
Endive	1.7	.09	.23	.12	3.7
Escarole	1.1	.14	?	.19	?
Figs, dried	3.0	.34	.34	.26	?
fresh	.6	.06	?	.12	1.5
Filberts	4.1	1.2	?	1.0	?
Fish, general	.61	.33	.02	.80	66.5 (salt water) 7.0 (fresh water)
Flour, buckwheat	1.0	.72	2.1	1.0	?
whole wheat	3.3	.47	4.3	1.9	?
rye, medium	2.6	.43	2.0	?	2.3
patent	.8	.14	.54	1.2	3.6
Garlic	?	.26	.46	.92	2.7
Goose	1.8	.33	.05	?	?
Gooseberries	.5	.10	.05	.1	?
Grapefruit	.2	.45	.01	?	1.3
Grapes	.6	.11	.08	.17	?
Grape juice	.3	.02	?	?	.9
Hazelnuts	4.3	1.2	3.6	.97	1.4
Hickory nuts	2.6	1.4	?	?	?
Hominy	1.0	.18	.11	?	?
Honey	.9	.15	.03	?	2.3
Huckleberries—*see* blueberries					
Kale	2.2	.52	.86	?	?
Kidney—*see* beef, lamb					
Kohlrabi	.6	.14	.12	?	?
Kumquats	.55	.09	.07	?	?
Lamb, general	2.4	.42	?	?	?
chop	2.2	.42	.04	?	15.0
kidney	9.2	.31	?	1.9	?
Lard	0	.02	?	?	9.3
Leeks	1.3	.17	?	.23	?
Lemons	.6	.04	.35	?	.5
Lemon juice	.1	.13	?	.17	5.2
Lentils (dried)	7.4	.59	3.3	5.4	?
Lettuce, head	.8	.11	1.0	.39	2.9
leaf	2.0	.14	.82	.44	2.7
Liver—*see* beef, etc.					
Lobster	.6	1.5	.04	.24	80.1

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Table A-3 (Continued)

Trace elements in foods

(Fresh basis unless otherwise indicated)

Food	*Milligrams per 100 g. of edible portion* Fe	Cu	Mn	Zn	*Micrograms per 100 g. of edible portion* Iodine
Loganberries	1.2	.14	?	.45	2.7
Macaroni	1.5	.07	?	?	?
Mangoes	.2	.04	?	?	1.6
Milk, cow's	.1	.02	.03	.36	3.8
Milk powder	.6	.34	?	?	32.0
Milk, human	1.3	.03	?	?	?
Molasses, cane, light	4.3	1.4	.44	?	?
Mushrooms	1.0	1.0	.12	.4	0
Muskmelon—*see* cantaloupe					
Mussels	?	.35	.46	4.5	80.2
Mustard greens	2.9	.12	1.2	?	5.4
Mutton, leg	4.8	.4	?	2.2	1.8
chop	1.0	.16	?	?	?
liver	?	1.6	?	4.1	3.3
Nectarines	.46	.06	?	?	?
Oatmeal	4.5	.38	3.3	?	4.2
Oats, grain	7.2	1.4	3.9	2.9	5.2
Okra	.7	.14	.56	?	5.6
Oleomargarine	0	.04	?	?	7.4
Olives	1.6	.25	.12	.3	?
Onions	.5	.11	.38	1.3	3.6
Oranges	.4	.18	.03	.17	.6
Orange juice	.2	.05	?	?	1.5
Oysters	5.6	3.4	.13	46.0	74.2
Oyster plant—*see* salsify					
Parsley	13.0	.23	1.2	?	?
Parsnips	.7	.12	.04	?	3.6
Peaches	.6	.07	?	.02	1.3
dried	6.9	.27	.68	?	?
Peanuts	1.9	1.1	.86	1.6	.7
Pears	.3	.16	.05	.16	.4
Peas, dried	4.7	1.1	1.8	4.0	?
fresh	1.9	.23	.3	1.1	2.1
Pecans	2.4	1.4	3.5	?	?
Peppers, green	.4	.11	.15	.06	?
red	.6	?	.19	?	2.3
Pimentos	1.5	.60	?	.23	.2
Pineapple	.3	.09	1.5	.28	16.0
Pistachio nuts	7.9	1.2	.67	?	?
Plums	.5	.14	.11	.03	4.7
Pork, general	1.8	1.5	?	1.4	7.6
chop	2.5	.31	.06	?	?
liver	18.0	1.3	.38	.79	14.0
Potatoes	.7	.17	.41	.31	3.9
Prunes, dried	3.9	.29	.16	.05	.12
Pumpkin	.8	.07	.04	.21	1.4
Quinces	.85	.13	.04	?	?
Radishes	1.0	.22	.17	.16	6.4
Raisins	3.3	.23	.34	.20	?
Raspberries	.9	.16	.67	.35	1.0
Rhubarb	.5	.09	.16	.16	26.0
Rice, brown	2.0	.26	1.9	2.1	25.0
white	.8	.2	1.1	.22	5.1
puffed	1.8	?	?	?	?
Rutabagas	.4	.12	.12	.30	6.7
Rye, grain	3.7	.63	3.8	1.8	6.7
Salsify (oyster plant)	1.4	.3	.41	.22	?
Salmon	1.2	.23	?	.8	29.1
Sardines	3.5	.04	.26	.94	27.0
Scallops	1.8	.23	3.9	?	47.5

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Table A-3 (Continued)

Trace elements in foods

(Fresh basis unless otherwise indicated)

Food	*Milligrams per 100 g. of edible portion*				*Micrograms per 100 g. of edible portion*
	Fe	Cu	Mn	Zn	Iodine
Shrimp	3.1	1.2	.23	1.4	35.5
Sirup	4.1	.09	?	?	?
Soybeans	8.0	1.1	2.9	1.8	6.3
Soybean flour	13.0	1.2	3.2	?	?
Spinach	3.0	.11	.73	.62	41.0
Squash, summer	.4	.08	.14	?	2.3
winter	.6	.10	.22	.21	?
Strawberries	.8	.07	.23	.09	?
Sweet potatoes	.7	.15	.3	.23	2.4
Tangerines	.4	.09	.04	?	?
Tapioca	1.0	.07	?	.04	?
Tea extract	.72	?	?	?	16.0
Tomatoes	.6	.09	.13	.24	1.5
Tuna fish	1.2	.5	?	?	30.5
Turkey	3.8	.17	.03	?	?
Turnips	.5	.08	.16	.08	7.5
Turnip greens	2.4	.08	1.9	.28	2.4
Veal, medium, lean	2.9	.20	.03	3.5	5.0
Vinegar	.5	.04	1.0	?	?
Walnuts, black	6.0	3.2	?	?	?
English	2.1	.88	2.4	2.3	?
Watercress	2.0	.1	.42	.56	3.6
Watermelon	.2	.07	.02	?	?
Wheat, grain	3.1	.8	3.7	5.4	7.6
bran	10.3	1.3	11.0	12.0	?
germ	8.1	2.7	13.0	14.3	?
puffed	3.0	?	?	?	?
shredded	3.5	?	?	?	?
Yams	.7	?	.05	?	4.7
Yeast, dried	13.0	3.0	.5	9.0	?

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Table A–4

Vitamin content of common foods

(Amount per 100 g., edible portion)

Food	*Vitamin A value* I.U.	*Ascorbic acid* mg.	*Thiamine* mg.	*Riboflavin* mg.	*Pantothenic acid* mg.	*Nicotinic acid* mg.
Almonds	0	trace	0.25	0.67	0.40	4.6
Apples, fresh	90	5	0.04	0.03	0.05	0.2
dried	0	12	0.10	0.10	0	1.0
Apricots, fresh	2,790	7	0.03	0.05	0	0.8
dried	7,430	12	0.01	0.16	0	3.3
canned *	1,350	4	0.02	0.02	0.09	0.3
Asparagus	1,000	33	0.16	0.19	0.50	1.4
Bananas	430	10	0.04	0.05	0.07	0.7
Barley, entire	70	0	0.50	0.20	0.95	6.0
dry pearled	0	0	0.12	0.08	0	3.1
Beans, dried	0	2	0.67	0.23	0.60	2.2
Lima, fresh	280	32	0.21	0.11	0	1.4
dried	0	2	0.48	0.18	1.2	2.0
string or green	630	19	0.08	0.11	0.15	0.5
green, canned †	500	5	0.04	0.05	0.06	0.4
baked, canned	80	2	0.05	0.04	0.09	0.5
Beef, medium fat	0	0	0.08	0.16	0.65	4.4
Beets	20	10	0.02	0.05	0.2	0.4
Beet greens	6,700	34	0.08	0.18	0.5	0.4
Brains	0	18	0.23	0.26	0	4.4
Bread, white	0	0	0.05	0.11	0.4	0.9
white enriched	0	0	0.24	0.15	0.4	2.2
whole wheat	0	0	0.30	0.13	0.8	3.0
rye (33%)	0	0	0.18	0.08	0.5	1.5
Broccoli	3,500	118	0.10	0.21	1.2	1.1
Brussels sprouts	400	94	0.08	0.16	0.6	0.7
Buckwheat, entire	0	0	0.46	0.15	1.3	2.0
Butter	3,300	0	trace	0.01	0	0.1
Cabbage	80	50	0.06	0.05	0.23	0.3
celery	260	31	0.03	0.04	0	0.4
Cantaloupe	3,420	33	0.05	0.04	0	0.5
Carrots	12,000	3	0.06	0.06	0.21	0.5
canned †	17,570	3	0.02	0.02	0.13	0.3
Cashew nuts	0	0	0.63	0.19	0	2.1
Cauliflower	90	69	0.11	0.10	0.80	0.6
Celery	0	7	0.05	0.04	0.3	0.4
Chard	2,800	38	0.06	0.07	0	0.4
Cheese, cheddar	1,400	0	0.02	0.42	0.28	trace
cottage	20	0	0.02	0.31	0.25	0.1
Swiss	1,450	0	0.01	0.40	0.35	0.1
Cherries	620	8	0.05	0.06	0	0.4
Chicken	0	0	0.08	0.16	0.60	8.0
Chocolate, bitter	60	0	0.05	0.24	0	1.1
Clams	110	0	0.10	0.18	0	1.6
Cocoa	30	0	0.12	0.38	0	2.3
Coconut, fresh	0	2	0.10	0.01	0	0.2
dried, sweetened	0	0	trace	trace	0	trace
Collards	6,870	100	0.11	0.27	0	2.0
Corn, entire, yellow	810	0	0.54	0.24	0.80	1.7
sweet, fresh	390 ‡	12	0.15	0.12	0	1.7
sweet, canned	230 ‡	5	0.03	0.06	0.20	0.9
Cottonseed, meal	44	0	0.4	0.5	1.0	3.0
Cowpeas, dried	30	2	0.32	0.16	1.8	2.2
Crabs	0	0	0.14	0.06	0	2.7
Cranberries	40	12	0.03	0.02	0	0.1
Cream, heavy	1,440	1	0.02	0.11	0	0.1
Cucumbers	0	8	0.03	0.04	0.39	0.2
Currants, fresh	120	36	0.04	0	0	0

* Solids and liquid. † Drained solids. ‡ Yellow sweet corn.

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Table A-4 (Continued)

Vitamin content of common foods

(Amount per 100 g., edible portion)

Food	*Vitamin A value* I.U.	*Ascorbic acid* mg.	*Thiamine* mg.	*Ribo-flavin* mg.	*Panto-thenic acid* mg.	*Nicotinic acid* mg.
Dandelion, greens	13,650	36	0.19	0.14	0	0.8
Dates	60	0	0.09	0.10	0	2.2
Eels	1,800	0	0.28	0.37	0	1.4
Eggplant	30	5	0.04	0.05	0	0.6
Eggs	1,140	0	0.10	0.29	2.7	0.1
Egg white	0	0	0	0.26	0.13	0.1
Egg yolk	3,210	0	0.27	0.35	6.0	trace
Endive	3,000	11	0.07	0.12	0.23	0.4
Figs, fresh	80	2	0.06	0.05	0	0.5
dried	80	0	0.16	0.12	0	1.7
Fish, various (av.)	375	9	0.08	0.15	0	8.2
Flour, white, enriched	0	0	0.44	0.26	1.0	3.5
whole wheat	0	0	0.48	0.07	1.0	5.5
rye (light)	0	0	0.15	0.07	0	0.6
Frog legs, raw	0	0	0.14	0.25	0	1.2
Gooseberries	290	33	0	0	0	0
Grapefruit	trace	40	0.04	0.02	0	0.2
Grapefruit juice, canned	trace	35	0.03	0.02	0.12	0.2
Grapes	80	4	0.06	0.04	0	0.2
Haddock	0	0	0.05	0.08	0	2.4
Heart	30	6	0.58	0.89	0	7.8
Honey	0	4	trace	0.04	0	0.2
Kale	7,540	115	0.10	0.26	0.40	2.0
Kidney	1,150	13	0.37	2.55	0	6.4
Kohlrabi	trace	61	0.06	0.05	0.16	0.2
Lamb (mutton)	0	0	0.14	0.20	1.0	4.5
Lemons	0	50	0.04	trace	0	0.1
Lentils (dried)	570	5	0.56	0.24	0	2.2
Lettuce (head)	540	8	0.04	0.08	0.13	0.2
Liver	43,900	31	0.26	3.33	5.5	13.7
Lobster	0	0	0.13	0.06	0	1.9
Macaroni, enriched	0	0	0.88	0.37	0	6.0
Milk, cow, fresh	160	1	0.04	0.17	0.30	0.1
evaporated	400	1	0.05	0.36	0	0.2
powder	1,400	6	0.30	1.46	2.5	0.7
goat	160	1	0.04	0.11	0	0.3
human	80	3	0.01	0.04	0.2	0.2
Mushrooms	0	5	0.10	0.44	0	4.9
Mustard greens	6,460	102	0.09	0.20	0	0.8
Oatmeal (rolled oats)	0	0	0.60	0.14	1.3	1.0
Oats, entire	83	0	0.62	0.17	1.3	1.4
Okra	740	30	0.08	0.07	0	1.1
Olives	300	0	trace	0	0	0
Onions	50	9	0.03	0.04	0.15	0.2
Oranges	190	49	0.08	0.03	0.07	0.2
Orange juice	190	49	0.08	0.03	0	0.2
Orange juice, canned	100	42	0.07	0.02	0.13	0.2
Oysters	320	0	0.15	0.20	0	1.2
Parsley	8,230	193	0.11	0.28	0.6	1.4
Parsnips	0	18	0.08	0.12	0	0.2
Peaches, fresh	880	8	0.02	0.05	0	0.9
dried	3,250	19	0.01	0.20	0	5.4
canned *	450	4	0.01	0.02	0.05	0.7
Peanuts	0	0	0.30	0.13	3.4	16.2
Pears	20	4	0.02	0.04	0	0.1
Peas, green	680	26	0.34	0.16	0.58	2.7
canned †	670	9	0.12	0.06	0.20	1.0

* Solids and liquid. † Drained solids.

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Table A-4 (Continued)

Vitamin content of common foods

(Amount per 100 g., edible portion)

Food	*Vitamin A value* I.U.	*Ascorbic acid* mg.	*Thiamine* mg.	*Ribo-flavin* mg.	*Panto-thenic acid* mg.	*Nicotinic acid* mg.
mature	370	2	0.77	0.28	1.8	3.1
Peppers, green	630	120	0.04	0.07	0.12	0.4
red	0	350	0	0	0	0
Persimmons	2,710	11	0.05	0.05	0	trace
Pineapple, fresh	130	24	0.08	0.02	0	0.2
canned *	80	9	0.07	0.02	0.10	0.2
Plums	350	5	0.06	0.04	0	0.5
Pork, fresh	0	0	0.58	0.14	0.70	3.1
Potatoes, white	20	17	0.11	0.04	0.4	1.2
Prunes, dried	1,890	3	0.10	0.16	0	1.7
Pumpkins	3,400	8	0.05	0.08	0	0.6
Radishes	30	24	0.03	0.02	0	0.3
Raisins	50	trace	0.15	0.08	0	0.5
Raspberries, red	130	24	0.02	0.07	0	0.3
Rhubarb	30	9	0.01	0	0	0.1
Rice, entire	0	0	0.32	0.05	0	4.6
polished	0	0	0.07	0.03	0.8	1.6
Rutabagas	330	36	0.07	0.08	0	0.9
Rye, entire	0	0	0.36	0.18	0	1.1
Salmon, red, canned *	230	0	0.04	0.16	0.75	7.3
Sardines, canned †	0	0	0.01	0.17	0.60	4.8
Sauerkraut, canned †	40	16	0.03	0.06	0	0.1
Shrimp, canned †	60	0	0.01	0.03	0.29	2.2
Soybeans, mature	110	trace	1.07	0.31	1.6	2.3
Spaghetti— *see* macaroni						
Spinach	9,420	59	0.11	0.20	0.20	0.6
Squash, winter	4,950	8	0.05	0.12	0	0.5
summer	260	17	0.05	0.09	0	0.8
Strawberries	60	60	0.03	0.07	0	0.3
Sweet potatoes	7,700	22	0.09	0.05	1.0	0.6
Tomatoes, raw	1,100	23	0.06	0.04	0.08	0.5
canned	1,050	16	0.06	0.03	0.23	0.7
Tomato juice, canned	1,050	16	0.05	0.03	0.25	0.8
Tuna, canned †	80	0	0.05	0.12	0.34	12.8
Turkey	trace	0	0.09	0.14	0	8.0
Turnips	trace	28	0.05	0.07	0.25	0.5
Turnip greens	9,540	136	0.09	0.46	0	0.8
Veal	0	0	0.14	0.25	1.3	6.4
Walnuts, English	30	3	0.48	0.13	0.88	1.2
Watercress	4,720	77	0.08	0.16	0.15	0.8
Watermelon	590	6	0.05	0.05	0	0.2
Wheat, entire	0	0	0.57	0.12	1.3	5.9
Wheat bran	0	0	0.68	0.25	3.0	33
Wheat germ	0	0	2.05	0.80	1.0	4.6
Yeast, brewer's, dried	0	0	9.69	5.45	20	36.2

* Solids and liquid. † Drained solids.

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