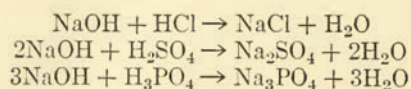their most outstanding property, their ability to react to form salts, the molecules react according to the number of replaceable hydrogens of acids and hydroxyl groups of bases. Note in the following equations that the reacting powers per mole of HCl, H_2SO_4, and H_3PO_4 are in the ratio 1:2:3.

$$NaOH + HCl \rightarrow NaCl + H_2O$$
$$2NaOH + H_2SO_4 \rightarrow Na_2SO_4 + 2H_2O$$
$$3NaOH + H_3PO_4 \rightarrow Na_3PO_4 + 3H_2O$$

Hence the complete reaction of one mole of sodium hydroxide requires one mole of hydrochloric acid, but only one-half mole of sulfuric or one-third mole of phosphoric acid. If these amounts of the respective acids be diluted to a common volume, for example, 1000 ml., the resulting solutions are of *equivalent concentrations* so far as their ability to react with alkalies is concerned. It is upon such a basis that normal solutions are prepared.

By definition a normal solution is of such concentration that *one liter* of the solution contains exactly *one gram equivalent weight* of the solute. The gram equivalent weight is that weight of a compound that contains one gram of replaceable (acid) hydrogen, or will react with one gram of replaceable hydrogen, or is *in any way equivalent to* this weight of hydrogen. To calculate the gram equivalent weight of acids divide the gram molecular weight by the number of replaceable hydrogen atoms in the molecule. Since one hydroxyl group requires one acid hydrogen for its neutralization, it follows that the gram equivalent weight of bases is obtained by dividing the gram molecular weight by the number of hydroxyl groups in the molecule. For a salt the divisor is the number of hydrogens that have been replaced in the formation of the salt from the corresponding acid. Obviously, one obtains a like result in the last two instances by dividing the gram molecular weight by the valence of the metal contained therein. Thus the divisor for NaOH is 1, but for $CaSO_4$ it is 2. The divisor in each case is termed the *hydrogen equivalent*. The use of these values in calculations involving normal solutions is illustrated in Table 7–1.

One gram equivalent weight of a chemical substance is frequently called simply an *equivalent* of that substance. One-thousandth of this amount similarly is termed a *milliequivalent* (abbreviation m.e.q.), which, if expressed in milligrams, is the same numerical figure as an equivalent expressed in grams. For example, in the case of acetic acid an equivalent is 60 g. (Table 7–1) and a milliequivalent is 60 mg. One liter of a normal solution always contains one equivalent of the solute, one milliliter containing one milliequivalent. Analogous fractions of a mole are also frequently used in biochemical work. Thus, one-thousandth of a mole is a *millimole*, and one-millionth is a *micromole*. Molarity

of solutions is indicated by a number followed by M, and normality by a number followed by N.

Table 7–1

Weights of typical reagents in representative standard solutions

REAGENT	Molecular weight	Hydrogen equivalent	Equivalent weight	GRAMS OF REAGENT Per liter of normal solution	Per ml. of normal solution
HCl	36.5	1	36.5	36.5	0.0365
$HC_2H_3O_2$	60	1	60	60	0.060
H_2SO_4	98	2	49	49	0.049
$H_2C_2O_4 \cdot 2H_2O$	126	2	63	63	0.063
$H_2C_4H_4O_6$	150	2	75	75	0.075
H_3PO_4	98	3	32.7	32.7	0.0327
$H_3C_6H_5O_7$	192	3	64	64	0.064
NaOH	40	1	40	40	0.040
$Ca(OH)_2$	74	2	37	37	0.037
NH_4OH	35	1	35	35	0.035
NaCl	58.5	1	58.5	58.5	0.0585
$Ba(NO_3)_2$	261.4	2	130.7	130.7	0.1307
$Al_2(SO_4)_3$	342	6	57	57	0.057
$K_2C_4H_4O_6$	226.2	2	113.1	113.1	0.1131
$KHC_4H_4O_6$	188.1	1	188.1	188.1	0.1881
$NaHCO_3$	84	1	84	84	0.084

Standardization of solutions

It is not always possible to prepare standard solutions by weighing out the amount of reagent theoretically required, because many substances are not obtainable in sufficient purity, and others take up water or carbon dioxide when exposed to the air during the time required for weighing. Solutions of such substances can, however, be "standardized" by titration against a solution of known concentration prepared from a "primary standard," that is, a substance that can be obtained in a high state of purity and conveniently weighed. Frequently, normal solutions are too concentrated for accurate measurement of the limited amount of acid or base in the substance that is being analyzed. In general practice, 0.1 normal solutions are quite satisfactory.

Titration is a process of measuring the volume of one solution that is required to react exactly with a definite amount of a second solution. In titrating acids and bases the point at which the reaction is completed is revealed by the color of an "indicator," which is added before the titration is started. The selection of a suitable indicator is explained below. *In practical work equal volumes of solutions of the same normality are considered to react exactly with one another.* If the two solutions do not have the same normality, it takes proportionately more

of the less concentrated to titrate a given quantity of the more concentrated. If solution A is titrated against solution B, the following relationship holds:

$$\text{volume of A} \times \text{normality of A} = \text{volume of B} \times \text{normality of B}$$

Thus if the normality of A is known, that of B may be calculated from the titration data, since from the above equation it may be seen that

$$\text{normality of B} = \frac{\text{volume of A} \times \text{normality of A}}{\text{volume of B}}$$

As an example of a typical standardization let us assume that 21.4 ml. of a solution of sodium hydroxide are required to neutralize 25 ml. of tenth-normal (0.1N) oxalic acid. The normality of the sodium hydroxide solution will then be equal to

$$\frac{25 \times 0.1}{21.4} = 0.117$$

We should say, therefore, that the sodium hydroxide solution is 0.117 normal, which means simply that it is 0.117 times as concentrated as a normal solution.

It is obvious that if solution A is one-tenth as concentrated as solution B, a given volume of A is equivalent to one-tenth of that volume of B. Likewise in the example just considered, 1000 ml. of the solution that is 0.117 normal is equivalent to only 117 ml. of normal solution. *The volume of a solution used in a titration when multiplied by its normality gives the equivalent volume of normal solution.* In other words, by means of this calculation one determines the volume that the solution would occupy if it were exactly normal.

Analysis of biological materials

In order to determine the percentage of a given constituent in any material, two things must be known, namely, the weight of sample taken and the amount of standard solution needed to titrate it. The following calculation of the citric acid content of lemon juice is typical:

Weight of sample	5.0 g.
Volume of alkali, normality 0.103, for titration	34.6 ml.
Molecular weight of citric acid ($H_3C_6H_5O_7$)	192
Hydrogen equivalent of citric acid	3
Equivalent weight of citric acid	64
Weight of citric acid per ml. of normal reagent	0.064 g.
Volume of normal citric acid equivalent to alkali used for titration (34.6 × 0.103)	3.56 ml.
Weight of citric acid in sample (3.56 × 0.064)	0.2278 g.
Concentration of acid in the juice $\left(\dfrac{0.2278 \times 100}{5} \right)$	4.56 per cent

Indicators

The ability to determine when sufficient reagent has been added in titration of an acid or base depends upon the sensitivity of certain dyes to changes in acidity. Such compounds are called *indicators*. Many dyes that are used as indicators change color in either slightly acidic or basic media rather than at exact neutrality. This is a desirable characteristic, as may be seen by a study of the salts formed through interaction of the respective acids and bases. Salts of strong bases and weak acids, *e.g.*, sodium carbonate, undergo hydrolysis when dissolved in water, producing basic solutions, whereas those salts formed by union of weak bases and strong acids, like ammonium sulfate, are somewhat acidic for a similar reason. Therefore when titrating an acid with a base, or vice versa, it is essential that the standard solution be added until the same degree of acidity or alkalinity is produced that would result by dissolving the corresponding salt in water. Choice of indicators is made accordingly rather than with the idea of determining the point of exact neutrality. Methyl orange, methyl red, bromthymol blue, and phenolphthalein are examples of indicators in common use. The first two are suitable for titration of weak bases, and the last one for weak acids.

HYDROGEN-ION CONCENTRATION

"Active" acidity as contrasted to "total" acidity is due solely to that portion of the total replaceable hydrogen that, under prevailing conditions, exists in the ionic state. As a commonplace illustration one may liken acidity of a solution to the wealth of an individual. Total acidity corresponds to total wealth, which includes currency, real estate, personal property, notes, bonds, and so on. Active acidity, on the other hand, is comparable only to currency, and just as the response of a ticket salesman is conditioned by the currency in the hand of a prospective purchaser, so the behavior of a cell is conditioned by the active hydrogen in the aqueous medium surrounding it. It is true that other forms of wealth are convertible into currency, and, likewise, acids tend to dissociate further as some of their hydrogen ions are used up by chemical reaction.

The hydrogen-ion concentration has much more to do with enzyme action and the maintenance of a normal colloidal structure in cells than has total acidity. A fatigued muscle may contain as much as 0.4–0.5 per cent lactic acid for a time without undergoing injury, but a like concentration of hydrochloric or sulfuric acid would result in death to the tissue. Consider also the supply of carbon dioxide—potentially carbonic acid—carried by the blood stream. Introduction into the blood

stream of an equivalent amount of other common acids, even such acids as citric and acetic, which the body normally oxidizes for energy, would doubtless be fatal. In these two instances it is not the concentration of total acid that determines whether or not injury results; it is the concentration of hydrogen ions. Figure 7–2 shows the effect of various hydrogen-ion concentrations on plants. Growth is poor when the active acidity is too high (pH too low).

Courtesy of Illinois 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. 7–2. The effect of increasing acidity (left to right) on growth of red clover. The poor growth results from calcium starvation, because high acidity interferes with the absorption and retention of calcium by the plant roots.

For these reasons measurement of the hydrogen-ion concentration frequently is of more significance than determination of titratable acidity or alkalinity of a given biological fluid or extract. But one must not conclude that active acidity is always of prime importance. A familiar illustration involves the use of soda and sour milk as a leavening agent in the making of corn bread. If the housewife should add only enough soda to react with the hydrogen ions initially present in the sour milk, most of the lactic acid, *i.e.*, the nonionized part, would not be neutralized, and sour bread would be the inevitable result. Sufficient soda to react with all of the acid must be added.

Water is a neutral substance because it yields an equal, though relatively small, number of hydrogen and hydroxyl ions. The concentration is known to be 0.0000001 (also expressed 10^{-7}) gram-ion per liter, which is equivalent to 0.0000001 g. of hydrogen ions and 0.0000017 g. of hydroxyl ions. It must be borne in mind that hydrogen ions exist even in basic solutions. Their concentration is reduced as basicity increases, but, theoretically, all are never entirely removed from a solution.

Concentration of hydrogen ions may be expressed directly in gram-ions per liter as above (comparable to moles per liter when considering a given reagent). Usually, however, when dealing with biological materials this necessitates the use of relatively small decimal fractions with attendant possibility of an error in writing. A more convenient method is that of pH, by which one merely expresses the logarithm of the reciprocal of the hydrogen-ion concentration. Thus the reciprocal of 0.0000001, the hydrogen-ion concentration of water, is 10,000,000 or 10^7, and the logarithm of this number is 7. The pH of pure water therefore is 7, and all solutions of such pH are said to be neutral.

That acidic solutions have pH values less than 7 is apparent in view of the fact that any concentration greater than 0.0000001 will have a correspondingly smaller reciprocal and consequently a smaller logarithm (pH value). Conversely, all basic solutions have pH values greater than 7. To those unfamiliar with this system of expressing active acidity, it may seem that small differences in pH correspond to unbelievably great differences in actual hydrogen-ion concentration. This can best be realized by a comparison of several hydrogen-ion concentrations and corresponding pH values simultaneously:

Concentration in gram-ions per liter	Reciprocal of concentration	pH
0.1	10	1
0.001	1,000	3
0.000001	1,000,000	6
0.00000001	100,000,000	8
0.0000000001	10,000,000,000	10

Thus far only a tenfold (or some power thereof) increase or decrease in hydrogen-ion concentration has been considered, but it is obvious that between any two such values, e.g., 0.1 and 0.01 gram-ions per liter, are countless possible concentrations with corresponding pH values. To make interpretation of pH values easy Table 7–2 has been included. Column 1 merely gives the approximate logarithms of some appropriate numbers between 1 and 10 (listed in column 2).

Table 7–2

Comparison of pH values

Change in pH	Approximate equivalent change in acidity
0.1	1.25 times
0.2	1.6 times
0.3	2.0 times
0.6	4.0 times
0.9	8.0 times
1.0	10.0 times

Example. Compare pH 6.6 and 5.1.

The difference between pH 6.6 and 5.1 is 1.5 units, which can be broken down into units found in the table, namely, 1.0, 0.3, and 0.2.

A difference of 1.0 equals 10 times.
A difference of 0.3 equals 2 'times.
A difference of 0.2 equals 1.6 times.

Hence a difference of 1.5 equals $10 \times 2 \times 1.6 = 32$.
Therefore pH 5.1 is 32 times as acid as pH 6.6.

Buffers

Although addition of a minute amount of hydrochloric acid, or any other strong acid, to water produces relatively a great change in pH, biological fluids, in general, do not undergo a comparable change when strong acid or base is added to them, because of the presence of certain compounds in these fluids. Such compounds which resist change in acidity or basicity are known as *buffers*.

In general, a buffer consists of a weak acid (or base) and its salt. The buffer is the *mixture* of the two substances. Examples are acetic acid—sodium acetate, carbonic acid—sodium bicarbonate, ammonium hydroxide—ammonium chloride. Frequently the second hydrogen of a di- or tri-basic acid serves as the weak acid, as in the buffer NaH_2PO_4—Na_2HPO_4. Other metals, such as potassium, are equally satisfactory in buffers provided they form water-soluble salts with the acids concerned.

Buffers exert their effect through chemical reactions that use up most of the hydrogen or hydroxyl ions that are added. This action depends, fundamentally, on the fact that the weak acid (or base) is only slightly ionized. A weak acid HA ionizes according to the equation:

$$HA \rightleftarrows H^+ + A^-$$
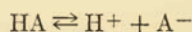
The A^- here represents the acid radical. Since this ionization is a reversible process, the addition of extra hydrogen ions shifts the reaction back to the left (law of mass action) and thereby converts most of the added H^+ into undissociated HA molecules. On the other hand, if a strong base is added to the buffer, the OH^- ions react with H^+ to form water, and more of the HA molecules ionize to replace most of the H^+ ions used. In either case the pH remains relatively constant.

The exact pH of any individual buffer solution and the pH change resulting from the addition to it of a certain quantity of strong acid or alkali may be calculated readily from a knowledge of the dissociation constant of the weak acid or base in the buffer. The mathematical expression for the dissociation constant K_a of a weak acid is based on the equation for its ionization. It is:

$$K_a = \frac{[H^+] \cdot [A^-]}{[HA]}$$

The brackets indicate concentrations expressed on a molar basis. Since weak acids ionize to only a slight degree, the numerical values of $[H^+]$ and $[A^-]$ are small, whereas $[HA]$ is large. Consequently K_a for weak acids is a small number, for example, 0.000018 in the case of acetic acid. The weaker the acid, the smaller is its K_a value, and vice versa. Strong acids like hydrochloric are considered to be completely ionized in water solution and, therefore, have no, or more exactly an infinitely large, K_a value.

Considerations exactly similar to those set forth above apply also to weak bases. The corresponding expressions are:
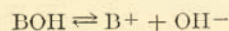
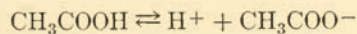$$BOH \rightleftharpoons B^+ + OH^-$$

and

$$K_b = \frac{[B^+] \cdot [OH^-]}{[BOH]}$$

To illustrate how pH values of particular buffers may be calculated, several typical problems will be worked out.

PROBLEM 1. What is the pH of a 0.1M acetate buffer solution?

The phrase "0.1M acetate buffer" means that both acetic acid and sodium acetate are present in 0.1M concentration. The ionization equation for acetic acid is:

$$CH_3COOH \rightleftharpoons H^+ + CH_3COO^-$$

and its dissociation constant has the numerical value 1.8×10^{-5}. The pH may be found from the expression for the dissociation constant:

$$K_a = 1.8 \times 10^{-5} = \frac{[H^+] \cdot [CH_3COO^-]}{[CH_3COOH]}$$

by inserting the proper values for $[CH_3COO^-]$ and $[CH_3COOH]$, and solving for $[H^+]$.

Let $X = [H^+]$. Then $[CH_3COOH] = (0.1 - X)$, since the original acetic acid concentration was 0.1M. The concentration of acetate ions is the sum of the concentrations resulting from the ionization of both acetic acid and sodium acetate. That from the acetic acid is obviously X, while that from sodium acetate is 0.1, because such salts are strong electrolytes and are completely ionized in aqueous solution. Therefore, $[CH_3COO^-] = (0.1 + X)$.

Substituting these values in the expression for K_a we have:

$$1.8 \times 10^{-5} = \frac{X \cdot (0.1 + X)}{(0.1 - X)}$$

Now X is very much smaller than 0.1, since we are dealing with a slightly ionized acid, so $(0.1 + X)$ and $(0.1 - X)$ are both very nearly equal to 0.1. Making this substitution, we have as a close approximation:

$$1.8 \times 10^{-5} = \frac{0.1X}{0.1}$$

or
$$X = 1.8 \times 10^{-5}$$

Since the pH is the negative logarithm [1] of the molar H^+ concentration,

$$\begin{aligned} pH &= -\log(1.8 \times 10^{-5}) \\ &= -\log 1.8 + (-\log 10^{-5}) \\ &= -0.26 + 5 \\ &= 4.74 \quad \text{(Answer to Problem 1)} \end{aligned}$$

Note that in this problem $[H^+] = K_a$, or $pH = pK_a$. This relation holds for any buffer where the acid and salt are present *in equal amounts*. When different amounts are present, the pH may be calculated from the equation:

$$[H^+] = K_a \times \frac{[\text{acid}]}{[\text{salt}]}$$

In the case of a base-type buffer, the corresponding equation is:

$$[OH^-] = K_b \times \frac{[\text{base}]}{[\text{salt}]}$$

If the composition of the buffer and the numerical value of K_b are known, $[OH^-]$ and, hence, pOH can be calculated. From this result the corresponding pH value can easily be found from the relation:

$$pH + pOH = 14$$

which holds for any aqueous solution at room temperature.

The use of the above equations in buffer calculations is illustrated below.

PROBLEM 2. What is the pH of 40 ml. of 0.1M acetate buffer to which has been added 10 ml. of 0.1N HCl?

The HCl added amounts to 1 m.e.q. (10×0.1), and the buffer originally contained 4 m.e.q. (40×0.1) each of acetic acid and sodium acetate. For purposes of calculation it may be assumed that the 1 m.e.q. of HCl reacts with 1 m.e.q. of sodium acetate to form 1 m.e.q. of additional acetic acid [2]:

$$HCl + CH_3COONa \longrightarrow NaCl + CH_3COOH$$
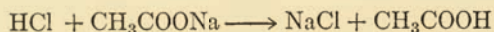
Consequently, the acid : salt ratio of the buffer has been changed from 1 (*i.e.*, 4 : 4) to 5 : 3. The pH may therefore be calculated by substituting known values in the equation above:
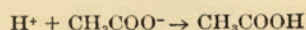
$$[H^+] = 1.8 \times 10^{-5} \times \frac{5}{3}$$

$$= 3 \times 10^{-5}$$

whence $pH = 4.52$ (Answer to Problem 2)

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[1] The small letter "p" is used to mean "the negative logarithm of."

[2] More precisely, since the HCl, NaCl, and CH$_3$COONa do not exist as such in water solution but are 100 per cent ionized at all times, the only reaction which actually occurs is:

$$H^+ + CH_3COO^- \rightarrow CH_3COOH$$

Note that the original pH of the buffer has dropped only 0.22 unit. This answer shows very clearly the effect of the buffer because if 10 ml. of 0.1N HCl are added to 40 ml. of plain water, without any buffer present, the resulting H^+ concentration is 1 m.e.q. in 50 ml., or 0.02M. Therefore the pH is $-\log 0.02$, or 1.7, a very much greater drop.

The salt-acid combination is most effective when the salt and acid are present in equal molecular proportions. Of course, there is a limit to the capacity of the buffer to take up hydrochloric acid or sodium hydroxide. For example, when about 85 per cent of the sodium acetate has been converted into acetic acid, or vice versa, the limit is close at hand. On adding more acid or alkali, the pH of the solution changes rapidly, and, hence, there is little buffer action.

The most widely used buffers are mixtures of sodium or potassium salts of relatively weak acids and the corresponding free acid. For most purposes, acids such as phosphoric, carbonic, acetic, and other organic acids are used. Carbonates, bicarbonates, and phosphates, together with proteins, form the most important buffers in the body. These maintain the pH within very narrow limits even though considerable acid or base is added.

Measurement of pH

Each hydrogen ion bears an electric charge, and the concentration of these ions can be measured most accurately by electrometric means. This method, however, requires the use of relatively expensive apparatus and an experienced operator. For many dyes there is a particular degree of acidity at which there is a very definite change in color, and fortunately the various dyes, or indicators, change color at different hydrogen-ion concentrations. This fact serves as a basis for a colorimetric method that is quite simple, as well as fairly accurate. The method consists of matching the color produced by an appropriate indicator in the unknown solution with the color of a standard solution of known hydrogen-ion concentration to which the same indicator has been added. If the color of the unknown is the same as that of the standard, the hydrogen-ion concentration likewise must be the same. Standard color charts and glass discs of appropriate colors that may be substituted for the standard solutions possess the added advantage of being more permanent than the solutions.

In Table 7–3 are given the approximate pH values of a number of biological materials.

Table 7–3

pH values of representative biological materials

Material	pH Value
Blood, normal limits	7.3–7.5
Blood, extreme limits	7.0–7.8
Enzymes, activity range of	
Amylopsin, optimum	7.0
Erepsin, optimum	7.8
Invertase, optimum	5.5
Lipase, pancreatic	7.0–8.0
Maltase, optimum	6.1–6.8
Pepsin, optimum	1.5–2.4
Trypsin, optimum	8–9
Fruit juices	
Apple	3.8
Banana	4.6
Grapefruit	3.0–3.3
Orange	3.1–4.1
Tomato	4.2
Gastric juice, adult	1.6–1.8
Milk, cows, limits	6.2–7.3
Milk, human	7.0–7.2
Muscle juice	6.8
Plants (extracted juice)	
Alfalfa tops	5.9
Carrot	5.2
Cucumber	5.1
Peas, field	6.8
Potato	6.1
Rhubarb, stalks	3.4
String beans	5.2
Saliva	6.2–7.6
Sweat	4.5–7.1
Tears	7.2
Urine, human, limits	4.2–8.0

REVIEW QUESTIONS ON ACIDITY

1. Explain the difference in meaning of "active" and "total" acidity. In what terms are concentrations of the two usually expressed?

2. Define: (1) molar solution, (2) normal solution, (3) hydrogen equivalent, (4) gram equivalent weight, (5) indicator.

3. What is the normality of a solution of NaOH if 25 ml. of it are required to neutralize 20 ml. of 0.1N oxalic acid?

4. What is the normality of a solution of acetic acid which contains 0.3 g. of this reagent in 50 ml.?

5. What volume of 0.5N NaOH would be required to neutralize the acetic acid mentioned in the preceding question?

6. What is a hydrogen ion? Represent by equations the ionization of (1) HNO_3, (2) NaCl, (3) $CH_3CHOHCOOH$, (4) NaOH, (5) H_2SO_4.

7. What is a buffer? Write equations to illustrate the reaction of a buffer with HCl and NaOH, respectively.

8. What do pH 5, 7, and 9 mean with respect to acidity, neutrality, and alkalinity?

9. How much more acid is the first member of the following pairs than the second member: pH 6 vs. 7, pH 4 vs. 7, pH 4.2 vs. 6.3, pH 4 vs. 8.3, pH 2 vs. 10?

10. Give the approximate pH values of six representative biological materials.

11. Give the names and structural formulas of ten organic acids (other than fatty acids) and five organic bases commonly found in biological materials.

REFERENCES AND SUGGESTED READINGS

Bonner, James, *Plant Biochemistry*, Academic Press, Inc., New York, 1950.

Clark, W. M., *The Determination of Hydrogen Ions*, 3rd ed., The Williams and Wilkins Company, Baltimore, 1928.

Kolthoff, I. M. and Laitinen, H. A., *pH and Electrotitrations*, 2nd ed., John Wiley and Sons, Inc., New York, 1941.

La Motte, F. L., Kenny, W. R., and Reed, A. B., *pH and Its Practical Application*, The Williams and Wilkins Company, Baltimore, 1932.

Pierce, W. C. and Haenisch, E. L., *Quantitative Analysis*, 3rd ed., John Wiley and Sons, Inc., New York, 1948.

Schmidt, C. L. A. and Allen, F. W., *Fundamentals of Biochemistry*, 1st ed., McGraw-Hill Book Company, Inc., New York, 1938.

Chapter 8

BIOCHEMICALLY IMPORTANT MINERAL ELEMENTS

Definition

The mineral elements of biochemical interest are all those chemical elements, except carbon, hydrogen, oxygen, and nitrogen, which are, or may be, present in the tissues of living organisms. They are frequently called inorganic, or ash elements, since, as a rule, they remain in the ash when biological materials are burned. Those which have been proved to be essential constituents of living tissues are listed below. They are classified as major and minor (or trace) elements on the basis of the amounts usually present in biological samples.

MAJOR MINERAL ELEMENTS

Metals	*Nonmetals*
Sodium	Sulfur
Potassium	Chlorine
Calcium	Phosphorus
Magnesium	

· MINOR MINERAL ELEMENTS OR TRACE ELEMENTS

Metals	*Nonmetals*
Iron	Iodine
Copper	Boron
Cobalt	
Zinc	
Manganese	
Molybdenum	

Most of these elements are required by living organisms, generally. However, boron is needed only by plants; sodium, chlorine, iodine, and cobalt, only by animals. Thus plants require 15 chemical elements in all (counting C, H, O, and N), and animals 18.

Aluminum, vanadium, arsenic, bromine, fluorine, silicon, and other mineral elements are widely distributed in living cells and may have important biological functions, but as yet their essential nature has not been demonstrated, except in a few isolated cases (*e.g.*, vanadium is apparently a normal, necessary constituent of a respiratory pigment in

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certain marine worms). Still other elements, for example, selenium, are found only in the tissues of plants or animals grown in certain restricted localities. Some of these elements which are of interest for one reason or another are discussed in more detail below.

Ashing

When it is desired to examine a biological sample for mineral elements, the first step is to dry the sample, then burn it to remove organic matter, and convert the mineral elements present into simple inorganic compounds. From the chemical standpoint the process of burning or ashing is essentially a very vigorous oxidation, which is carried out in the air at a temperature of about 600–800°C. The organic substances present are decomposed as the sample is heated and turn black on account of the formation of free carbon. As the heating continues this carbon is oxidized to carbon dioxide, which escapes. Disappearance of the black color, therefore, indicates that the ashing is complete. The hydrogen in the original organic matter is converted to water vapor, and the nitrogen escapes in the form of nitrogen gas.

The mineral elements are contained in biological materials partly in complex organic combinations such as sulfur in methionine, phosphorus in lecithin, iron in hemoglobin, etc. (see Table 8–2). As these organic substances are destroyed during the ashing process, the mineral elements in them combine with each other—metals with nonmetals—and frequently also with oxygen to form inorganic salts such as the chlorides, sulfates, phosphates and silicates of sodium, potassium, calcium, and magnesium. The ash, then, consists largely of these salts.

If the sample happens to contain relatively more metals than nonmetals, as in vegetables, fruits, milk, only a part of the metals present can be converted into such salts because there will not be enough nonmetals to go around. In this case the excess metals combine with oxygen or carbon dioxide, which is always available from the burning organic matter, to form oxides and carbonates. Sodium and potassium form the carbonates, and calcium and magnesium the oxides, since their carbonates are unstable at the high temperatures used. Such ash, therefore, is strongly alkaline and, because of the carbonates present, effervesces when dissolved in mineral acid.

On the other hand, if the sample contains a larger amount of nonmetals than metals, as in meats, cereals, eggs, the excess nonmetals will be converted into the corresponding oxides, most of which are volatile, and therefore escape (e.g., SO_2). Thus, 99 per cent of the sulfur in rice and in corn meal is lost during burning. Silicon is an exception since its oxide, SiO_2, is very nonvolatile. This loss may be prevented by adding to the sample before ashing a reagent which will shift the balance of metals

versus nonmetals in favor of the metals. The oxides, peroxides, hydroxides, carbonates, or nitrates of sodium, potassium, calcium, or magnesium are suitable for this purpose. The peroxides or nitrates are especially useful because they are also strong chemical oxidizing agents and help to complete the ashing in a shorter time or at a lower temperature.

Once the ash has been obtained it is usually taken up in an acid solution such as nitric, the insoluble silica filtered off, and the solution tested for metallic and nonmetallic ions by the usual methods of qualitative analysis.

Occurrence

The amount of inorganic material contained in foodstuffs varies with the material, and in the case of plants with the type of soil, fertilizer, etc. Approximately 4.4 per cent of the total weight of the body consists of inorganic compounds. The bones contain from 22 to 82 per cent, whereas the muscles and the body fluids contain about 1 per cent. Plant material varies in ash content from 1 to 10 per cent. More is contained in the stems and leafy portions of the plants than in the seeds.

Although the concentration of any element in a given food material may vary markedly, depending upon the conditions under which it is produced, the table given below and the tables in the Appendix afford a fairly accurate estimate of the relative concentrations of these elements in various foodstuffs. In Table 8–1 note the low calcium content of cereals in comparison with the large demands for this element in the animal body. Observe that the milling process results in a concentration of the mineral elements in the bran and, hence, a depletion of the same in the flour.

Table 8–1

Mineral composition of some typical foodstuffs

(Fresh basis)

percentage of:	Ca	Mg	K	Na	P	Cl	S	Fe
Wheat, whole	0.055	0.163	0.409	0.106	0.342	0.088	0.175	0.006
Wheat, flour, white	.021	.021	.137	.053	.096	.079	.155	.0012
Wheat, bran	.065	.420	1.25	.007	1.43	.042	.245	.014
Corn meal	.016	.084	.213	.039	.152	.146	.111	.0011
Cabbage	.054	.016	.217	.038	.031	.034	.074	.00066
Turnips	.042	.019	.193	.104	.032	.054	.048	.00061
Cow's milk	.123	.019	.129	.047	.088	.114	.031	.00024
Animal body (Ox).	1.24	.030	.117	.089	.682			

More extensive tables are given in the Appendix. The table on trace elements (p. 443) shows the small amounts contained in foodstuffs. Of these elements iodine is the least abundant. It should be noted that

Table 8–2

Some specific organic compounds of mineral elements known to exist in plant or animal materials

Element	Compound name and formula		Contained in
Potassium	Acid salt of tartaric acid	$KHC_4H_4O_6$	Grapes, cucumbers
	Salts of citric acid	$K_2HC_6H_5O_7$	Fruits, vegetables
	Salts of malic acid	$KHC_4H_4O_5$	Fruits, vegetables
Calcium	Acid salt of tartaric acid	$Ca(HC_4H_4O_6)_2$	Grapes
	Salts of phytic acid	$C_6H_6(CaPO_4)_6$	Bran of wheat, rye, etc.
	Calcium caseinate	Not known	Milk
Magnesium	Salts of phytic acid	$C_6H_6(MgPO_4)_6$	Bran of wheat, rye, etc.
	Chlorophyll	$C_{55}H_{72}N_4MgO_5$	Green plants
Iron	Hemin	$C_{34}H_{32}N_4FeO_4Cl$	Hemoglobin of blood
	Ferratin	Not known	A protein of spleen and intestinal wall
Sulfur	Cystine	$C_6H_{12}N_2S_2O_4$	Proteins
	Glutathione	$C_{10}H_{17}N_3SO_6$	Animal tissues
	Insulin	$(C_{45}H_{69}N_{11}SO_{14})_n$	A hormone, secreted by Isles of Langerhans
	Thiamine chloride	$C_{12}H_{17}N_4SCl$	Yeast, pork muscle, etc.
	Allyl isothiocyanate	C_3H_5NCS	Mustard, onions
	Allyl sulfide	$(C_3H_5)_2S$	Garlic, radishes, cabbage, turnips, etc.
Phosphorus	Lecithins _e.g._,	$C_{44}H_{88}NPO_9$	Egg yolk, brain, nerves, etc.
	Cephalins _e.g._,	$C_{41}H_{80}NPO_8$	Blood
	Nucleic acids _e.g._,	$C_{20}H_{45}N_5PO_{26}$	Nuclear tissue, _e.g._, thymus
	Phosphoproteins	Not known	Egg yolk, milk
	Hexosemonophosphate	$C_6H_{11}O_5(H_2PO_4)$	Yeast, muscle
	Hexosediphosphate	$C_6H_{10}O_4(H_2PO_4)_2$	Yeast
	Phytic acid	$C_6H_6(H_2PO_4)_6$	Bran of wheat, rye, etc.
	Creatine phosphate	$C_4H_{10}N_3PO_5$	Muscle
Chlorine	Chloromycetin	$C_{11}H_{12}O_5N_2Cl_2$	_Streptomyces venezuela_
Iodine	Thyroxine	$C_{15}H_{11}I_4O_4N$	A hormone secreted by thyroid gland
Copper	Hemocyanins	Not known	Respiratory protein in lower animals (_e.g._, lobster)
Cobalt	Vitamin B₁₂	$C_{61-64}H_{86-92}N_{14}O_{12}PCo$	Animal tissues
Zinc	Carbonic anhydrase	Not known	Red blood cells

the unit (micrograms) in which the iodine is expressed is only one-thousandth as large as that used for the other elements. Cereals, as a class, contain relatively high percentages of all these elements, but it must be borne in mind that these high values are due largely to the high percentage of dry matter in cereals. Conversely, vegetables do not rank as high on a percentage basis because of the large amount of water contained in them. Perhaps a more correct basis for comparison would be percentages of the foods as eaten, that is, in the cooked condition. Differences resulting from water content would then be largely eliminated.

A second consideration that must be kept in mind is the quantity of a particular food that is eaten. A food may be conspicuously high in some element, but if it is eaten only occasionally, it contributes very little toward the actual supply of that element in the diet. Lobster, for example, is high in copper (1.5 mg. per 100 g.), but since lobster is eaten rarely or not at all by the majority of people, it is a relatively unimportant factor in supplying copper to the average diet. Another example will illustrate the converse situation. Although milk is low in iron, it is, because of the large quantity consumed, one of the largest contributors of iron in the diet of a small child.

On the basis of both composition and consumption, cereals and vegetables are the chief sources of supply of the trace elements.

The mineral elements exist in living tissues partly in the form of inorganic ions (K^+, Na^+, Ca^{++}, Mg^{++}, Fe^{++}, Cl^-, SO_4^{--}, HPO_4^{--}, $H_2PO_4^-$, etc.) dissolved in tissue fluids, and partly as components of various organic molecules. Many of the latter are of particular biological importance and are therefore given special attention. In Table 8–2 are a few organic compounds that are known to exist in plant or animal materials.

Unusually large or small amounts of particular mineral elements occur in the soil in various regions of the world, and this distribution often causes the vegetation and drinking water in these areas to contain correspondingly high or low amounts of the elements concerned. As a result, domestic animals and human beings consume abnormal quantities, frequently with serious, and even fatal, consequences. The best known cases involve deficiencies of copper, cobalt, zinc, boron, and iodine in some localities, and dangerously high concentrations of selenium, molybdenum and fluorine in others. These cases will be discussed below in greater detail in connection with the individual elements. In general, quantities of the mineral elements much larger than the physiologically required amounts are likely to be toxic. This is particularly true of the trace elements.

General functions

Considerable importance has been attached by certain investigators to a proper balance in the diet between basic and acidic elements. Such emphasis is based on the idea that an excess of either type is not desirable. As a result of metabolism the basic and acidic elements will be combined to form salts in much the same way that these elements combine when the foodstuff is burned to form salts in the ash. To illustrate this result more clearly: the sulfur of cystine and the magnesium of chlorophyll may, as a result of metabolism, be combined and excreted as magnesium sulfate. Since in the body a number of organic acid radicals, *e.g.*, the citrate and malate, may be oxidized completely to carbon dioxide and water, the corresponding metallic salts frequently contribute only to the supply of basic elements in the diet. It is for this reason that most fruit juices, in spite of their actual acidity, exert a basic effect in the body. A few organic acids, however, are not so oxidized and, hence, contribute to the total acidity (which normally is caused by the three elements, chlorine, phosphorus, and sulfur). In this class are benzoic and quinic acids. The presence of these acids in cranberries, plums, and prunes is responsible for the acidic effect of these fruits in the body.

In order to determine whether there is an excess of basic or acidic mineral elements in foodstuffs, the quantities of each are expressed in terms of the equivalent volume of $0.1N$ base or acid per 100 g. of the food. If the basic elements together are equal to a larger volume of $0.1N$ solution than the total of the acidic elements, the foodstuff is said to be basic, and vice versa; *e.g.*, 100 g. of beef will contain 120 ml. of $0.1N$ acid in excess of the basic elements. Milk, on the other hand, contains 22.5 ml. excess of base over acidic elements. Fruits, vegetables, milk, and legumes contain an excess of basic elements, while cereals, meat, and eggs have a preponderance of the acidic elements. Certain investigators have assumed that people naturally combine foodstuffs high in basic material with foodstuffs high in acidic compounds. It has been suggested that perhaps we eat vegetables with meat for this reason. To have an *exact balance* between the two may not be as important as has been assumed, but it is probable that a *large excess* of one over the other is not a desirable condition.

The remarkable constancy of the pH of blood, 7.3–7.5, is made possible through the systems of buffers contained therein. In addition to the proteins, especially hemoglobin, the chief buffer systems are the carbonates, H_2CO_3 and $NaHCO_3$, and the phosphates of potassium and sodium, *e.g.*, NaH_2PO_4 and Na_2HPO_4.

The *osmotic pressure* existing between body cells is due in part to the presence of salts of the mineral elements. The movement of liquids from

one part of the cell to the other or through the walls of a cell may be brought about by the presence of these salts. If a high concentration of salts exists in a given locality, water tends to flow in that direction to dilute the solution, while the salts tend to move in the direction of the water, thus equalizing conditions. Similarly, the water-holding capacity of the blood is in part due to this action of its inorganic salts.

Irritability of muscles and nerves, that is their ability to respond to physiological stimulation in a normal manner, is dependent upon the proper kind and amount of inorganic ions in the body fluids which bathe them. Many enzymes are inactive unless some particular inorganic ion is present. Examples are given below and in Chap. 10.

Functions of specific elements

Sodium and Potassium. These elements exist in living tissues almost exclusively in the form of Na^+ and K^+ ions. They make up the basic portion of several buffer systems which maintain the physiological pH values not only of the blood, as mentioned above, but also of such body fluids as saliva and the pancreatic and intestinal juices. Potassium ions constitute the main base inside the cells of the body, whereas sodium is characteristically more concentrated in the blood plasma and interstitial fluids, that is, tissue fluids outside the actual cells. Sodium ions, in fact, make up over 90 per cent of all the cations (positively charged ions) of these fluids (Table 8–3) and thus, together with Cl^- ions, are mainly responsible for their osmotic pressure.

Table 8–3

Approximate electrolyte distribution in human blood plasma *

Cations		Anions	
(Milliequivalents per liter of plasma water)			
Sodium (Na^+)	154	Chloride (Cl^-)	106
Potassium (K^+)	5	Bicarbonate (HCO_3^-)	28
Calcium (Ca^{++})	5	Protein	17
Magnesium (Mg^{++})	3	Others	17

* Data from Hawk, Oser, and Summerson.

Sodium and potassium ions play a vital role in the process by which carbon dioxide is carried by the blood stream from the muscles, where it is produced, to the lungs, where it is eliminated from the body (see p. 187). Both tend also to promote muscle relaxation.

Common salt, sodium chloride, supplies most of the sodium and chlorine in the diet. The daily salt requirement for normal adult persons is in the neighborhood of 5 g., but the exact amount needed depends on the water intake, because salt is carried out of the body in the urine

and, especially, in sweat. The average American diet furnishes 10–15 g. of salt a day, which is more than adequate, except during profuse sweating. An extra gram of salt (above the 5 g. minimum) should be consumed for each liter of water intake in excess of 4 l. per day. This may amount to as much as 20–30 g. in extreme cases. So-called heat prostration after hard work in hot weather is often merely the result of uncompensated salt losses and may be prevented by proper attention to salt intake.

Among the symptoms characterizing potassium deficiency in rats are lethargy, distension of the abdomen, edematous kidneys, discoloration of the skin owing to improper blood supply, failure of growth, and early death. Pathological changes may be detected in the intestines, pancreas, kidneys, and hearts of such animals.

The potassium requirement for human beings is unknown, but the element is present in nearly all foods in such large amounts that the daily intake appears to be entirely adequate.

Calcium and Phosphorus. Both of these elements are essential constituents of all living cells. Calcium is present in the animal body in larger amounts than any other mineral element. About 99 per cent of the total is in the bones and teeth, which are made up of approximately one-half moisture and organic matter and one-half inorganic or mineral matter. The latter consists essentially of calcium phosphate together with smaller amounts of calcium carbonate. Magnesium and other elements are also present in minor amounts.

The remainder of the calcium exists mostly as Ca^{++} ions in the body fluids, where it is of fundamental importance for the normal activity of nerves, muscles, and heart, for the clotting of blood, and for maintaining the permeability of cell membranes. Thus the clotting of freshly drawn blood may be prevented, or greatly retarded, by the addition of a reagent, such as sodium oxalate or citrate, which removes the Ca^{++} ions by forming an insoluble (oxalate) or nonionized (citrate) product. Coagulation of milk also requires calcium, and the cementing substances which hold cells together in tissues appear to involve this element. Normally, the total calcium content of man's blood ranges from 9 to 11 mg. per 100 ml. of plasma. In the young it is slightly higher. About half the total is inorganic Ca^{++} ions. If the blood calcium falls below certain levels, depending upon the species of animal, tetany (generalized spasmodic muscle contractions) results, and death may follow unless restorative measures are employed. Both vitamin D and the secretion of the parathyroid gland operate in controlling the calcium content of the blood, the former through an increased "net absorption" of food calcium and the latter by mobilization of the calcium in certain labile structures of the skeleton.

The bulk of the phosphorus of the body, about 80 per cent, is also

contained in the bones and teeth.   About half the remainder is combined with the organic constituents of the muscles, while the rest is distributed throughout the blood and other tissues of the body.   Mention was made above of the buffer action of the alkali phosphates contained in the blood, and it will be recalled that phosphorus is also a constituent of various organic compounds previously studied, such as lecithins, certain proteins, and nucleic acids, as well as a whole series of substances involved in intermediary carbohydrate and fat metabolism and in muscle contraction (Chaps. 10 and 16).   Without a supply of phosphorus none of these essential materials could be formed by the living cell, so it is easy to understand why this element is vital to normal health and development.

The amounts of calcium and phosphorus needed daily by human beings depend on many factors, one of which is the Ca : P ratio in the food eaten.   When either element is consumed in a large excess, the excretion of the other is increased, so it is desirable that this ratio be about 0.7–1.0. In other words, the food eaten should contain about equal quantities of calcium and phosphorus, or slightly more of the phosphorus.   Furthermore, unless enough vitamin D is furnished (see p. 211) absorption of calcium through the intestinal wall is greatly reduced.

A third factor affecting calcium requirements is the presence in the food eaten of oxalic acid, or soluble salts of oxalic acid, which produce insoluble calcium oxalate.   This compound is not utilized as a source of calcium in the body.   The concentration of soluble oxalates in some plants reaches toxic levels (see p. 161).

For these and other reasons there is a tremendous variation among individuals in their ability to make use of dietary calcium, some absorbing as little as 5 per cent of the amount eaten.   There is evidence, however, that the efficiency of utilization increases when the calcium content of the food is low and the needs of the body are acute.

The amounts of various dietary essentials that should be supplied daily by a good diet have been carefully studied by the Food and Nutrition Board of the National Research Council.   Their recommended daily allowances, which are designed to provide an excess over the bare minimum requirements for life, were announced in 1941 and revised in 1948.   For calcium the recommended amounts are: adults 1.0 g., pregnancy (latter half) 1.5 g., lactation 2.0 g., children up to 10 years 1.0 g., adolescents 1.0 to 1.4 g.   The phosphorus intake should be at least equal to that of calcium for children, adolescents, and women during pregnancy and lactation, and for other adults about 1.5 times the calcium intake.

Calcium is the one essential mineral element which is most likely to be supplied in inadequate amounts by the average American diet.   The reasons for this situation are the facts that two of the principal types of foodstuffs, meats and cereals, are notably deficient in this element and

A. Symptoms of nitrogen deficiency appear in uninoculated soybeans growing in a soil which does not contain the appropriate nitrogen-fixing nodule bacteria. Note the pale green to yellowish color and the lower height of the plants in the uninoculated check strip.

B. Cotton boll showing potassium-deficiency symptoms. Left, normal, large, well opened boll. Right, small, immature, partly opened boll resulting from a deficiency of potassium.

Plate I. Nitrogen and potassium deficiencies in plants.

Plate II. Phosphorus hunger causes purpling of the leaves of many strains of corn.

that foods, in general, contain far less calcium than the animal body (Table 8–1). Milk is one of the best food sources, a quart supplying approximately one gram of both calcium and phosphorus. Cheese, egg yolk, and green leafy vegetables are also excellent sources; most other vegetables contain moderate amounts. Ground bone is well utilized by the animal body as a source of calcium and phosphorus, and, in fact, bone meal is commonly used as a supplement to the feed of domestic animals. The bones in most types of canned fish should be regarded as valuable food rather than being carefully picked out and thrown away as is usually the case.

On the other hand, the phosphorus supply needs no special attention. A diet providing the recommended amounts of protein and calcium will almost certainly contain sufficient phosphorus, at least for human beings. Cattle, however, occasionally become phosphorus deficient, but only when their feed consists almost entirely of forage grown in certain areas where the soil is low in this element (*e.g.*, parts of Wisconsin, Montana, Texas, and Florida).

Diets inadequate or imbalanced in their calcium, phosphorus, or vitamin D contents lead to rickets in children and to osteomalacia (softening of the bones) in adults (see chapter on Vitamins). All three factors are also obviously essential for sound, well-formed teeth, although many other influences likewise play important roles in dental health.

Magnesium. This element is also an indispensable constituent of all living cells. Since it is a part of the chlorophyll molecule (p. 388), magnesium is essential for photosynthesis and, hence, indirectly responsible for the production of all our foodstuffs. About three-fourths of the magnesium in the animal body is contained in the skeleton, the rest being present in the blood and other body fluids as Mg^{++} ions. The normal concentration of Mg^{++} in human blood serum is 2–3 mg. per 100 ml. Marked increases of up to 10–20 mg., resulting, for example, from the injection of soluble magnesium salts, lead to generalized anesthesia, complete muscle relaxation, and eventual death. If animals or human beings are deprived of magnesium until the blood level falls to 0.6–0.8 mg. per 100 ml., or less, symptoms of magnesium deficiency appear. These include dilation of capillaries, extreme nervousness, convulsions (tetany), and death.

Magnesium ions are necessary for the normal activity of several enzymes in the body, particularly peptidase, carboxylase, enolase, hexokinase, and others. In some cases the Mg^{++} may be replaced by other ions, such as Mn^{++}. However, for many biological functions there exists marked antagonism between various inorganic ions. Calcium and magnesium offer an outstanding illustration of this antagonism. Thus the enzyme adenosine triphosphatase, which is activated by Ca^{++} ions, is inhibited by Mg^{++}. Likewise Ca^{++} is the best antidote for the

anesthesia and paralysis of animals caused by excess Mg^{++} ions in the body fluids.

The magnesium requirement of human beings is not known with certainty, but it has been estimated to lie in the range of 0.2 to 0.4 g. per day for a normal adult. Studies of American dietaries indicate that the daily magnesium intake per 3000 calories varies from 0.17 to 0.53 g. This amount evidently meets the normal needs since cases of human magnesium deficiency are almost never encountered.

Iron and Copper. The iron contained in the animal and human body is mostly present as a component of certain conjugated proteins, of which the best known is *hemoglobin.* The iron is contained in the prosthetic group of hemoglobin, which is called *heme.* Like chlorophyll, heme belongs to the porphyrin class of substances and carries its iron atom in the center of the porphyrin ring.

Iron is also an essential component of several physiologically important enzymes such as *catalase, peroxidase,* the *cytochromes,* and *cytochrome oxidase.* Like hemoglobin, these enzymes are conjugated proteins with an iron-porphyrin type of prosthetic group. The iron content of hemoglobin and of the above enzymes lies in the range of 0.1 to 0.4 per cent. There is also present in the animal body another iron containing protein, *ferritin,* which, in contrast to the above materials, contains as much as 23 per cent of iron. Ferritin is present in the spleen and in the intestinal wall, where it is probably involved in the metabolism of iron in the body, particularly in absorption and storage.

Copper also is known to be associated with certain proteins in living tissues. It is an essential component of several enzymes and of a respiratory pigment, *hemocyanin.* This substance is present in the blood of certain lower animals, for example, the lobster, snail, and other invertebrates, and acts as an oxygen carrier just as hemoglobin does in higher forms. Hemocyanins from various species contain about 0.2 to 0.4 per cent of copper and range in molecular weight from 350,000 to several million. The copper is easily removed on acidification, being fully utilized as a source of food copper by animals. It has not been established whether the copper is attached to a prosthetic group of the porphyrin type. Although this might be expected by analogy with hemoglobin, the easy removal of the metal argues against this possibility, and no porphyrin derivative has been obtained from hemocyanin. However, *turacin,* a feather pigment of the turaco bird (South Africa), is a copper-porphyrin derivative.

The copper-containing enzymes include *ascorbic acid oxidase, polyphenol oxidase, laccase,* and several other oxidases. Each of these enzymes is a protein which contains a small amount of copper, ranging from 0.15 to 0.34 per cent, as an integral part of the molecule. The blood of the ox, sheep, and horse has been found to contain another

copper protein, *hemocuprein*, which has been purified and obtained in the form of blue crystals containing 0.34 per cent of copper. The copper in hemocuprein and in a similar substance, called *hepatocuprein*, in the liver accounts for nearly all the copper in the bodies of higher animals and, presumably, also in human beings.

The function of iron in the body is to serve as one of the essential raw materials for the various physiologically important iron-containing substances listed above. Given an adequate dietary supply of iron, the body can synthesize whatever amounts of hemoglobin and the iron-porphyrin enzymes it may need, provided, however, that sufficient amounts of copper are also supplied by the food intake. Just how copper functions in this regard is not understood, but it is an observed fact that copper is needed for the proper absorption and utilization of iron. It is not surprising, therefore, that diets low in either copper or iron lead to the development of nutritional anemia, that is, an abnormally low amount of hemoglobin in the blood, which is corrected by consuming a complete diet. An average family diet contains about 2 mg. of copper per person per day.

The tissues of the body are dependent almost entirely upon hemoglobin for their oxygen supply. In the lungs, where relatively large amounts of oxygen are available from the inhaled air, hemoglobin is converted into oxyhemoglobin. The oxygen is held in a rather loose combination and is easily given off whenever the oxyhemoglobin reaches a place where the prevailing oxygen pressure is low, that is, where little oxygen is present. This occurs normally, of course, in the muscles.

Hemoglobin also functions indirectly in the transportation of carbon dioxide to the lungs. Oxyhemoglobin is a stronger acid than hemoglobin itself and when it is formed in the lungs combines with a certain amount of potassium. As the oxyhemoglobin changes to hemoglobin in the muscles, this potassium is released and combines with carbon dioxide to form potassium bicarbonate. This substance is then carried back to the lungs where the potassium is taken up by freshly produced oxyhemoglobin, and the bicarbonate radical becomes free carbonic acid. The last step in the process of eliminating carbon dioxide involves the decomposition of this newly formed carbonic acid. Here still another mineral element, namely zinc, plays an essential role, since it is a component of *carbonic anhydrase*, an enzyme, present in red blood cells, which greatly speeds up the breakdown of carbonic acid:

$$H_2CO_3 \quad \underset{\text{anhydrase}}{\overset{\text{carbonic}}{\rightleftarrows}} \quad H_2O + CO_2$$
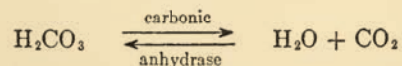
As a result of the action of this enzyme, carbon dioxide is released in gaseous form and exhaled as rapidly as it is brought to the lungs.

The iron-containing enzymes, such as cytochrome, are very widely

distributed in both plant and animal cells, and in the various lower forms of life, where they are concerned with biological oxidation processes (see Chap. 13). A good daily allowance of iron for human beings has been estimated by the Food and Nutrition Board to be as follows: normal adult, 12 mg.; woman during pregnancy and lactation, 15 mg.; infants, 6 mg.; children 1 to 12 years old, 7 to 12 mg.; and adolescents 13 to 15 years old, 15 mg. The need for iron is, of course, greatest whenever hemoglobin is being formed in the body in relatively large amounts, as during rapid growth and after the loss of blood. For this reason women should have more iron than men to compensate for blood lost during menstruation. In fact, there is evidence that adult men, and women after the menopause, get along quite satisfactorily even when they receive much less than the above amounts of iron.

An adequate copper intake is provided by an amount equal to about one-tenth that of iron. For adults, this is in the range of 1 to 2 mg. per day. A good diet containing enough of the other essential food factors may be depended upon to contain sufficient copper, except in a few areas where the copper content of the soil is abnormally low. Such copper-deficient areas have been reported in Holland, Florida, New Zealand, and parts of Great Britain and Australia.

Iron, on the other hand, is one of the mineral elements which is apt to be supplied in too small amounts by ordinary dietaries. This is partly due to the modern process of refining cereals, which removes much of the iron (see Table 8–1), and partly to the fact that the iron contained in many foods is not well assimilated by the body. Thus the iron in the iron-porphyrin substances listed above is not utilized, although most simple, inorganic iron salts, and even metallic iron itself if finely divided, are able to meet bodily needs very well. It is important, therefore, to consider not only the total iron content of various foods, but also the proportion of it which is physiologically available. The best food sources of iron are liver and egg yolk. Muscle meats, fish, green leafy vegetables, and dried peas and beans are also good sources, since they are high in total iron, and about half of it is available. White flour is low in iron, but is now being enriched approximately to whole grain levels so that it probably contributes materially to the total intake. Milk is deficient not only in iron, but also in copper and manganese.

Cobalt. That cobalt also plays a role in hemoglobin formation is evidenced by the fact that cattle and sheep in certain areas of New Zealand, Australia, and Florida sometimes suffer from a nutritional anemia that can be cured only by administration of small amounts of this element. Cobalt deficient areas have also been reported in Wisconsin, Michigan, New Hampshire, North Carolina, Great Britain, and Scotland. The occurrence of deficiency symptoms is limited to cows, sheep, and other ruminants. Feeding trials with cobalt-low rations have failed to

demonstrate a need for this element in rats or rabbits but showed that the requirement, if any, of the rat for cobalt is less than 0.6 μg. per day.

However, vitamin B_{12} has recently been found to contain cobalt, and since it plays a vital role in hemoglobin formation in many species, including man, the need for cobalt is evident. Since vitamin B_{12} contains only slightly more than 4 per cent of cobalt, and only a few micrograms of the vitamin are needed daily, it is not surprising that requirement for this element was difficult to demonstrate.

Manganese. This element seems to be essential for both plant and animal life, although as yet no specific manganese-containing substance of biological importance is known. Since plants deprived of manganese become chlorotic (contain too little chlorophyll), it is probable that the element plays some role in chlorophyll synthesis (Fig. 8–1). Lack of iron has a similar result (Fig. 8–2). In animals, manganese deficiency leads to poor growth, failure of reproduction, and abnormal bone development. Chickens suffer from a condition known as perosis (slipped tendon), which results from poorly shaped leg bones and is cured by manganese plus choline. Eggs produced by hens on a low-manganese ration hatch poorly.

Although the detailed manner in which lack of manganese brings about these difficulties is not known, it seems most probable that the explanation will be found to lie in the effect of Mn^{++} ions on various enzymes. Arginase is activated by a number of metal ions, of which Mn^{++} is probably the most important under natural conditions. Carboxylase requires either Mg^{++} or Mn^{++} for activity. Certain peptidases and phosphatases also are known to be activated by Mn^{++}.

The human requirement for manganese is not definitely known, but it is estimated to be in the neighborhood of a few mg. per day for the adult. Whole cereals, and especially cereal brans, are high in manganese. Tea is an outstanding source (150–900 parts per million in the dry leaves), contributing several milligrams daily to those who use this beverage. The daily intake on ordinary American diets has been calculated to be at least 2.5 mg., which evidently meets all bodily needs, since human manganese deficiency does not occur, as far as is known.

Zinc. The essential nature of zinc for animal life has been demonstrated by feeding young rats a ration very low in this element (Hove, Elvehjem, and Hart). Poor growth and various abnormalities resulted. It has also been found that about 0.3 per cent of zinc is present in the enzyme carbonic anhydrase, which occurs in red blood cells, as well as in the pancreas and stomach lining of animals. This enzyme plays a vital role in the elimination of carbon dioxide from the body, as explained above (p. 187). It has been suggested that carbonic anhydrase also functions in the secretion of hydrochloric acid by the stomach lining. Zinc likewise is a constituent of crystalline insulin, although zinc in-

Courtesy of New Jersey 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. 8–1. Right, manganese deficient leaves showing diminished chlorophyll content. Left, normal leaf.
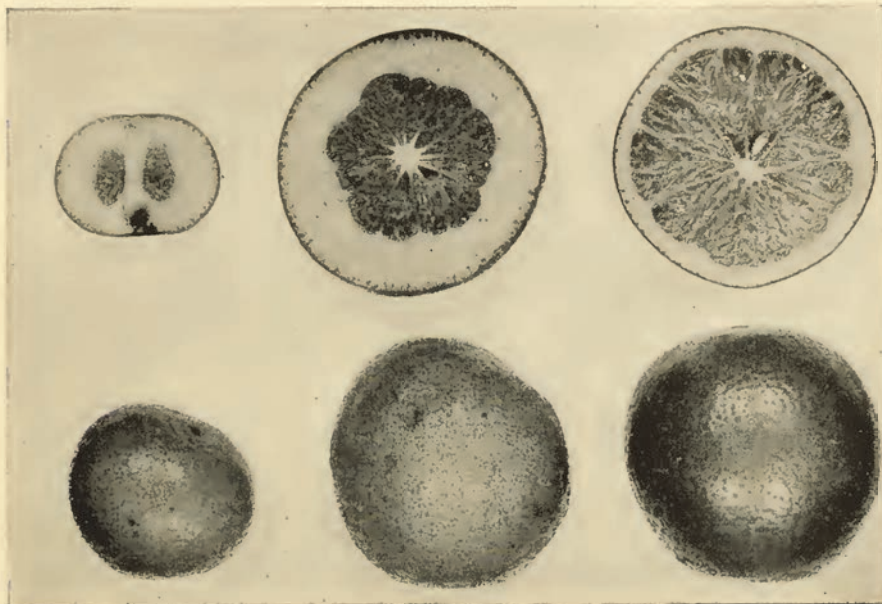

Courtesy of California Agricultural Experiment Station. Reproduced from *Hunger Signs in Crops*, a publication of the American Society of Agronomy and the National Fertilizer Association, Washington, D. C.

Fig. 8–2. Iron deficiency symptoms on lemon leaves. Left, normal leaf. The other leaves show increasing lack of iron from left to right.

sulinate is apparently not the active form of this hormone. A combination of zinc and protamine with insulin is widely used because it acts much longer than insulin alone.

Plants also require zinc. Fertile soils contain 1 to 5 parts per million

or more. Zinc-deficient soils are well known in the southern United States, where the lack of zinc causes "rosette" disease of pecan and cherry trees, "mottled leaf" of citrus fruits, and "bronzing" of tung oil trees. The stunting effect of zinc deficiency on grapefruit is shown in Fig. 8–3.

Courtesy of California 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. 8–3. Grapefruit showing, on the left, the effects of acute zinc deficiency; center, fruit from similar tree two months after treatment, showing discolored tough areas impregnated with gum in the thick rind; and, on the right, fruit from a similar tree treated 15 months previously.

The amount of zinc needed by human beings is not well established, but it has been estimated to be 0.3 mg. per kilogram of body weight for growing children. Adult persons on an average diet in this country probably consume about 12 mg. per day, and this amount appears to be sufficient for all bodily needs. Foods high in zinc include wheat bran, wheat germ, oysters, liver, egg yolk, cocoa, and others (see Appendix, Table A–3). In human tissues the highest concentrations (ca 200 ppm.) are found in the nails and hair.

Sulfur. Sulfur is present in more organic compounds of biochemical importance than any other mineral element, with the possible exception of phosphorus. A partial list is given in Table 8–2. Plants have the ability to manufacture from inorganic sulfates all the organic sulfur

compounds needed for their tissue structures and metabolism. Animals, on the other hand, must be supplied with several preformed sulfur-containing organic substances. These include methionine, thiamine, biotin, and possibly others.

Glutathione, a tripeptide of glutamic acid, cystine, and glycine, is apparently universally present in living tissues, and by virtue of the ability of the cystine constituent to change from the oxidized to the reduced state, cysteine, and vice versa, the compound may function in the oxidation-reduction processes of the tissues.

Chlorine. Chlorine, in the form of hydrochloric acid, imparts to the gastric juice the proper acidity for the reaction of the digestive enzymes found therein. Only a very few organic chlorine compounds have been discovered in nature. One example is chloromycetin, an antibiotic. However, thousands of organic chlorine compounds have been produced synthetically, and many have important industrial uses.

The chlorine required by animals and human beings is supplied almost exclusively by common salt. So much salt is ordinarily consumed that the need for chlorine is amply filled.

Iodine. This element is essential to the proper development and functioning of the thyroid gland. A lack of iodine results in an enlargement of the thyroid, a condition known as simple goiter. McLendon made an extensive study of the distribution of iodine in food and water. He showed that in the areas where the iodine content of the materials was low, simple goiter was prevalent. In some regions, over 70 per cent of the girls of high school age had goiter. It might almost be said that goiters were as common there, and as lightly regarded, as freckles. Prophylactic measures—use of iodized salt, addition of iodides to drinking water, distribution of iodine tablets to school children—are now being adopted in many parts of the United States and are rapidly decreasing the incidence of this disfiguring disease. The ocean is the great source of iodine and its derivatives. Sea foods and foods grown near the sea commonly contain relatively large amounts, whereas foods coming from far inland are low in iodine.

A low iodine content in plants and water affects, not only human beings, but also farm animals. Swine, sheep, and cattle develop enlarged thyroid glands. The young may be born with little or no hair, and, if alive, usually die shortly after birth.

It is estimated that the normal human adult requires from 0.15 to 0.30 mg. of iodine daily. Fortunately, the body is able to store this element quite readily, and the administration of iodide for two to four weeks twice yearly is effective in preventing the development of simple goiter. Iodized salt, which also is employed as a prophylactic measure, normally contains 0.015 per cent of iodine.

Fluorine. Fluorine has not been proved to be an essential element for either plant or animal life, although it is normally present in many living tissues. The element tends to be accumulated in the bones and teeth of animals in the form of the mineral, fluoroapatite, $[Ca_3(PO_4)_2]_3 \cdot CaF_2$. Normal bones of animals and human beings contain about 0.02–0.1 per cent of fluorine, but much higher percentages (up to 1 per cent or over) have been found in cases of excessive fluorine intake. Much interest has been aroused in fluorine because of its effect on the skeleton of animals and man and because of its ability to reduce human caries (tooth decay).

Fluorine is unevenly distributed in the soils of various parts of the world, being found particularly in phosphate rock and in cryolite (a sodium aluminum fluoride). The amount in drinking water is likewise variable, ranging from none to 15 parts per million (ppm.), or more. In those areas where the water supply contains 1 to 2 ppm., or over, many people are afflicted with "mottled" teeth, a condition characterized by soft, chalky areas in the tooth enamel which gradually become discolored and pitted. The threshold level for mottling appears to be near 1 ppm., since this amount of fluorine in the water supply produces mild mottling in about 10 per cent of the growing children. The first teeth are not affected in this condition, but the damage is done to the permanent teeth before eruption.

Larger fluorine intakes, corresponding to about 5 ppm. or more in the water, lead to thickening and other malformation of the bones—both in animals and in human beings. Severe malformation of the spine has been reported among people living in certain areas in India, where about 5 ppm. of fluorine is naturally present in the water, and has been attributed to abnormally high fluorine intake.

It has been well established that increasing amounts of fluorine (or more exactly, soluble fluorides) in drinking water up to about 2 ppm. result in decreased tooth decay in children, provided they have received such water since infancy. In order to avoid, insofar as possible, any danger of mottling, the amount of fluorine added to the water supplies of communities experimenting with this method of controlling tooth decay is ordinarily such as to raise the level to 1 ppm., but no higher. It is probable that fluorine acts to reduce caries by inhibition of bacterial enzymes, possibly by combining with and rendering unavailable such metals as zinc, cobalt, manganese, copper, calcium, and others, which activate various enzymes. The fact that fluoroapatite is considerably harder than normal calcium phosphate may also be a factor in the reduction of tooth decay by fluorine.

Boron, Molybdenum, and Silicon. The essential nature of boron for plant growth was discovered in rather recent times. It was first shown to be needed by corn in 1915, broad beans in 1923, tomatoes and potatoes

in 1928, and still more recently by a long list of agriculturally important plants. It is now generally recognized as an essential component of commercial fertilizers for use on low-boron soils. Various plants need from about 0.1 to 0.5 ppm. of available boron in the soil, and many soils, particularly in the more humid regions, have less than this amount. As a result, such crops as sugar beets and alfalfa have frequently suffered from "diseases," which are now known to be due simply to boron deficiency and are easily controlled by proper fertilization. Some effects of boron deficiency are shown in Figs. 8–4 and 8–5.

Courtesy of South Carolina 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. 8–4. Left to right: Ear from healthy boron-treated corn plant, and three ears from boron-deficient plants. Note one-sided shriveling of kernels on the deficient ears.

Molybdenum was not recognized as an essential element for plants until 1939 (Arnon and Stout), but it is now known to be required, for example, by tomatoes, oats, and barley. It is also needed by such lower forms as molds *(Aspergillus niger)* and the nonsymbiotic (free-living) nitrogen-fixing bacteria *(e.g., Azotobacter chroococcum).*

Most soils contain not more than 5 ppm. of molybdenum, but a few areas are known (in Scotland, California, and Wyoming) where the amount is as high as 20–200 ppm. Livestock grazing these areas receive enough of the element from the forage to cause serious poisoning,

which, however, can be alleviated by feeding small amounts of soluble copper salts.

Although silicon is a normal constituent of many plants, no absolute requirement for it has been well enough established to be generally accepted. Silicon tends to be concentrated particularly in fibrous plant tissues such as wheat straw, cereal brans, rice hulls, timothy hay, and the like. Such plant materials have a high ash content (3–7 per cent or more), and from one-fourth to two-thirds or more of the ash consists of silicates and silicon dioxide.

Courtesy of A. B. Burrell, Cornell University. Reproduced from *Hunger Signs in Crops*, a publication of the American Society of Agronomy and the National Fertilizer Association, Washington, D. C.

Fig. 8–5. Oldenburg apples about five weeks after petal fall, showing symptoms of both internal and external cork due to boron deficiency.

Selenium. Although this element is not among those needed by living things, it has attracted much attention because of its presence in the plants grown in certain localities and its toxic effect on animals consuming such plants. The so-called "alkali disease" of horses and cattle, known since pioneer days in parts of Kansas, Nebraska, South Dakota, and Wyoming, is a chronic form of selenium poisoning. The disease, which also affects sheep, pigs, dogs, rats, and poultry, follows consumption of a diet containing 5–10 ppm. or more of selenium. Symptoms include soreness and sloughing of the hoofs, Fig. 8–6, loss of hair, stiff joints, and atrophy of the heart and liver. Poor hatching of eggs is one of the earliest signs in poultry. A more acute form of the disease known as "blind staggers," resulting from higher selenium intake, is quickly fatal. The most practical antidote or control measure for range cattle, sur-

prisingly enough, is the feeding of arsenic in the salt supply at a concentration of 25 ppm.

Availability of organic and inorganic combinations

Much work has been done to determine whether inorganic forms of the mineral elements can supply the needs of the body for these elements. With respect to phosphorus and iron the answer today seems to be clearly in the affirmative. Moreover, inorganic phosphorus and iron not only

Courtesy of A. L. Moxon and the South Dakota Agricultural Experiment Station.
Fig. 8–6.   Hind feet of a cow suffering from selenium poisoning.

can supply the requirements of the body but seem actually to be more useful than some organic forms of the elements. The iron of hemin and similar porphyrin compounds is unavailable to the body. In the case of sulfur it is clearly established that some of the sulfur must be present in the form of methionine, thiamine, and biotin. These substances cannot be synthesized from inorganic sulfur, and, hence, a part of the sulfur supply must be furnished to the body in organic form. Sulfates and other forms of sulfur, however, are utilized. The observation that milk is the best available source of calcium probably can be attributed to the high digestibility of this food rather than to the presence of a particular calcium compound. Extremely poor utilization of the calcium of spinach is due, doubtless, to its reaction with the oxalic acid in this foodstuff to give insoluble calcium oxalate. In general, it may be said that, with the exception of sulfur as noted above, and probably cobalt which is needed in the form of vitamin B_{12} (p. 249), the bodily needs for the various mineral elements may be met entirely satisfactorily by simple inorganic salts of these elements.

Excretion

Sodium, potassium, calcium, magnesium, phosphorus, and chlorine are excreted mainly as inorganic salts in both urine and feces. Those salts that are readily soluble are excreted chiefly in the urine; those salts that are less readily dissolved are excreted largely in the feces. Iron, copper, zinc, manganese, and cobalt are excreted mainly in the feces.

The excretion of sulfur is almost entirely by way of the kidneys. In this respect it resembles the excretion of nitrogen. Several forms of sulfur are contained in the urine. From 60 to 90 per cent of the total sulfur is excreted in the form of sulfates; from 5 to 15 per cent as ethereal sulfates; and 5 to 20 per cent as neutral or unoxidized sulfur. Taurine and cystine are forms of unoxidized sulfur contained in the urine.

Variations and losses of mineral elements in foods

Unless the food that is eaten is analyzed, it is practically impossible to say whether or not the requirements listed above have been satisfied. Different samples of food vary so greatly in their mineral content that any calculation based on existing data is extremely uncertain. For example, Peterson, Elvehjem, and Jamison found that 18 samples of cabbage from various parts of the United States showed variations (from the lowest to the highest) of 96 per cent for calcium, 118 per cent for phosphorus, and 246 per cent for iron. Such extreme variations would probably not appear in a mixed diet over a long period of time.

A second difficulty in the calculation of the mineral content of the diet results from losses incurred in the cooking of the food. Such losses vary with the kind of food, the method of cooking, and the length of time of cooking. If cabbage is boiled and the water is discarded, 72 per cent of the calcium, 60 per cent of the phosphorus, and 66 per cent of the iron may be lost. The average losses for 16 vegetables reported by Peterson and Hoppert were:

Method of cooking	Calcium per cent	Phosphorus per cent	Iron per cent	Protein per cent
Steaming	10.7	16.7	21.3	16.0
Pressure-cooking	12.0	19.4	17.4	19.1
Boiling	20–32	30–46	40–48	31–43

Differences in the requirements of individuals, variations in the vitamin D content of the diet, variations in the composition of the food, and losses in the preparation of food make it imperative that a liberal allowance be made in excess of any calculated figures.

REVIEW QUESTIONS ON MINERAL ELEMENTS

1. Give the names and sources of two organic forms of (1) phosphorus, (2) calcium, (3) sulfur; one organic and one inorganic form of (4) magnesium, (5) iron, (6) iodine, (7) copper, (8) zinc, (9) cobalt.

2. Discuss the mineral element calcium with reference to (1) its function in the body, (2) the daily requirement of the body, (3) its distribution in food materials. Repeat the above discussion for phosphorus and iron.

3. Discuss the relative availability to the body of organic and inorganic forms of the mineral elements important in nutrition.

4. Name five important trace mineral elements. Are any of these essential to the body? If so, in what way do they serve it?

5. Calculate the acid-base balance between 0.210 gram Ca and 0.168 gram S. $Ca = 40, S = 32$.

6. About how much copper and manganese are contained in the food eaten daily by an adult on an average diet? Name three foods rich in copper; three rich in manganese.

7. How much calcium is contained in a quart of milk; a one-pound loaf of bread; a five-pound head of cabbage?

8. How much calcium may be lost if the water in which vegetables are boiled is discarded? Name some practical means by which the mineral elements extracted in the cooking of vegetables may be conserved and utilized.

9. Which are the main types of chemical change that occur during ashing?

10. Of what does the ash from biological material consist? List five typical substances that you would expect to be present in wood ashes.

11. How could the loss of sulfur or chlorine during ashing be prevented?

12. List all the elements now thought to be essential for the normal nutrition of higher animals; of higher plants.

13. Which of the nutritionally important mineral elements are associated with the action of certain enzymes? List the enzymes concerned.

14. Discuss the effect of abnormal concentrations of specific mineral elements in the soil of certain areas on the animals and people living in those areas.

REFERENCES AND SUGGESTED READINGS

Arnon, D. I. and Stout, P. R., "Molybdenum as an Essential Element for Higher Plants," *Plant Physiology*, **14**, 599 (1939).

Berger, K. C., "Boron in Soils and Crops," *Advances in Agronomy*, **1**, 321 (1949).

Elvehjem, C. A., "The Biological Significance of Copper and Its Relation to Iron Metabolism," *Physiol. Rev.*, **15**, 471 (1935).

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

Hambidge, G., *Hunger Signs in Crops*, 2nd ed., American Society of Agronomy, Washington, D. C., 1949.

Hart, E. B. and Elvehjem, C. A., "Mineral Metabolism," *Ann. Rev. Biochem.*, **5**, 271 (1936).

Hawk, P. B., Oser, B. L., and Summerson, W. H., *Practical Physiological Chemistry*, 12th ed., The Blakiston Company, Philadelphia, 1949, p. 1002.

Heilbrunn, L. V., *An Outline of General Physiology*, 2nd ed., W. B. Saunders Company, New York, 1943.

Hoagland, D. R., *Inorganic Nutrition of Plants*, Chronica Botanica Company, Waltham, Mass., 1948.

Hove, E., Elvehjem, C. A., and Hart, E. B., "The Physiology of Zinc in the Nutrition of the Rat," *Am. J. Physiol.*, **119**, 768 (1937).

Lunde, G., "The Geochemistry of Iodine and Its Circulation in Nature," *Chem. Rev.*, **6**, 45 (1929).

McClendon, J. F., "The Distribution of Iodine with Special Reference to Goiter," *Physiol. Rev.*, **7**, 189 (1927).

Monier-Williams, G. W., *Trace Elements in Food*, John Wiley and Sons, Inc., New York, 1949.

Moxon, A. L., "Alkali Disease, or Selenium Poisoning," *South Dakota Agricultural Experiment Station, Bulletin 311* (1937).

Peterson, W. H., Elvehjem, C. A., and Jamison, Lida A., "Variations in the Mineral Content of Cabbage and Sauerkraut," *Soil Science*, **20**, 451 (1925).

Peterson, W. H. and Hoppert, C. A., "The Loss of Minerals and Other Constituents from Vegetables by Various Methods of Cooking," *J. Home Econ.*, **17**, 265 (1925).

Salter, W. J., *The Endocrine Function of Iodine*, Harvard University Press, Cambridge, Mass., 1940.

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

Shohl, A. T., *Mineral Metabolism*, Reinhold Publishing Corporation, New York, 1939.

Stiles, W., *Trace Elements in Plants and Animals*, The Macmillan Company, New York, 1946.

Underwood, E. J., "The Significance of the Trace Elements in Nutrition," *Nutrition Abstracts and Reviews*, **9**, 515 (1940).

Chapter 9

VITAMINS

The various vitamins differ so greatly in composition that it is impossible to define them in terms of their chemical structure, as is done with other classes of compounds such as carbohydrates, fats, and proteins. From the physiological viewpoint, which is probably the best for an accurate characterization, vitamins may be defined as organic compounds that are essential constituents of the diet but are required in only minute amounts for the normal functioning of the body. Thus they may be seen to differ from hormones in that the body cannot synthesize them, from trace elements in that they are organic compounds, and from the main structural and energy-yielding materials of the diet (the carbohydrates, fats, and proteins) in that they are required in extremely minute amounts. For example, an average adult person consumes about 600 g. (dry basis) of the major food materials daily, whereas his total vitamin requirements are only about 0.1–0.2 g. per day. The reason why such small amounts are effective is that their role in the chemistry of life processes is essentially catalytic. Several such as thiamine, riboflavin, nicotinic acid, pantothenic acid, and pyridoxine have already been shown to form a part of, or to act with, various enzymes, and in all probability the remainder will be found to function in a similar manner.

The development of our knowledge of the vitamins has come from two main lines of investigation: the study of nutritional diseases, mainly beriberi, scurvy, rickets, and pellagra, and the feeding of "simplified" or "purified" diets to experimental animals.

Until about 1900 it was rather generally believed that an adequate diet for animals or man need contain only purified protein, carbohydrate, fat, and mineral salts. However, earlier work of Lunin (1881) and, particularly, the work of Hopkins (1906) demonstrated conclusively that animals on such diets did very poorly unless small amounts of certain additional foods, such as milk, were also given them. Thus the idea arose that besides the four major constituents of the diet "accessory food factors" were needed in small amounts for normal nutrition.

What are now known to be vitamin deficiency diseases have been studied for centuries, but with relatively little progress until recent times. The first and one of the most difficult steps was to prove that a disease was caused by faulty diet and not by some infection or other agent. Some of the observations which gradually led to the acceptance of this

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idea were the curing of scurvy by fresh fruits and vegetables (Lind 1757, Budd 1841), the prevention of beriberi by using other foods in place of polished (white) rice, and the demonstration by Eijkman (1897) that a disease of fowls similar to human beriberi could be produced or prevented at will by feeding the birds various diets. Eijkman thus introduced the use of experimental animals as test subjects for such studies, without which the modern development of the vitamin field would probably have been impossible.

Funk (1912) was the first investigator to formulate and state clearly the concept that each of the then known deficiency diseases was caused by the absence from the diet of a separate definite chemical substance. He had been trying to obtain in pure form from rice polishings the substance capable of preventing or curing beriberi, and since it appeared to have the chemical properties of an amine, he proposed as a name for all such essential substances the term "vitamine," a contraction of "vital amine."

At approximately the same time McCollum and other investigators discovered that certain fats such as butter and cod-liver oil contained a substance that in small amounts was capable of promoting the growth of young rats and preventing the development of an eye disorder known as xerophthalmia. Since the beriberi preventing substance was soluble in water, McCollum concluded there were two "accessory dietary factors." He designated the one "fat soluble A" and the other "water soluble B." Several years later (1920), when it had become evident that there were at least three factors, two of which were in no way related to amines, Drummond suggested that the terms of Funk and McCollum be combined by dropping the letter "e" from the word "vitamine" and adding the letters A, B, and C to give the terms "vitamin A," "vitamin B," and "vitamin C." As new vitamins have been discovered they have been designated by letters added to the class name such as vitamin D, or by use of subscript numbers, $e.g.$, vitamin B_1, B_6, etc.

The number of known vitamins has grown rapidly from the early abbreviated list composed of vitamins A, B, and C to one comprising some fifteen to twenty factors that apparently deserve to be included in this category. Only those substances that have been generally accepted by research workers in this field are considered in this brief treatment. For convenience they may be classified as either fat-soluble or water-soluble. In the former class belong A, D, E, and K, and in the latter, ascorbic acid (or vitamin C) and the vitamin B group. This group includes thiamine (vitamin B_1), riboflavin, nicotinic acid (or niacin), pantothenic acid, pyridoxine (vitamin B_6), biotin, pteroylglutamic acid (abbreviated PGA; also called folic acid), vitamin B_{12} (antipernicious anemia vitamin), choline, inositol, and para-aminobenzoic acid (PABA).

Unfortunately, in the earlier work the experimental diets lacked more than one vitamin with the result that symptoms ascribed to a deficiency

of one vitamin were often in reality due to a lack of several. To illustrate, the original vitamin B has been found to consist of some ten or more factors. This accounts, in part, for the lack of a systematic nomenclature, which is so apparent in the list just given. Now that each of the above vitamins is well known as a distinct chemical substance, much confusion can be avoided if they are designated by their scientific names rather than by mere letters. All of the vitamins listed have been isolated in pure form, and the chemical structures have been worked out, except for vitamin B_{12}. In addition, all the vitamins are produced commercially, mainly by synthetic processes, and hence are available in practically unlimited amounts at much lower prices than formerly.

Since chemical processes in widely differing organisms are frequently closely related, one might be led to assume that in all higher animals the vitamin requirements would be practically the same. That such an assumption is without foundation, however, is evidenced by the fact that the rat synthesizes its own supply of ascorbic acid. So far as this animal is concerned, therefore, this compound is not a vitamin. Skin disorders resembling those of pellagra in man can be produced in the rat and chick, but they do not respond to treatment with nicotinic acid, as in the case of man, and neither is pellagra in man relieved by the vitamins that effect cures in the above-named species. Other examples of such differences in dietary requirements may be noted in discussions of the various vitamins.

The relative prevalence of vitamin and other dietary deficiencies in the United States is difficult to estimate closely, but is probably much greater than has been supposed. Sebrell has commented on the situation as follows:

. . . Today it is recognized that all of the known nutritional diseases probably exist to some extent in the United States. Those that appear to be of most importance are anemia, due to iron or cobalt deficiency; nutritional edema, due to protein deficiency; hyperkerataosis [1] and night blindness, due to vitamin A deficiency; beriberi and peripheral neuritis,[2] due to thiamine (vitamin B_1) deficiency, frequently secondary to such conditions as alcoholism, pregnancy and diabetes; lip lesions, seborrhea [3] and keratitis,[4] due to riboflavin deficiency; pellagra or encephalopathy,[5] due to nicotinic acid deficiency; swollen bleeding gums, skin and subperiosteal hemorrhages,[6] due to ascorbic acid deficiency; rickets and osteomalacia,[7] due to vitamin D deficiency; hemorrhagic disease of the newborn, due to vitamin K (phthiocol) deficiency; tetany, due to hypocalcemia,[8] and probably many other at present less well identified conditions with a nutritional background. . . .

[1] Excessive growth of the cornea.
[2] Inflammation of the nerve endings.
[3] A chronic disease of the sebaceous, or oil-secreting glands of the skin.
[4] Inflammation of the cornea.
[5] Any disease of the brain.
[6] Bleeding under the periosteum, the fibrous membrane covering bones.
[7] Softening of the bones.
[8] Abnormally small amounts of calcium in the blood.

An interesting sidelight on this question is furnished by the estimate that during 1948–1950 the people of the United States spent over $500,-000,000 annually for vitamin preparations. This figure does not include the value of vitamins used in enriched foods and food supplements, which is also estimated to approximate $500,000,000 a year.

VITAMIN A
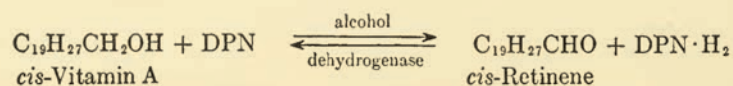
Physiological function

Vitamin A was first recognized and studied as a growth-promoting substance, but it was soon discovered that it plays other equally important roles in the body. In the absence of adequate amounts of vitamin A the epithelial cells of the mucous membranes lose their ability to secrete normally and, as a consequence, become dry and hardened. This hardening process is known technically as cornification or keratinization (production of a horny layer). In many species the eye seems to be particularly susceptible to this change, and a disease known as xerophthalmia is produced. When the tear glands cease to secrete, the eyes are not cleansed properly, they become noticeably irritated, and infection ensues. As the exuding pus dries, movement of the eyelids becomes increasingly difficult until eventually they become closed tightly. In the later stages ulcers form on the cornea. When these ulcers rupture, the lens pops out, thus permanently destroying sight. Keratinization likewise occurs extensively in the respiratory tract (including the lungs), the alimentary tract, the urino-genital tract, and the salivary and ductless glands. Infants suffering from xerophthalmia also exhibit a dry, scaly skin.

Because keratinization of the mucous membranes opens those parts of the body to bacterial invasion, some have ascribed to vitamin A an anti-infective role, particularly valuable in warding off colds and other respiratory diseases. However, vitamin A does not appear to be toxic to organisms that cause these diseases. Only to the extent that it helps maintain a healthy condition of the mucous surfaces and increases the general resistance of the body is it "anti-infective."

In addition to keratinization of epithelial tissue there are a number of other symptoms of vitamin A deficiency. Urinary calculi and deposits of calcium phosphate have been observed in rats suffering from vitamin A deficiency. Although xerophthalmia seldom develops in the chick, immense deposits of urates accumulate in the kidney and renal tubules as a result of vitamin A deficiency. The vitamin is also essential to reproduction, for on diets deficient in this vitamin, female rats cease to ovulate normally, and males show testicular degeneration, even though the vitamin E intake may be adequate. Sherman has shown that fertility and longevity of rats is greater on a ration rich in vitamin A than on one that is low in this factor.

Vitamin A plays a vital role in connection with vision. The rods in the retina of the eye contain a rose-colored pigment, *rhodopsin*, which is a conjugated chromoprotein. When light falls on the retina, rhodopsin is broken down into a simple protein, *opsin*, plus *trans-retinene*, a yellow substance now known to be vitamin A aldehyde. Associated with this chemical change is the production of a stimulus which, being imparted to the optic nerve, results in vision. These changes are very rapid and are probably completed within a few hundredths of a second.

Subsequently, a slower process, not dependent on light, brings about the regeneration of rhodopsin so that an adequate supply for normal vision is continuously present in the retina. Rhodopsin is formed from opsin plus *cis*-retinene, a geometric isomer of the *trans*-retinene released by the action of light on rhodopsin.[1] The *cis*-retinene needed may be obtained by isomerization of the *trans* form, or from *cis*-vitamin A through the action of alcohol dehydrogenase and DPN (p. 275):

$$C_{19}H_{27}CH_2OH + DPN \underset{\text{dehydrogenase}}{\overset{\text{alcohol}}{\rightleftarrows}} C_{19}H_{27}CHO + DPN \cdot H_2$$

$$\text{\textit{cis}-Vitamin A} \qquad\qquad\qquad\qquad \text{\textit{cis}-Retinene}$$

Since the breakdown and resynthesis of rhodopsin are probably not perfectly efficient processes, some retinene derived from vitamin A no doubt must be continually supplied.

The retinas of fresh-water fish do not contain rhodopsin but a closely similar, light-sensitive pigment, *porphyropsin*. This substance fulfills the same functions for the vision of these fish as rhodopsin does for higher animals and man. The outstanding difference lies in the retinene and vitamin A associated with it, which are different from those derived from rhodopsin. To distinguish the two varieties of the vitamin, that from mammals is designated A_1 and the other, A_2. The corresponding retinenes are similarly designated $retinene_1$ and $retinene_2$. Fresh-water fish have vitamin A_2, not only in the retina, but also in the liver and other organs.

Since vitamin A is needed for rhodopsin formation, it would be expected that persons deficient in this vitamin would have subnormal amounts of rhodopsin in the retina. In any event, it has been observed that such individuals cannot see as well in dim light or adapt themselves to changes in light intensity as readily as those whose supplies of vitamin A are ample for immediate resynthesis of the light-sensitive pigment. By means of the biophotometer it is possible to measure accurately one's ability to adapt to darkness after the eyes are exposed to a bright light for a few minutes. Adaptation is determined by the amount of light necessary for the individual to recognize the number of openings in a screen

[1] For a brief explanation of *cis*- and *trans*-isomers see p. 79. Since vitamin A and retinene each contain five double bonds, theoretically $(2)^5$ such isomers of each can exist. It is not known which particular *cis*-isomer is involved in rhodopsin formation.

through which dim light is transmitted. Pronounced inability to see in dim light is termed "night-blindness."

Prevalence of vitamin A deficiency

Because the young of mammals, including man, are born with limited stores of vitamin A, we find them more susceptible to a deficiency than adults. Consequently, xerophthalmia is most often observed in young children of the extremely poor classes, or in children at times of great food shortage. Although considered to be a rare disease in the United States, 13 cases were reported in 1931. Of every five children of the poor classes in Yucatan one is said to be suffering from xerophthalmia. Reports from Ceylon state that the disease is very prevalent there, especially in asylums and charity boarding schools. If, as is reported, the diets in the latter institutions furnish only 30 per cent of the estimated minimum requirement of vitamin A, one can readily understand why two-thirds of the children are suffering to some extent. In China, India, Java, and Sumatra the disease is said to be very common. Biophotometer tests reveal that diets of the poorer classes in our country frequently are inadequate with respect to vitamin A, and some indicate that diets of those in the higher economic brackets are likely to show suboptimal amounts of vitamin A.

Chemical nature

The vitamins A are closely related to a group of yellow, orange, and red pigments called *carotenoids*, present in many plants and animals. The group name is derived from one of the best known and most important members, *carotene*, the orange coloring matter of carrots. Lycopene of the red tomato, zeaxanthine of yellow corn, and xanthophyll of egg yolk are other common carotenoids. There are several isomers of carotene, but the one most closely related to vitamin A_1 is *beta-carotene*, of which the formula is given below:

Beta-carotene, $C_{40}H_{56}$
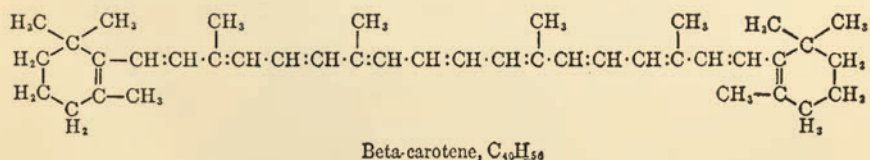
A high degree of unsaturation, in which the double bonds occur alternately with single bonds along the carbon chain, is characteristic of carotene, as well as of vitamin A and the whole carotenoid group. Pure beta-carotene is a dark red, crystalline solid (Fig. 9–1).

The relationship of vitamin A to carotene was first established by

Fig. 9–1. Carotene.

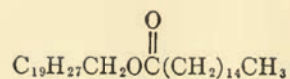Fig. 9–2. Vitamin A (with one molecule of solvent of crystallization). Yellow crystals first isolated by Prof. Harry N. Holmes and Dr. Ruth E. Corbet, Oberlin College, 1936.

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animal feeding experiments, which proved that carotene is converted into the vitamin in the animal body. Later, pure vitamin A_1 was obtained from fish liver oils in the form of lemon-yellow crystals (Fig. 9–2). When the chemical formula of the purified vitamin had been established, its relation to carotene became even more apparent:

$$H_3C \diagdown {} \diagup CH_3$$

Vitamin A_1, $C_{19}H_{27}CH_2OH$

It may be seen that the carbon structure of vitamin A_1 is exactly half that of the beta-carotene molecule and that the vitamin is a primary alcohol containing five double bonds. The exact chemical formula of vitamin A_2 has not yet been determined.

Of the many carotenoids of known chemical structure only four (alpha-, beta-, and gamma-carotene, and cryptoxanthin) of common occurrence in foodstuffs are convertible into vitamin A. It was long supposed that this transformation occurred in the liver, but more recent information tends to discredit this view. Carotene injected into the blood stream of rats deprived of vitamin A does not prevent the animals from dying of vitamin A deficiency, even though the livers may contain considerable amounts of carotene at the time of death. Increased vitamin A contents in the intestinal wall and in the lymph, following the injection of carotene, make it appear most probable that the conversion occurs during absorption of the carotene through the intestinal wall.

The fact that vitamin A_1 is almost exactly one-half of a beta-carotene molecule suggests that two molecules of the vitamin might arise from the conversion of one molecule of carotene. This, however, does not seem to be the case, since on a weight basis beta-carotene has only about one-half (or less) of the biological value of vitamin A. Therefore, the conversion is probably not a splitting of the carotene molecule in the middle, but rather a degradation of one end of the molecule until one molecule of vitamin A is produced.

Other related substances which possess vitamin A activity have been prepared synthetically, as has the vitamin itself. These include the corresponding aldehyde ($C_{19}H_{27}CHO$, retinene), acid ($C_{19}H_{27}COOH$), and methyl ether ($C_{19}H_{27}CH_2OCH_3$). In a natural state the primary alcohol group of vitamin A_1 is frequently involved in ester linkage, and such esters as the acetate

$$\overset{O}{\overset{\|}{C_{19}H_{27}CH_2OCCH_3}}$$

and palmitate

$$C_{19}H_{27}CH_2O\overset{\overset{\displaystyle O}{\|}}{C}(CH_2)_{14}CH_3$$

have also been prepared artificially. They are fully active, and yet considerably more stable than the free vitamin.

The vitamin A value of a given food sample may be due either to the actual vitamin itself, an ester of it, one of the carotenoids listed above, or to a mixture of these. The carotenoids which are capable of conversion to vitamin A are often designated as provitamins. Unless otherwise specified, the collective meaning, that is, precursors as well as the true vitamin, is implied in the subsequent discussion.

Preparations of vitamin A rapidly lose their potency in the presence of oxidizing agents. Atmospheric oxygen at room temperature or above brings about noticeable destruction of vitamin A unless the vitamin is protected by antioxidants. These antioxidants, or inhibitors, are associated with lipides in nature and hence stabilize the vitamin in foodstuffs. Vitamin E is one of the most important of these antioxidants, which act to minimize the destruction of vitamin A by oxidation. Cooked foods retain the greater part of their original vitamin A potency. Since vitamin A is fat-soluble, it is extracted from foodstuffs along with the lipides by the common fat solvents. Saponification of these lipides does not destroy the vitamin.

The vitamin A value of biological samples may be determined by animal feeding tests, but this laborious method has been almost entirely supplanted by quicker chemical methods. However, the animal assay measures the precursors as well as true vitamin A, whereas each chemical test measures either one or the other, but not both. The chemical measurement of true vitamin A depends on reacting it with antimony trichloride in dry chloroform (Carr-Price reaction). A clear, deep blue color is produced which is compared with the intensity of the color from known amounts of the pure vitamin to estimate the potency of the sample. The carotenoid pigments, many of which have no vitamin A value, all respond to some extent to this same test, and so interfere unless a suitable correction is applied. This method is widely used for determining the true vitamin A content of butter, cheese, fish oils, pharmaceuticals, and many animal tissues such as blood and liver.

The vitamin A value of plant samples is usually determined by measuring the carotene content. This is accomplished by extracting with a fat solvent, purifying the extract to remove interfering pigments, and then comparing the yellow color of the solution with that of known concentrations of carotene in the same solvent.

Occurrence

The carotenoid pigments possessing vitamin A activity are synthesized in the plant, where they are found associated with such pigments as xanthophyll and chlorophyll, which have no vitamin A potency. From available information it appears that all animals are ultimately dependent upon the carotenoid pigments for their supply of vitamin A, for in no instance has the real vitamin been reported to occur in plant products. Neither has any animal been observed to possess the ability to synthesize the vitamin from other compounds. The potency of animal products may be due either to stored carotenoids or to the vitamin that the body has formed from these ingested pigments.

The most potent sources of vitamin A are the fish-liver oils. Of these oils, that of the cod has been most widely used. The oil from the liver of the halibut has been shown to possess 75 to 125 times the potency of cod-liver oil. Foods containing relatively high concentrations of vitamin A include: butter, cream, cheese, milk, egg yolk, liver, the green vegetables such as spinach, escarole, string beans, and leaf lettuce, apricots, bananas, oranges, peaches, prunes, pumpkins, sweet potatoes, and tomatoes. The green outer leaves of lettuce are reported to contain 30 times as much vitamin A as the crisp inner portion. The potency of cow's milk has been found to vary with the breed and ration. Milk remains a good source of the vitamin, even in winter, since the cow is able to draw upon her reserves when confined to feeds deficient in vitamin A. In some milk (*e.g.*, Guernsey) the larger part of the potency is due to carotene, while in others (*e.g.*, Holstein) there is more vitamin A than carotene. Since the vitamin A activity of colostrum (the first milk after parturition) is 10–100 times that of ordinary milk, it is apparent that nature has provided for immediate supplementation of the meager supply with which the young are provided at birth. Human milk is 3–5 times richer in this factor than cow's milk. Glandular organs, like liver, contain much more vitamin A than muscles. Cereals and cereal grains (with the exception of yellow corn), roots, and tubers are generally conspicuously deficient in the vitamin.

Requirements

According to the Food and Nutrition Board of the National Research Council, the vitamin A requirement of the average adult or adolescent is satisfied by the consumption of approximately 5000 international units daily. The needs of smaller children are somewhat less. Pregnancy may increase the demand for vitamin A to 8000 international units per day. Quantities of representative foods that would supply approximately

5000 international units are: ¼ lb. of butter, ½ lb. of carrots, 1 to 1½ oz. of spinach, or 1–2 teaspoonfuls of cod-liver oil.

The international unit (I.U.) is defined as the vitamin A potency of 0.6 microgram (μg.) of pure beta-carotene. Pure vitamin A has a biological potency of one unit in about 0.25 μg. Thus 5000 I.U. would correspond to only 1.2 mg. of true vitamin A, but 3 mg. of beta-carotene would be needed to supply this number of units. The other carotenoid precursors of vitamin A are only about one-half as effective as beta-carotene, and so about 6 mg. would be required if the total vitamin A supply were to come from this source. The above recommended daily allowances are based on the assumption that approximately two-thirds of the total vitamin A value of the diet will be contributed by one or more of the precursors and one-third by vitamin A itself.

A greatly excessive intake, for example, several million units taken over a period of a few days or weeks, is apt to produce symptoms of toxicity such as nausea and vomiting, headache, peeling of the skin, and general prostration. The amounts necessary to cause such sickness, however, are so much greater than the normal requirements of the body that in the ordinary course of events no such disorder should ever arise.

VITAMIN D

Physiological function

Lack of vitamin D manifests itself largely in the form of a disease known as rickets, and for this reason it is sometimes called the antirachitic vitamin. The most obvious symptoms of rickets are bowed legs, enlarged joints, and malformation of the head and chest. These abnormalities result from improper calcification of the growing bones, as may be readily detected by comparing X-ray photographs of rachitic and normal bones. Analyses of the bones of rachitic rats show that the ash content on the fat-free dry basis may be as low as 25–30 per cent as compared with approximately 50 per cent for the bones of normal rats of the same age. Such bones are necessarily weak and eventually assume the characteristically bowed shape. Enlargements occur also where the rib bones join the cartilages, and to this chain of bead-like formations has been given the name "rachitic rosary." Weak abdominal muscles, together with malformation of the chest, are responsible for a pot-bellied appearance. Since bones and teeth are so closely related chemically, it is not surprising that rachitic children also have defective teeth. It is hardly necessary to state that growth is retarded with the onset of rickets and eventually ceases. The disease is seldom fatal, but it predisposes the individual to other diseases, of which broncho-pneumonia is said to be most common.

The physiological action of vitamin D in preventing or curing rickets is thought to be due largely to the fact that it promotes the absorption of calcium from the food through the intestinal wall and into the blood stream. In addition, some investigators feel that it has some direct action, at present of an obscure nature, in promoting the actual deposition of calcium and phosphorus in the growing bone. It may be that these beneficial effects result from an interaction of the vitamin with

Courtesy of the Wisconsin Alumni Research Foundation.
Fig. 9-3. Rickets.

alkaline phosphatase, a type of enzyme which is concerned with phosphorus absorption in the intestine and reabsorption in the kidney (a process which reduces loss of phosphates in the urine). This enzyme is especially active in bone where it breaks up organic phosphates of the blood and thus provides inorganic phosphate ($PO_4^{\equiv}$) for bone formation. A water-soluble derivative of vitamin D has recently been shown to stimulate the action of alkaline phosphatase very markedly,

and this observation, if confirmed, would go a long way toward explaining the action of vitamin D in rickets.

Prevalence of vitamin D deficiency

No other deficiency disease has been as noticeable in the United States as that due to lack of vitamin D. Infants from 3 to 24 months of age are most susceptible. Not only is rickets seasonal in appearance, but it is observed more frequently in the North than in the South, where more sunshine is available throughout the year. In fact, it is almost inevitable during the cold Northern winters unless the diets of the young are supplemented with vitamin D.

Rickets is said to be most prevalent in Great Britain, France, Belgium, Germany, European Russia, Austria, Hungary, and the United States. Sebrell cites reports of the U. S. Census Bureau to the effect that 244 deaths resulted from rickets in 1938. Before vitamin D therapy was used (1928) the Ministry of Health reported 87 per cent of London elementary school children as giving evidence of having had some degree of rickets. A survey of children in Sydney, Australia, revealed that about one-half of them either were or had been rachitic. In 1943 Follis and co-workers reported the results of examining 230 consecutive cases of children from 2 to 14 years of age who had died from various causes in Baltimore, Maryland. No less than 46.5 per cent were found on autopsy to have had some degree of rickets. It will be noted that countries of the extreme north are not included in the above list, a fact which is probably due to the important place that fish and glandular organs assume in the diets of these peoples. Young girls of the upper Mohammedan classes in India suffer from rickets as a result of religious tenets, now rapidly disappearing, that require the women to remain in seclusion after becoming 12 years of age. Lower classes fare better, even though their diet is less satisfactory, because they live more in the open air.

Chemical nature

Although the existence of as many as ten different antirachitic compounds has been rather well established, only two of the D vitamins have been proved to be of any practical importance so far as antirachitic medicines and foods are concerned. These may be produced in the laboratory from ergosterol and from 7-dehydrocholesterol, respectively, by irradiation with ultraviolet light. The principal structural changes associated with acquisition of antirachitic potency are shown by comparison of the formulas of ergosterol and calciferol. Ring B is opened,

and a methylene group (CH_2=) is formed from the methyl group attached to rings *A* and *B*. Similar changes are wrought in the structure of 7-dehydrocholesterol and quite likely in all other sterols during their activation. Vitamin D_2, or calciferol as the irradiation product of ergosterol is known, is a white crystalline substance that differs from

Ergosterol, $C_{28}H_{43}OH$

Calciferol, $C_{28}H_{43}OH$
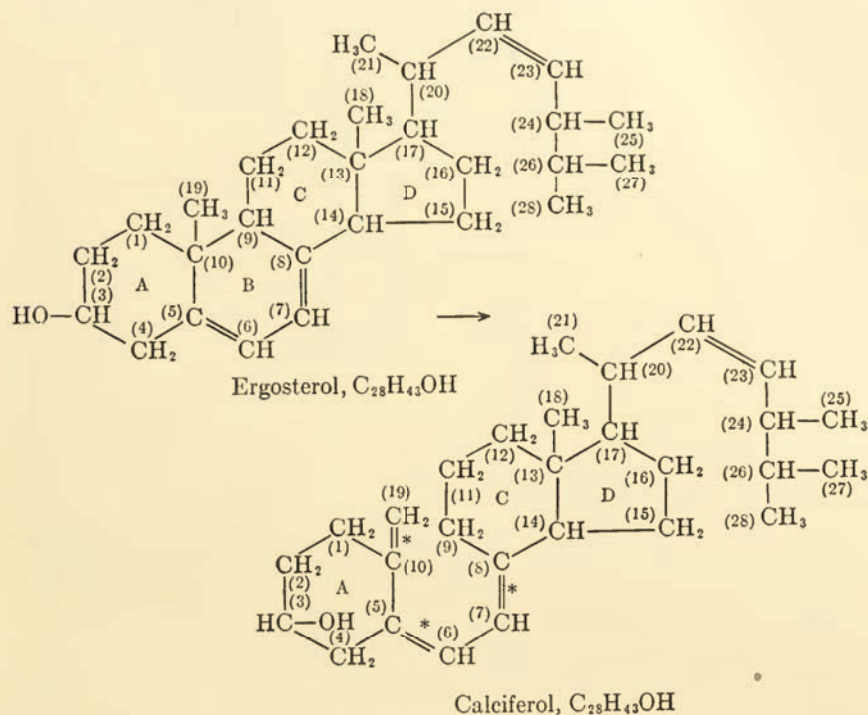
the parent sterol in several properties such as melting point, optical rotation, solubility, and precipitability.

It seems quite probable that the three double bonds between carbons 10 and 19, 5 and 6, and 7 and 8, respectively, (designated by * in the above formula) are necessary for vitamin activity, since this grouping is present in each of the D vitamins whose chemical structures have been accurately determined. In spite of the rather large number of D vitamins that have been produced in the laboratory, only two of natural occurrence (D_2 and D_3) have been isolated thus far. Vitamin D_3, which is obtained from fish-liver oils, is identical with the vitamin formed by the action of ultraviolet light upon 7-dehydrocholesterol. Vitamins D_2 and D_3 have approximately the same potency toward mammals, including man, but D_3 is about 30 times as effective as D_2 is for fowls. The D vitamins are so resistant to high temperatures that practically no loss of activity is suffered when a food is cooked.

Relationship of light and mineral content of diet to vitamin
D requirements

Sunlight prevents rickets by the action of its ultraviolet rays on some provitamin in the skin, presumably 7-dehydrocholesterol. Practically none of the effective ultraviolet rays penetrate ordinary window glass, and, hence, much transmitted light possesses little or no antirachitic value.

Calcification of growing bone, the process in which vitamin D is so vitally concerned, consists primarily in the deposition of calcium and phosphorus as a complex calcium phosphate in the organic matrix. No amount of vitamin D in the diet, therefore, can take the place of these inorganic constituents. Not only must the two elements be present in adequate amounts, but their relative proportions influence greatly the need for vitamin D. Rations employed in producing rickets experimentally in rats are made especially effective through the addition of large amounts (three per cent usually) of calcium carbonate. Rickets so produced is said to be of the low phosphorus type (common clinically) since the inorganic phosphate content of the blood serum of animals on such a ration is abnormally low. In human beings the normal amount of inorganic phosphorus in the blood plasma is approximately 4 mg. per 100 ml., and the calcium content is 10 mg. per 100 ml. If either or both of these values becomes low enough so that their product (milligrams inorganic P $\times$ milligrams Ca per 100 ml.) drops to 30 or less, rickets is almost certain to develop. A value of 40 or above is considered normal. A low calcium type of rickets also occurs, and a third type in which serum calcium and phosphorus are both low is observed occasionally.

Occurrence

The most potent natural sources of D vitamins are the fish-liver oils. Fish in which considerable oil is distributed throughout the body, *e.g.*, sardines and salmon, are the richest food sources. The concentration in salmon averages approximately 12 μg. per 100 g., while several other fish are nearly as high.

Next in order of concentration are egg yolk and butter with 4.6 and 2.3 μg. per 100 g., respectively. Although milk normally contains only small amounts of the vitamin—reports vary from 0.008 to 0.106 μg. per 100 g.—it may be enriched by irradiation so as to contain 0.3–1.1 μg. per 100 g., which is equivalent to about 135–400 I.U. per quart. Milk produced by cows fed irradiated yeast also contains about 400 I.U. per quart. Direct irradiation of the animals is without effect in increasing the concentration of vitamin D above the normal level in milk. The amount of vitamin D in a number of typical foods is indicated in the

following table. It will be noted that, except for vitamin D milk and certain fish, most native foods contribute only negligible amounts of this vitamin.

Table 9–1
Vitamin D content of foods
(The values are expressed as micrograms of vitamin D per 100 g. of edible portion, fresh basis.)

Food	Vitamin D	Food	Vitamin D
Beef liver	0.72	Egg yolk	4.6
Beef steak	0.33	Halibut-liver oil	3500
Beet greens	0.004	Lamb liver	0.43
Bread, vitamin D	1.7	Mackerel	4–6
Butter	2.3	Milk, whole	0.11
Cabbage	0.005	Milk, vitamin D, irradiated	1.1
Carrots	0.004	Milk, vitamin D, fortified	1.1
Carrot tops	0.075	Pork liver	1.1
Cheese	0.83	Salmon	12
Cod-liver oil	250	Sardines	6–8
Corn oil	0.22	Spinach	0.005
Cream	0.42	Tuna	5–8
Crisco	0.22	Veal liver	0.24

Precursors of vitamin D are widely distributed in plant and animal materials. Therefore, if a food is subjected for a few seconds to the light from an ultraviolet lamp, it becomes potent as an antirachitic agent through the conversion of its provitamin into vitamin D. Certain brands of cereals, cookies, evaporated milk, yeast, and bread containing vitamin D are now commercially available. In some cases the potency is due to the addition of small amounts of irradiated ergosterol rather than to irradiation of the food.

Since foodstuffs can be rendered antirachitic through treatment with light from an ultraviolet lamp, one might assume that plant products would possess considerable potency as a result of the effect of the ultraviolet rays of the sun. Such has not been found to be true. At least by the time foods are prepared for consumption little, if any, vitamin D is present.

In addition to the fish-liver oils and foods that contain the D vitamins, extremely potent preparations such as (1) liver oils fortified with additional vitamin D, (2) liver oil concentrates, (3) calciferol dissolved in an inert substance such as corn oil are on the market. Viosterol is a general name for preparations of irradiated ergosterol. Cod-liver oil contains on the average about 250 μg. per 100 g., a concentration which is exceeded by the liver oils of a number of other species. Thus halibut-liver oil is 10–20 times richer, and the liver oil of one species of tuna fish has been reported to contain 150,000 μg. per 100 g. Since with massive doses of vitamin D, calcification may occur in tissues other

than bone, *e.g.*, in the aorta, kidneys, and intestines, the extremely potent preparations of vitamin D should be used with care and preferably in accordance with the advice of a competent physician.

Requirements

Because of the various factors that affect the individual's need for vitamin D, it is difficult to determine exactly the required amount. The Food and Nutrition Board of the National Research Council recommends 10 μg. per day for infants under one year and for women during lactation and the latter half of pregnancy. This is equivalent to 400 I.U. since 0.025 μg. provides one unit. Older children should also receive 10 μg. (400 units) daily, but adults probably require no vitamin D in the diet unless they are seldom or never exposed to direct sunlight. This amount, 10μg., would be contained in approximately 1–2 teaspoonfuls of cod-liver oil, 2 lb. of butter, 2.5 gal. of whole milk, or 1 doz. eggs. It should be emphasized that no ordinary diet can be relied upon to meet the requirements of a growing child for vitamin D unless special precautions are taken to include adequate amounts of a fish-liver oil, or other suitable source.

One fortunate aspect of the problem of supplying adequate amounts of vitamin D to growing children is that if more than the day's requirement is consumed at one time, the extra amount is stored in the body (mostly in the liver) and prevents the development of any deficiency for a correspondingly longer time. Thus a single dose of 20,000 I.U. of vitamin D per kilogram of body weight was found to protect puppies from rickets for as long as 12–14 months.

This ability of vitamin D to be stored extensively in the body is also shared by the other fat-soluble vitamins (A, E, and K), but not by the water-soluble B vitamins or vitamin C.

VITAMIN E

Physiological function

Because the outstanding function of vitamin E seems to be that of promoting reproduction, it has become known as the "antisterility factor" or "vitamin of reproduction." Such a term is somewhat misleading in view of the fact that the vitamin performs other functions in the body, including that of promoting growth. Moreover, it was noted previously that vitamin A also is essential to reproduction.

The most detailed investigations regarding the functions of vitamin E have been carried out with rats. In the male rat on a diet deficient in vitamin E the germ cells undergo a permanent degeneration, as evidenced

by failure to recover even when the animal is fed large amounts of the vitamin. The females, on the other hand, continue to ovulate normally, mating occurs, and the foetuses grow until about the twelfth or thirteenth day of gestation, at which time they die and are resorbed by the mother's body. Injury to the female is less severe than to the male, for normal young may be born in the next gestation period if an abundance of vitamin E is supplied. However, repeated resorptions may lead to paralysis and other serious injury. Similar experiments with mice indicate that females respond to vitamin E deficiencies as do rats, but the males do not show testicular damage after prolonged lack of the vitamin.

Deficiency of vitamin E produces muscle dystrophy in several species. This is a condition of impaired nutrition of the cell that results in degenerative changes in the muscle fibers. Eventually the fibers atrophy and are replaced by connective tissue and fat. Such changes necessarily result in paralysis, although the two do not always correspond in degree. The chemical composition of the involved muscles is altered in that lipide phosphorus and cholesterol increase greatly, whereas there is a marked decrease in glycogen and creatine. Abnormally large amounts of creatine are excreted in the urine. Muscle dystrophy has been observed in dogs, rabbits, guinea pigs, and rats, and cures have been effected through administration of vitamin E. Muscle dystrophy is also a disease of man, but unfortunately human patients are not cured by vitamin E. The vitamin E content of eggs coincides closely with their hatchability and with the vitality of the young chicks. Because of the function of hormones in reproduction, it has been suspected that a relationship exists between tissue concerned with their production, for instance, the pituitary gland, and vitamin E supply. Experimental data to support such a view, however, are still lacking. Ability to store vitamin E is pronounced.

Evidence is accumulating which indicates that the chief physiological function of vitamin E may be associated with its pronounced antioxidant properties. The muscles of vitamin E deficient animals show a greatly increased respiration (oxygen uptake) as compared with normal muscles, and the respiration is dramatically reduced by administration of vitamin E to such animals. It has recently been found, also, that feeding adequate amounts of vitamin E greatly reduces the oxidation of carotene and vitamin A in the intestinal tracts and body tissues of animals so that they are able to remain in good condition on much less vitamin A than would otherwise be required.

Chemical nature

Vitamin E activity in foodstuffs has been traced to four phenols (hydroxy derivatives of benzene), which are known as alpha-, beta-, gamma-, and delta-tocopherol, respectively. The alpha form has much

greater vitamin E activity than the others; it is the form usually meant when the term vitamin E is used. Alpha-tocopherol has the structure represented by the following formula:
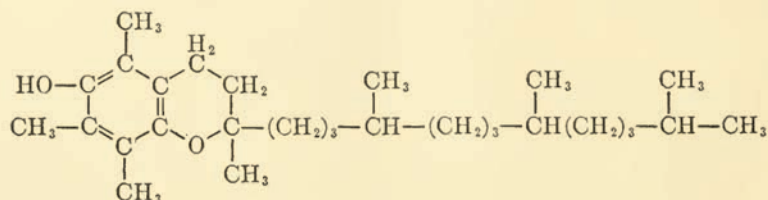
$$\alpha\text{-Tocopherol, } C_{29}H_{50}O_2$$

The E vitamins are remarkably stable to heat, alkali, and many ordinary chemical reagents, but they are unstable to ultraviolet light and oxidizing agents. The potency of food materials containing these vitamins is rapidly destroyed by oxidation in the presence of certain fats (notably lard) and iron salts. The E vitamins are also destroyed in rancid fats.

Occurrence

The amounts of alpha-tocopherol and of all the various tocopherols present in some common food materials are listed in Table 9–2. Certain

Table 9–2

Tocopherol (vitamin E) content of foods

Food	Tocopherol content*	
	Total	Alpha
Bread, white	0.23	
Butter	2.40	
Cheese, American	1.00	
Chocolate, unsweetened	11.1	5.3
Corn oil	87	7·
Cottonseed oil	90	56
Eggs, hen's	2.00	1.16
Fruits, various	0.24–0.74	0.23–0.72
Lard	2.7	2.3
Margarine	54	28
Meat, fish, and poultry, various	0.25–1.40	0.21–1.40
Milk, cow's, whole	0.12	
Oatmeal	2.10	1.94
Peanuts	9.30	4.60
Rice, brown	2.40	1.20
Rice, polished	0.57	0.35
Soybean oil	140	10
Vegetables, various	0.06–4.0	0.1–4.0
Wheat, whole	2.20	
Wheat germ oil	200–300	130–195
Yeast, brewer's, dried	0	0

* Milligrams per 100 g.

vegetable oils, particularly wheat germ oil, are the richest sources. Whole grain cereals, eggs, and peanuts are other rather good sources of vitamin E. Fruits and vegetables as a class are rather poor sources, as are most meats, fish, poultry and other animal products, except butter. Harris, Quaife, and Swanson estimate that the average daily intake of alpha-tocopherol in the United States is about 14 mg. per capita, of which nearly 8 mg. are obtained from fats and oils in the diet.

Prevalence of vitamin E deficiency

Various breeding troubles in farm animals such as horses and sheep have been thought to result from inadequate intake of vitamin E, but it is now practically certain that this is not the cause. Vitamin E deficiency most probably does not occur except when the vitamin is deliberately excluded from the diet.

Claims have been made that vitamin E deficiency occurs quite commonly in women during pregnancy and that miscarriages, sterility, and similar reproductive disorders of human beings have been helped by administration of extra vitamin E. Other reports, however, contradict these claims, so that at present the matter is still unsettled.
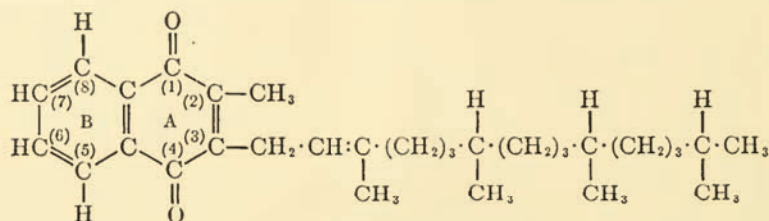
VITAMIN K

Physiological function

When chicks or other fowls are fed a ration deficient in vitamin K, eventually the blood ceases to clot within normal time with the result that there is a marked tendency for hemorrhages to occur. This condition is characterized by bleeding from the pinfeathers and hemorrhages into the subcutaneous tissues and muscles. The concentration of pro-thrombin, one of the factors involved in clotting of blood, invariably is found to be reduced in this deficiency disease. Just how vitamin K influences the formation of prothrombin remains to be determined. Since the sequence of changes involved in clotting of blood very likely is the same for all species, it will not be surprising if further investigations reveal a universal need of vitamin K. Experiments have demonstrated that man, rabbits, mice, and perhaps cattle require this vitamin, although deficiencies do not normally occur because adequate amounts usually are provided by the food intake, or are synthesized by intestinal bacteria.

Chemical nature

Several substances exhibiting vitamin K activity have been found in nature, and many more have been synthesized in the chemical laboratory.

The K vitamins are derivatives of 1,4-naphthoquinone, and this structure, or one readily convertible into it in the body, is essential for vitamin K activity. In the accompanying formula of vitamin K_1 the 1,4-naphthoquinone structure consists of rings A and B without the side chains

Vitamin K_1, $C_{31}H_{46}O_2$

attached at positions 2 and 3. Synthetic 2-methyl-1,4-naphthoquinone is even more active, gram for gram, than vitamin K, but the two are of about equal activity when compared on a molecular weight basis. It may be, as some have suggested, that in the body the K vitamins are degraded to 2-methyl-1,4-naphthoquinone or, as others believe, perhaps the latter is used in the body to synthesize these vitamins. A number of bacteria have been found to synthesize compounds possessing vitamin K activity. Phthiocol (2-methyl-3-hydroxy-1,4-naphthoquinone), a yellow pigment found first in the tubercle bacillus, is one such compound.

Water-soluble derivatives of vitamin K (*e.g.*, the sodium salt of 2-methyl-1,4-naphthohydroquinone diphosphate) have been prepared synthetically and are medically useful in certain cases (see below). Both *menadione*, as 2-methyl-1,4 naphthoquinone is called, and the water-soluble derivatives are in common use.

The K vitamins are fat-soluble and, therefore, are dissolved by the usual fat solvents such as ether and petroleum ether. They are stable to heat, but are destroyed by alkalies, strong acids, oxidizing agents, and sunlight. Solutions of vitamin K gradually lose their activity when subjected to light from an ordinary electric light bulb.

Prevalence of vitamin K deficiency

The general distribution of vitamin K in foodstuffs doubtless is responsible for the fact that deficiencies seldom are encountered in the adult. The vitamin apparently is also produced in the intestinal tract through the agency of the bacteria normally present. Vitamin K preparations were used on man first in treatment of obstructive jaundice, a condition in which a low concentration of prothrombin in the blood always prevailed. Deficiency of vitamin K in this instance is related to lack of bile, without which the vitamin is poorly absorbed from the intestinal tract. Surgical removal of the obstruction was formerly accompanied

by severe hemorrhages, but this is now very effectively prevented by prior administration of vitamin K, either by injection or by use of a water-soluble derivative which is easily absorbed. The deficiency of prothrombin associated with certain intestinal conditions—for example, obstruction and severe diarrheal diseases such as ulcerative colitis, sprue, and celiac disease—also responds to treatment with compounds possessing antihemorrhagic activity.

Since the concentration of prothrombin in the blood of a newborn baby is quite low, a large percentage of deaths during the first few days of life are due to hemorrhages resulting from injuries suffered at birth. Fortunately, however, investigations have revealed that prothrombin levels may be markedly increased within a short time by administration of vitamin K to the newborn. Administration of vitamin K to the mother prior to childbirth has been shown to raise the level of prothrombin in the foetus. These treatments should effect a drastic reduction in mortality of infants due to hemorrhage. The hereditary disease, hemophilia, does not respond to treatment with vitamin K.

A substance, *dicumarol*, which has a specific antagonistic effect toward vitamin K has been found in improperly cured sweet-clover hay. If freshly cut, partially dried, sweet clover is piled up for a few days, it undergoes a fermentation during which coumarin, a normal component of the hay, is converted into dicumarol. The chemical formulas of these substances are indicated below:

Coumarin   Dicumarol
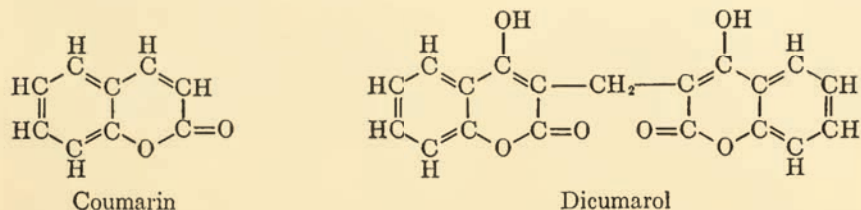
The blood of cattle eating such hay soon loses its normal clotting power, with the result that any injury is followed by severe and often fatal bleeding. Dicumarol was discovered by Link and co-workers.

The administration of dicumarol to experimental animals or man interferes with the formation of prothrombin, so that the result is similar to that of vitamin K deficiency. In fact, it is now well established that extra doses of the vitamin will counteract the effect of dicumarol, and vice versa. Neither substance affects the clotting power of blood *in vitro*, that is, if added after the blood is removed from the body. Dicumarol has been put to good use clinically to reduce the danger of the formation of blood clots inside the blood vessels following surgery.

A chemically related substance, sold under the trade name "Warfarin," is an effective poison for rats and mice. It acts by causing fatal hemorrhages. Warfarin's advantages for this purpose are that the effects are

so slow that the rats do not become "bait-shy" and that higher animals are proportionately less sensitive to it than are rodents.

$$O=C-CH_3$$

"Warfarin"
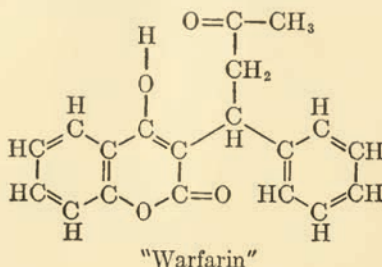
Occurrence

Vitamin K is particularly concentrated in green tissues of plants. Alfalfa, kale, spinach, carrot tops, chestnut leaves, and oat sprouts are some of the most potent sources of the vitamin. It also occurs in soybean and other vegetable oils. Cereals and seeds, however, are generally poor sources.

ASCORBIC ACID (VITAMIN C)

Physiological function

A pronounced lack of vitamin C in the diet leads to a deficiency disease known as scurvy. The onset is gradual and is characterized by loss of weight, a sallow or pallid complexion, tendency to fatigue, and shortness of breath. The gums become swollen, bleed easily, and ulcers may form. In later stages the teeth loosen and may drop out. Hemorrhages into the mucous membranes, skin, joints, limbs, and bone marrow, together with fragility of the bones are noted upon autopsy. These hemorrhages into the skin are quite obvious during life because blue-black spots occur after trivial injury, or they may appear spontaneously. The joints become swollen, and fleeting pains are noted in them. Death may eventually follow the headache, convulsions, and delirium that are seen in the later stages, or it may be caused directly by such complications as heart failure or pneumonia. Only two species other than man, namely, guinea pigs and monkeys, have been found susceptible to scurvy. Other animals seem to possess the power to synthesize vitamin C. However, the amount

Plate III. Clinical symptoms of vitamin C deficiency (scurvy). A. Inflammation and congestion about hair follicles. B. Petechiae (multiple small hemorrhages under the skin) in a scorbutic patient after application of blood pressure cuff. Increased pressure below the cuff has caused rupture of weakened capillaries. C. Purpura (purple blotches caused by bleeding under the skin) in a scorbutic patient, from the pressure of shoes. D. Purpura and petechiae in a scorbutic patient. E. Bleeding gums in an infant with scurvy. F. Scorbutic gums in an adult. (Reproduced with permission of Paul Hoeber, Inc., from Clinical Nutrition by Jolliffe, Tisdall, and Cannon, New York, copyright 1950.)
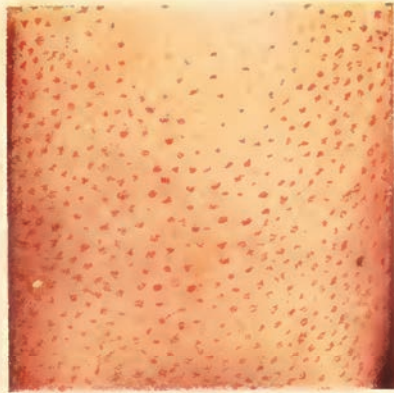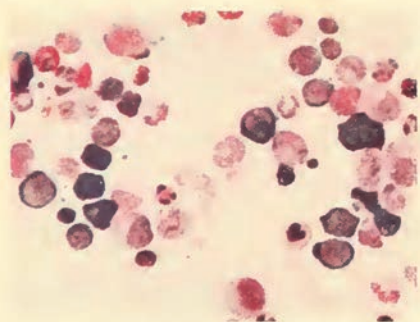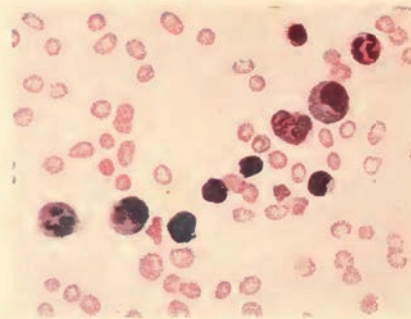
BEFORE TREATMENT
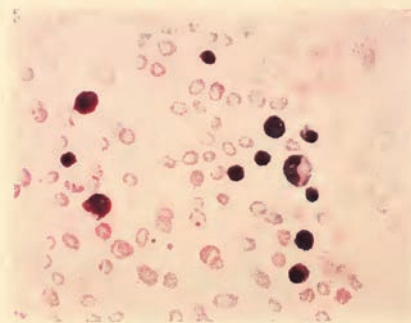

18 HOURS AFTER VITAMIN B_{12}

A. Hemopoietic (blood-cell forming) response of bone marrow after injection of 25 micrograms of vitamin B_{12} in a patient with pernicious anemia in relapse. The large blue and pink structures are red blood cells whose development has been arrested by lack of vitamin B_{12}. Note the formation of normal cells (small pink bodies) after treatment.

90 HOURS AFTER VITAMIN B_{12}

B. Regeneration of lingual papillae (normal tongue surface) after vitamin B_{12} administration.

Plate IV. Response of pernicious anemia patients to treatment with vitamin B_{12}.

synthesized or the rapidity with which the synthesis is carried out may not always be great enough to supply all the physiological needs of the animal. This fact has been strikingly demonstrated by the discovery that injections of vitamin C are of marked value in overcoming various breeding difficulties of cattle that have long been a source of loss to the dairy industry.
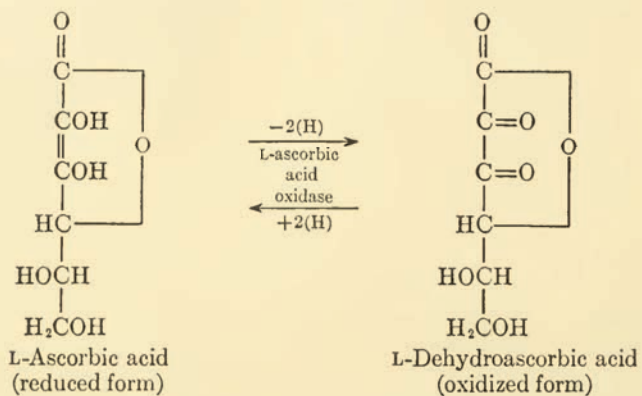
Since the average human dietary is not likely to be completely lacking in vitamin C, the symptoms of the earlier stages of scurvy, subacute or latent scurvy as it has been termed, are of more significance to us. The formation of intercellular substances which tend to acquire properties resembling a gel seems to be under the influence of vitamin C. In the absence of adequate amounts of the vitamin these intercellular substances, which normally act as a cementing medium, are not formed properly or may even be resorbed. Such changes may be observed microscopically in connective tissue, bones, and teeth, and are believed to occur in blood vessels as well. Since the tooth changes are rather pronounced and readily observed, a study of the extent of such changes offers a method for determining vitamin C. Hemorrhagic tendencies are noticeable even in subacute scurvy. In fact, the "capillary resistance" test, which has been devised to detect the beginning stages of scurvy, has as its basis the resistance to development of hemorrhagic spots, through rupture of the surface capillaries, when a tourniquet is used to produce intravascular pressure. This condition and other symptoms of scurvy are shown in Plate III, opposite p. 222.

Prevalence of vitamin C deficiency

Although scurvy in its worst form was quite prevalent in early exploration days, its occurrence at the present time is rather limited. It is said to be fairly common among the natives in South Africa, and certain tribes of aborigines in central Australia are reported to suffer considerably from it. The subacute form seems to be more prevalent than had been suspected prior to development of the "capillary resistance" test. For example, students in the university at Upsala, Sweden, showed a decreased resistance to hemorrhage in the spring corresponding to a proportionate decrease in vitamin C in the diet. The test has not been employed so extensively in the United States. However, American investigators have shown that administration of large quantities of vitamin C is beneficial in promoting normal tooth formation in the growing child. Such results suggest a probable deficiency in the diets of many American people. Some experimental results even point to the probability that certain rheumatic pains, particularly noticeable in late winter, may actually have their origin in a lack of vitamin C. A rather interesting observation, reported in 1939, was the marked improvement that vitamin C produced in men suffering from lead poisoning.

Chemical nature

Vitamin C isolated in crystalline form from natural sources has been named L-ascorbic acid. Its chemical constitution is shown by the structural formula in the following equation, which also indicates how it is converted into an oxidized form:

$$
\begin{array}{ccc}
\text{O} & & \text{O} \\
\parallel & & \parallel \\
\text{C} & & \text{C} \\
\mid & & \mid \\
\text{COH} & \rlap{\hspace{1em}\text{O}} & \text{C}{=}\text{O} \\
\parallel & \xrightarrow{\substack{-2(\text{H})\\ \text{L-ascorbic}\\ \text{acid}\\ \text{oxidase}}} & \mid \\
\text{COH} & \xleftarrow{+2(\text{H})} & \text{C}{=}\text{O} \quad\text{O} \\
\mid & & \mid \\
\text{HC} & & \text{HC} \\
\mid & & \mid \\
\text{HOCH} & & \text{HOCH} \\
\mid & & \mid \\
\text{H}_2\text{COH} & & \text{H}_2\text{COH}
\end{array}
$$

L-Ascorbic acid (reduced form) | L-Dehydroascorbic acid (oxidized form)

A close resemblance to the formulas of hexose sugars is evident.

Vitamin C is readily oxidized by mild oxidizing agents, and, in fact, its tendency to lose hydrogen atoms is so great that it is actually a strong reducing agent. The vitamin may function in living tissues by virtue of
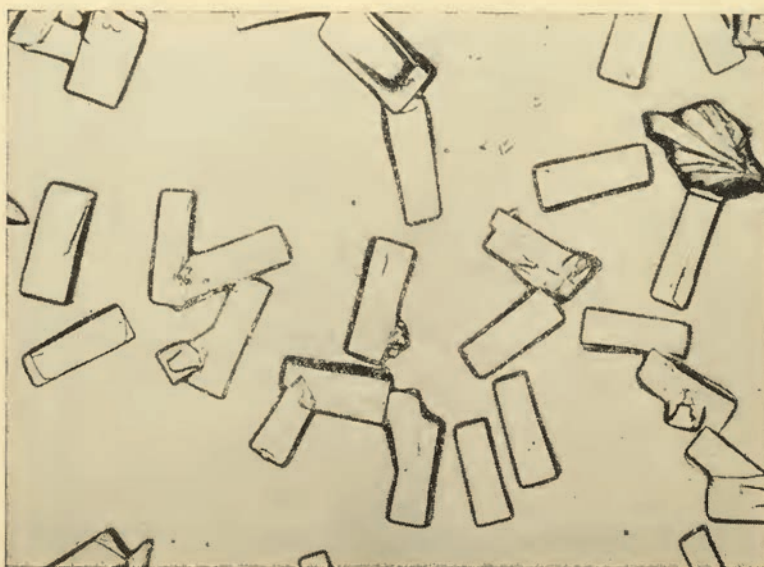
Courtesy of Merck & Co., Inc.

Fig. 9-4. L-Ascorbic acid.

its reversible oxidation and reduction, as shown in the equation above. These reactions are catalyzed by ascorbic acid oxidase, which, however, has been found so far only in plant tissues. The reducing power of vitamin C has also been utilized in determining by chemical means the amount of ascorbic acid in a given material. This is accomplished by use of a dye, 2,6-dichlorophenolindophenol, which is reduced by vitamin C to a colorless form. Hence the quantity of dye that is reduced is a measure of the vitamin potency of the food. The chemical method for determining vitamin C agrees well with the results obtained with feeding experiments, and since it takes but a few minutes to make a determination, chemical analysis has largely superseded biological assay. Atmospheric oxygen reacts quite readily with ascorbic acid to destroy it, especially at slightly elevated temperatures and in alkaline solution.

Occurrence

Although paprika exhibits the highest individual concentration of vitamin C hitherto observed in nature, as a class, the citrus fruits such as oranges, limes, lemons, and grapefruit, probably deserve to be ranked first in vitamin C content. Other good sources that find extensive use in the diet include tomatoes, strawberries, raw cabbage, fresh spinach, leaf lettuce, turnips, rutabagas, carrots, bananas, apples, and potatoes. The large consumption of potatoes in the average American dietary rather than a high content of vitamin is responsible for its contribution to the total intake of vitamin C. Milk is too low in vitamin C to furnish an adequate supply, and, hence, pediatricians recommend administration of fruit juices early in the life of a baby to compensate for the deficiency. The bottle-fed baby receives only about one-fourth as much vitamin C from cow's milk as it would from its mother's milk. Meat and eggs contain very little vitamin C. Cereal grains likewise contain little, if any, although considerable amounts are formed during germination.

Because of the ease with which vitamin C is destroyed by oxidation and also because it is water-soluble and therefore lost if the cooking water is discarded, cooked foods are apt to contain much less than the original raw materials. Studies made in a Canadian army camp during World War II revealed that of every 100 mg. of vitamin C in the food brought into the kitchens only 15 mg. reached the table! Boiling cabbage for 20–30 minutes and discarding the water may cause the loss of over 80 per cent of its vitamin C potency. Even when vegetables such as broccoli or spinach are allowed to remain at room temperature for 24 hours, 50 to 70 per cent vitamin destruction occurs. The principles to be followed if loss of this sensitive but vital food factor is to be avoided or minimized may be summarized thus: (1) eat the food raw if possible, (2) if the food is to be cooked, use the minimum amount of water, heat quickly to

the boiling point (to destroy ascorbic acid oxidase) and cook no longer than necessary, (3) use fresh fruits and vegetables immediately after harvesting, (4) if the food must be stored, keep it cold and protected from air as much as possible (*e.g.*, wrapped in paper, with the original husks or skins on, not cut or sliced up, etc.), (5) avoid the use of materials, especially copper, which catalyze the oxidation of vitamin C. Many of these same principles apply equally well to the preservation of other sensitive vitamins. In general, if food is so handled that its vitamin C is largely retained, all other food factors will most probably be preserved also.

Certain tissues of the body are rich in vitamin C. It is particularly concentrated in the cortex of the adrenal glands, in the lens, aqueous humor, and vitreous humor of the eye, and in the pituitary gland. In fact, concentration seems to be correlated with metabolic activity. Although liver contains more than other animal tissues used for food, this organ does not seem to function so prominently in the storage of vitamin C as it does in retaining vitamins A and D, and riboflavin.

Requirements

The needs of various persons for this vitamin are shown in Table 9–3, from which it can be seen that relatively large amounts are required. Relying upon one food source for the day's supply, one would need to consume three-fourths cup of orange juice, four to five quarts of milk, one-fourth pound of raw cabbage, or three-fourths pound of raw tomatoes.

THIAMINE (VITAMIN B_1)

Physiological function

An inadequate intake of this vitamin eventually leads to the onset of a disorder known in human beings as beriberi and in the experimental animal as polyneuritis. There are two distinct forms of beriberi: (1) the dry type, in which there is excessive muscular atrophy, anaesthesia of the skin, and paralysis of the legs, arms, diaphragm, and intercostal muscles; (2) the wet type, which is characterized by swelling of the arm, leg, and trunk muscles; by effusion of fluid into the body cavities; by congestion of the liver; and by dilatation of the heart with resultant heart failure. The wet type is somewhat more common in infants, but symptoms of both types frequently appear in the same patient. In general, the symptoms appear first in the feet and lower parts of the legs, and progress upward toward the trunk.

It is now generally agreed that thiamine functions in the animal body

in the form of its pyrophosphoric acid ester, which is known as cocarboxylase. This substance is required for the action of various enzymes (for example, carboxylase and pyruvic oxidase), which function in the breakdown of pyruvic acid formed during normal carbohydrate metabolism. Thus when the body is deprived of an adequate amount of thiamine, pyruvic acid accumulates in the blood, heart, and nervous tissues until toxic concentrations are reached, and the visible symptoms of beriberi result. Degeneration of nerve tissue formerly attributed to a lack of thiamine is apparently the result of other dietary deficiencies. Animals that receive adequate amounts of vitamin A and riboflavin but an insufficient amount of thiamine show no degeneration of nerve tissue.

Another characteristic symptom in thiamine deficient animals is anorexia, or loss of appetite. Administration of the vitamin to such animals

Courtesy of the Dept. of Biochemistry, University of Wisconsin.
Fig. 9–5. Thiamine deficiency in the chick. The posture is typical of polyneuritis in birds.

results in a phenomenal stimulation of the desire to eat, and the animals may consume as much food in the succeeding twenty-four hours as they had previously in an entire week. The growth of young animals on thiamine deficient diets is subnormal long before the severe symptoms of polyneuritis become apparent. Thiamine has also been used with fair success as a stimulant to the appetite in human beings. However, it should be emphasized that many other factors also affect the appetite and that thiamine is probably only effective in those cases where the anorexia is actually due to a lack of this particular vitamin. Decreased

motility of the stomach and intestinal tract with resultant constipation are also caused by this deficiency.

Perhaps because of its intimate relation to carbohydrate metabolism, thiamine plays an important role in the life of plants and microorganisms, as well as higher animals. Many microorganisms cannot be grown without it, and others, although able to synthesize it, make a much faster growth if it is contained in the nutrient medium. The need for thiamine seems to be universal, and it probably plays a part in the activities of all living cells. Plants, however, seem able to synthesize adequate amounts of thiamine to supply their own needs. Claims regarding the beneficial effect of thiamine and other B vitamins when applied to growing plants as yet are unsubstantiated, and the evidence is conflicting. It seems quite certain that these claims have been grossly exaggerated.

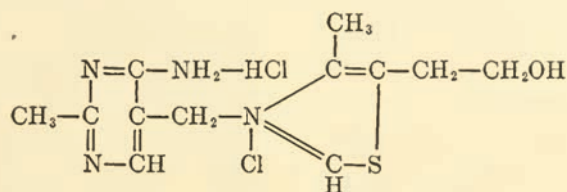### Prevalence of thiamine deficiency

Historically, beriberi has been associated with the Orient, where polished rice constitutes such a large part of the diet. In spite of the knowledge for many years that it is a deficiency disease, beriberi is still prevalent in India, China, Japan, the Philippine Islands, and other Oriental countries. According to Salcedo and co-workers, beriberi is second only to tuberculosis as a cause of death in the Philippines. Mortality figures for 1947 were 132 per 100,000 population, and 13 per cent of the population showed evidence of thiamine deficiency.

Although the disease in its worst form has been considered rare in the United States, recent studies of various forms of neuritis have revealed that a number of these exhibit a clinical picture comparable to that of classical beriberi. Diets in the United States are seldom restricted to foods so low in thiamine as is polished rice, yet it now seems fairly certain that the food ordinarily consumed by many people in this country fails to furnish an "optimal" amount of this vitamin and that a slight degree of thiamine deficiency is much more widespread than was formerly supposed. Furthermore, a number of conditions which either increase the thiamine requirement or in some way interfere with the utilization of the normal supply probably contribute to the appearance of this deficiency. Excessive consumption of alcohol is an important factor in this regard, for the caloric value of the alcohol leads to a diminished food intake, and, in addition, gastrointestinal disorders incident to excessive use of alcohol may reduce the absorption of the vitamin. In one of our large hospitals 61.6 per cent of 1000 consecutive male patients admitted to the alcoholic ward in 1935 were suffering from polyneuritis. Whereas during pregnancy the requirements of the body for thiamine are materially increased, the nausea and vomiting frequently associated with this condition may actually curtail the food intake and, with it, the

vitamin supply, thereby inducing a form of neuritis. Since infectious diseases likewise increase the needs of the body for this vitamin, they may be causal agents in the production of thiamine deficiencies.

Chemical nature

This vitamin, which has been synthesized by two distinctly different methods, contains the familiar pyrimidine ring (cytosine, thymine, and uracil of the nucleic acids are pyrimidines) together with the thiazole ring, in which the sulfur is contained. In addition to these two rings note in the formula below that this vitamin is an amine.

$$CH_3-\overset{\displaystyle N=C-NH_2-HCl}{\underset{\displaystyle N-CH}{\overset{\displaystyle |}{\underset{\displaystyle \parallel}{C}}}\overset{\displaystyle |}{\underset{\displaystyle \parallel}{C}}-CH_2-\overset{\displaystyle |}{\underset{\displaystyle Cl}{N}}} \overset{\displaystyle CH_3}{\underset{\displaystyle \overset{C-S}{H}}{\overset{\displaystyle |}{C}=C-CH_2-CH_2OH}}$$

Thiamine chloride hydrochloride, $C_{12}H_{18}N_4SCl_2$

In addition to being water-soluble, thiamine dissolves in acids and dilute alcohol. It is fairly stable to heat in acid solution, but in an alkaline medium it is rapidly destroyed by heat. Although the evidence is somewhat conflicting, it appears to be fairly well established that appreciable destruction of thiamine occurs during the cooking of food. Thus it has been reported that up to 57 per cent of the thiamine may be lost by stewing meat, about an equal amount by roasting or baking, and 10 to 30 per cent by frying. Baking bread causes about 15 to 20 per cent loss. The destruction of thiamine by boiling vegetables has been reported to amount to as much as 22 per cent, with an additional 15 per cent present in the cooking water. The amount of destruction increases rapidly at temperatures above 100° C. Pressure cooking, therefore, causes considerably more destruction of thiamine than cooking at ordinary pressure.

An enzyme (thiaminase) in certain fish, mostly of fresh water origin, has the specific property of destroying thiamine. Foxes fed diets containing raw carp develop a paralysis which was found to be actually a thiamine deficiency, brought about through the agency of thiaminase. Cooking the carp, or feeding extra amounts of thiamine to the animals prevented the disease.

In the last few years several relatively quick chemical methods of assaying foods for their thiamine content have been proposed. Perhaps the most widely used is the "thiochrome method," which is based on the oxidation of thiamine, in an extract of the food, to thiochrome, which shows a characteristic bluish fluorescence in ultraviolet light. The in-

tensity of this fluorescence serves as a measure of the amount of thiamine that was present in the food. Such assay methods are now used much more extensively than the longer animal feeding methods, although the latter are still relied upon as the final test.

Courtesy of Merck & Co., Inc.

Fig. 9–6. Thiamine.

Occurrence

The richest known source of thiamine is brewer's yeast. It has been found, however, by Kingsley and Parsons that the greater part of the thiamine (and riboflavin) in yeast is not utilized by human beings unless the yeast is given some previous treatment such as cooking which kills the yeast cells. Next in order of concentration follow pork muscle, rice polishings, and bran of grains. Obviously, cereals can constitute a good source of this vitamin only when the entire grain is used. If considered on the dry basis, most vegetables and fresh fruits are fairly potent sources. Eggs and most meats contain appreciable amounts of the vitamin. Synthetic thiamine is being employed to an increasing extent for the fortification of commercial foods, particularly white flour.

Requirements

The amount of thiamine needed daily by a normal person depends on the amount and kind of food that he consumes. As indicated above, this vitamin functions in the metabolism of carbohydrate in the body,

and it probably also is involved in the utilization of excess dietary protein as a source of energy. It is not apparently needed for the metabolism of fat, and the inclusion of a large amount of fat in the diet exerts a thiamine-sparing action. In short, it may be said that the thiamine requirement is related to the nonfat calories provided by the food consumed. Abnormal conditions affecting the thiamine requirement of the body have been considered on pp. 228 and 229.

Table 9–3

Daily human requirement for water soluble vitamins*

SUBJECT	Thiamine mg.	Riboflavin mg.	Nicotinic acid mg.	Ascorbic acid mg.
Man (154 lb., 70 kg.)				
Moderately active	1.5	1.8	15	75
Very active	1.8	1.8	18	75
Sedentary	1.2	1.8	12	75
Woman (123 lb., 56 kg.)				
Moderately active	1.2	1.5	12	70
Very active	1.5	1.5	15	70
Sedentary	1.0	1.5	10	70
Pregnancy (latter half)	1.5	2.5	15	100
Lactation	1.5	3.0	15	150
Children				
Under 1 year	0.4	0.6	4	30
1–3 years (27 lb., 12 kg.)	0.6	0.9	6	35
4–6 years (42 lb., 19 kg.)	0.8	1.2	8	50
7–9 years (58 lb., 26 kg.)	1.0	1.5	10	60
10–12 years (78 lb., 35 kg.)	1.2	1.8	12	75
Girls				
13–15 years (108 lb., 49 kg.)	1.3	2.0	13	80
16–20 years (122 lb., 55 kg.)	1.2	1.8	12	80
Boys				
13–15 years (108 lb., 49 kg.)	1.5	2.0	15	90
16–20 years (141 lb., 64 kg.)	1.7	2.5	17	100

* As recomended by the Food and Nutrition Board of the National Research Council, 1948.

Detailed estimates of the amount of thiamine needed daily by various persons are shown in Table 9–3. For most people approximately 1–2 mg. daily constitute a safe intake. Quantities of representative foods that would supply an amount within these limits are: 1 lb. brown rice, 4 lb. cabbage, 2 lb. asparagus, 1/4 lb. lean pork chop, 2 qt. whole milk, or 5 lb. white flour. If desired, the above amounts of thiamine may be expressed as international units, on the basis that 3 μg. of the pure vitamin correspond to one unit.

One of the largest dietary sources of thiamine, as well as of niacin and riboflavin, would be bread and other grain products, except for the great losses of these vitamins which occur on milling (Table 9–4). To com-

pensate for these losses white flour and bread are now usually enriched
with the three pure vitamins, at least to the levels.

Table 9–4

B vitamins in bread and flour

VITAMIN	Whole wheat flour *	White flour *	Enriched white flour *†	Enriched white bread *†
Thiamine	0.55	0.066	0.44	0.33
Riboflavin	0.12	0.033	0.26	0.26
Niacin	5.56	0.77	3.52	2.75

* Milligrams per 100 g.   † Minimum standards.

RIBOFLAVIN

Physiological function

The outstanding symptom in young animals or birds fed on a ration
low in riboflavin is retarded growth. In poultry, deprivation of this
vitamin leads to diminished egg production and especially to a failure
of the eggs to hatch. Continued deficiency in the chick causes a condi-
tion known as "curled toe paralysis," in which the bird is unable to walk
and eventually dies. Examination of such chickens reveals extensive
nerve degeneration.

The necessity of riboflavin has also been demonstrated for rats, dogs,
pigs, and man. In a series of outstanding studies Sebrell and his co-
workers of the United States Public Health Service have shown that
when adult persons subsist for an extended period of time on a diet low
in riboflavin, they contract an illness of which characteristic symptoms
are soreness and inflammation of the tongue (glossitis), and cracks and
sores on the lips and at the corners of the mouth (cheilosis). Further-
more, such patients nearly always suffer from various disorders of the
eye. These include abnormal sensitivity to light, dimness of vision, and
inflammation and development of blood vessels in the cornea. All of
these symptoms respond promptly to the daily administration of 5 to
10 mg. of pure riboflavin. It is probable that human riboflavin deficiency
often accompanies pellagra, although masked somewhat by the more
striking symptoms of the latter disease, and that it is rather widespread
among the population of America. It has also been reported in India,
the West Indies, and many parts of Africa. A children's disease known
as "perleche," occasionally seen in the southern United States, is actually
a riboflavin deficiency.

In living tissues riboflavin is built up into more complex substances,
namely, *riboflavin phosphate* and *flavin-adenine-dinucleotide* (abbreviated
FAD, p. 277), which serve as coenzymes for a series of enzymes involved
in metabolism. These enzymes, which are called flavoproteins or "yellow

enzymes" because of the color imparted to them by the flavin group, have the function of catalyzing the removal of hydrogen atoms from certain metabolites and passing the hydrogen to some acceptor, such as cytochrome c or molecular oxygen. Xanthine oxidase, D- and L-amino acid oxidases, and cytochrome c reductase are examples of flavoproteins. As a result of its participation in the make-up of these enzymes, riboflavin fulfills a vital role on the "main line" of the oxidation processes by which food energy is made available to living tissues. Like the other B vitamins, it apparently is needed by all living cells.
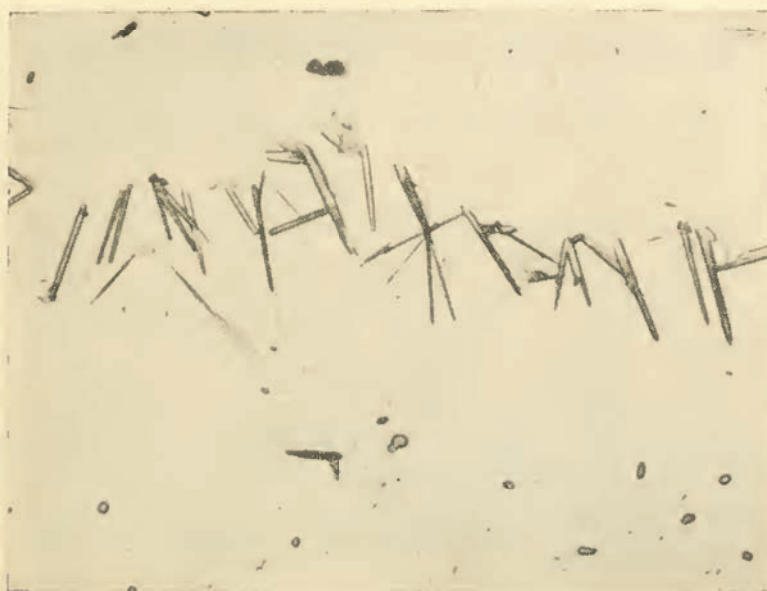

Courtesy of Merck & Co., Inc.

Fig. 9–7.   Riboflavin.

Chemical nature

Riboflavin, which previously has also been called vitamin B_2 or vitamin G, is a water-soluble pigment widely distributed in plant and animal materials, although in very minute concentrations. Dilute solutions of it have a greenish yellow color and in ultraviolet light show a characteristic greenish yellow fluorescence. It is, for example, the substance mainly responsible for the rather faint but distinct color of whey and of egg white It was first obtained in the pure state in 1933 and was synthesized in 1935. The pure vitamin, which is an orange-red powder, is now readily available. It is produced mainly by fermentation with special riboflavin-producing microorganisms, for example, *Eremothecium ashbyii*.

The structural formula of riboflavin is:

$$\text{Riboflavin, } C_{17}H_{20}N_4O_6$$
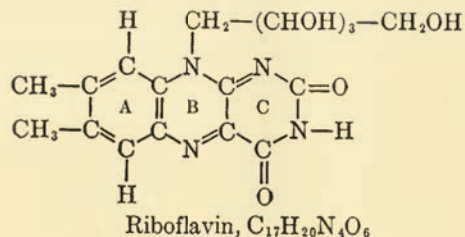
Note the benzene A and pyrimidine C rings, as well as the sugar-like side chain, which is related to the rare pentose sugar, ribose.

Riboflavin is very stable to heat, acids, and oxidizing agents, but is easily destroyed by light and by alkalies. Losses attendant on the cooking of food may range from 0 to 60 per cent. A quart of milk in an ordinary clear glass bottle, if set in direct sunlight, may lose as much as 50 per cent of its riboflavin content in two hours. Brown glass bottles or paper cartons prevent this destruction. Although riboflavin is a water soluble vitamin, surprisingly little—perhaps 10 to 20 per cent—is removed by boiling vegetables in water. This is probably attributable to the fact that in tissues it exists largely combined with various proteins.

Occurrence

Riboflavin is formed primarily in green leaves of actively growing plants; hence, green leafy vegetables constitute a good source of this vitamin. Brewer's yeast is a highly potent source. Of the various meats, liver contains the greatest concentration of the vitamin and is followed closely by kidney. The muscle meats likewise contain appreciable amounts. Milk and eggs are quite satisfactory sources, and in the well-planned diet the former contributes largely to the total riboflavin intake.

At present several fairly reliable methods other than animal assay are available for determining the amount of riboflavin in foodstuffs. Chemical methods depend on the measurement of the characteristic color or fluorescence in suitably clarified extracts of the food. Still another method of assay has been based on the fact that certain lactic acid bacteria require riboflavin for normal growth and acid production. An aqueous suspension of the sample is fermented by the organism, and the amount of lactic acid produced (as determined by titration of the entire culture) serves as a measure of the riboflavin present. Similar assay methods are used for most of the other B vitamins and for amino acids.

Requirements

Detailed information regarding the riboflavin requirement of man is given in Table 9–3. In general, a daily intake of 1.5–3.0 mg. would appear to be adequate. However, Sherman concludes, from a series of experiments on rats carried out over a long period of time, that the optimum intake, as indicated by a higher level of health and general well-being, may be several times as great as the minimum amount needed to prevent deficiency symptoms. It does not seem likely that harmful effects will result from too large an intake of riboflavin, since excessive doses are rapidly excreted in the urine.

Amounts of representative foods that would supply approximately 2 mg. of riboflavin are: 1½ lb. lean beef, 2 oz. liver, 1 lb. kale, 1 qt. whole milk, 1 lb. eggs, 13 lb. white flour.

NICOTINIC ACID (NIACIN)

Physiological function

Extreme deficiency of nicotinic acid produces a disease in man known as *pellagra*. This disease is characterized by roughness and pigmentation

Courtesy of J. M. Ruffin, D. T. Smith, and *The Southern Medical Journal.*
Fig. 9–8. Typical dermatitis of pellagra.

of the skin, particularly on the hands, arms, face, and back of the neck. Apparently the sun exerts an irritating effect, causing exposed areas of the skin to exhibit first this abnormality. The mouth becomes sore because of lesions which develop on the mucous membrane, and the tongue may become sore, red, and swollen. Indigestion and diarrhea are associated conditions, and in severe cases insanity may result. The symptoms of pellagra have been summed up by the "three D's": dermatitis, diar-

Courtesy of J. M. Ruffin, D. T. Smith, and *The Southern Medical Journal.*
Fig. 9-9. Effect of nicotinic acid on dermatitis of pellagra.

rhea, and dementia. In dogs the disease is known as black-tongue. Pigs and monkeys have also been shown to develop a similar disease when deprived of nicotinic acid. Rats, on the other hand, apparently do not require this vitamin in the diet.

Nicotinic acid, like riboflavin, is an essential constituent of several enzyme systems. In fact, it is a definite part of triphosphopyridine nucleotide (TPN, p. 275), without which one cytochrome reductase cannot function. In addition to the amide of nicotinic acid this coenzyme contains adenine, two molecules of ribose, and three molecules of phosphoric acid. A compound differing from this only in that it contains one less phosphoric acid molecule is diphosphopyridine nucleotide (DPN), which is essential to the breakdown of glucose by animals and to fermentation by yeast. Its function as a part of oxidation-reduction systems is the

important role played by nicotinic acid, or nicotinamide, in many, if not all, living cells.

Prevalence of nicotinic acid deficiency

Next to rickets, pellagra is probably the most common vitamin deficiency disease in the United States. Although doubtless occurring prior to the twentieth century, it was not recognized here until about 1907 or 1908. The disease is confined largely to the southern states where the diet of the poorer classes is derived chiefly from corn meal, molasses, and fat pork. The United States Public Health Service estimated that in 1929 there were 200,000 cases of pellagra in the country. In 1930 pellagra was reported to have caused 7146 deaths, of which 98 per cent were in the cotton belt. In contrast to the above figures, there were reported in 1940 only 8688 cases of pellagra. Pellagra also occurs in Egypt, Rumania, and South Africa, always in areas where corn is a main article of diet. Over 1000 cases annually were reported in South Africa during 1944–1947.

Symptoms of niacin deficiency in animals are relieved by administering tryptophan, and it is now known that tryptophan can be converted into nicotinic acid in the animal body (p. 347). Since corn contains little tryptophan, this relationship helps to explain why pellagra develops especially among corn-eating populations. It has been suggested also that corn may contain an antivitamin (p. 256) antagonistic to niacin. In addition to these complicating factors, it is probable that pellagra also involves deficiencies of other vitamins besides niacin. Nearly all cases of pellagra require treatment with thiamine and riboflavin, in addition to nicotinic acid, in order to clear up all of the symptoms, and some have also been reported to benefit from the use of pyridoxine. For this reason it is very probable that the pellagra problem cannot be solved by the use of nicotinic acid alone, but that the answer lies much more in a general improvement of the diet. It is probable that this deficiency disease will not be eradicated in the South until the prevailing diet of corn bread, molasses, and fat meat is properly supplemented. This constitutes an economic problem, since practically all potent sources of the vitamin are relatively expensive foods, which the poorer classes are unable to buy.

Chemical nature

Nicotinic acid is a relatively simple derivative of pyridine and possesses a structure represented by the accompanying formula. The corresponding amide is also effective as a vitamin:

$$HC \overset{H}{\underset{\underset{N}{||}}{\overset{C}{\underset{HC}{C}}} \overset{O}{\underset{||}{C}} - OH$$
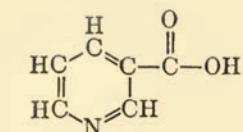
Nicotinic acid, $C_6H_5NO_2$
(Niacin)

$$HC \overset{H}{\underset{\underset{N}{||}}{\overset{C}{\underset{HC}{C}}} \overset{O}{\underset{||}{C}} - NH_2$$
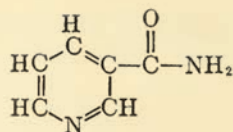
Nicotinamide, $C_6H_5ON_2$
(Niacinamide)

As its name implies, the compound is closely related to nicotine, from which it can be easily formed by oxidation in the laboratory. This vitamin is soluble in dilute alcohol and in water; it is very stable to heat, light, acids, alkalies, and oxidizing agents. In fact, it is more resistant to chemical attack than any of the other vitamins. Nicotin-amide is preferred for therapeutic use since it is less likely to cause a burning sensation of the face that is often noted after doses of nicotinic acid.

Occurrence

Nicotinic acid is a substance that was known to organic chemists for many years before its usefulness as a vitamin was discovered. It is now prepared synthetically on a rather large scale and is among the most easily obtainable and cheapest of the vitamins. An amount sufficient to cure a pellagrin costs only about ten cents. The synthetic material is now being used in the enrichment of white flour. The term "niacin" has

Fig. 9-10. Nicotinic acid.