CHEMISTRY OF FOOD AND NUTRITION

SHERMAN

THIRD EDITION

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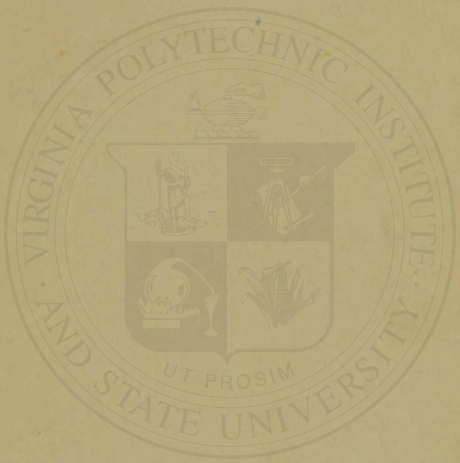

CHEMISTRY OF FOOD AND NUTRITION

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CHEMISTRY OF FOOD AND NUTRITION

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PREFACE TO THE THIRD EDITION

THE purpose of this book is to present the principles of the chemistry of food and nutrition with special reference to the food requirements of man and the considerations which should underlie our judgment of the nutritive values of foods. Food is here considered chiefly in its relations to nutrition, the more detailed description of individual articles of food and the chemical and legal control of the food industry having been treated in another volume.

The present work is the outgrowth of several years' experience in teaching the subject and is published primarily to meet the needs of college classes. It is hoped that the book may also be of service to other readers who appreciate the importance of food and nutrition as factors in health and are interested in the scientific foundations which have been so greatly broadened and strengthened by the investigations of recent years.

While the small size, to which the book is limited by its main purpose, permits little of either historical or technically critical treatment, yet a limited number of original investigations and of controverted views have been discussed in order to give an idea of the nature of the evidence on which our present beliefs are based, and in some cases to put the reader on guard against theories which, while now outgrown, are still sometimes encountered.

Special attention has been given to the difficult task of attempting to present the striking results of some of the most recent investigations in nutrition in such a manner as to make clear their importance without giving exaggerated impressions,

and with due emphasis upon the fact that on many significant points any interpretation which can now be offered is necessarily tentative.

The reader is especially urged to keep in mind the importance of a well-balanced knowledge of all the four main factors of nutritive requirement — energy, protein, mineral elements, and vitamins.

The activity of research work in this field has made necessary a complete rewriting of most of the text; the addition of several new chapters; and a considerable extension of the lists of references at the ends of chapters, in order that these may continue to serve to put the reader in touch with the more significant literature.

The author desires to express his great indebtedness to the colleagues and former students who have contributed many helpful suggestions, and especially to Dr. M. L. Caldwell, Miss H. L. Campbell, Miss M. M. Heseltine, Professor Grace MacLeod, Dr. E. J. Quinn, Professor M. S. Rose, Professor A. W. Thomas, and Professor M. W. Weeks for constructive criticism and active collaboration in the preparation of the present revision of the work.

H. C. S.

May 1, 1926.

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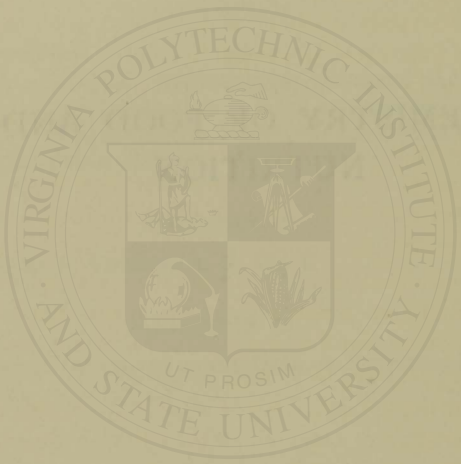
CHEMISTRY OF FOOD AND NUTRITION



CHEMISTRY OF FOOD AND NUTRITION

INTRODUCTION

THE activities on which the life of the body depends involve a continuous expenditure of energy and a constant exchange of material. Ultimately the body is dependent upon food for the fuel materials which supply energy, for the substances which are transformed in the body, and for those whose presence regulates and controls these transformations. The materials leaving the body are to be regarded not merely as wastes but as end products of an orderly and coördinated series of chemical reactions which occur in the body and by virtue of which its functions are performed. Thus the chief functions of food are: (1) to yield energy, (2) to build tissue, (3) to regulate body processes.

These functions involve reactions which are dependent upon the chemical composition and constitution of the food. "The assemblage of processes concerned in the growth, maintenance, or repair of the living body as a whole, or of its constituent parts or organs" constitutes *nutrition* in the broader meaning of the term; therefore, any food constituent which takes part in any of these processes may be regarded as having nutritive value and serving as a nutrient.

Most of the nutrient material contained in food requires more or less change to bring it into the exact forms most useful in nutrition. These changes as a rule take place in the digestive tract and together constitute the process of digestion.

The changes which take place in the foodstuffs, after they have been absorbed from the digestive tract, are included un-

der the general term "metabolism." Although the chemical changes and the energy transformations are of course inseparable, it has become customary to speak of the metabolism of matter and the metabolism of energy, and to regard the extent of the metabolism of any material substance as measured by the amount of its end products eliminated, and the extent of the energy metabolism as measured by the amount of heat, or of heat and external muscular work, which the body gives off.

The metabolism of matter and the metabolism of energy are normally supported by the food; but if no food is taken, they continue at the expense of the body substance. The expenditure of energy can never cease in the living body because it includes the work involved in carrying on the internal processes which are essential to life itself; and the expenditure of matter cannot cease because the energy for this necessary work is obtained by the breaking down of the organic compounds of the food or of the body substance into simpler compounds, many of which are of no further use to the body and must be eliminated. When the food supplies sufficient energy, the body substance is protected; when the food is insufficient, body substance is burned as fuel. In order, then, to consider intelligently the nutritive requirements of the body as regards the substances of which it is composed, it is necessary first to know whether the fuel requirements (the requirements of the energy metabolism) have been fully met.

The carbohydrates, fats, and proteins of the food all serve as fuel to yield the energy required for the activities of the body, and the proteins serve also as material for the maintenance or growth of body tissue. But of the fifteen or more chemical elements which are essential to the structure and functions of the body, simple proteins furnish only five. The remaining elements are largely constituents of the ash of the food and are known as ash constituents, inorganic foodstuffs, mineral matter, or salts.

The chemistry of food and nutrition obviously includes the study not only of both the ultimate and proximate composition of foods but also of the utilization of these foods by the body, as determined by feeding experiments. Experimentation on human subjects must necessarily be limited in some respects, both because of the difficulties in submitting human beings to laboratory conditions over long periods, and because the life-time of the investigator would hardly suffice for observations upon the complete life history of his subject. Fortunately, carefully selected laboratory animals have been found to be highly satisfactory "reagents" for this *chemistry in vivo* (sometimes referred to as the "biological method of food analysis"). Sufficient biometric data have been compiled for the albino rat in particular to make possible the selection from this species of individuals of unquestioned normality. The albino rat of the present-day nutrition laboratory resembles man in his environment, his omnivorous feeding habits, and the chemical nature of his nutritional processes. Added to these qualifications is the great advantage that the cycle of development takes place about thirty times as rapidly in the rat as in man, enabling observations on the entire period of growth or even the entire life span to be completed within a comparatively brief period. For these reasons and many others, the rat has proven to be the most generally useful of all the various experimental animals. As a result of many and careful studies by a number of investigators experienced in both this and other methods, it can now be said with confidence that, under proper conditions, the rat can safely and wisely be used for nutrition experiments the findings of which are to be applied to human problems.

Recent investigations have developed the fact (explained more fully in Chapter XIV) that food of sufficient energy value and containing ample amounts of each of the chemical elements known to be essential to the body is not necessarily adequate to meet all the requirements of nutrition. Thus it appears that

certain substances occurring in natural foods but not yet chemically identified are also to be included among the nutritive requirements of the body and therefore among the factors which determine the nutritive values of foods. The substances, still in process of being identified chemically, but whose nutritional relationships have been extensively studied, are called the vitamins.

The essentials of a chemically adequate food supply may therefore be summarized as follows: (1) sufficient of the organic nutrients in digestible forms to yield the needed energy; (2) protein, sufficient in amount and appropriate in kind; (3) adequate amounts and proper proportions of the various ash constituents or inorganic foodstuffs; (4) sufficient of each of the essential vitamins.

In attempting to give in the following pages a general view of the chemistry of food and nutrition it has seemed best to discuss first the chemical nature and nutritive functions of the carbohydrates, fats, and proteins; second, the nutritive requirements of the body in terms first of energy, then of protein; third, the inorganic or mineral elements in food and nutrition; and fourth, the vitamins. In general this permits us to progress from the topics which are more familiar to those which are newer or less well worked out.

It is important to keep clearly in mind the fact that the "newer knowledge of nutrition" supplements but does not supplant the knowledge which had been gained before vitamins were discovered or the importance of the mineral elements was appreciated.

The endeavor of the present-day student should be to gain and hold a thorough grasp and a well-balanced knowledge and appreciation of all four of the main factors of nutritive requirement — energy, protein, mineral elements, and vitamins.

CHAPTER I

CARBOHYDRATES[1]

Of the constituents of the ordinary mixed food of man the carbohydrates are usually the most abundant and the most economical sources of energy. They are also considered to be the first of the three great groups of foodstuffs to be formed by synthesis from simple inorganic substances in plants; "in the long run, all the energy of living matter comes from them." The synthesis of carbohydrates in nature is therefore a logical starting point for the study of the organic foodstuffs.

In the chlorophyll cells of the leaves of green plants the energy of the sun's rays brings about reaction between carbon dioxide and water which results in the liberation of oxygen and the formation of organic compounds. This is known as *photosynthesis*. There is still doubt as to the mechanism of the process and no certainty that it is the same in all cases. While the ratio varies with the respiration of the plant, yet as a general average result it is found that the volume of oxygen liberated is equal to that of carbon dioxide consumed. The simplest possible representation of the reaction would be

$$CO_2 + H_2O \longrightarrow CH_2O + O_2$$

according to which the first product of the synthesis would be formaldehyde. One theory which has long been debated is that formaldehyde is thus produced and then polymerized into a simple sugar; another is that a simple sugar is formed without the intermediate production of formaldehyde. The

[1] The term *glucides* has been proposed as a group name for the carbohydrates by the International Union of Pure and Applied Chemistry.

older conceptions of photosynthesis as a formation of carbohydrate by a relatively simple and direct reaction or reactions seem now to be giving way to the view that the carbohydrate formed may really be the outcome of a much more complex process or series of processes, perhaps involving the fundamental metabolism of the protoplasm of the chlorophyll cell. Spoehr's extensive recent work, relating photosynthesis to the respiration, and both to the protein metabolism, of the leaf, tends strongly in this direction.

Whatever the steps in the process, there is a relatively early production of carbohydrate. Usually the first product which can be demonstrated as accumulating in the plant as the result of photosynthesis is a sugar (glucose or sucrose) or starch. Assuming glucose as a typical product and neglecting the intermediate stages, the photosynthesis of carbohydrate may be represented thus:

$$6\,CO_2 + 6\,H_2O \longrightarrow C_6H_{12}O_6 + 6\,O_2$$

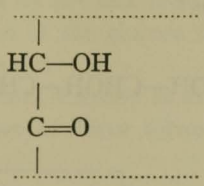
Glucose is the most familiar representative of a group of simple sugars (monosaccharides or monosaccharoses) which are in composition direct polymers of formaldehyde (CH_2O) and which are classified, according to the number of carbon atoms in the monosaccharide molecule, as trioses, pentoses, hexoses, etc.

Structure and Classification

The term carbohydrates is generally used to include the simple sugars and their anhydrides. Definitions of the term simple sugar vary somewhat, depending chiefly upon the views of different authors as to how simple a compound may properly be called a sugar.

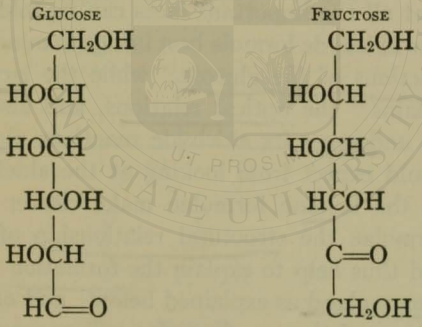
According to Browne, a simple sugar or monosaccharide may be defined as an aldehyde alcohol or ketone alcohol of the aliphatic series, the molecule of which contains one carbonyl

and one or more alcohol groups, one of the latter being always adjacent to the carbonyl group. All simple sugars contain, therefore,



as a characteristic group upon the presence of which the chief chemical properties of the sugars depend.

The simplest possible sugar according to this definition is glycolaldehyde, $CH_2OH—CHO$, which (in analogy with the nomenclature of the familiar sugars) may also be called *glycolose*. The structural formulae of glucose and fructose, the most familiar representatives of the aldehyde-alcohol (aldose) and ketone-alcohol (ketose) sugars, respectively, are as follows:



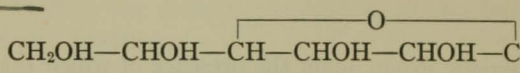
Since glucose gives aldehyde reactions but not so readily as the above structural formula would lead one to expect, it is believed that in ordinary solutions of glucose the substance exists partly in the condition indicated by the aldehyde formula and partly in a tautomeric form represented by the lactone or " oxygen bridge " formula.

Following are the aldehyde and lactone formulae written without reference to the spatial relationships of the hydrogen and hydroxyl groups:

Aldehyde form:

$$CH_2OH—CHOH—CHOH—CHOH—CHOH—CHO$$

Lactone form:



It has frequently been asked, "Which is right, the aldehyde or the lactone formula?"

Perhaps the best answer is that each has its place. Structural formulae are not so much matters of right or wrong as they are a kind of chemical shorthand which serves to picture in a small space and bring to mind at a glance a condensed summary of certain phases of our knowledge of the substance. In the case of glucose not all the important facts can be told by one such picture. The aldehyde formula best brings to mind the ordinary chemical reactions of the glucose; while the lactone formula better "explains" the optical relations, and also reminds us that glucose undergoes its aldehyde reactions more sluggishly than we should expect from looking at the aldehyde formula alone. Also the lactone formulae make clearer than do the aldehyde formulae the structural relationship of glucose and galactose and thus help to explain the formation of milk sugar in the mammary gland as explained below; and other instances of the usefulness of the two formulae will appear with further study.

Moreover, the aldehyde and lactone formulae are probably both right in the further sense that in a solution of pure glucose a part probably exists in what we call the aldehyde form and another (probably much larger) part in the chemically less active condition which we designate as the lactone form. In

turn, the lactone glucose doubtless exists in two forms distinguished in name as α and β, and in structural formulae by the spatial relations suggested by the fact that the lactone formula makes the terminal carbon of the glucose asymmetric whereas the aldehyde formula does not.

Thus glucose in pure water solution is now believed to exist as an equilibrated mixture of three forms as represented in the following diagram:

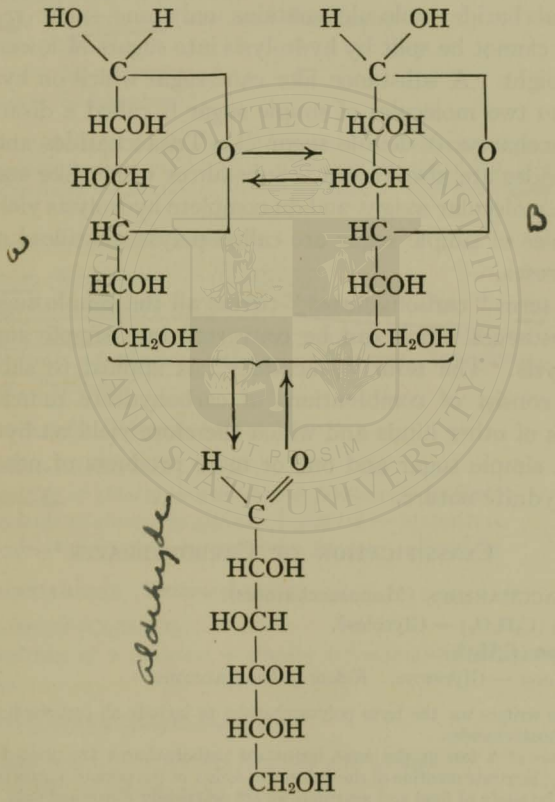


Diagram showing probable forms in which glucose exists in solution.

For discussion of the structural formulae of the carbohydrates the reader must be referred to textbooks on organic chemistry or to special works such as those listed at the end of the chapter. Our present space suffices only for a bare outline of the classification of the substances belonging to this large group of chemical compounds and very brief summaries of a few of the properties of the more important members.

The name monosaccharide (" single sugar ") implies that the monosaccharide molecule contains only one sugar radicle — that it cannot be split by hydrolysis into sugars of lower molecular weight. A substance like cane sugar which on hydrolysis splits to two molecules of simple sugar is called a disaccharide or disaccharose (" double sugar "). Trisaccharides and tetrasaccharides are also known. Substances which like starch are of high molecular weight and on complete hydrolysis yield many molecules of simple sugar are called polysaccharides [1] or polysaccharoses.

The term " carbohydrates " covers all the simple sugars and all substances which can be converted into simple sugars by hydrolysis. The term " glucosides " is applied to substances which consist of combinations of carbohydrate radicles with radicles of other kinds and which therefore yield on hydrolysis both a simple sugar and one or more products of other than carbohydrate nature.

Classification of Carbohydrates [2]

MONOSACCHARIDES (Monosaccharoses).

Diose ($C_2H_4O_2$) — Glycolose.

Trioses ($C_3H_6O_3$).

Aldoses — Glycerose. *Ketose* — Dioxyacetone.

[1] Some writers use the term polysaccharides to include all carbohydrates other than monosaccharides.

[2] Names of a few of the most important carbohydrates are printed in small capitals. Separate mention of the *d*, *l*, and *dl* forms of the various sugars is omitted, since in the study of food and nutrition we are practically concerned only with that one of the three forms which is found in or derived from natural products.

Tetroses ($C_4H_8O_4$).
Aldoses — Erythrose,‡ Threose.‡
Ketose — Erythrulose.†
Pentoses ($C_5H_{10}O_5$).
Aldoses — Arabinose,† Xylose,† Ribose,† Lyxose.‡
Ketoses — Araboketose,‡ Xyloketose (ketoxylose).†
(Methyl pentoses ($C_6H_{12}O_5$) — Rhamnose,† Fucose†).
Hexoses ($C_6H_{12}O_6$).
Aldoses — GLUCOSE,* Mannose,† GALACTOSE,† Gulose,‡ Idose,‡ Talose,‡ Allose,‡ Altrose.‡
Ketoses — FRUCTOSE,* Sorbose,† Tagatose.‡
Heptoses ($C_7H_{14}O_7$).**
Aldose — Mannoheptose.*
Ketose — Sedoheptose.*

NOTE. — No attempt is here made to summarize the occurrence of any but the tetroses, pentoses, hexoses, and heptoses. Glycolose and the trioses, if formed in nature, are probably too reactive to accumulate sufficiently for identification.

DISACCHARIDES (Disaccharoses).

Dihexoses (Hexobioses) — ($C_{12}H_{22}O_{11}$).
Anhydride of glucose + fructose — SUCROSE.
Anhydrides of glucose + galactose — LACTOSE, Melibiose.
Anhydrides of glucose + glucose — MALTOSE, Isomaltose, Trehalose, Turanose.

TRISACCHARIDES (Trisaccharoses).

Trihexoses ($C_{18}H_{32}O_{16}$).
Anhydride of glucose + galactose + fructose — Raffinose.
Anhydride of glucose + glucose + glucose — Melezitose.
Anhydride of fructose + fructose + fructose — Secalose.

TETRASACCHARIDES (Tetrasaccharoses).

Tetrahexoses ($C_{24}H_{42}O_{21}$).
Anhydrides of 2 galactose + glucose + fructose — Stachyose, Lupeose.

POLYSACCHARIDES (Polysaccharoses).

Pentosans (chief constituents of gums and mucilages).
Anhydrides of xylose — Xylans.
Anhydrides of arabinose — Arabans.

Hexosans

Anhydrides of glucose — STARCH, CELLULOSE, GLYCOGEN, DEXTRIN (and other "dextrans").

Anhydrides of mannose — Mannans.

Anhydrides of galactose — Galactans (pectins).

Anhydrides of fructose — Inulin (and other "levulans").

** Although the heptoses have not been found to play any part of practical importance in food and nutrition, the discovery of the occurrence of sugars of this group in nature is relatively recent and the student may be interested to read the original papers and note the nature of the experimental evidence which constitutes such a discovery. — See La Forge, *Journal of Biological Chemistry*, Vol. 28, page 511 (1916), and LaForge and Hudson, *Ibid.*, Vol. 30, page 61 (1917).

* Occurs free in nature.

† Not yet found free in nature (or only in negligible amounts, at least so far as known with certainty) but obtained by hydrolysis or fermentation of natural product.

‡ Known only (with certainty) as a laboratory product.

N.B. Beyond the simple sugars, only carbohydrates occurring in nature are included in the table. Hence the above notations are carried through the simple sugars (monosaccharides) only.

PROPERTIES OF THE CHIEF CARBOHYDRATES OF FOOD

Monosaccharides

The monosaccharides are all soluble, crystallizable, diffusible substances, unaffected by digestive enzymes, and if not attacked by bacteria in the digestive tract, they are absorbed and enter the blood current unchanged. All of the three hexoses described below are utilized for the production of glycogen in the animal body and the maintenance of the normal glucose content of the blood. A few of the leading facts regarding the occurrence in food and the nutritive relations of individual monosaccharides are given below.

Glucose (*d*.glucose, dextrose, grape sugar, starch sugar, diabetic sugar) is widely distributed in nature, occurring in the blood of all animals in small quantity (usually about 0.1 per cent) and more abundantly in fruits and plant juices, where it is usually associated with fructose and sucrose. It is especially abundant in grapes, of which it often constitutes 20 per cent of the total weight or more than half of the solid matter. Sweet corn, onions, and unripe potatoes also contain glucose.

Glucose is also obtained from many other carbohydrates by hydrolysis either by acids or by enzymes as in natural digestion and thus becomes the principal form in which the carbohydrate of the food enters into the processes of nutrition. In the healthy body the glucose of the blood is constantly being burned and replaced. In diabetes the body loses to a greater or less degree the power to burn glucose, which then accumulates in excessive amount in the blood, from which it escapes through the kidneys. A temporary and usually unimportant loss of glucose in the urine may occur as the result of feeding large quantities at a time. This condition is known as alimentary glycosuria. Ordinarily any surplus of glucose absorbed from the digestive tract is converted into glycogen which, as described beyond, is readily reconvertible into glucose. Thus, while other carbohydrates occur in food in greater quantity, glucose occupies a very prominent place, partly because it is more widely distributed than any other carbohydrate, being a normal constituent of both plants and animals, and partly because it is the form in which most of the carbohydrate material of the food comes actually into the service of the body tissues (Chapter V). It is estimated that over half the energy manifested in the human body is derived from the oxidation of glucose.

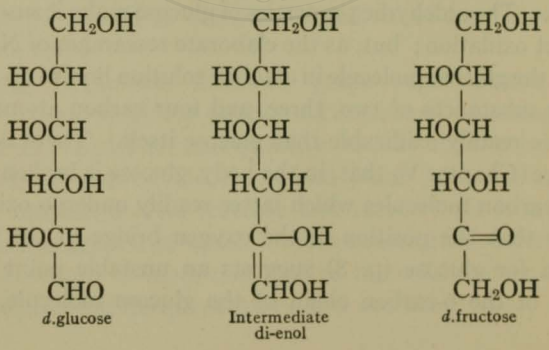
It is not to be inferred from the foregoing statement that the body obtains the energy of the glucose by oxidizing it directly as such. The aldehydic properties of glucose make it susceptible to direct oxidation; but, as the elaborate researches of Nef have shown, the glucose molecule in alkaline solution breaks up to form simpler substances of two, three, and four carbon atoms which are more readily oxidizable than glucose itself. There is strong evidence (Chapter V) that, in the body, glucose is broken chiefly into 3-carbon molecules which latter readily undergo oxidation.

Note that the position of the oxygen bridge in the lactone formula for glucose (p. 8) suggests an unstable point at the middle of the 6-carbon chain of the glucose molecule, which

correlates with the experimental evidence of a breaking of glucose into 3-carbon derivatives in nutrition. This is therefore another instance in which the lactone formula appears to greater advantage than the aldehyde formula in explaining the behavior of glucose in the body.

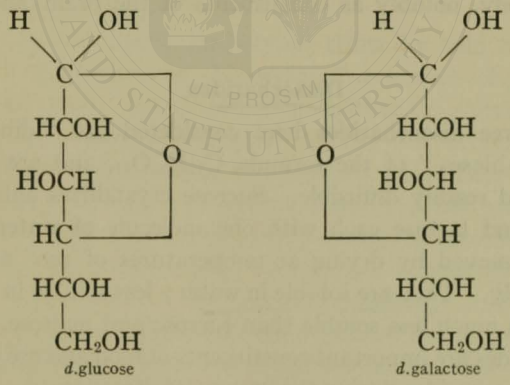
Fructose (*d*.fructose, fruit sugar, levulose) occurs with more or less glucose in plant juices, in fruits, and especially in honey, of which it constitutes about one half the solid matter. It results in equal quantity with glucose from the hydrolysis of cane sugar and in smaller proportion from some other less common sugars. Fructose may occur in normal blood, but probably only in insignificant amounts. It serves, like glucose, for the production of glycogen; and the fructose which enters the body either through being eaten as such or as the result of the digestion of cane sugar is mainly changed to glycogen on reaching the liver, so that it does not enter largely into the blood of the general circulation. Glucose and fructose are partially convertible, either one into the other, under the influence of very dilute alkalies. It is not surprising, therefore, that fructose should be converted in the liver into glycogen, which on hydrolysis yields glucose.

A convenient chemical explanation of the mutual interconvertibility of glucose and fructose is that the transformation occurs through the intermediate formation of a di-enol as follows:



It will be noted that the aldehyde formula for glucose serves well for the purpose of representing its formation from fructose in the body, whereas its breakdown in the body, as mentioned above, and its transformation into galactose, as shown below, are more readily pictured if we start with the representation of the glucose molecule in the lactone form. Thus we find additional reason for the belief that both the aldehyde and lactone formulae are valid as representing ways in which glucose actually exists and enters into reactions in the body.

Galactose is not found free in nature, but results from the hydrolysis of milk sugar, either by acids or by digestive enzymes, and appears to have the same power as glucose and fructose to promote the formation of glycogen in the animal body. If glucose and galactose be represented by their lactone formulae, it will be seen that a very direct stereochemical relationship exists between them so that the fact just noted that galactose goes to form the same glycogen as is formed from glucose, and the further fact that galactose is evidently formed from glucose in the mammary gland, both become readily intelligible.



The difference between the lactone formulae of the glucose and galactose thus lies only in the spatial relation of the oxygen

bridge to the rest of the molecule, and this is evidently changed in the mammary gland with resulting transformation of glucose into galactose which then combines with some of the remaining glucose to form lactose. The transformation probably occurs through the enzymic action of something in the gland cell which first forms a combination with glucose and then splits off galactose, the stereoisomeric shift of the oxygen bridge occurring in the course of the formation and subsequent cleavage of the complex intermediate compound which the sugar radicle forms with the protoplasmic enzyme molecule — this latter being itself doubtless a very complex and optically active substance.

The transformation of glucose into galactose-radicles in the mammary gland is thus the converse of what takes place in the liver when galactose goes to form glycogen which later is hydrolyzed into glucose.

Anhydrides of galactose, known as galactans, occur quite widely distributed in plants; and galactosides, which are compounds containing galactose in chemical combination with radicles of other than carbohydrate nature, are found in the animal body, notably as constituents of the brain and nerve tissues.

Disaccharides

The three disaccharides here considered are "dihexoses" or "hexo-bioses" of the formula $C_{12}H_{22}O_{11}$, and are crystallizable and readily diffusible. Sucrose crystallizes anhydrous; maltose and lactose each with one molecule of water, which can be removed by drying at temperatures of 100° and 130° respectively. They are soluble in water; less soluble in alcohol. Lactose is much less soluble than sucrose and maltose. These disaccharides are important constituents of food and are changed to monosaccharides during the process of digestion.

Sucrose (saccharose, cane sugar) is widely distributed in the vegetable kingdom, being found in considerable quantity,

generally mixed with glucose and fructose, in the fruits and juices of many plants. The commercially important sources of sucrose are the sugar beet, the sugar and sorghum canes, the sugar palm, and the sugar maple; but many of the common fruits and vegetables contain notable amounts. For example, sucrose is said to constitute at least half the solid matter of pineapples and of some roots, such as carrots.

On hydrolysis each molecule of sucrose yields one molecule each of glucose and fructose. These sugars all rotate the plane of vibration of polarized light, sucrose and glucose to the right (+), and fructose to the left (−). The terms "dextrose" and "levulose," synonyms for glucose and fructose respectively, arose from this behavior of the sugars in rotating the plane of polarized light to the right and left. Since at ordinary temperatures the fructose rotates more strongly to the left than the glucose does to the right, the result of the hydrolysis of sucrose is to change the sign of rotation from + to −. For this reason the hydrolysis of cane sugar is often called "inversion," and the resulting mixture of equal parts glucose and fructose is known as "invert sugar."

Sucrose is very easily hydrolyzed either by acid or by the sucrase ("invertase" or "inverting" enzyme) of yeast or of intestinal juice. So far as known neither the saliva nor the gastric juice contains any enzyme capable of hydrolyzing cane sugar, and the slight amount of hydrolysis which takes place in the stomach is believed to be due simply to the presence of hydrochloric acid (or to the regurgitation of intestinal juice into the stomach, which may occur more commonly than is generally realized). Under normal conditions the sucrose of the food passes mainly into the intestine unchanged and is there split by the sucrase of the intestinal juice, and the resulting glucose and fructose are absorbed into the portal blood.

When large amounts of sucrose are fed, some absorption takes place in the stomach; but the unchanged sucrose thus absorbed

appears to be largely, if not wholly, lost through the kidneys, as it is when injected directly into the blood current. Sugar eaten in concentrated form or in considerable quantities at a time is apt to cause irritation of the stomach either directly, or as the result of undergoing an acid fermentation, or in both of these ways. According to Herter, sucrose and glucose are more likely to ferment in the stomach than is lactose. In cases where fermentation does not occur and the sucrose itself has no irritating effect, it may be especially useful as a rapidly available foodstuff. However, it is not known that sucrose has any advantage over maltose and lactose in this respect, and the latter are less apt to irritate the stomach and cause indigestion.

Lactose (milk sugar) occurs in the milk of all mammals, constituting usually from 6 to 7 per cent of the fresh secretion in human milk and 4.5 to 5 per cent in the milk of cows and goats. At the time of parturition or if the milk is not withdrawn from the udder, some lactose may occur in the urine. If in such a case the mammary glands are removed, the percentage of glucose in the blood increases, and glucose (but no lactose) may appear in the urine. The observations (due chiefly to experiments made by Moore and Parker at the suggestion of Schaefer and of Lusk) indicate that lactose is formed in the mammary gland and probably from the glucose brought by the blood. The chemistry of the process has been discussed briefly in the section on galactose above (p. 15).

Lactose is less sweet and much less soluble than sucrose, dissolving only to the extent of about 1 part in 6 parts of water.

When hydrolyzed either by heating with acids or by an enzyme, such as the lactase of the intestinal juice, each molecule of lactose yields one molecule of glucose and one of galactose. In normal digestion, probably none of the lactose eaten is absorbed as such, for lactose injected into the blood is eliminated quickly and almost completely through the kidneys, whereas large amounts of lactose can be taken by the mouth without

any such loss. As already noted, Herter found lactose to be less subject to fermentation in the stomach than is sucrose. Also, because of the much lower solubility, there is less danger of direct irritation of the stomach membrane by lactose than by sucrose. Mathews has suggested that the occurrence in milk of lactose, a sugar having the galactose radicle, may be of special significance as a source of material for the synthesis of the galactosides of the brain and nerve tissues of the rapidly growing young mammal.

Recent research has shown that the taking of liberal quantities of lactose in the food is for many people a very important aid to the maintenance of good intestinal conditions, because the lactose is especially favorable to the development of desirable types of intestinal bacteria, notably the *Bacillus acidophilus*. (For fuller discussion see the article on lactose by Whittier, cited among the references at the end of this chapter; also the discussion of bacteria in the digestive tract, in the works of Herter and of Rettger and Cheplin.)

Maltose (malt sugar) is formed from starch by the action of diastatic enzymes (amylases) and is therefore an important constituent of germinating cereals, malt, and malt products. It is also formed as an intermediate product when starch is digested in the human or other animal body, or hydrolyzed by boiling with dilute mineral acid, as in the manufacture of commercial glucose.

In animal digestion, maltose is formed by the action of the ptyalin of the saliva (salivary amylase) or of the amylopsin of the pancreatic juice (pancreatic amylase) upon starch or dextrin. The maltose-splitting enzyme of the intestinal juice readily hydrolyzes maltose to glucose. Maltose is also readily and completely hydrolyzed by boiling with dilute mineral acids. In either case each molecule of maltose yields two molecules of glucose.

While it is probable that little if any maltose is absorbed as

such from the digestive tract under ordinary conditions, it is possible that such absorption may occur and that maltose as such may play a part in the normal carbohydrate metabolism; for when injected into the blood it appears to be utilized to better advantage than either sucrose or lactose, and it may be obtained from glycogen by the action of diastatic enzymes in much the same way as from starch and dextrin.

Polysaccharides

The polysaccharides are all colloids insoluble in alcohol. Some dissolve in water in the sense that they form colloidal dispersions which will pass through filter paper; some swell and become gelatinous; some are unchanged. The members of greatest importance in nutrition are starch and glycogen, the typical reserve carbohydrates of plants and animals respectively.

Pentosans, $(C_5H_8O_4)_x$, occur in the greatest variety of plants and in various parts of the plant organism. As a rule, however, they are abundant only in the fibrous tissues and gummy exudations and not in the starchy and succulent parts which are more commonly used for human food. Moreover, experiments have not yet succeeded in demonstrating in man or other mammals any enzyme capable of digesting the pentosans (Swartz). It is therefore believed that, notwithstanding their wide distribution in plants, the pentosans can play only a very small, if appreciable, part in the nutrition of man.

Starch, approximately $(C_6H_{10}O_5)_x$, is the form in which most plants store the largest part of their carbohydrate material, and is of great importance as a constituent of many natural foods and as the source of dextrin, maltose, commercial glucose, and many fermentation products. Starch is found stored in the seeds, roots, tubers, bulbs, and sometimes in the stems and leaves of plants. It constitutes one half to three fourths of the solid matter of the ordinary cereal grains and at least three fourths of the solids of mature potatoes.

Unripe apples and bananas contain much starch which is to a large extent changed into sugars as these fruits ripen, while, on the other hand, young tender corn (maize) kernels and peas contain sugar which is transformed into starch as these seeds mature.

Unchanged starch occurs in distinct granules, and those formed in different plants vary in size and structure,[1] so that in most cases the source of a starch which has not been altered by heat, reagents, or ferments can be determined by microscopical examination. Starch granules are scarcely affected by cold water; on warming they absorb water and swell. Finally the starch passes into a condition of colloidal dispersion or semi-solution, "starch paste." Starch which has been heated in water (either admixed or naturally present with the starch as in a potato) until the granules are ruptured and the material more or less dispersed is very much more rapidly hydrolyzed by digestive ferments than is raw starch. This is one of the ways (perhaps one of the most important) in which cooking improves the digestibility of food. That raw starch may, nevertheless, be quite thoroughly digested is also a well-established fact recently emphasized anew by the work of Langworthy and his collaborators.

To colloids such as starch, the usual methods of determining molecular weight are not applicable. It is certain, however, from the chemical complexity of some of the dextrins which result from hydrolysis of starch, that the molecular weight of starch must be very high and its chemical constitution very complex. Probably the value of x in the formula $(C_6H_{10}O_5)_x$ is very large, perhaps in the neighborhood of 200, corresponding to a molecular weight of about 32,000.

This estimate, originally arrived at through consideration of

[1] A very detailed study of the starch granules of different species of plants has been made by Reichert and published by the Carnegie Institution of Washington. (See references at end of chapter.)

the probable molecular weights of the dextrins which the starch yields on enzymic hydrolysis, is now confirmed by the finding of about 0.09 per cent of phosphorus in starch as an essential constituent (Taylor and Iddles). If the molecule of starch contains one atom of phosphorus and this constitutes 0.09 per cent of the weight, then the minimum molecular weight of the starch would be approximately 34,000, which is as close an agreement with the above estimate as could be expected in view of the very small percentage of phosphorus involved in the calculation —

$$0.09 : 100.00 :: 31 : x$$
$$x = 34{,}400 \text{ (about)}.$$

For a full discussion of the more important facts bearing on the chemical constitution of starch, see the paper by Thomas cited in the list of references at the end of the chapter.

Starch either in the solid or in the " soluble " (dispersed) form is colored intensely blue when treated with iodine.

The term " starch," as we ordinarily use it, probably covers at least two substances. The more abundant of these, α-amylose (also called " amylopectin "), which forms on heating in water a viscous opalescent paste, gives a somewhat purplish blue color with iodine and is evidently of great molecular complexity. The less abundant component of starch, β-amylose (also called "amylose"), forms when heated in water a clear, limpid solution which gives a pure blue color with iodine. The starch-digesting enzymes hydrolyze both α-amylose and β-amylose but not always with equal facility. (See paper by Sherman and Baker in list of references at the end of the chapter.)

Recently Taylor and Iddles have described the separation of the amyloses by electrophoresis and by ultrafiltration.

Starch on hydrolysis by means of acid gives glucose as end-product, dextrin and maltose being intermediate products. The most satisfactory hydrolysis of starch to glucose is accomplished by boiling or heating in a boiling water bath with hydro-

chloric acid of a concentration of about 2.5 per cent (about 0.7 Molar).

When brought in contact with saliva, starch is hydrolyzed by the enzyme ptyalin (salivary amylase) with the formation of dextrin and maltose. A similar hydrolysis is effected by "amylopsin," the starch-splitting enzyme of the pancreatic juice, preferably known as pancreatic amylase (see terminology of enzymes, Chapter IV).

"Soluble starch," largely used for laboratory experiments, is commonly prepared by soaking raw starch in cold hydrochloric acid (about 7 per cent HCl) for several days, and then washing with cold water, or by treating starch with alkali under carefully regulated conditions. The work of Taylor and Iddles suggests the use of β-amylose as prepared by their method in place of "soluble starch" as hitherto prepared.

Dextrins, approximately $(C_6H_{10}O_5)_x$ or $(C_6H_{10}O_5)_x \cdot H_2O$, are formed from starch by the action of enzymes, acids, or heat. The term, even if used in the singular, must be understood as belonging to a group rather than an individual substance. Small amounts of dextrin are found in normal, and larger amounts in germinating, cereals. Malt diastase, acting for some time upon starch in fairly concentrated solution, yields usually about one part of dextrin to four of maltose. Commercial dextrin, the principal constituent of "British gum," is obtained by heating starch, either alone or with a small amount of dilute acid.

The dextrins are much more soluble than the starches; and dextrin molecules while doubtless very large and complex are probably not over one fifth the size of the starch molecules from which they are derived.

The digestion of dextrin has already been mentioned in connection with that of starch, both saliva and pancreatic juice forming dextrin during the digestion of starch and acting upon it with the production of maltose. Complete hydrolysis of

dextrin, as by boiling with acid, yields glucose as the final product.

The work of Rettger indicates that dextrin is, like lactose, a favorable medium for the nourishment of the *Bacillus acidophilus* in the digestive tract, so that liberal proportions of dextrin and lactose in the food are conducive to good intestinal hygiene.

Glycogen, $(C_6H_{10}O_5)_x$, plays much the same rôle in animals which starch plays in plants, and is sometimes called " animal starch." It is a white, amorphous powder, odorless and tasteless, which swells up and apparently dissolves in cold water to an opalescent colloidal dispersion which is not cleared by repeated filtration, but loses its opalescence on addition of a very small amount of potassium hydroxide or acetic acid. Water solutions (dispersions) of glycogen are readily precipitated by alcohol. When treated with iodine they react yellow-brown, red-brown, or deep red. Hydrolysis of glycogen yields glucose only, as an end-product.

Glycogen occurs in the lower as well as the higher animals, and in all parts of the body, but is especially abundant in the liver. The amount of glycogen in the liver depends to a great extent upon the condition of nutrition of the animal. In the average of seven experiments by Schöndorff, in which dogs were fed for the production of as much glycogen as possible, 38 per cent of that found was in the liver, 44 per cent in the muscles, 9 per cent in the bones, and the remaining 9 per cent in the other tissues of the body. But the distribution of glycogen in the body as shown by these experiments was quite variable, even among animals of the same species which had been fed in the same way. It is well known, too, that some species store glycogen in their muscles to a greater extent than others, attempts even having been made to distinguish analytically between horseflesh and beef by the difference in their glycogen content. The storage of glycogen in the body is promoted by rest as well as

by liberal feeding, and stored glycogen is used up rapidly during active muscular work.

Cellulose, $(C_6H_{10}O_5)_x$, the chief constituent of wood and of the walls of plant cells generally, is an anhydride of glucose and can be made to yield the latter when hydrolyzed by suitable treatment with strong acid. Typical cellulose of mature fiber (such as cotton, linen, or wood fiber) is, however, quite resistant to the action of dilute acids or of ordinary enzymes, and passes through the digestive tract for the most part unchanged. The toughness of the cellulose differs with the stage of growth or maturity, and some of the less resistant forms of cellulose, such as that of tender white cabbage, may disappear from the digestive tract in appreciable amounts. Experiments to determine whether the cellulose thus disappearing is digested to a sugar and absorbed or merely decomposed by bacteria in the digestive tract have not given conclusive results. According to Swartz: "In any event, the quantities of cellulose which the alimentary tract of man is capable of absorbing are, apparently, too small for it to play a rôle of any importance in the diet of a normal individual." The cellulose in the food may, however, serve a very useful purpose in giving bulk to the food residues and thus facilitating their passage along the digestive tract.

Hemicelluloses is a term somewhat loosely applied to polysaccharides, usually occurring as constituents of cell walls in plants, which are not digested by the starch-splitting enzymes but are usually much more readily hydrolyzed by acid than is cellulose. In many plant tissues the hemicellulose consists chiefly of pentosans; in other cases it is largely mannan or galactan.

Mannans, $(C_6H_{10}O_5)_x$, anhydrides of mannose, are widely distributed in the vegetable kingdom and, as Swartz points out, show great differences in solubility, ranging from the readily soluble mucilaginous forms found in certain legumes to the horny matter of such seeds as the date, a form of mannan which

was long confused with true cellulose. The experiments of Swartz (Dr. Mary Swartz Rose) upon the mannan of salep showed it to disappear completely in its passage through the human digestive tract although tests with individual digestive enzymes gave negative results. In what way and to what extent the mannan thus disappearing from the digestive tract becomes available in nutrition is still a subject of investigation. (See SWARTZ and ROSE in references at the end of the chapter.)

Galactans, $(C_6H_{10}O_5)_x$, anhydrides of galactose, are widely distributed in plants. They occur in the seeds of legumes and to a slight extent in the cereals also, in by-products of beet sugar manufacture and abundantly in several of the algae and lichens, including Chinese moss, agar-agar, and Irish moss. *The pectins* are said to consist largely of galactans, apparently either in combination or admixture with pentosans and perhaps other complexes as well. The galactans differ in their solubilities and apparent digestibility when eaten by man or other animals, but on the whole do not appear to be of much nutritional value. Those of agar-agar and Irish moss, which are most used as food, are not digested but serve to give bulk to the intestinal contents and thus to assist the normal mechanical movement of the food residues and fecal material along the intestinal tract.

Levulans is the term under which a number of polysaccharides of the composition $(C_6H_{10}O_5)_x$, and yielding fructose (levulose) on hydrolysis, have been described. The most important of these, at least so far as is at present known, is *inulin*, a white, powdery substance occurring in the tubers of the Jerusalem artichoke and to a less extent in the bulbs of onions and garlic as well as in various parts of plants not commonly used for food. By the action of acids inulin is very readily hydrolyzed to levulose, but the digestive juices do not seem to contain enzymes capable of hydrolyzing inulin and it appears to be of practically no importance as human food.

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CHAPTER II

FATS AND LIPOIDS[1]

Fats

ALMOST as widely distributed in nature as the carbohydrates, and constituting a much more concentrated form of fuel to supply energy in nutrition, are the fats. Fats are glyceryl esters of fatty acids, and since glycerol is a triatomic alcohol and the fatty acids are monatomic, a normal glyceride is a triglyceride and on hydrolysis yields three molecules of fatty acid and one molecule of glycerol. Thus, for example:

$$\underset{\text{Stearin (glyceryl tristearate)}}{C_3H_5(C_{18}H_{35}O_2)_3} + 3\ H_2O \longrightarrow \underset{\text{Glycerol}}{C_3H_5(OH)_3} + 3\ \underset{\text{Stearic acid}}{C_{18}H_{36}O_2}$$

When, as is usual, the splitting of the fat is brought about by means of an alkali, the corresponding products are glycerol and three molecules of the alkali salt of the fatty acid; and since alkali salts of the fatty acids are commonly known as soaps, this reaction is usually called the saponification of the fat.

The fats are, as implied in the above definition, a structurally distinct group of chemical compounds, and the term applies equally to the solid and the liquid members of this group. As a matter of convenience, however, the liquid fats are often called "fatty oils." The fatty oils are also sometimes called "fixed oils," since a spot made by dropping a fatty oil on paper cannot be removed by drying (as can a volatile oil), nor by washing with water (as can glycerol).

[1] *Lipides* is the group name recommended by the International Union of Pure and Applied Chemistry to cover the fats and related substances.

Another property which helps to characterize the fats is that glycerol, or the glyceryl radicle of a fat, when heated to a high temperature (300° C. or over), decomposes with production of acrolein, an aldehyde of characteristic odor and very irritating to the mucous membranes. Doubtless fatty acid radicles also may sometimes contribute to the production of irritating fumes when fat is overheated.

The fats, including fatty oils, are lighter than water, their specific gravities ranging between 0.90 and 0.97. They are poor conductors of heat and therefore tend to conserve the heat of the body, while they show on oxidation a much higher fuel value than any of the other foodstuffs.

All of the fats are practically insoluble in water, and all except those of the castor oil group are sparingly soluble in cold alcohol, but dissolve readily in petroleum ether and mix in all proportions with light petroleum oils. Light petroleum distillate ("petroleum ether") is often used as a solvent for fat. All of the fats are readily soluble in ether, carbon bisulphide, chloroform, carbon tetrachloride, and benzene. Since neither carbohydrates, proteins, nor ash constituents are soluble in ether, it follows that the fat of a food may be readily separated from the other chief components by drying the food and extracting the dry material with this solvent (pure ether). After the fat has been completely dissolved away from the other foodstuffs, it can be recovered from the solvent by evaporating the latter at a relatively low temperature. This is the method commonly used to estimate the percentages of fat in foods and to obtain small portions of fat for examination. It must be noted, however, that the fat thus obtained is not always pure in the sense of consisting entirely of substances meeting the definition of fat as given above. Obviously such an extract will contain, along with the fat, any other ether-soluble substances which were present in the food, and may contain substances which, while not appreciably soluble in ether alone, are dissolved by the mixture of

ether and fat. It is, therefore, somewhat more accurate to speak of the material extracted by ether as "ether extract" rather than as "fat," and it will be found so designated in some statements of analytical results. In most human foods — at least most of those which are important as sources of fat — the constituents of the ether extract other than true fat are for the most part fat-like substances and we shall therefore be sufficiently accurate in most cases if we designate the material extracted by ether by the simple term "fat," remembering, however, that we may thus include along with the glycerides (and any free fatty acids which may be present) small amounts of *fat-like* substances or *lipoids*, and of fat-soluble or other ether-soluble matter.

The food fats of commerce have been separated from the materials in which they naturally occurred, not usually by solvents as above described, but by mechanical means such as churning (butter) or pressing (olive or cottonseed oil); but even in this case the naturally occurring fat-soluble substances will still remain dissolved in the separated fat. Recent investigations have shown that some of these fat-like and fat-soluble substances, although occurring only in small quantities, have very important functions in nutrition. We shall have occasion to study them in that connection later.

Shortages of food fats during the World War brought about an increased appreciation of the food values of certain fats which previously had been applied chiefly to industrial uses. Thus it has recently been pointed out [1] that about three fourths of all corn (maize) oil produced is now used as food, and that in the United States more than three fourths of the total production of cottonseed oil is used in making "lard substitutes" (cooking fats).

The actual glycerides of any common natural fat, with the

[1] Grinnell Jones, *Journal of the American Chemical Society*, Vol. 47, page 2067 (July 1925).

probable exception of butter, would if obtained absolutely pure be colorless, tasteless, and odorless. The colors, tastes, and odors of fats are, therefore, ordinarily due to substances present in small amount which might be removed by refining processes. All of the quantitative differences among the fats are to be accounted for by the kinds and the amounts of the fatty acids which enter into the composition of the glycerides.

Fatty Acids

The greater number of the fatty acids belong to a few homologous series. The series to which stearic acid belongs may be represented by the general formula, $C_nH_{2n}O_2$. The principal members of physiological importance are as follows:

ACIDS OF THE SERIES $C_nH_{2n}O_2$

Butyric acid, $CH_3(CH_2)_2COOH$, occurs as glyceride to the extent of about 5 to 6 per cent in butter and in very small quantities in a few other fats.

Caproic acid, $CH_3(CH_2)_4COOH$, is obtained from goat and cow butter and coconut fat.

Caprylic acid, $CH_3(CH_2)_6COOH$, is obtained from coconut oil, goat and cow butter, and human fat.

Capric acid, $CH_3(CH_2)_8COOH$, is obtained from coconut oil, goat and cow butter, and the fat of the spice bush.

Lauric acid, $CH_3(CH_2)_{10}COOH$, occurs abundantly as glyceride in the fat of the seeds of the spice bush, and in smaller proportions in butter, coconut fat, palm oil, and some other vegetable oils.

Myristic acid, $CH_3(CH_2)_{12}COOH$, is obtained from nutmeg butter, coconut oil, butter, lard, and many other fats, as well as from spermaceti and wool wax.

Palmitic acid, $CH_3(CH_2)_{14}COOH$, occurs abundantly in a great variety of fats, both animal and vegetable, including many

fatty oils, and also in several waxes, including spermaceti and beeswax.

Stearic acid, $CH_3(CH_2)_{16}COOH$, is found in most fats, occurring more abundantly in the solid fats and especially in those having high melting points.

Butyric acid is a mobile liquid, mixing in all proportions with water, alcohol, and ether, boiling without decomposition, and readily volatile with steam. With increasing molecular weight the acids of this series regularly show increasing boiling or melting points, decreasing solubility, and loss of volatility with steam. For ordinary temperatures the dividing line between liquids and solids falls at about capric acid. Stearic acid is a crystalline solid, insoluble in water, and only moderately soluble in alcohol and ether.

ACIDS OF THE SERIES $C_nH_{2n-2}O_2$

These are unsaturated compounds. Each molecule contains one ethylene linkage or "double bond," and can take up by addition two atoms of halogen to form a saturated compound.[1] These unsaturated acids have, as a rule, much lower melting points than the saturated acids containing the same number of carbon atoms. The glycerides show correspondingly lower melting points than those of the saturated fatty acids and are therefore found more largely in the soft fats and the fatty oils. Such soft fats or fatty oils can be hardened to any desired consistency (up to that of stearin) by hydrogenation, which changes the unsaturated fatty acid radicles into the corresponding members of the saturated series. In recent years this process has been exploited commercially and large quantities of refined cottonseed oil are now hydrogenated to the consistency of lard

[1] The relative number of double bonds is measured analytically by determining the percentage of iodine which the fat or fatty acid will absorb. This is called the "iodine number." Thus pure oleic acid (mol. wt. 282) absorbs 2 atoms of iodine, giving an "iodine number" of 90; pure linoleic acid would absorb 4 atoms of iodine to the molecule, giving an "iodine number" about twice as great.

and sold under trade names as "lard substitutes." Other oils are also hardened by hydrogenation.

Phycetoleic acid ($C_{16}H_{30}O_2$) is obtained from seal oil and sperm oil; an isomeric acid, *hypogaeic*, occurs in peanut oil.

Oleic acid ($C_{18}H_{34}O_2$) occurs as glyceride in nearly all fats and fatty oils and is much the most important member of the series. Many of the typical oils of both animal and vegetable origin, such as lard oil and olive oil, consist mainly of olein.

Erucic acid ($C_{22}H_{42}O_2$) is obtained from rape seed and mustard seed oils, and is not found in animal fats except when oils which contain this acid have been fed to the animal.

OTHER UNSATURATED FATTY ACIDS

Acids of the series $C_nH_{2n-4}O_2$, $C_nH_{2n-6}O_2$, and $C_nH_{2n-8}O_2$ have been found to occur as glycerides in some of the fats. *Linoleic acid*, $C_{18}H_{32}O_2$, and *linolenic acid*, $C_{18}H_{30}O_2$, are the best known of these acids. They are found abundantly in linseed oil. Fatty acids having the same number of double bonds are found in some fatty oils of animal origin, especially those obtained from marine animals and from fishes. Since the acids of this series have still lower melting points than the corresponding acids of the oleic series, and since the physical properties of the glycerides follow those of the fatty acids which they contain, a fat containing an acid isomeric with linoleic or linolenic acid will remain fluid at a lower temperature than one containing oleic acid in the same proportion. Hence, it is apparent that glycerides of the highly unsaturated and more fluid acids are physiologically adapted to the cold-blooded animals, in which they are especially abundant.

Simple and Mixed Triglycerides

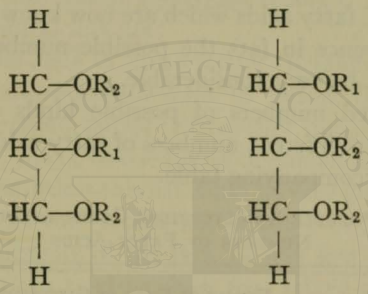
Triglycerides in which the three fatty acid radicles are of the same kind are known as *simple triglycerides*. Tristearin, triolein, tripalmitin, are examples of simple triglycerides. A *mixed triglyceride* is one in which the three fatty acid radicles are

Why does butter not melt as quickly as a mixture of the substances of which it

is made?

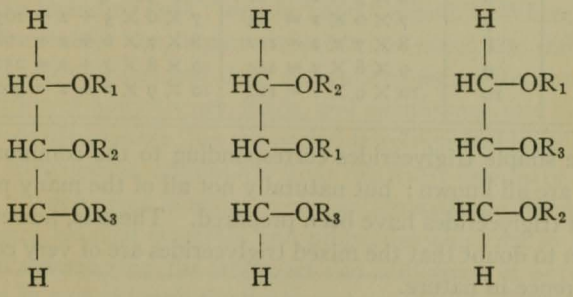
not all of the same kind. For example, distearo-olein (having two radicles of stearic and one of oleic acid), dioleo-palmitin (having two of oleic and one of palmitic), or stearo-oleo-palmitin (having one radicle each of stearic, oleic, and palmitic acids) is each a mixed triglyceride.

It is evident from the chemical structure of glycerol that there can be only one simple triglyceride of any given fatty acid, but that with two fatty acid radicles alike (R_2) and one different (R_1) the triglyceride may have either of the following forms:



That is, the two radicles of the same kind may be on the terminal carbons or may be adjacent. It will be noted that the two substances here represented have exactly the same composition, but different constitution.

If now the triglyceride contains one each of three different acid radicles (R_1, R_2, R_3), there are plainly three possible forms:



Each of these three substances has exactly the same composition, though the constitution is different for each.

It should be noted that these five formulae represent *types* of structure and that the *actual number* of triglycerides possible from three fatty acid radicles is greater, since we may have substances corresponding to either of the first two in which R_3 replaces either R_1 or R_2; and it is plain that as the number of fatty acids is increased beyond three the number of possible mixed triglycerides increases rather rapidly so that with the large number of fatty acids which are now known to be of fairly common occurrence in fats the possible number of mixed triglycerides must be very considerable.

The calculated numbers of possible kinds of triglycerides corresponding to different numbers of fatty acids up to ten are shown in the accompanying table.

THEORETICALLY POSSIBLE TRIGLYCERIDES CORRESPONDING TO DIFFERENT NUMBERS OF FATTY ACIDS

Number of Kinds of Fatty Acids	Number of Kinds of Glycerides			
	"Simple"	"Mixed," using 2 kinds of fatty acid	"Mixed," using 3 kinds of fatty acid	Total
3	3	$3 \times 2 \times 2 = 12$	$3 \times 2 \div 2 = 3$	18
4	4	$4 \times 3 \times 2 = 24$	$4 \times 3 \times 2 \div 2 = 12$	40
5	5	$5 \times 4 \times 2 = 40$	$5 \times 4 \times 3 \div 2 = 30$	75
6	6	$6 \times 5 \times 2 = 60$	$6 \times 5 \times 4 \div 2 = 60$	126
7	7	$7 \times 6 \times 2 = 84$	$7 \times 6 \times 5 \div 2 = 105$	196
8	8	$8 \times 7 \times 2 = 112$	$8 \times 7 \times 6 \div 2 = 168$	288
9	9	$9 \times 8 \times 2 = 144$	$9 \times 8 \times 7 \div 2 = 252$	405
10	10	$10 \times 9 \times 2 = 180$	$10 \times 9 \times 8 \div 2 = 360$	550

The simple triglycerides corresponding to the common fatty acids are all known; but naturally not all of the many possible mixed triglycerides have been prepared. There is, however, no reason to doubt that the mixed triglycerides are of very common occurrence in nature.

Berthelot in 1869 suggested that ordinary fats probably contain mixed glycerides and in 1889 Blyth and Robertson reported a palmito-stearo-olein in butter, but it is only since Kreis and Hafner (1903) described the preparation of *palmito-distearin* from beef tallow and Bömer (1909) separated *stearo-dipalmitin* from mutton tallow and *palmito-distearin* from lard that the widespread occurrence of mixed glycerides in the familiar fats has been generally accepted.

Among the other mixed glycerides reported as having been isolated from natural fats are:

Myristo-palmito-olein in cacao butter (Klimont, 1902), *dipalmito-olein* and *stearo-palmito-olein* in tallow (Hansen, 1902), *distearo-olein* in cacao butter (Fritzweiler, 1903) and in Borneo tallow (Klimont, 1905), *stearo-diolein* in human fat (Partheil and Ferie, 1903). Doubtless this list could be very greatly extended.

According to Bloor's recent statement (1925): "Far from being rare constituents of the fats, as was first believed, more recent investigations tend to show that mixed glycerides form the bulk of many of the natural fats."

Assuming a given number of fatty acid radicles of given kinds combined with glycerol to form triglycerides, it will make no difference to the food value of the fat as ordinarily regarded, *i.e.*, as fuel to yield energy in the body, whether there be a relatively small number of simple glycerides or a much larger number of glycerides including both "simple" and "mixed." When, however, we consider the important extent to which the different kinds of triglycerides and their derivatives may enter into the composition of body tissues, fluids, and membranes, and modify their properties, we see that the probable existence of large numbers of triglycerides obtainable from relatively few fatty acids may be of very great moment in connection with the differences in physico-chemical structure and properties which are so influential in determining the transport and utilization of nutritive material in and through the fluids and membranes of the body.

Formation and Composition of Natural Fats

Fats are formed both in plants and in animals. The conditions which determine fat formation, and the character of the fat formed in different species and under different conditions, are better known than the chemical steps involved in the process. It is hardly necessary to mention the fact that the true fats are composed of the same three chemical elements of which the carbohydrates are composed (carbon, hydrogen, and oxygen), and that since the fats contain less oxygen and more carbon and hydrogen than the carbohydrates, they constitute a more concentrated form of fuel or a more compact and lighter medium for the storage of energy for future use. The question therefore presents itself whether plants and animals have the power to change carbohydrate material into fat.

Formation of Fat from Carbohydrate

In plants there are many indications of the formation of fat from carbohydrate, as when decrease of starch and increase of fat go on simultaneously in a ripening seed, or when sugars are found to be constantly brought to a tissue in which fat is forming and there disappear as the formation of fat progresses. It is probably because no one has doubted the formation of fat from carbohydrate in plants that the process has not been more rigorously investigated.

In animals it is certain that fat may be formed from carbohydrate. From the standpoint of our present knowledge it would seem that the readiness with which farm animals are fattened on essentially carbohydrate food should have been sufficient to convince early observers; but this evidence appears to have been overlooked formerly because of the idea, for a long time prevalent, that simpler substances are built up into more complex compounds only in the plant, and not in the animal organism. In recent years it has become necessary to abandon this

latter assumption completely, and there is now abundant evidence that the animal body synthesizes fat from carbohydrate.

The most obvious method of demonstrating the conversion of carbohydrate into fat is that followed by Lawes and Gilbert. Several pigs of the same litter and of similar size were selected; some were killed and analyzed as "controls," while the others were fed on known rations and later weighed, killed, and analyzed to determine the kinds and amounts of material stored in the body. In several cases the amounts of fat stored during such feeding trials were found to have been much larger than could be accounted for by all of the fat and protein fed, so that at least a part, and in some cases the greater part, of the body fat must have been formed from the carbohydrate of the food. Many similar experiments have been made, and the transformation of carbohydrate into fat has been demonstrated by this method in carnivorous as well as herbivorous animals.

It has also been shown that carbohydrates contribute to the production of milk fat. Jordan and Jenter kept a milch cow for fifty-nine days upon food from which nearly all the fat had been extracted. During this period about twice as much milk fat was produced as could be accounted for by the total fat and protein of the food, and in addition the cow gained in weight and her appearance showed that she had more body fat at the end than at the beginning of the experiment.

Instead of determining directly the fat formed in the animal fed on carbohydrate, the production of fat from carbohydrate may be demonstrated by keeping the animal experimented upon in a respiration chamber so arranged that the total carbon given off from the body may be determined and compared with the total carbon of the food. If in such a case the body is found to store more carbon than it could store as carbohydrate or protein, it is safe to infer that at least the excess of stored carbon is held in the form of fat. Many such experiments upon dogs, geese, and swine have shown storage of carbon very much greater than

could be accounted for on any other assumption than that a part of the carbon of the carbohydrates eaten remained in the body in the form of fat.

Further evidence of the transformation of carbohydrate into fat in the animal body is obtained from the *respiratory quotient.* The discussion of this quotient and the significance of the information which it furnishes, as also the study of the chemical steps through which the transformation of carbohydrate into fat may take place, will be taken up in connection with the general study of the fate of the foodstuffs in metabolism (Chapter V).

Composition and Properties of Animal Fat

Just as we found that the character of the fat of the cold-blooded animals is adapted to the maintenance of a fluid or plastic consistency at the low temperature to which it is exposed, so to a less degree the character of the fat of warm-blooded animals appears to vary with its position in the body and with the temperature to which the body is subjected during the time that the fat is in process of formation. Thus Henriques and Hansen concluded from experiments with pigs that the thick layer of subcutaneous fat on the back, where it was not thoroughly warmed by the blood and therefore had an average temperature considerably below that of the interior of the body, was richer in glycerides of the unsaturated fatty acids and had a lower melting point than the fat of the body as a whole; while the subcutaneous fat from animals which had always lived in a warm room, or which had been heavily jacketed so that the skin was never exposed to cold, was of more nearly the same composition with the fat from the interior of the body.

Moulton and Trowbridge have observed that the fat in beef animals becomes richer in olein and therefore softer with age, with fatness, and with nearness to the surface of the body.

Usually, however, the nature of the fat found in the body is more or less characteristic of each species or group of closely

related species. Herbivora contain as a rule harder fats than carnivora, land animals have harder fat than marine animals, and all warm-blooded animals have fats which are decidedly harder than those found in fishes. The fats of different mammals were investigated by Schulze and Reineke, whose results [1] showed little variation from an average of carbon, 76.5 per cent; hydrogen, 12 per cent; oxygen, 11.5 per cent, as may be seen from the following:

Kind of Fat	Carbon	Hydrogen	Oxygen
Human fat *	76.62	11.94	11.44
Beef fat	76.50	11.91	11.59
Mutton fat	76.61	12.03	11.36
Pork fat	76.54	11.94	11.52

* Benedict and Osterberg (*American Journal of Physiology*, Vol. 4, page 69) found in 8 samples of human fat an average of 76.08 per cent carbon and 11.78 per cent hydrogen.

The foregoing statements refer to the fat of the adipose tissues. In the fat extracted from the liver, kidney, and heart, Hartley [2] finds fatty acids of the series $C_nH_{2n-4}O_2$, $C_nH_{2n-6}O_2$, and possibly $C_nH_{2n-8}O_2$.

A partial explanation of this difference between the fat of the adipose tissues and of the actively functioning organs is to be found in the greater reactivity of the unsaturated fatty acid radicles. However, the formation of unsaturated fatty acid radicles such as oleic and linoleic does not, according to our present knowledge, seem essential to the "β-oxidation theory" which is now generally held as most probably representing the main course of fatty acid metabolism (Chapter V). It is therefore entirely possible that the highly unsaturated fatty acids found, for example, in the liver, may be present as constituents

[1] Armsby's *Principles of Animal Nutrition*, page 61.
[2] *Journal of Physiology*, Vol. 36, page 17.

of the protoplasm of these cells, essential to the properties which enable them to carry out some of their functions but not necessarily connected with the metabolism of fat itself.

Butter fat differs from body fat in containing fatty acids of lower molecular weight (particularly butyric acid, which is fairly characteristic of butter), and so shows a higher percentage of oxygen and lower percentages of carbon and hydrogen. The most abundant acids of butter fat are, however, palmitic, oleic, and myristic, and the ultimate composition is not very greatly different from that of body fats. A sample of butter fat analyzed by Browne [1] showed 75.17 per cent carbon, 11.72 per cent hydrogen, and 13.11 per cent oxygen.

Storage of Food Fat in the Body

In discussing the formation of body fat from carbohydrate it was shown that often the greater part of the fat stored is manufactured in the body from carbohydrate. So striking were the results of some of the experiments demonstrating the synthesis of fat from carbohydrate, that physiologists came to question for a time whether any of the fat deposited in the tissues comes from the fat in the food.

Abundant evidence that food fats may be deposited in the body has been obtained by feeding characteristic fats to dogs and showing that these fats can afterwards be recognized in the tissues of the animals. Experiments of this kind have been made, using linseed oil, rapeseed oil, or mutton tallow, any of which is easily distinguishable by its chemical and physical properties from the fat normally found in the body of the dog. For example, Munk starved a dog for 19 days, and then for 14 days fed a mixture of the fatty acids obtained from mutton tallow, as a consequence of which about one half of the weight lost by fasting was regained. The dog was then killed and

[1] *Journal of the American Chemical Society*, Vol. 21, page 823 (1899).

yielded on dissection 1100 grams of fat melting at 40°, which is about the melting point of mutton tallow, whereas normal dog fat melts at about 20°. In another experiment by Munk, rape-seed oil was fed and the fat obtained from the dog was found to contain 82.4 per cent of oleic and erucic acids and 12.3 per cent of solid acids, whereas normal dog fat had only 65.8 per cent oleic, no erucic, and 28.8 per cent of solid fatty acids.

The occurrence in the body fat of properties usually characteristic of some particular fat which has been fed is now very well known and is recognized in establishing standards of purity for fats of animal origin. Thus, the lard obtained from swine which have been fed cottonseed meal shows the characteristic color reactions of cottonseed oil, and more elaborate tests must be made in order to determine whether cottonseed fat has actually been mixed with the lard.

European food officials sought to establish an easy method of distinguishing between butter and its substitutes by requiring manufacturers of any butter substitute to use a certain proportion of sesame oil in the preparation, sesame oil having a characteristic color reaction which can be very easily demonstrated. It was found, however, that the same sesame oil reaction might be exhibited by a perfectly pure butter fat from cows which had been fed upon sesame meal.

Evidence of the formation of body fat from food fat has been obtained also by experiments upon the total amount of fat formed in the body when the amount and composition of the food eaten was accurately known. Hoffmann starved a dog until its weight had decreased from 26 to 16 kilograms, so that it must have been almost devoid of fat. He then fed small amounts of meat and large amounts of fat for five days, after which the dog was killed and analyzed. The body contained 1353 grams of fat, of which not over 131 grams could have come from proteins, and only a few grams at most from the small amount of carbohydrate in the meat fed, so that about nine

tenths of the fat which the animal had stored must have come from the fat of the food.

Thus there is abundant experimental evidence that both the carbohydrate and the fat of the food may serve as sources of body fat. In a later chapter it will be shown that protein also may contribute to the production of fat in the body.

A question naturally arises as to how, if proteins, fats, and carbohydrates of food may all contribute to the production of body fat, the nature of the fat can still be to any significant degree characteristic of the species in which it is found. A partial explanation appears to be furnished by the work of Bloor, who finds that when the fat of the food has been split to glycerol and fatty acids in the course of digestion and these digestion products are taken up and re-synthesized to fat in the intestinal wall, there may go into the re-synthesized fat not only the fatty acid radicles of the food fat but also fatty acid radicles formed in the body. These latter, entering into the constitution of the re-formed fat, tend to give it some of the properties characteristic of the species while at the same time some of the characteristics of the food fat may be retained. Thus when a dog is fattened by feeding mutton tallow which contains more stearin and less olein than ordinary dog fat the organism may, if the fattening is gradual, furnish enough oleic acid radicles to bring the re-synthesized fat to the consistency ordinarily found in dog fat; but if the fattening is more rapid, the oleic acid radicles may not be supplied at a sufficiently rapid rate to yield this result and the dog will then lay on fat of a character somewhere between that of mutton tallow and ordinary dog fat, the influence of the food fat upon the character of the stored fat being more pronounced the more rapidly the fattening is carried out. It will be noted that, even if the fatty acid radicles synthesized in the body are built into the absorbed fat to such an extent as to make its consistency and other physical properties normal for the species, yet such body fat may still contain some radicles of fatty acids

characteristic of the experimental food and not ordinarily found in the fat of the animal, as in the case of erucic acid in the experiment cited above (page 43).

Fats and Lipoids as Body Constituents

From what has been stated above, fat is seen to be a form of reserve fuel to which any of the organic foodstuffs may contribute (see also the discussion of fate of the foodstuffs in Chapter V). It is as reserve fuel that the large deposits of body fat are chiefly significant, but it should not be forgotten that even this "depot fat" may function as a protection to the body from mechanical injury and too rapid a loss of heat when exposed to cold, and as a packing and support to the visceral organs, particularly the kidneys. In recent years it has come to be recognized that modified fats and fat-like substances (lipoids) are essential constituents of body tissues. Thus cell membranes are not simply walls of protein matter but are composed of both proteins and lipoids of different kinds and in varying proportions, and protoplasm is to be thought of as an emulsion of proteins and lipoids rather than as a jelly of proteins alone.

Taylor, writing in 1912, says: "Fat plays two rôles within the body. Fat represents the ultimate form of the storage of fuel, and the depot fats are quite the most inert and dead of any of the body structures. On the other hand, fats joined with protein, and in complex combinations of still unknown composition, represent the most essential structures in cellular protoplasm, cell membranes, and in the central nervous system. The subjects of fat in its cellulometabolic relations and fat in the energy metabolism are almost as distinct as though different substances were under consideration. Our information on the two subjects is not equal; we know much concerning fat as fuel; we know little concerning fat in cellular structure." [1]

[1] *Digestion and Metabolism*, page 342.

Mathews, in 1915, writes: "It will be recalled that all living matter contains a larger or smaller amount of organic substances which are soluble in alcohol, ether, and other fat solvents. These substances help to give to protoplasm its properties of containing large amounts of water but not dissolving; and also the power of taking up readily and in large amounts chloroform, ether, and other substances soluble in fats but not readily soluble in water. They are among the fundamental and ever-present constituents of living matter."

Bloor has recently (*Chemical Reviews*, 1925, Vol. 2, p. 244) reviewed present knowledge in this field. Using, as recommended by the International Union of Pure and Applied Chemistry, the general group name lipides to cover the fats and related substances, he defines lipides as: "Substances having the following characteristics: (*a*) Insolubility in water and solubility in the fat solvents, such as ether, chloroform, benzene. (*b*) Relationship to the fatty acids as esters, either actual or potential. (*c*) Utilization by living organisms." He classifies them as follows:

LIPIDES

Simple lipides. Esters of the fatty acids with various alcohols.
Fats — esters of the fatty acids with glycerol.
Waxes — esters of the fatty acids with alcohols other than glycerol.
Compound lipides. Esters of the fatty acids containing groups in addition to an alcohol and fatty acid.
Phospholipides — substituted fats containing phosphoric acid and nitrogen — lecithin, cephalin, spingomyelin.
Glycolipides — compounds of the fatty acids with a carbohydrate and containing nitrogen but no phosphoric acid — cerebrosides.
Aminolipides, sulpholipides, etc. — groups which are at present not sufficiently well characterized for classification.
Derived lipides. Substances derived from the above groups by hydrolysis.
Fatty acids of various series.
Sterols — mostly large molecular alcohols, found in nature combined with the fatty acids and which are soluble in the fat solvents — cholesterol ($C_{27}H_{43}OH$), myricil alcohol ($C_{30}H_{61}OH$), cetyl alcohol ($C_{16}H_{33}OH$), etc.

Bloor comments on this classification as follows:

"Almost all the known lipides are found in living organisms, so that the general characteristics of the group are in the main those of naturally occurring substances. But such substances as have been produced synthetically behave, as far as is known, like the natural ones, and there is no reason to believe that the characteristics of the group will have to be altered to suit synthetic members. Thus this classification which was intended for biochemical purposes answers very well in the wider sense as a chemical classification.

"The most general characteristic of the group is the solubility in fat solvents such as ether, chloroform, benzene, as contrasted with the insolubility in water. This of itself is sufficient to set it off from the other great groups of biological substances — the carbohydrates, proteins, and mineral salts. The property is not absolute, since certain members of the group such as the lecithins form dispersions on mixing with water, which are at least colloidal and may approach true solubility. On the other hand, many members of the group are not soluble in all fat solvents. For example, most of the lecithins are insoluble in acetone, the cephalins are mainly insoluble in alcohol, while sphingomyelin and the cerebrosides are difficultly soluble in ether.

"In order to exclude organic compounds which have no biochemical relationship to the fats or fatty acids, but which from their solubilities alone would be included in the group the limitations in (*b*) and (*c*) have been applied. The substances included in the group must be either ester-like combinations of the fatty acids or capable of forming such combinations, and they must be capable of performing some useful functions in living organisms."

Elsewhere Bloor says also

". . . there is always a large proportion of the fatty materials of tissues which cannot be seen with the microscope, cannot be stained by the usual fat stains and cannot be extracted by the ordinary fat solvents such as ether, chloroform, or benzene, but may be removed, although much of it with difficulty, by alcohol either cold or hot. The nature of this lipide material and its relationship to the fat of food and stores constitutes one of the important problems in the field of fat metabolism at the present time.

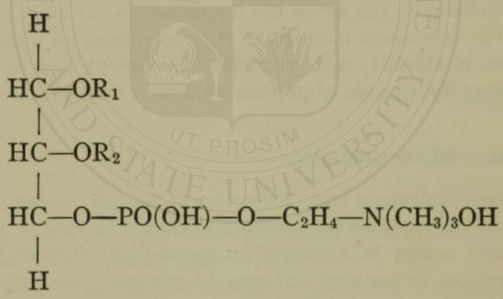
"The most widely distributed of these constituents are the phospholipides of various composition which are present in practically all tissues and in blood. Their function (see below) is assumed to be in connection with the intermediary metabolism of the fatty acids since they carry in combination the more unsaturated acids found in the body. Cholesterol, either as such

or as its numerous isomers, is also universally distributed and in the form of its esters with various fatty acids is one of the main lipides of blood plasma. Cholesterol esters are found in tissues and organs but only in such small amounts that their presence there may be accidental as the result of adherent blood plasma. Practically all the other known lipides have been prepared from animal and plant tissues."

Prominent among the lipoids (or fat-like substances other than true fats) are the *phospholipins*, or *phosphatids*, and the *sterols* (solid alcohols). The former are substances which contain a substituted phosphoric acid radicle in place of one or more of the fatty acid radicles of a fat.

Phospholipins or phosphatids are widely distributed in living cells and doubtless essential to their structure and functions.

Of the phospholipins or phosphatids the best-known are the lecithins which are abundant in egg yolk and occur also in significant quantities in brain and nerve tissue, blood, lymph, milk, many seeds, and other plant and animal tissues. The structure of lecithin has usually been represented by the formula



in which R stands for a fatty acid radicle. Bloor states that, as far as present information goes, each lecithin contains one saturated and one unsaturated fatty acid radicle.

On hydrolysis such a compound would yield glycerol, fatty acids, phosphoric acid, and the nitrogenous base choline (trimethyl oxyethyl ammonium hydroxide). If one of the radicles

be that of oleic and the other that of palmitic acid the hydrolysis may be represented thus:

$$C_{42}H_{84}NPO_9 + 4\,H_2O \longrightarrow C_3H_8O_3 + C_{18}H_{34}O_2 + C_{16}H_{32}O_2 + H_3PO_4 + C_5H_{15}NO_2$$

Glycerol — Oleic acid — Palmitic acid — Phosphoric acid — Choline

Some investigations appear to throw doubt upon the view that the nitrogen of lecithin is present only as choline groups.

A phosphatid which, like lecithin as represented above, contains one atom of nitrogen and one of phosphorus to the molecule is classified as a monoamino-monophospholipin or monoamino-monophosphatid. Monoamino-diphospholipins, diamino-monophospholipins, and triamino-monophospholipins have also been described.

The fat of the active tissues of the body, as distinguished from that of the adipose tissue, seems to consist largely of lipoids rather than true fats. Thus MacLean and Williams found 84 per cent of the total ether extract of pigs' liver to consist of phospholipins.

Bang holds that it is "no mere coincidence that the most highly organized cells are always richest in lipoids."

Sterols occur, at least in small amounts, in all natural fats. The best-known sterols are cholesterol and phytosterol. These are apparently isomeric substances to which some writers assign the formula $C_{27}H_{44}O$ and others $C_{27}H_{46}O$.

Cholesterol occurs in animal fats and phytosterol (or the closely related sitosterol) in those of vegetable origin. One method of determining whether vegetable fat is present in butter or lard is to examine for the presence of phytosterol, since phytosterol is not, like the substances to which the color reactions of cottonseed and sesame oils are due, carried over from the fat of the food to that of the animal body.

Although its functions are not yet clearly defined, cholesterol appears to be a substance of much physiological significance.

As a constituent of the blood, cholesterol acts to protect the red blood cells against the action of hemolytic substances, which unless counteracted would tend to cause anemia through excessive destruction of red corpuscles. According to Mathews, cholesterol is one of the most abundant lipins of the brain and occurs in nearly all living tissues: as a constituent of waxes and the sebum of the skin it protects the dermal structures; it, or its degradation products, aids the other lipins in giving to cells their power of holding large quantities of water without dissolving or losing their peculiar semifluid characters; it is believed to be the mother substance from which the bile acids are derived and so plays an important part in the intestinal digestion and absorption of fat; and, on the other hand, cholesterol itself appears to check the action of fat-splitting enzymes in the body and thus to function as a regulator in the metabolism of the fats and fat-like substances of the cells.

Of even greater interest is the discovery, made independently by Hess and by Steenbock in 1924, that cholesterol and phytosterol under the influence of ultraviolet rays (from direct sunlight or artificial sources such as the mercury-vapor quartz lamp) may be so changed as to acquire the property of "antirachitic vitamin."

The presence of cholesterol in the skin also acquires a new and greatly enhanced significance from the discovery that it may be changed by irradiation into an important vitamin.

Fuller discussion of these questions must be deferred until later chapters.

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CHAPTER III

PROTEINS

CARBOHYDRATES and fats are the chief sources of energy for the activities of the body but not the chief constituents of which the active tissues are composed. Muscle tissue, for instance, is almost devoid of carbohydrate and often contains very little fat. The chief organic constituents of the muscles, and of the protoplasm of plant and animal cells generally, are substances which contain nitrogen and sulphur in addition to carbon, hydrogen, and oxygen. Mulder, in 1838, described a nitrogenous material which he believed to be the fundamental constituent of tissue substances and gave it the name *protein*, derived from a Greek verb meaning "to take the first place." While Mulder's chemical work did not prove to be of permanent value, the term which he introduced has been retained, and in the plural form, *proteins*, is now used as a group name to cover a large number of different but related nitrogenous organic compounds which are so prominent among the constituents of the tissues and of food that they may still be accorded some degree of preëminence in a study of the chemistry of food and nutrition.

Proteins are essential constituents of both plant and animal cells. There is no known life without them. Plants build their own proteins from inorganic materials obtained from the soil and air. Animals form the proteins characteristic of their own tissues, but in general they cannot build them up from simple inorganic substances such as suffice for the plants, and must depend upon the digestion products obtained from the proteins of their food. Since animals must have proteins for

the construction and repair or maintenance of their tissues, and since, broadly speaking, they cannot make their proteins except from the cleavage products of other proteins, it follows that proteins (or their cleavage products, the amino acids) are necessary ingredients of the food of all animals.

Chemical Nature and Physical Properties of Proteins in General

Generally speaking, the proteins of different kinds of tissue, and even of the corresponding tissues of different species, are not identical substances. The total number of different proteins occurring in nature must therefore be very great. Of these, somewhat over fifty have been sufficiently isolated and studied to warrant description as chemical individuals. All of these have proven to be very complex substances and in no case has the chemical structure of a natural protein been fully determined. It has, however, been shown that the typical proteins are essentially anhydrides of the following amino acids:

Chemical Structure of the Amino Acids

Monaminomonocarboxylic acids

Glycine, amino-acetic acid, $CH_2(NH_2) \cdot COOH$.

Alanine, α-amino-propionic acid, $CH_3CH(NH_2) \cdot COOH$.

Valine, α-amino-isovaleric acid,

$$(CH_3)_2CH \cdot CH(NH_2) \cdot COOH.$$

Leucine, α-amino-isocaproic acid (α-amino-isobutyl-acetic acid), $(CH_3)_2CH \cdot CH_2 \cdot CH(NH_2) \cdot COOH$.

Phenylalanine, phenyl-α-amino-propionic acid,

$$C_6H_5CH_2 \cdot CH(NH_2) \cdot COOH.$$

Tyrosine, oxyphenyl α-amino-propionic acid,

$$C_6H_4(OH) \cdot CH_2 \cdot CH(NH_2) \cdot COOH.$$

Serine, α-amino-β-hydroxy-propionic acid,

$$CH_2(OH) \cdot CH(NH_2) \cdot COOH.$$

Cystine (dicysteine), or di-(α-amino-β-thio-lactic acid),

$$\begin{array}{l} S{-}CH_2{-}CH(NH_2) \cdot COOH. \\ | \\ S{-}CH_2{-}CH(NH_2) \cdot COOH. \end{array}$$

Monaminodicarboxylic acids

Aspartic acid, amino-succinic acid,

$$COOH \cdot CH_2 \cdot CH(NH_2) \cdot COOH.$$

Glutamic (glutaminic) acid, α-amino-glutaric acid,

$$COOH \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH.$$

Diaminomonocarboxylic acids

Ornithine, α, δ, diamino-valeric acid,

$$CH_2(NH_2) \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH.$$

Arginine, δ-guanidino-α-amino-valeric acid,

$$\begin{array}{l} \overset{\cdot\cdot}{N}H \\ (H_2N)C \cdot NH \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH. \end{array}$$

Lysine, α, ϵ, diamino-n-caproic acid,

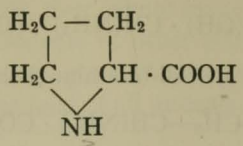
$$CH_2(NH_2) \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH.$$

Heterocyclic amino acids

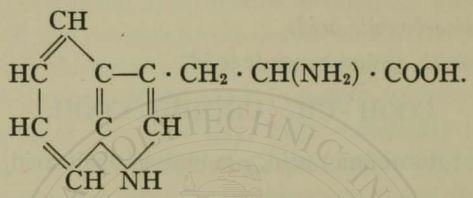
Histidine, α-amino-β-imidazol-propionic acid,

$$\begin{array}{l} HC{=}C \cdot CH_2 \cdot CH(NH_2) \cdot COOH. \\ \quad | \quad | \\ \quad HN \quad N \\ \quad \diagdown \; // \\ \quad\quad CH \end{array}$$

Proline, pyrrolidin-carboxylic acid,

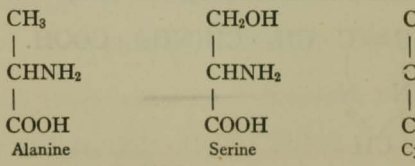


Tryptophane, α-amino-β-indol-propionic acid,



It will be noted that these constituents of the protein molecule differ much in structure among themselves. They are, however, all α-amino acids, *i.e.*, the amino group (or one of them if there be more than one) is attached to the carbon atom adjacent to the carboxyl.

In view of the wide occurrence of the alanine radicle in proteins and the frequency with which we shall have occasion to discuss the behavior of alanine (as a typical amino acid) in metabolism, it may be of interest to point out that several of the amino acids, even including some of unique constitution, may be regarded as derived from alanine by the substitution of a simple or complex radicle for one of the hydrogens on the β carbon of alanine. Thus by the substitution of an OH or SH group one obtains serine or cysteine, respectively; by substituting the phenyl or oxyphenyl group, there results phenylalanine or tyrosine; by the imidazole ($C_3H_3N_2$), histidine; by the indol (C_8H_6N) radicle, tryptophane.



$CH_2C_6H_5$	$CH_2C_6H_4OH$	$CH_2C_3H_3N_2$	$CH_2C_8H_6N$
$CHNH_2$	$CHNH_2$	$CHNH_2$	$CHNH_2$
COOH	COOH	COOH	COOH
Phenylalanine	Tyrosine	Histidine	Tryptophane

Amino Acids in the Protein Molecule

The linkage of the amino acid radicles in the protein molecule is chiefly through the carboxyl group of one amino acid reacting with the amino group of another. Thus two molecules of glycine combined by elimination of one molecule of water yield glycyl-glycine,

$$CH_2NH_2—CO$$
$$|$$
$$CH_2NH—COOH,$$

which is the simplest of an immense group of anhydrides of amino acids, all of which are called "peptids." Dipeptids contain two amino acid radicles, tripeptids contain three, etc. Fischer, by uniting 7 to 19 amino-acid radicles, has produced synthetic polypeptids which in some of their properties resemble the peptones, the simplest substances usually regarded as true proteins. Peptones are formed in nature by the digestive hydrolysis of ordinary proteins whose structure is doubtless considerably more complex.

A certain analogy between carbohydrates and proteins may be noted. As starch on hydrolysis yields the polysaccharide dextrins, the disaccharide maltose, and finally as end product the monosaccharide glucose, so the native protein is hydrolyzed through peptones, polypeptids, and di- or tri-peptids, to amino acids. Thus the amino acid bears the same general relation to the protein which glucose bears to starch; and just as the molecular weight of starch is very high and a single starch molecule yields a large (unknown) number of monosaccharide molecules,

so the molecular weight of the protein is very high and the protein molecule yields a large (unknown) number of amino acid molecules. There is, however, this important difference: the molecules of monosaccharide resulting from complete hydrolysis of starch are all alike (glucose), whereas the complete hydrolysis of any typical protein yields several of the above-mentioned kinds of amino acids, in the case of most proteins from twelve to twenty.

In view of the marked differences in chemical structure and nutritive function existing among these amino acids, it becomes important to know the relative proportions in which the various amino acid radicles exist in the different proteins. This is studied by hydrolyzing the protein and separating and recovering as completely as possible the amino acids resulting from the hydrolysis. Since the recovery of the amino acids cannot be accomplished without loss, the results obtained are not strictly quantitative and our knowledge of the radicles which make up the protein molecule remains incomplete. It is believed by the investigators who have given most attention to the question that the failure of the recovered amino acids to show a summation of one hundred per cent is more largely due to unavoidable losses in estimating the known amino acids than to the presence of other amino acids not yet identified. The table on the opposite page shows the percentages of amino acids obtained from four proteins occurring in different food materials.

From the data given in the table it will be seen that the proportions in which a given amino acid radicle occurs in various proteins may be quite different. The four proteins here shown yield from 0.0 to 25.5 per cent of glycine; from 1.8 to 13.4 per cent of alanine; from 1.0 to 7.9 per cent of valine; from 6.6 to 19.6 per cent of leucine. Of lysine, zein yields none, gliadin about 1 per cent, gelatin 6 per cent, and casein about 8 per cent. Of tryptophane, zein and gelatin yield none, gliadin about 1 per cent, casein about 2.2 per cent.

gelatin comes from connective tissues of animals
Gliadin } form gluten -
Glutenin }
Zein comes from corn -

PERCENTAGES OF INDIVIDUAL AMINO ACIDS OBTAINED FROM FOUR DIFFERENT PROTEINS

	CASEIN	GELATIN	GLIADIN	ZEIN
Glycine	0.45	25.50	0.00	0.00
Alanine	1.85	8.70	2.00	13.39
Valine	7.93	1.00	3.34	1.88
Leucine	9.70	7.10	6.62	19.55
Proline	7.63	9.50	13.22	9.04
Oxyproline	0.23	14.10	?	?
Phenylalanine	3.88	1.40	2.35	6.55
Glutamic acid	21.77	5.80	43.66	26.17
Oxyglutamic acid	10.50	0.00	2.40	2.50
Aspartic acid	4.10	3.50	0.58	1.80
Serine	0.50	0.40	0.13	1.02
Tyrosine	6.50	0.01	3.50	3.55
Cystine	0.50	0.31	2.32	0.85
Histidine	2.84	0.90	3.35	0.82
Arginine	3.81	8.22	3.14	1.82
Lysine	7.62	5.92	0.92	0.00
Tryptophane	2.20	0.00	1.14	0.00
Ammonia	1.61	0.49	5.22	3.64
Summation	93.62	92.85	93.89	92.58

For more detailed comparisons of the percentages of amino acids in different proteins and also in the flesh of five widely separated species, the more extended table further on in this chapter may be consulted. Whether it be essential that the proteins of the food shall furnish all the amino acids which the body proteins contain will naturally depend upon whether the body is able to make individual amino acids or not. Experimental evidence has shown that the animal body can make glycine readily so that the absence of glycine radicles in the food proteins does not detract from their nutritive value. On the other hand, the animal body does not seem able to make tryptophane and as this is an essential constituent of body tissue the food protein must always furnish tryptophane if it is to meet

the needs of animal nutrition. Feeding experiments have also shown that the rate of growth of young animals may be largely influenced by the lysine content of the proteins fed and that a small amount of lysine is also required for maintenance. Gliadin, the alcohol-soluble protein of wheat, contains only a little lysine and for this reason suffices as a sole protein for maintenance of either adult or young animals but not for the support of normal growth in the young.

Such facts as these make it important that we study the proteins not only as a group but also individually and that we learn as much as possible about the kinds and amounts of amino acid radicles in the individual proteins.

The ultimate composition of the proteins shows a general similarity throughout the group. All contain carbon, hydrogen, oxygen, nitrogen, and sulphur; some also phosphorus or iron.

COMPOSITION OF SOME TYPICAL PROTEINS ACCORDING TO OSBORNE

	CARBON PER CENT	HYDROGEN PER CENT	NITROGEN PER CENT	OXYGEN PER CENT	SULPHUR PER CENT	IRON PER CENT	PHOSPHORUS PER CENT
Egg-albumin .	52.75	7.10	15.51	23.024	1.616		
Lact-albumin .	52.19	7.18	15.77	23.13	1.73		
Leucosin . . .	53.02	6.84	16.80	22.06	1.28		
Serum-globulin	52.71	7.01	15.85	23.32	1.11		
Myosin . . .	52.82	7.11	16.67	22.03	1.27		
Edestin . . .	51.50	7.02	18.69	21.91	0.88		
Legumin . . .	51.72	6.95	18.04	22.905	0.385		
Casein . . .	53.13	7.06	15.78	22.37	0.80	—	0.86
Ovo-vitellin . .	51.56	7.12	16.23	23.242	1.028	—	0.82
Gliadin . . .	52.72	6.86	17.66	21.733	1.027		
Zein	55.23	7.26	16.13	20.78	0.60		
Oxyhemoglobin	54.64	7.09	17.38	20.165	0.39	0.335	—

From the accompanying table of ultimate composition of twelve typical proteins, it will be seen that all these proteins contain 51 to 55 per cent carbon, about 7 per cent hydrogen, 20

to 23 per cent oxygen, 15.5 to 18.7 per cent nitrogen, 0.3 to 2.0 per cent sulphur. Other typical proteins thus far studied have shown ultimate composition within these same limits.

Similarity of elementary composition is entirely consistent with the belief that there may be an enormous number of chemical individuals among the proteins of nature.

Fischer has recently illustrated the vast number of isomers which may exist among polypeptids and proteins by pointing out that a synthetic 19-peptid obtained by linking 15 glycine and 4 leucine molecules is only one of 3876 possible isomers, without considering the tautomerism of the peptid linking. When more than two kinds of amino acids are involved, the possible number of isomers increases very rapidly. If a protein be imagined made up of 30 molecules of 18 different amino acids, one taken twice, one 3 times, another 3, one 4, one 5 times, and 13 taken once each, there would be 10^{27} isomers even if there were no tautomerism of the peptid group and if the linking took place only in the simple way as with monamino-monocarboxylic acids.

It is easy to see that when one considers not only isomerism but the vast number of compounds of slightly different composition which can be obtained by varying the kinds and proportions of the amino acid radicles in the protein molecule, the possible number of different proteins of very similar elementary composition is practically unlimited.

Probable Molecular Weights of Proteins

From the results of ultimate analysis an approximate indication of the minimum molecular weight may often be obtained by a very simple calculation. Thus, oxyhemoglobin contains only 0.335 per cent of iron, and since there must be at least one iron atom in the molecule, it is obvious from a simple proportion making use of the atomic weight of iron,

$$0.335 : 56 :: 100 : x$$

that the molecular weight of hemoglobin must be in the neighborhood of 16,800 or a multiple of this.

To take an example from the simple proteins, zein contains 0.60 per cent of sulphur, of which one third is much more readily

split off than the other two thirds, from which it appears that the molecule contains three, or a multiple of three, sulphur atoms. Then by the proportion,

$$0.60 : (32 \times 3) :: 100 : x$$

it is found that about 16,000 or a multiple thereof is the probable molecular weight of zein.

Estimates of the same order of magnitude are obtained if we base our calculations on the proximate rather than the ultimate analyses of the purified protein preparations. Osborne, Van Slyke, Leavenworth, and Vinograd concluded from a very searching investigation that the lysine content of gliadin must lie between 0.64 and 1.20 per cent. Since the molecular weight of lysine is 146 it follows that the corresponding minimum estimate of the molecular weight of gliadin must fall between 12,000 and 23,000. The experimental facts do not permit the assumption of any lower molecular weight but are not inconsistent with the view that the true molecular weight may be some multiple of this.

Physico-chemical Properties of Proteins

In only a few cases have proteins been obtained in crystalline form. Generally speaking, the proteins may be regarded as typically colloidal substances. This does not preclude the belief that in the tissues and fluids of the body the proteins may exist largely in combination with electrolytes. In view of the fact that the behavior of proteins in the tissues is largely dependent upon their colloidal character it is of interest to bear in mind the very high molecular weights of the proteins as mentioned in the last paragraph. These enormously high molecular weights make it possible that in the case of a typical native protein the individual molecule is so large as to constitute a colloidal particle, so that all solutions of such proteins must necessarily be colloidal, so long at least as the protein remains intact.

The amino acids of which the proteins are composed are, by virtue of their amino and carboxyl groups, amphoteric substances or ampholytes, capable of dissociating either as cations or as anions, depending upon the reaction (hydrogen ion concentration) of the solution. The proteins also are amphoteric substances. This is believed to be due to the presence of free amino and carboxyl groups in the molecules. Thus if a typical protein be represented by the simple formula $R\begin{cases}NH_2\\COOH\end{cases}$ it can easily be seen that it is capable of reacting with a base such as sodium hydroxide to form a salt $R\begin{cases}NH_2\\COONa\end{cases}$ which would ionize into the cation Na^+ and the anion $R\begin{cases}NH_2\\COO^-\end{cases}$ or with an acid such as hydrochloric acid to form a salt, similar to ammonium chloride, $R\begin{cases}NH_3Cl\\COOH\end{cases}$ which would ionize into the cation $R\begin{cases}NH_3^+\\COOH\end{cases}$ and the anion Cl^-.

This amphoteric nature of the proteins is very important. Because of it proteins can combine with either acids or bases, according to conditions, and thus play an important part in the regulation of neutrality in the body. (Chapter IX.)

As the protein molecule may dissociate either as a cation or as an anion, depending upon the hydrogen ion concentration of the solution, it is evident that there must be some hydrogen ion concentration at which the tendencies toward the acidic and basic dissociations are equal. This is known as the *iso-electric point* because at this reaction the protein, or other ampholyte, does not migrate in an electrical field.

At this hydrogen ion concentration the dissociation of the ampholyte in solution is at a minimum and it is therefore in its (chemically) most inert condition; is least soluble and most easily precipitated by neutral salts or alcohol. These facts have been extensively used recently in work dealing with the

purification of proteins and other amphoteric substances and in attempts to gain more knowledge of their chemical nature.

The proteins are insoluble in all of the solvents for fats (ether, acetone, chloroform, carbon disulphid, carbon tetrachlorid, benzene, and petroleum distillate). They differ in their solubilities in water, salt solutions, and alcohol, and these differences play a considerable part in the present schemes of classification.

Classification

There was formerly considerable confusion in the classification and terminology of the proteins and some differences of usage will still be met in the literature. At present, however, the majority of writers follow the recommendations made by a joint committee of the American Physiological Society and the American Society of Biological Chemists in December, 1907. The full text of these recommendations will be found in the appendix. The following is an outline of the classification thus recommended; to which have been added examples covering most of the food proteins thus far described as chemical individuals.

I. Simple Proteins. Protein substances which yield only amino acids or their derivatives on hydrolysis.

(*a*) *Albumins.* Simple proteins soluble in pure water and coagulable by heat. Examples: egg albumin, lactalbumin (milk), serum albumin (blood), leucosin (wheat), legumelin (peas).

(*b*) *Globulins.* Simple proteins insoluble in pure water, but soluble in neutral salt solutions. Examples: muscle globulin, serum globulin (blood), edestin (wheat, hemp seed, and other seeds), phaseolin (beans), legumin (beans and peas), vignin (cow peas), tuberin (potato), amandin (almonds), excelsin (Brazil nuts), arachin and conarachin (peanuts).

(*c*) *Glutelins.* Simple proteins insoluble in all neutral solvents, but readily soluble in very dilute acids and alkalies. The best-known and most important member of this group is the glutenin of wheat.

(*d*) *Alcohol-soluble proteins.* Simple proteins soluble in relatively strong alcohol (70–80 per cent) but insoluble in water, absolute alcohol, and other neutral solvents. Examples: gliadin (wheat), zein (corn), hordein (barley), kafirin (kafir corn).

(*e*) *Albuminoids.* These are the simple proteins characteristic of the skeletal structures of animals (for which reason they are also called scleroproteins) and also of the external protective tissues, such as the skin, hair, etc. None of these proteins is used for food in the natural state, but collagen when boiled with water yields gelatin.

(*f*) *Histones.* Soluble in water, and insoluble in very dilute ammonia, and in the absence of ammonium salts insoluble even in an excess of ammonia; yield precipitates with solutions of other proteins and a coagulum on heating which is easily soluble in very dilute acids. On hydrolysis they yield several amino acids, among which the basic ones predominate. The only members of this group which have any considerable importance as food are the thymus histone and the globin derived from the hemoglobin of the blood.

(*g*) *Protamins.* These are simpler substances than the preceding groups, are soluble in water, not coagulable by heat, possess strong basic properties, and on hydrolysis yield a few amino acids among which the basic amino acids greatly predominate. They are of no importance as food.

II. Conjugated Proteins. Substances which contain the protein molecule united to some other molecule or molecules otherwise than as a salt.

(*a*) *Nucleoproteins.* Compounds of one or more protein molecules with nucleic acid. Examples of the nucleic acids thus found united with proteins are thymo-nucleic acid (thymus gland), tritico-nucleic acid (wheat germ).

(*b*) *Glycoproteins.* Compounds of the protein molecule with a substance or substances containing a carbohydrate group other than a nucleic acid. Example: mucins.

(*c*) *Phosphoproteins.* Compounds in which the phosphorus is in organic union with the protein molecule otherwise than in a nucleic acid or lecithin. Examples: caseinogen (milk), ovovitellin (egg yolk).

(*d*) *Hemoglobins.* Compounds of the protein molecule with hematin or some similar substance. Example: hemoglobin of blood.

(*e*) *Lecithoproteins.* Compounds of the protein molecule with lecithins or related substances.

III. Derived Proteins.

1. *Primary protein derivatives.* Derivatives of the protein molecule apparently formed through hydrolytic changes which involve only slight alterations.

(*a*) *Proteans.* Insoluble products which apparently result from the incipient action of water, very dilute acids, or enzymes. Examples: casein (curdled milk), fibrin (coagulated blood).

(*b*) *Metaproteins.* Products of the further action of acids and alkalies whereby the molecule is sufficiently altered to form proteins soluble in very weak acids and alkalies, but insoluble in neutral solvents. This group includes the substances which have been called " acid proteins," " acid albumins," " syntonin," " alkali proteins," " alkali albumins," and " albuminates."

(*c*) *Coagulated proteins.* Insoluble products which result from (1) the action of heat on protein solutions, or (2) the action of alcohol on the protein. Example: cooked egg albumin, or egg albumin precipitated by means of alcohol.

2. *Secondary protein derivatives.* Products of the further hydrolytic cleavage of the protein molecule.

(*a*) *Proteoses.* Soluble in water, not coagulable by heat, precipitated by saturating their solutions with ammonium sulphate or zinc sulphate. The products commercially known as " peptones " consist largely of proteoses.

(*b*) *Peptones.* Soluble in water, not coagulable by heat, and

not precipitated by saturating their solutions with ammonium sulphate or zinc sulphate. These represent a further stage of cleavage than the proteoses.[1]

(*c*) *Peptids.* Definitely characterized combinations of two or more amino acids. An anhydride of two amino acid radicles is called a "dipeptid"; one having three amino acid radicles, a "tripeptid"; etc. Peptids result from the further hydrolytic cleavage of the peptones. As was mentioned above, many peptids have also been made in the laboratory by the linking together of amino acids.

Substances simpler than the peptones but containing several amino acid radicles are often called "polypeptids."

Properties of Some Individual Proteins as Nutrients

Several members of each group have also been studied to determine the kinds and amounts of amino acid radicles which they contain, with the results shown in the table on pages 72 and 73. It is of interest to compare the amino acid make-up of typical proteins with their adequacy in nutrition. Rats have been chiefly used as the experimental animal. (See page 3.)

Egg albumin, perhaps the most familiar of all proteins and the one most often chosen to illustrate, in the laboratory, the properties of proteins in general, will be seen to yield no glycine but to furnish all the other usual amino acids in quite appreciable proportions. The feeding experiments show that with a diet adequate as regards all other factors animals may be maintained in normal nutrition and young animals may make normal growth with egg albumin as the sole protein food.

Lactalbumin shows this same property in even greater degree. It appears to be the most efficient in supporting growth of all the proteins which have been studied, and this is believed to be

[1] The term "peptone" was formerly applied to all digestion products not coagulated by boiling, and is still popularly used in the same sense, the best commercial "peptones" consisting largely of proteoses.

due primarily to its high content of lysine, tryptophane, and other nutritionally essential amino acids.

Among the globulins, *edestin*, which is found in several of the cereal grains, *excelsin* from Brazil nuts, and *glycinin* from soy beans, have each been found adequate to meet the protein requirements of maintenance and growth. In fact Osborne and Mendel have kept one family of rats through three generations with edestin as their sole protein food.

Phaseolin, a globulin of the Lima and navy beans, was found to require cooking before it could be well utilized by the experimental animals (rats). Also the fact that it contains but a small amount of the nutritionally essential amino acid, cystine, tended to limit its nutritive efficiency. When cooked phaseolin was supplemented with cystine it was found to constitute a satisfactory sole source of protein for the support of normal growth in young rats.

Glutelins and the *alcohol-soluble proteins* (*prolamins*) are important as constituents of the cereal grains. The best-known examples of the respective groups are glutenin and gliadin of wheat flour. These proteins resemble each other in ultimate composition, but differ not only in solubilities, but also in their cleavage products. They are much the most important of the proteins of the wheat kernel, the gliadin making up about 50 per cent and the glutenin about 40 per cent of the total protein present. The gliadin and glutenin together constitute the gluten of wheat flour.

Glutenin (wheat glutelin) and *maize glutelin* have each been shown capable, in the rat-feeding experiments cited above, of meeting the requirements not only of maintenance but also of normal growth when fed as the sole protein food in diets adequate in other respects.

Gliadin, *hordein*, and the *prolamin* of rye, when fed singly in the same manner, are found capable of maintaining grown rats but not of supporting normal growth. *Zein*, fed alone in similar

experiments, did not suffice either for maintenance or for growth. Osborne and Mendel concluded from these experiments that the failure even to maintain the grown animals was due to the absence of tryptophane; while the failure of the rats to grow on gliadin, hordein, or rye prolamin was due to the fact that these proteins either lack lysine [1] or contain it in insufficient quantity. This interpretation was confirmed by later experiments in which they found that adding tryptophane to the zein food made it adequate for maintenance, and adding lysine to the gliadin food made it adequate to support growth.

Gelatin, the only member of the *albuminoids* (*scleroproteins*) which is of practical importance as food, has long been known to be unable to support protein metabolism when fed as the sole protein food. This inadequacy now appears to be due to the absence of tryptophane and tyrosine and perhaps in part also to the fact that some of the other amino acids, cystine and histidine, are furnished by gelatin in only very small proportion. As early as 1905 Kauffmann tried the experiment of living upon a diet in which gelatin was the sole protein, but was supplemented by additions of tyrosine, tryptophane, and cystine. So far as could be determined by a short experiment the addition of these amino acids seemed to make good the deficiencies of the gelatin.

Nucleoproteins are the characteristic proteins of cell nuclei, and are therefore especially abundant in the highly nucleated cells of the glandular organs, such as the thymus, the pancreas, and the liver. They are compounds of simple proteins with nucleic acid or nuclein. The chemical nature of the latter and their behavior in metabolism will be considered in Chapter V.

Phosphoproteins occur especially in milk and eggs, which obviously function in nature to provide the material for growth and development of new animal tissue. The phosphorus, while

[1] Lack of lysine in the protein fed would mean that the food of these experiments was deficient in this amino acid but probably not absolutely devoid of it; for very small amounts of lysine were probably contained in the material which was fed to supply water-soluble vitamin.

probably present in the form of a more or less modified phosphoric acid radicle, appears to be more closely bound in these than in the nucleoproteins. Casein of milk and the vitellin of egg yolk (ovovitellin) are the most prominent members of the group. Formerly these were sometimes classed with the simple proteins under the name nucleo-albumins. Phosphopro-

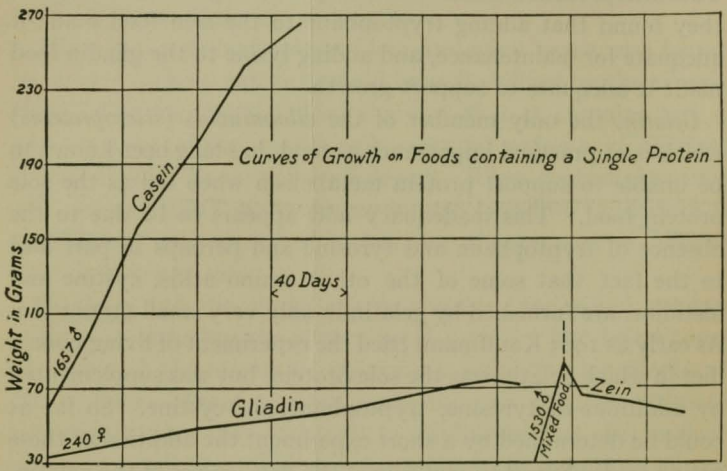


FIG. 1. — Showing typical curves of growth of rats on diets otherwise similar and adequate but containing in each case only a single protein, casein, gliadin, or zein. Courtesy of Dr. L. B. Mendel and the Journal of the American Medical Association.

tein preparations show on analysis small amounts of iron, which has usually been neglected as an impurity but which is not improbably an essential constituent.

Casein and *ovovitellin* fed singly as the sole protein of the ration in the experiments by Osborne and Mendel described above have each been found capable of supporting both maintenance and normal growth, as their amino acid make-up and their place in nature would lead us to expect. The curves in Fig. 1 illustrate the rapid growth on casein as compared with the very slow

growth (virtual suspension of growth) on gliadin and the loss of weight when zein was the sole protein food. The rations were alike except for the nature of the protein fed, and the percentage of protein in the ration was the same in each case.

It will be seen that the rat receiving casein grew over 200 grams in 140 days while the one fed with gliadin grew only 20 grams during the same period. The third rat, which had been growing rapidly on mixed food, began at once to lose weight when put on a ration of which zein was the sole protein.

Quantitative Studies of the Amino Acid Make-up of Proteins

In recent years there has been a rapid simultaneous development of knowledge of the differing values or efficiencies of individual proteins in nutrition and their differences in chemical structure as shown in the relative proportions of the different amino acids which they yield on hydrolysis. Illustrative data of the latter sort are shown in the table on page 59, and a fuller compilation of the amino acid make-up of proteins is tabulated on pages 72 and 73.

Attempts to determine the percentage of each amino acid obtainable from a given protein are so costly and time-consuming, and require such large quantities of the purified protein material, that data of this sort can be accumulated only very slowly. If this were the only means of obtaining an insight into the amino acid make-up of proteins, progress in this direction would often be indefinitely delayed. Van Slyke has, however, devised a method of fractioning the nitrogen of a protein into eight parts, four of which are measures of nutritionally important amino acids.

In order to accomplish this the protein is hydrolyzed, the basic amino acids precipitated by means of phosphotungstic acid, and this precipitate fractioned to show how much of its nitrogen exists in each of the four forms, arginine, histidine, lysine, and

Percentages of Amino Acids from Hydrolysis of Various Proteins

	Albumins				Globulins								Glutelins	
	Egg albumin	Lactalbumin	Legumelin	Leucosin	Amandin (almonds)	Coconut globulin	Edestin (hemp seed)	Excelsin (Brazil nut)	Glycinin (soy bean)	Legumin (peas)	Phaseolin (navy bean)	Vicilin (peas)	Vignin (cow pea)	Glutenin (wheat)	Maize glutelin
Glycine	0.00	0.37	0.50	0.94	0.51	trace	3.80	0.60	0.97	0.38	0.55	0.00	0.00	0.89	0.25
Alanine	2.22	2.41	0.92	4.45	1.40	4.11	3.60	2.33	*	2.08	1.80	0.50	0.97	4.65	*
Valine	2.50	3.30	0.69	0.18	0.16	3.57	6.20	1.51	0.68	*	1.04	0.15	0.34	0.24	*
Leucine	10.71	14.03	9.63	11.34	4.45	5.96	14.50	8.70	8.45	8.00	9.65	9.38	7.82	5.95	6.22
Proline	3.56	3.76	3.96	3.18	2.44	5.54	4.10	3.65	3.78	3.22	2.77	4.06	5.25	4.23	4.99
Oxyproline	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Phenylalanine	5.07	1.25	4.79	3.83	2.53	2.05	3.09	3.55	3.86	3.75	3.25	3.82	5.27	1.97	1.74
Aspartic acid	2.20	9.30	4.11	3.35	5.42	5.12	4.50	3.85	3.89	5.30	5.24	5.30	3.97	0.91	0.63
Glutamic acid	9.10	12.89	12.96	6.73	23.14	19.07	18.74	12.94	19.46	16.97	14.54	21.34	16.89	23.42	12.72
Oxyglutamic acid	*	10.00	*	*	*	*	*	*	*	*	*	*	*	1.80	*
Serine	*	1.76	*	*	*	1.76	0.33	*	*	0.53	0.38	*	*	0.74	*
Tyrosine	1.77	1.95	1.56	3.34	1.12	3.18	2.13	3.03	1.86	1.55	2.84	2.38	2.26	4.50	3.78
Cystine	2.13†	1.73	*	*	0.85	1.54	0.97	1.84	1.18	0.83	0.58	0.57	0.52	1.80	*
Arginine	4.91	3.47	5.45	5.94	11.85	15.92	14.17	16.10	8.07	11.71	4.87	8.91	7.20	4.72	7.06
Histidine	1.71	2.61	2.27	2.83	1.58	2.42	3.92	2.50	2.10	1.69	2.62	2.47	3.08	1.76	3.00
Lysine	3.76	9.87	3.03	2.75	0.70	5.80	3.76	1.64	9.06	4.98	4.58	5.40	4.31	1.92	2.93
Tryptophane	3.64†	3.00	present	present	1.37	1.25	2.48	2.59	1.66	1.76	0.94	0.15	1.65	1.80	present
Ammonia	1.34	1.31	1.26	1.41	3.70	1.57	2.28	1.80	2.28	2.05	2.06	2.03	2.32	4.01	2.21
Summation	54.62	83.01	51.13	50.27	61.22	78.86	88.57	66.63	67.30	64.80	57.71	66.46	61.85	65.31	45.53

* Not determined.

† Means of data given by Jones for ovoalbumin and conalbumin of egg white.

PERCENTAGES OF AMINO ACIDS FROM HYDROLYSIS OF VARIOUS PROTEINS (*Continued*)

	Alcohol-soluble Proteins				Albuminoid	Phosphoproteins		Muscle Tissues (not single proteins)				
	Gliadin (wheat)	Hordein (barley)	Prolamin of rye	Zein (maize)	Gelatin	Casein (cow's milk)	Ovovitellin (hen's eggs)	Beef	Chicken	Halibut	Scallop	Shrimp
Glycine	0.00	0.00	0.13	0.00	25.50	0.45	0.00	2.06	0.68	0.00	0.00	*
Alanine	2.00	0.43	1.33	13.39	8.70	1.85	0.75	3.72	2.28	*	*	*
Valine	3.34	0.13	*	1.88	1.00	7.93	1.87	0.81	*	0.79	*	*
Leucine	6.62	5.67	6.30	19.55	7.10	9.70	9.87	11.65	11.19	10.33	8.78	*
Proline	13.22	13.73	9.82	9.04	9.50	7.63	4.18	5.82	4.74	3.17	2.28	*
Oxyproline	*	*	*	*	14.10	0.23	*	*	*	*	*	*
Phenylalanine	2.35	5.03	2.70	6.55	1.40	3.88	2.54	3.15	3.53	3.04	4.90	*
Aspartic acid	0.58	*	0.25	1.80	3.50	4.10	2.13	4.51	3.21	2.73	3.47	6.98
Glutamic acid	43.66	43.20	38.05	26.17	5.80	21.77	12.95	15.49	16.48	10.13	14.88	15.00
Oxyglutamic acid	2.40	*	*	2.50	0.00	10.50	*	*	*	*	*	*
Serine	0.13	*	0.06	1.02	0.40	0.50	*	*	*	*	*	*
Tyrosine	3.50	1.67	1.19	3.55	0.01	6.50	3.37	2.20	2.16	2.39	1.95	4.88
Cystine	2.32	1.55	*	0.85	0.31	0.50	0.83	1.55	*	1.32	*	1.78
Arginine	3.14	2.89	2.22	1.82	8.22	3.81	7.46	7.47	6.50	6.34	7.38	10.24
Histidine	3.35	2.14	0.39	0.82	0.90	2.84	1.90	1.76	2.47	2.55	2.02	3.78
Lysine	0.92	1.01	*	0.00	5.92	7.02	4.81	7.59	7.24	7.45	5.77	7.60
Tryptophane	1.14	1.05	present	0.00	0.00	2.20	2.42	1.25	present	1.25	present	1.21
Ammonia	5.22	4.84	5.11	3.64	0.49	1.61	1.25	1.07	1.67	1.33	1.08	*
Summation	93.89	83.34	67.55	92.58	92.85	93.62	56.33	70.10	62.15	52.82	52.51	*

* Not determined.

cystine. The nitrogen of the filtrate is fractioned into amino and non-amino nitrogen. The two other forms of nitrogen determined are the amide nitrogen of the protein which appears as ammonia in the hydrolysis, and the humin or melanin nitrogen which is the nitrogen found in the humus-like insoluble material formed during the hydrolysis of the protein. The amino nitrogen of the filtrate corresponds to that of the glycine, alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, aspartic acid, glutamic acid, and one half of the tryptophane which the protein contained. The other half of the nitrogen of tryptophane appears with the nitrogen of proline and oxyproline in the non-amino nitrogen fraction of the filtrate. The results of a Van Slyke analysis of a protein are expressed, not in terms of percentage of amino acid or acids, but as the percentage distribution of the total nitrogen of the protein among the eight fractions just mentioned. The results of applying this method to some typical proteins is shown in the accompanying table.

VAN SLYKE DISTRIBUTION OF NITROGEN IN SOME TYPICAL PROTEINS

	CASEIN	LACTALBUMIN	GLIADIN	EDESTIN
Arginine nitrogen	7.41	7.20	5.45	27.05
Histidine nitrogen	6.21	4.57	3.39	5.75
Lysine nitrogen	10.30	12.24	1.33	3.86
Cystine nitrogen (estimated from sulphur in phosphotungstate precipitate)	0.20	1.30	0.80	1.49
Amino nitrogen of the filtrate . .	55.81	62.00	51.95	47.55
Non-amino nitrogen of the filtrate	7.13	2.00	10.70	1.70
Amide nitrogen	10.27	8.57	24.61	9.99
Humin or melanin nitrogen . . .	1.28	2.32	0.58	1.98

Since the Van Slyke method consists in fractioning the total nitrogen without attempting to isolate individual substances,

it is not subject to the losses discussed above. The tendency is rather toward possible overestimates through assigning to known substances the whole of the nitrogen found, whereas in fact some of it may have been in unknown forms.

It has been suggested that perhaps the best approximation to the truth may be obtained by taking the mean of the data obtained (1) by the Van Slyke method and (2) by the method of actual isolation of each amino acid. Thus gliadin has yielded 0.64 per cent lysine by actual isolation but has shown 1.21 per cent when examined by the method of Van Slyke; so the mean of these figures, 0.92 per cent, is generally accepted as representing the best present knowledge as to the lysine content of gliadin. In some of the compilations included on pages 72 and 73, figures for arginine, histidine, lysine, and cystine obtained by the Van Slyke method have been used.

Relation between Chemical Constitution of Proteins and Their Food Value

Several facts bearing upon the relation between the feeding values of individual proteins and their amino acid make-up have been cited in the preceding pages.

Students of this important subject are now generally agreed that, except in the cases of a few proteins difficult of digestion, the relative values of individual proteins in nutrition are primarily due to their contents of certain amino acids. Those amino acids which are essential to the maintenance of the adult body or to maintenance and growth in the young and which the body apparently cannot synthesize for itself (at least not in the amounts needed) are commonly spoken of as the "essential" or "nutritionally essential" amino acids.

Early in their work in this field, Osborne and Mendel pointed out that growth will be limited by a shortage of any amino acid needed for the synthesis of new body protein, and that the lack

of any needed amino acid which cannot be formed in suitable amounts in the body constitutes such a growth-limiting factor.

Through investigations making use of growth experiments, therefore, data are gradually being accumulated as to which amino acids are, and which are not, nutritionally essential in the sense that they must be supplied to the body by the food. The following statements regarding individual amino acids are intended to summarize our knowledge on this subject as it stands at the time of writing (1925).

Glycine, although an essential constituent of body tissue, need not be furnished by the food, for proteins which do not yield glycine on hydrolysis have been shown to be adequate when fed as sole protein of an experimental ration. It appears, therefore, that supplies of glycine fully adequate to meet all normal needs may be formed within the body itself.

Alanine is probably also formed in the body in ample amounts. Feeding experiments with food supplies adequate in other respects and lacking alanine have not been made because alanine is a constituent of all the food proteins which have thus far been studied in detail with reference to their amino acid make-up.

Tryptophane, on the other hand, apparently must always be supplied to the animal body; food furnishing no tryptophane has always proven inadequate even for maintenance of full-grown animals. Apparently the animal body is unable to make tryptophane (or at least to make it at the rate required for normal metabolism), and proteins lacking the tryptophane radicle must be regarded as always inadequate as a sole protein food.

Lysine is also to be regarded as a nutritionally essential amino acid, needed both for maintenance and for growth; although the amounts required for mere maintenance appear to be relatively small so that this phase of the question was not made entirely clear by the earlier experiments. Lysine has, therefore, often been referred to as especially important in connection with growth. Proteins which yield little lysine (and which are

otherwise adequate in their amino acid make-up) appear to suffice as the sole protein food in the maintenance of full-grown animals (rats) but not to support a normal growth of the young.

As regards the influence of the presence or absence of glycine, lysine, and tryptophane radicles in the protein molecule, it is possible to correlate the chemical structure and the nutritive value of the proteins quite definitely. In establishing this correlation, Osborne and Mendel made one of the most important

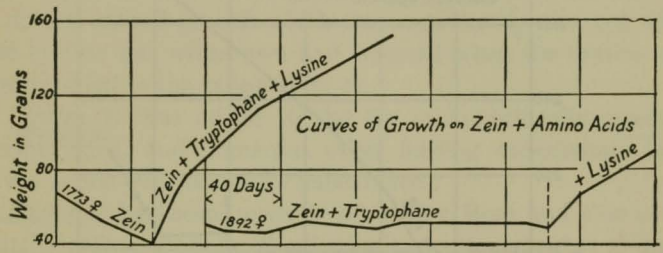


FIG. 2. — Showing the effect of adding tryptophane or tryptophane and lysine to a diet containing zein as the sole protein. Courtesy of Dr. L. B. Mendel and the Journal of the American Medical Association.

advances in the entire development of the chemistry of food and nutrition.

That the inadequacy of zein for maintenance is essentially due to the lack of tryptophane they demonstrated by feeding a ration with zein as sole protein but with tryptophane added. This mixture permitted maintenance without growth (rat 1892, middle portion of Fig. 2). As explained above, the animals on this diet doubtless received a very small amount of lysine from the material which was fed to supply them with water-soluble vitamin. Then by the addition of lysine to the zein and tryptophane diet, normal growth was induced as shown by the continuation of the weight curve of rat 1892 at the right of Fig. 2. In another case (rat 1773, at the left of Fig. 2) a rat which was rapidly losing weight on the zein diet was restored to a condition

of normal growth by the addition of tryptophane and lysine to the food.

As Mendel expresses it: "If we analyze the situation as revealed in the charts of some actual experiments, it becomes apparent that both lysine and tryptophane are unquestionably necessary as constructive units in growth. The decline brought

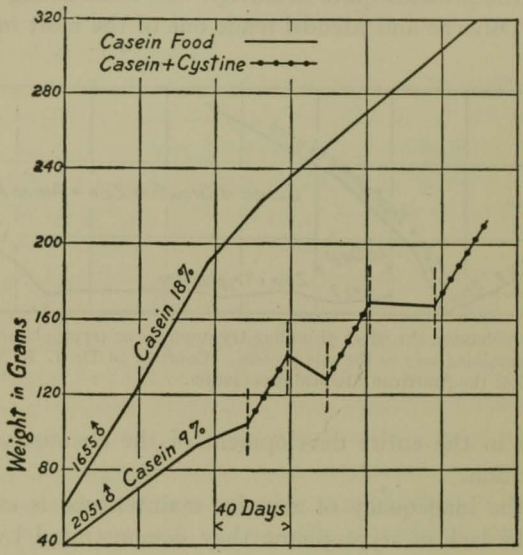


FIG. 3. — Showing that the insufficiency of a low-casein diet was essentially due to its relative deficiency in cystine. Courtesy of Dr. L. B. Mendel and the Journal of the American Medical Association.

about by the zein food can be stopped by the addition of tryptophane, as such, to the diet. This results in maintenance; but no growth ensues until lysine also is added."

Osborne and Mendel also showed that the addition of lysine to the gliadin ration made it adequate to support normal growth. And they have shown that retardation of growth may sometimes be due to restricted intake of some amino acid other than lysine.

In the experiments above described the rations always contained a liberal amount (usually 18 per cent) of protein. If, on the other hand, the percentage of protein in the food be sufficiently reduced, the growth may be retarded even though the protein be of a kind which is entirely adequate when liberally fed. Thus on a ration containing 9 per cent of casein the rats grew only about half as rapidly as when they received 18 per cent; and in this case the limiting factor was not lysine but cystine, for the addition of cystine to the low-casein diet induced a normal rate of growth which was immediately checked when the cystine was withdrawn and resumed when the cystine was again added to the ration (Fig. 3).

Cystine is thus added to the list of nutritionally essential amino acids, and numerous other feeding experiments have given added evidence of its importance.

Histidine has been recently shown by Rose and Cox to be nutritionally essential. Previously it was thought that arginine and histidine were interchangeable. While definitely demonstrating that arginine cannot take the place of histidine, Rose and Cox have not yet (1925) determined whether arginine can be entirely replaced by histidine or not.

Phenylalanine seems to yield *tyrosine* in the body and it appears that either tyrosine or phenylalanine must be fed.

Proline, recently studied in this connection by Sure, is thought also to be a nutritionally essential amino acid.

Other amino acids remain to be investigated as to whether or not they are nutritionally essential.

Growth Experiments as a Means of Determining an Amino Acid

In the experiments cited above it was seen that under certain conditions an increase in the growth rate can be induced by the addition of a single amino acid to the diet. This implies that there is in the basal diet a margin of all other essential amino

acids. If then the one " limiting " amino acid be fed in different proportions there should result different rates of growth, of increased gain in weight, until a rate of gain is reached at which some other amino acid becomes the limiting factor. In recent experiments [1] (with a basal diet adequate in all other respects but in which the protein was derived essentially from dried whole milk over-diluted with starch so that the percentage of protein was brought too low to support normal growth) it was found that cystine was the " limiting " amino acid, and that graded small additions of cystine gave quantitatively proportional increases in gain up to a point at which evidently the cystine became no longer the limiting factor. Beyond this point further additions gave only a much slower rate of increase.[2]

Fig. 4, which, it will be observed, does not present growth curves like those in the previous figures but *diagrams* of the *increased gains* induced by additions of cystine and casein respectively, shows well the break in the response to increasing amounts of cystine at a point where about 4 parts per 10,000 (0.04 per cent) of cystine had been added to the basal diet. Up to this point the relation of the increased gain to the proportion of cystine added was linear, and within this first linear region the results of added cystine were beautifully paralleled by the results of additions of 40 times as much casein. Inasmuch as cystine was the sole limiting factor in this region, the casein must (in this region) have functioned simply by virtue of its cystine content. This implies that the cystine content of casein is about one fortieth of its weight, or about 2.5 per cent. This

[1] Sherman and Woods. *Journal of Biological Chemistry*, Vol. 66, page 29 (1925).

[2] If it be asked why, if cystine had ceased to be the "limiting" amino acid, the further additions of cystine should increase the rate of gain at all, the answer is to be found in the fact that in such experiments the animals are not limited, except by their own appetites, in the amount of basal diet which they may consume. The increased growth induced by the cystine naturally resulted in an increased consumption of the basal diet and thus of the "second limiting" amino acid in this diet, so that beyond the region in which the cystine additions were the sole determining factor in the rate of gain they still had a slight influence in increasing the rate of growth.

is less than the percentage which the casein would contain if all of its sulphur were in the form of cystine but more than has been found by any other method yet devised. Since casein supplies all the other nutritionally essential amino acids as well as cystine,

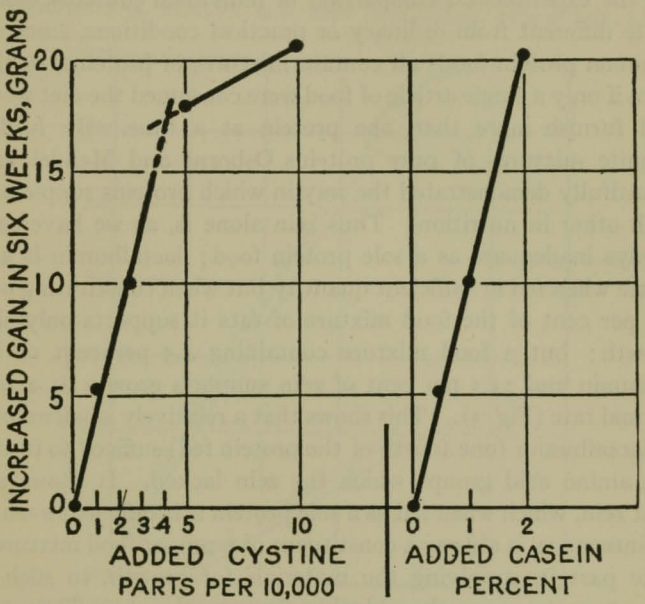


FIG. 4. — These are not growth curves but charts of average rates of increased gain induced by the addition of quantitatively graded proportions of pure cystine or of casein to a basal ration in which cystine was the growth-limiting factor. As explained in the text, the cystine curve continues linear as long as cystine is the sole limiting factor; then shows a distinct break in its direction. The casein curve continues linear longer, as casein furnishes the other nutritionally essential amino acids as well as cystine. (Courtesy of the *Journal of Biological Chemistry*.)

the additions of casein continue to give increases in the rate of growth beyond the point at which the cystine curve of Fig. 4 "breaks." Hence only observations falling within the first linear portion of the cystine curve can indicate correctly the ratio of cystine to casein or the cystine content of the casein.

Supplementary Relations between Proteins in Nutrition

In the feeding experiments shown in Fig. 1, each ration contained only a single isolated protein. This is the ideal condition for the experimental comparison of individual proteins, but is quite different from ordinary or practical conditions, since our common protein foods all contain mixtures of proteins, so that even if only a single article of food were consumed the diet would still furnish more than one protein at a time. By feeding definite mixtures of pure proteins Osborne and Mendel have beautifully demonstrated the way in which proteins supplement each other in nutrition. Thus zein alone is, as we have seen, always inadequate as a sole protein food; lactalbumin is adequate when fed in sufficient quantity but when constituting only 4.5 per cent of the food mixture of rats it supports only slow growth; but a food mixture containing 4.5 per cent of lactalbumin and 13.5 per cent of zein supports growth at a fully normal rate (Fig. 5). This shows that a relatively small amount of lactalbumin (one fourth of the protein fed) sufficed to furnish the amino acid groups which the zein lacked. It shows also that zein, which when fed as a sole protein is insufficient even for maintenance, is able as a constituent of a proper food mixture to take part in supplying the materials for growth, to such an extent as to more than double the growth-rate. Thus zein, although inadequate for either maintenance or growth when isolated and fed alone, may nevertheless take an important part in both maintenance and growth when fed as a part of a proper mixed diet. Moreover, it may not even be necessary to resort to a mixture of food materials in order to make good the deficiencies of the individual incomplete protein. Corn (maize) itself contains, along with zein, an almost equal amount of another protein, maize glutelin, which Osborne and Mendel have shown to be capable of supporting a normal rate of growth — not to mention the proteins in the embryo of the maize kernel which

appear to have a still higher nutritive efficiency (Hart and Humphreys; McCollum and Davis).

Similarly the other proteins of the wheat kernel supply in more liberal proportion the lysine in which gliadin is poor, so that (as Osborne and Mendel and also McCollum, Simmonds, and

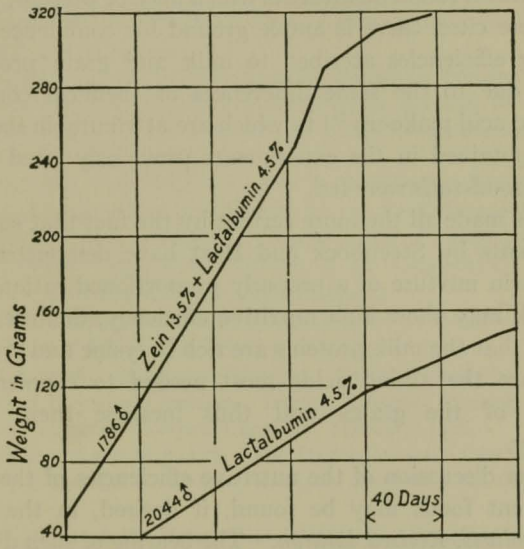


FIG. 5. — Showing the efficiency of lactalbumin as a supplement to zein, and also that zein may take an important part in growth although zein *alone* is inadequate either for growth or maintenance. Courtesy of Dr. L. B. Mendel and the Journal of the American Medical Association.

Parsons have shown) with ground whole wheat as the sole source of protein good growth can be obtained.

Thus it is plain that the mixtures of proteins contained in different articles of food as we eat them do not differ in such a striking way as do the individual proteins when isolated and fed singly; but neither is it true that the proteins of different articles of food are equivalent for all practical purposes. Hart, McCollum, and their associates have shown that the natural

protein mixture of milk is much more efficient than an equal weight of the mixed proteins of grain both for the support of growth and as food for the production of milk in dairy cattle. While it is always possible that in comparisons between natural food materials the results *may* be influenced by differences in the unknown food constituents which may be present, yet in the cases here cited there is ample ground for confidence that the differing efficiencies ascribed to milk and grain proteins are mainly due to the same differences of chemical constitution (" amino acid make-up ") to which are attributable the striking results obtained in the experiments previously cited in which isolated foodstuffs were fed.

This is made all the more certain by the fact that subsequent experiments by Steenbock and Hart have demonstrated that the protein mixture of a properly proportioned ration of grain and milk may show high nutritive efficiency, doubtless due to the fact that the milk proteins are rich in lysine and trytophane which are the amino acids most needed to supplement the proteins of the grains and thus increase their nutritive efficiency.

Further discussion of the nutritive efficiencies of the proteins of different foods may be found, if desired, in the author's *Food Products, Revised Edition.* The bearing of such differences upon the practical problem of protein requirement in dietetics is discussed in Chapter VIII of the present volume.

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CHAPTER IV

ENZYMES AND DIGESTION

THE carbohydrates, fats, and proteins as they exist in foods are in most cases not of a nature to be used by the body tissues in the exact form in which they are eaten, but must usually undergo more or less alteration in the digestive tract to fit them for absorption and utilization. In so far as the changes which the food undergoes in the alimentary tract are chemical they are brought about mainly by the action of digestive enzymes; but the efficiency of the digestive process is also largely dependent upon the mechanical factors of digestion which therefore will also be briefly considered in this chapter.

Historical

The idea that changes comparable to fermentation are involved in the processes of digestion apparently originated with von Helmont about 300 years ago. Sylvius, half a century later, cited alcoholic and acetous fermentations to illustrate the type of process by which he believed the foodstuffs to be digested. Descartes held that as the result of a peculiar fermentation there was generated in the stomach " an acid of great potency, comparable to nitric acid." From the standpoint of our present knowledge these early scientists appear to have made considerable progress toward a correct interpretation of the digestive process; but in their own times, before the beginning of the scientific development of organic or physiological chemistry, the views which they advanced appeared hazy and unscientific compared with those of the physiologists who were studying

digestion from the mechanical point of view and by supposedly exact methods. Thus Dr. Archibald Pitcairn (1652–1713) proposed to explain gastric digestion, " without the aid of a Daemon or a Stygian Liquor," as due entirely to the triturating action of the stomach, the power of whose muscular walls he estimated as " equal to 12,951 pounds " (Gamgee).

The view that the digestion of food in the stomach is due solely to the mechanical action of the stomach walls was refuted by Réaumur, working with birds, and by Stevens, who experimented with a man who was accustomed to swallow small stones and regurgitate them at will. In Stevens' experiments this man swallowed hollow silver balls filled with food and perforated to permit access of the gastric juice but strong enough to resist the muscular contractions of the stomach walls. Food thus introduced was found to undergo digestion in the stomach although it was entirely protected from the triturating action of the stomach walls. Furthermore Stevens found that gastric juice obtained from a dog was able to digest meat outside of the stomach. At about the same time Spallanzani also showed clearly that gastric juice can act outside of the body. In addition, he pointed out its antiseptic properties and emphasized the difference between the digestive process and that of alcoholic, acid, or putrefactive fermentation.

About fifty years after the work of Spallanzani, came the classical observations (1825–1833) of Dr. Beaumont upon Alexis St. Martin, who, as the result of a gunshot wound, was left after recovery from his injury with a gastric fistula which permitted both the collection of human gastric juice and the direct observation of the processes going on in the stomach of a healthy man " active, athletic, and vigorous, exercising, eating, and drinking, like other healthy and active people." Dr. Beaumont's full and interesting account of his experiments with St. Martin [1]

[1] W. Beaumont. *Experiments and Observations on the Gastric Juice and the Physiology of Digestion.* Plattsburg, 1833.

greatly extended the knowledge both of the muscular behavior of the stomach and of the conditions governing the secretion of the gastric juice and the " chymification " of the food in the stomach. The year after the publication of Beaumont's observations, Eberle showed [1] that by extracting the mucous membrane of the stomach with dilute hydrochloric acid he could obtain an artificial juice which showed the same digestive action which Spallanzani and Beaumont had observed with the natural secretion, and two years later Schwann [2] concluded that gastric juice owed its peculiar activity to a substance presumably different from any substance previously known and to which he gave the name *pepsin.* Schwann did not claim to have isolated this peculiar substance in a pure state but did effect a partial separation. Subsequently several other investigators attempted to isolate pepsin.

The Chemical Nature of Some Typical Enzymes [3]

In 1902 Pekelharing prepared what has generally been regarded as probably the purest pepsin of which we have record. This product contained carbon, hydrogen, nitrogen, and sulphur in proportions within the range of variation found among ordinary proteins.[4] It also behaved like ordinary proteins in the xanthoproteic test and Millon reaction and in showing the presence of the tryptophane group.

Dezani, in 1910, carried forward the work upon the chemical nature of pepsin by preparing what was believed to be a substantial duplicate of Pekelharing's product and submitting this to hydrolysis, followed by search for individual hydrolytic

[1] Eberle. *Physiologie der Verdauung nach Versuchen.* Würzburg, 1834.

[2] Schwann. *Ueber das Wesen der Verdauungsprocesse.* Müller's *Archiv.*, 1836, pages 90–138.

[3] Those students not yet familiar with the names of the common enzymes should perhaps read first the sections on classification and terminology below.

[4] A small amount of chlorine shown by Pekelharing's preparation was later found by Dezani to be not an essential constituent but probably due to incomplete removal of the hydrochloric acid with which pepsin is associated in the gastric juice.

products according to the methods which had recently been developed in the study of the structure of the proteins. He demonstrated the presence of leucine, tyrosine, arginine, histidine, and lysine, and also found evidence of other amino acids which the limitations of his material and methods did not permit him to identify.

Thus pepsin as prepared by Pekelharing and by Dezani is a nitrogenous material not identical with any other known substance but complying with the criteria of our present conception of a protein in elementary composition, in color reactions, and especially in yielding the familiar amino acids upon hydrolysis. Recent studies by Aldrich also indicate that the chemical nature of pepsin is that of a protein.

It must be borne in mind that the criteria of purity usually applied in chemical investigations are not applicable to enzyme preparations because of their colloidal nature and the readiness with which their characteristic properties are destroyed. Yet in view of the fact that, with very few if any exceptions, the changes by which the organic foodstuffs are prepared for absorption in the digestive tract and are utilized in the body tissues are dependent upon the presence of enzymes the material for whose synthesis must in the long run be furnished by food, we should not be deterred by the inherent difficulties of the subject, nor are we at present so much in the dark regarding the chemical nature of enzymes as the statements in most textbooks would seem to indicate.

Several years earlier than Pekelharing's work on pepsin, Osborne [1] had published an investigation of the chemical nature of diastase (malt amylase), which may be regarded as marking the beginning of our modern knowledge in this field. From this work it appeared that the enzymic activity is a property of a definite fraction of the protein material of the malt, or in other

[1] T. B. Osborne. *Journal of the American Chemical Society*, Vol. 17, page 587 (1895); Vol. 18, page 536 (1896).

words that the enzyme is protein in its chemical nature. Although criticized by some, Osborne's findings have been confirmed and extended by recent investigations.

Of the two amylases concerned in the digestive process, ptyalin of saliva and amylopsin of the pancreatic juice, only the pancreatic amylase has been studied by modern methods with reference to its chemical nature.

In an investigation [1] in which the attempts at purification were guided and their success largely judged by quantitative determinations of the starch-digesting action of the products, there was developed a method of purification which in numerous independent experiments yielded a product that was not only extraordinarily active in the hydrolysis of starch but was essentially uniform both in digestive activity and in chemical nature. This result indicates strongly that the product represents at least some approximation to an actual isolation of the enzyme. These preparations show the composition and color reactions of typical proteins and, like Osborne's malt amylase, the material when heated in water solution yields an albumin coagulum and a proteose or peptone which remains in solution. Moreover, on hydrolysis the material yields the same groups of amino acids which are yielded by typical proteins such as casein, which it also resembles in elementary composition.

While the chemical nature of the lipases of the digestive tract has not been studied, Falk and Sugiura have shown that the purified lipase preparations made from castor beans are, like the proteases and amylases above mentioned, essentially protein material.[2]

The materials obtained in attempts to isolate enzymes are

[1] *Journal of the American Chemical Society*, Vol. 33, page 1195; Vol. 34, page 1104; Vol. 35, page 1790.

[2] Falk has also suggested that the lipolytically active grouping is the tautomeric enol-lactim form of the peptide linking which becomes inactive on rearrangement to the keto form. Experiments testing this view resulted in the production of lipolytically active substances by the action of alkali on castor bean globulin, casein, and gelatin.

here called merely products or preparations; it is not stated that any enzyme has been perfectly separated and purified. As already explained, the familiar criteria of purity are not applicable to these unstable colloidal substances. It is possible that the enzymes in the purified preparations mentioned above may still be mixed with considerable amounts of other substances, and it has even been suggested that the protein material of which the above-mentioned enzyme preparations are chiefly composed may be present only as a carrier and that the actual enzyme may be a substance of a different nature. There is, however, no real evidence in favor of this suggestion, while there is much evidence of several independent kinds supporting the view of the protein nature of enzymes.

Thus, in addition to the large amount of evidence derived from investigations upon the purification of typical enzymes and study of the chemical nature of the purified preparations, the enzyme activity has been found to depend upon keeping the protein intact, the activity being lost when the enzyme solution is subjected to treatment which coagulates, hydrolyzes, or otherwise induces chemical change in the protein matter. Also, in the case of the typical enzyme malt amylase, the enzymic activity has been found to be so inseparable from the particular protein in which it resides as to travel with it under the influence of the electric current in either direction according to the hydrogen ion concentration of the solution. Thus this enzyme shows, like other protein substances, a characteristic iso-electric point. For further discussion of the significance of this observation, both as added evidence of the protein nature of the enzyme and as throwing light upon the conditions which influence the enzymic activity, the reader is referred to the original paper (Sherman, Thomas and Caldwell, *Journal of the American Chemical Society*, Vol. 46, page 1711 — July, 1924).

While much of the evidence is too recent to be reflected in the statements now generally current, there is, in the judgment

of the writer, no good reason to doubt and abundant reason to accept the conclusion that the typical enzymes concerned in the utilization of the foodstuffs either are proteins in their chemical nature or contain protein as an essential component.

Hence it is now believed that food protein must furnish material for body enzymes as well as for body tissue.

Classification and General Properties of Enzymes

The word " enzyme " (from the Greek " in yeast ") was introduced by Kühne as a general designation for the substances formed in plants or animals which had previously been called " soluble " or " unorganized " ferments to distinguish them from " organized " ferments (fermentation organisms). As more and more of the activities previously regarded as characteristic of organisms have been found to be due to enzymes, the conception of enzyme action has broadened until now the term enzyme is applied by most writers to all organic catalysts formed in plant or animal cells. Those which are ordinarily secreted from the cell and exert their activities outside of it (as in the case of the digestive ferments) are sometimes called *extracellular* enzymes, and those which normally perform their functions within the cells in which they are formed (as in yeast or in muscle cells) may be called *intracellular* enzymes even though it be possible by artificial means to cause them to act independently of living matter. Although each enzyme is generally supposed to be a definite chemical substance, the identification and classification of enzymes is based upon the changes which they bring about. Some of the better-known groups of enzymes are as follows:

1. The hydrolytic enzymes.
   - *a.* Proteolytic or protein-splitting enzymes (proteases).
   - *b.* Lipolytic or fat-splitting enzymes (lipases).
   - *c.* Amylolytic or starch-splitting enzymes (amylases).
   - *d.* Sugar-splitting enzymes (sucrase, maltase, lactase).

2. The coagulating enzymes, such as thrombin or thrombase (the fibrin ferment), and rennin, which causes the clotting of milk.

3. The oxidizing enzymes, or " oxidases " (which, if the oxidation be accompanied by a splitting off of amino groups, may be called " deamidizing " or " deaminizing " enzymes).

4. The reducing enzymes or " reductases."

5. Those which, like the zymase of yeast, produce carbon dioxide without using free oxygen.

6. Enzymes causing a breaking down of a larger into a smaller molecule of the same composition, as in the production of lactic acid from glucose.

7. Enzymes causing chemical rearrangement without breaking down of larger into smaller molecules, " mutases."

Terminology of the Hydrolytic Enzymes. Except in so far as some familiar enzymes continue to be known by their old established names (pepsin, rennin, trypsin, etc.), scientific usage now generally follows the suggestion of Duclaux that each hydrolytic enzyme be designated by a name indicating the kind of substance on which it acts, together with the suffix *ase*. Thus starch-splitting enzymes are called *amylases;* fat-splitting enzymes, *lipases;* protein-splitting enzymes, *proteases*. The name showing the activity of the enzyme is often preceded by an adjective to indicate its source; *e.g., salivary amylase* (ptyalin), *pancreatic amylase* (amylopsin). Such designation does not necessarily imply that the amylase found in the saliva either is or is not the same substance as the amylase of the pancreatic juice.

In discussions of enzyme action the substance on which the enzyme acts is commonly called the *substrate*.

Within the cell producing it, an enzyme often exists in an inactive form known as the *zymogen*, or antecedent of the active enzyme. The zymogen may be stored in the cell in the form of material which is converted into active enzyme at the time of secretion, or the secretion may be poured out with the zymo-

gen not yet completely changed to active enzyme, or sometimes in a form which requires the presence of some other substance in order to render it active. In this case the latter substance is said to *activate* the enzyme.

Influence of Hydrogen Ion Concentration. The activity of most enzymes is largely dependent upon the exact acidity or alkalinity of the medium. This is now usually expressed in terms of hydrogen ion concentration. Thus a normal solution of hydrochloric acid would contain, if the HCl were completely ionized, 1 gram of hydrogen ions per liter; and in a thousandth-normal solution in which the ionization actually is almost complete (actually about 99 per cent of the HCl in such a solution is ionized at ordinary temperatures) the concentration of hydrogen ions is 0.001 gram per liter or 1×10^{-3}. Pure water, according to the usually accepted estimates, has a hydrogen ion concentration of 1×10^{-7} and the same concentration of hydroxyl ions. Thus water which is pure and strictly neutral may also be regarded as being equivalent to a ten-millionth-normal acid and at the same time a ten-millionth-normal alkali. In order to avoid cumbersome numbers Sorensen proposed to indicate hydrogen ion concentration by writing merely the *negative exponent* (*i.e.*, the negative logarithm) as a whole number calling this abbreviation "pH," *e.g.*, in the case of pure water pH = 7.0; in thousandth-normal hydrochloric acid pH = 3.0. Thus a number lower than 7 shows acidity and the more acid the solution the lower the number; a number higher than 7 shows alkalinity and the greater the alkalinity the higher the pH number, since this is the negative exponent of the hydrogen ion concentration.

It must be remembered that the Sorensen exponent, or pH number, varies with the hydrogen ion concentration not arithmetically but logarithmically: 1×10^{-6} = pH 6.0; 2×10^{-6} = pH 5.7.[1]

[1] As pH numbers are logarithms, it is difficult to compare acidities in pH values unless one has had much practice with them. It is much easier to think arithmeti-

The hydrogen ion concentrations most favorable to the action of certain well-known enzymes have recently been measured with the following results:

Enzyme	Optimum H Ion Concentration as pH
Invertase (Sucrase)	4.4 (Nelson)
Pepsin	1.5 (Okada)
Trypsin	8.0–8.3 when acting on fibrin (Long)
Trypsin	5.6–6.3 when acting on casein (Long)
Malt amylase	4.4 (Sherman and Thomas)
Pancreatic amylase	7.0 (Sherman, Thomas and Baldwin)

Activity of the Digestive Enzymes

That the typical digestive enzymes are very pronounced catalysts may be judged from the relatively large amounts of material which they are capable of digesting under favorable conditions. Thus Hammarsten's rennin coagulated 400,000 to 800,000 times its weight of casein; Petit described a pepsin powder which dissolved 500,000 times its weight of fibrin forming 1000 times its weight of peptone; the pancreatic amylase preparation of Sherman and Schlesinger digested 4,000,000 times its weight of starch with the production of 2,300,000 to 2,800,000 times its weight of maltose.

This marked enzymic activity was exhibited by the preparation at a dilution of 1 : 100,000,000 parts of water. The most delicate tests for proteins are not valid at dilutions greater than about 1 : 100,000. The preparation reacted like typical protein to the usual protein tests, but its own enzymic activity constituted a test for its presence which was 1000 times more delicate. Thus the failure of protein reactions in solutions enzymically

cally than logarithmically. The pH values of 6.0 and 5.7 off hand do not appear widely different, but these logarithms are, when converted to simple numbers, 1×10^{-6} and 2×10^{-6} respectively. Thus we see that the first hydrogen ion concentration is half of the second.

active does not show that no protein is present or that the enzyme is of other than protein nature in its chemical composition, although this negative conclusion has been erroneously drawn by some investigators and is repeated by many writers.

A catalyzer is usually considered to alter the velocity of a reaction but not to initiate it. Thus hydrogen peroxide decomposes spontaneously into water and oxygen. In a pure aqueous solution this change goes on slowly, but it is very greatly accelerated by the presence of a minute amount of colloidal platinum. Blood and tissue extracts contain enzymes which accelerate the decomposition of hydrogen peroxide apparently in much the same way as does platinum, and the present tendency is to regard the enzymes generally as acting quite like the inorganic catalyzers in altering by their presence the velocity of certain reactions. Some of the best-known enzyme actions, however, fit into this view only theoretically; for if the enzyme be considered as simply accelerating a reaction already taking place, it must also be considered that in the absence of the enzyme the reaction is so slow that it cannot be demonstrated.

It may perhaps be asked why, if enzymes act by catalysis, there should be any limit to the amount of substrate which the enzyme can hydrolyze. One reason that enzymes cannot hydrolyze infinite amounts of substrate is that they are themselves unstable organic substances which undergo decomposition when kept in solution. In most cases the purer the enzyme the more rapidly its solutions lose their activity. Another reason that an enzyme does not continue to hydrolyze substrate indefinitely is that the reaction is progressively retarded by the accumulation of the products formed.

The activity of an enzyme may be stopped, even when all other conditions are favorable, by the accumulation of the product of its action; and in certain circumstances the action of the enzyme may be reversed so as to accelerate a change in the opposite direction to that in which it ordinarily acts. Thus

Croft Hill showed it to be possible to reverse the ordinary action of maltase so as to make it bring about a conversion of mono- into di-saccharide; Pottevin synthesized triolein by means of the pancreas ferment; Taylor and others have demonstrated a partial reversion of the tryptic digestion of proteins; and recently Wasteneys and Borsook have carried out a very extended and systematic investigation of the reversion of peptic and tryptic digestion. While the exact significance of these experiments upon the reversibility of the actions brought about by the digestive enzymes is still a subject of active research, there seems to be no doubt that hydrolytic enzymes are widely distributed in the body and that many of the transformations which take place in the course of metabolism are best explained on the ground of the reversibility of enzyme action. Consideration of the tissue enzymes will be left until the study of the fate of the foodstuffs in metabolism is taken up. At this point it may be convenient to summarize in tabular form the occurrence and action of the chief digestive enzymes.

SUMMARY OF CHIEF DIGESTIVE ENZYMES

ENZYMES		WHERE CHIEFLY FOUND	ACTION
Act on Carbohydrates	Ptyalin (salivary amylase)	Salivary secretions	Converts starch to maltose
	Amylopsin (pancreatic amylase)	Pancreatic juice	Converts starch to maltose
	Invertase (Sucrase)	Intestinal juice	Converts sucrose to glucose and fructose
	Maltase	Intestinal juice	Converts maltose to glucose
	Lactase	Intestinal juice	Converts lactose to glucose and galactose

SUMMARY OF CHIEF DIGESTIVE ENZYMES (*Continued*)

ENZYMES		WHERE CHIEFLY FOUND	ACTION
Act on Fat	Lipases	Gastric (?) and pancreatic juices	Split fats to fatty acids and glycerol
Act on Proteins	Pepsin	Gastric juice	Splits proteins to proteoses and peptones
	Trypsin	Pancreatic juice	Splits proteins to proteoses, peptones, polypeptids and amino acids
	Erepsin	Intestinal juice	Splits peptones to amino acids and ammonia

With this brief sketch of the nature and action of the digestive enzymes, the adequate discussion of which would require a volume in itself, we may now pass to a review of the digestive process, following the course of the food through the human alimentary tract and noting briefly both the mechanical and chemical treatment to which it is subjected.

Salivary and Gastric Digestion

Since the muscular movements of the digestive tract, particularly of the stomach when empty, play an important part in bringing about the sensations which lead to the taking of food, it may be well to note at this point the results obtained by Cannon and Washburn and more recently by Carlson in their investigations of hunger. Lest hunger be confused with appetite, it is essential to clearness that these terms be defined. Some have thought that the two experiences differ only quantitatively, appetite being regarded as a mild state of hunger; but it is now held that hunger and appetite are fundamentally different. In the view of Cannon and Washburn:

" Appetite is related to previous sensations of the taste and smell of food; it has therefore, as Pawlow has shown, important psychic elements. It may exist separate from hunger, as, for example, when we eat delectable dainties merely to please the palate. Sensory associations, delightful or disgusting, determine the appetite for any edible substance, and either memory or present stimulation can thus arouse desire or dislike for food.

" Hunger, on the other hand, is a dull ache or gnawing sensation referred to the lower midchest region or epigastrium. It is the organism's first strong demand for nutriment, and, not satisfied, is likely to grow into a highly uncomfortable pang, less definitely localized as it becomes more intense. It may exist separate from appetite, as, for example, when hunger forces the taking of food not only distasteful but even nauseating."

According to Carlson, normal functioning of the digestive tract and of the nervous system constitutes a necessary background for the development of appetite. " Given this background, the central and essential element in appetite is the memory processes of past experience (sight, smell, taste) with palatable foods. These memories are reinforced by present stimulation of these nerves by the food, since everyone knows that appetite, unless intense at the outset, is increased by the very act of eating. . . . In the normal individual hunger and appetite are usually experienced simultaneously." (*Control of Hunger in Health and Disease*, pages 98–99.)

Hunger is not due merely to emptiness of the stomach. It is true that under ordinary conditions hunger is apt to appear soon after the last food has passed from the stomach to the intestine, but if the stomach be artificially emptied, the sensation of hunger may not be felt until some hours afterward. Nor is hunger due to hydrochloric acid secreted into an empty stomach, for if the empty stomach of a hungry person be washed out, but little if any acid is found.

The explanation of hunger, advanced by Cannon and Washburn, is that it is due to the muscular contractions of the walls of the empty stomach.

In order to learn whether direct proof of this might be secured experimentally in man, one of the investigators accustomed himself to swallowing a small soft rubber balloon attached to the end of a rubber tube by means of which it could be withdrawn when desired. The tube and bulb were habitually carried thus in the esophagus and stomach for two or three hours at a time until the experience ceased to have any disturbing effect. Experiments were then made in which the balloon, thus held in the stomach, was partially inflated with air and connected with a manometer and recording apparatus by means of which any pressure exerted upon the balloon was recorded automatically. In the actual experiments, the subject sat at rest with his hand on a key which he pressed whenever he experienced the sensation of hunger. This key was connected with a recording device which, like the apparatus recording the muscular contractions of the stomach upon the rubber balloon, was out of sight of the subject.

Before hunger was experienced the recording apparatus revealed no evidence of muscular activity in the stomach. The records of hunger "pangs" and of muscular contractions of the stomach were always approximately simultaneous, that is, when the subject of the experiment felt hungry, powerful contractions of the stomach were always being registered. The contractions were about 30 seconds in duration, with pauses of 30 to 90 seconds between. It was found in almost every case that the contraction reached its greatest intensity just before the record of the hunger sensation began, and that the feeling of hunger disappeared when the contraction ceased although no food or drink had been taken. Cannon considers the evidence conclusive that hunger is caused by the contractions, and not vice versa, as Boldireff had thought. Other observations in the course of Cannon's experiments showed that the lower end of the esophagus also contracts periodically in hunger, an explanation of the fact that sensations of hunger may be felt in cases where the stomach has been removed. Furthermore Cannon considers that vague sensations of hunger may also originate from muscular contractions in the intestine.

Bulatao and Carlson have shown that the stomach contractions occur when the content of glucose in the blood drops below the normal level. Increasing the glucose content of the blood above the normal level (experimental hyperglycemia) in-

hibits hunger contractions in normal animals but not in diabetic animals which have lost their power to burn glucose.

The evidence seems to justify the view that the strong muscular contraction of the empty stomach may be regarded as an indication that the condition which causes the first sensation of hunger is that in which the stomach is in the best state of readiness to receive the food. There is also direct experimental evidence that the stomach digests more expeditiously the food which is " eaten with hunger " (Hudek and Stigler, cited by Carlson). The description of the digestive process which follows presupposes that the food is eaten under favorable conditions and received by a digestive tract which has been permitted to form good and regular habits.

The eating of food induces a flow of saliva from great numbers of minute glands in the lining membrane of the mouth and from the three pairs of large salivary glands. That saliva is secreted in response to psychic as well as chemical stimulation is shown by the fact that actual contact with the food is not necessary, since secretion may be started by the sight or odor or even the thought of food. Mixed human saliva has usually a faintly alkaline reaction and always contains ptyalin (salivary amylase), although its amylolytic power appears to vary considerably with individuals and with the same individual at different times of the day. As the food comes in contact with saliva, the digestion of starch and dextrin under the influence of the ptyalin begins at once; but as mastication is an entirely voluntary act, the thoroughness with which the food becomes mixed with saliva is subject to wide variations.

Usually the food stays too short a time in the mouth for the starch to be acted upon *there* to any great extent, and until recently it was supposed that salivary digestion must cease almost as soon as the food reaches the stomach, since the activity of ptyalin is quickly checked by even small amounts of free hydrochloric acid. It was supposed that the food mass must

soon be mixed with the gastric juice under the influence of the " churning " of the stomach contents by the muscular contraction of the stomach walls, which was so interestingly described by Dr. Beaumont in the account of his classical researches already referred to. From the nature of the case Dr. Beaumont's observations were made entirely at one point in the stomach. Here he found during digestion a vigorous muscular churning and mixing of the food mass with the gastric juice. For a long time this was supposed to represent the state of the entire stomach contents. This view has now been abandoned as the result of a number of later investigations, among which those of Cannon and of Grützner are of especial interest.

When a small amount of an inert metallic compound such as bismuth subnitrate is mixed with the food, it becomes possible to photograph the food-mass within the body by means of the Roentgen rays. By the use of this method Cannon has carried out an extended series of observations upon the movements of the stomach and intestines during digestion,[1] upon the results of which the statements concerning the mechanism of digestion in this chapter are chiefly based.

Cannon's observations, confirmed by those of other investigators, show that the vigorous muscular movements described by Beaumont, occur only in the middle and posterior, or pyloric, portion of the stomach, while the anterior portion, or fundus, which serves as a reservoir for the greater portion of the food, is not actively concerned in these movements and does not rapidly mix its contents with the gastric juice.

That there is no general circulation and mixing of the entire stomach contents during or immediately following a meal is further shown by the experiments of Grützner, who fed rats with foods of different colors and, on killing and freezing the animals and examining the stomach contents, found that the portions

[1] These and other investigations are fully discussed in Cannon's *Mechanical Factors in Digestion*. See also Carlson's *Control of Hunger in Health and Disease*.

which had been eaten successively were arranged in definite strata. The food which had been first eaten lay next to the walls of the stomach and filled the pyloric region, while the succeeding portions were arranged regularly in the interior in a concentric fashion (Fig. 6). In describing this result Howell says: "Such an arrangement of the food is more readily understood when one recalls that the stomach has never any empty space within; its cavity is only as large as its contents, so that the first portion of food eaten entirely fills it, and successive portions find the wall layer occupied and are therefore received into the interior."

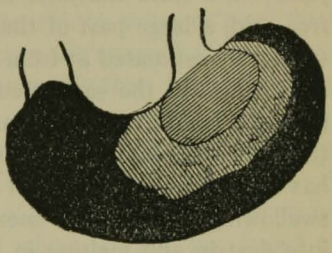

FIG. 6. — Section of frozen stomach of rat during digestion to show the stratification of food given at different times. (*Grützner.*) The food was given in three portions and colored differently. Reproduced from Howell's *Textbook of Physiology*, by permission of the W. B. Saunders Co.

The character of the gastric juice secreted in different parts of the stomach varies considerably, especially as regards its acidity. In the middle region the secretion is rich in acid, while both in the cardiac region and at the extreme pyloric end, the "border cells" or "cover cells" (from which the secretion of the acid appears to take place) are few in number or entirely lacking, and the juice secreted in these regions may be neutral or, according to Howell, even slightly alkaline.

The nature and extent of the muscular movements also vary greatly in the different regions of the stomach. The peristaltic waves of muscular constriction which bring about the thorough mixing of the food with the gastric juice begin in the middle region and travel toward the pylorus. Over the pyloric part of the stomach when food is present constriction waves are continually coursing toward the pylorus. The food in this region is first pushed forward by the running wave and then by

pressure of the stomach wall is returned through the ring of constriction. Thus the food *in this portion* of the stomach is thoroughly mixed with the gastric juice and is forced by an oscillating progress toward the pylorus.

The food in the cardiac end of the stomach is not moved by peristalsis, and so comes only slowly into contact with the gastric juice; and since the juice secreted here contains little if any free acid, a large part of the food mass remains for some time (variously estimated at from 30 minutes to 2 hours or more) in approximately the same neutral or faintly alkaline condition in which it was swallowed, and salivary digestion continues in this part of the stomach without interruption. Thus, if the food has been thoroughly chewed and well mixed with saliva before swallowing, much if not most of its starch may be converted into dextrin and maltose in the cardiac region of the stomach before the activity of the ptyalin is stopped by contact with the acid of the gastric juice.

The fundus, however, is not entirely inactive, but acts as a sort of elastic pouch which is distended by and slowly contracts upon the food mass, thus gradually tending to move the posterior portions and particularly the more fluid portion into the pyloric region. At intervals which are apparently related to rhythm of muscular contraction of the stomach walls, the pylorus opens and permits passage of chyme (the fluid or semi-fluid mixture of food material mixed with, and partly digested by, the saliva and the gastric juice) into the small intestine. Normally, the chyme, because of the gastric juice which it contains, is distinctly acid.

As digestion proceeds, the pylorus opens more frequently and the stomach tends to empty itself more and more freely, until finally the pylorus may open to allow the passage of particles which have not been acted upon by the gastric juice. Whether the stomach will thus completely empty itself of one meal before the eating of the next will depend of course upon the length of the interval and the amount and character of the

food composing the meal. Small test meals may disappear in from 1 to 4 hours, but meals approximating one third of the day's food may not disappear entirely from the stomach during 6 or 7 hours.

Ordinarily when each is fed separately, protein food stays longer in the stomach than carbohydrate; fat longer than protein. Mixtures of fat and protein leave the stomach more slowly than either when fed alone. In general the softer or more fluid the fat the more rapidly it will leave the stomach; also emulsified fats tend to pass on more promptly than fat of the same kind taken in larger masses.

The most important constituents of gastric juice are free hydrochloric acid and pepsin. While other acids may be found in stomach contents, the acidity of gastric juice appears to be due entirely to hydrochloric acid which Carlson has found to constitute an average of 0.40 to 0.50 per cent of normal human gastric juice. The acid has an antiseptic action on the stomach contents. When through any cause the acidity of the gastric juice is abnormally decreased, the numbers of bacteria in the stomach contents may increase greatly. As will be seen later, the acidity of the chyme as it passes the pylorus has an important influence upon the secretion of the pancreatic juice.

Carlson distinguishes between three types of normal gastric secretion. There is a slight but continuous secretion of juice in the empty stomach. The flow is augmented by both psychical and chemical stimulation. The psychic secretion (appetite juice) is brought about to some extent by the sight, odor, or memory of food, but principally by the tasting and chewing of food. The rate of this secretion is directly proportional to the palatability of the food. It may be checked entirely by unpleasant feelings, such as fear, anger, or pain, and under normal conditions it usually ceases within 15 to 20 minutes after the completion of mastication. Since the continuous secretion is sufficient to initiate gastric digestion, the psychic secretion is

not indispensable but may be advantageous. During and after any psychic effects (pleasurable or otherwise), stimulations arising within the stomach itself provide for the continuance of gastric digestion. This third type of secretion is brought about by the presence of food in the stomach and also by the stimulating effect of certain substances, notably meat extracts, water and dilute acids, which act through some local secretory mechanism in the walls of the stomach and duodenum.

Under normal conditions, the amount of nutritive material absorbed from the stomach is insignificant as compared with the amount absorbed from the intestine. Nearly all the food eaten is passed from the stomach into the intestine in the form of chyme, having been more or less liquefied and acidulated by its thorough mixing with the gastric juice in the middle and pyloric regions of the stomach.

The stomach therefore has several functions. It serves (1) as a storage reservoir receiving food in relatively large quantities, say three times a day, and passing it on to the intestine in small portions at frequent intervals, (2) as a place for the continuation of the salivary digestion of starch, and (3) for the beginning of the digestion of proteins and perhaps fats, and finally (4) as a disinfecting station of somewhat doubtful value since the food is subjected to the acidity of the gastric juice, but usually only for a relatively short time while in the pyloric region.

Intestinal Digestion

Digestion in the small intestine. When the pylorus opens, food, now reduced to liquid chyme, is projected into the upper part of the small intestine, where it usually lies for some time in the curve of the duodenum, until several additions have been made to it from the stomach. While the food rests here the bile and pancreatic juice are poured out upon it, and here also, as well as in other parts of the small intestine, a certain amount of intestinal digestive juice (" succus entericus ") is secreted

by the glands of the lining membrane and mixed with the intestinal contents. While for purposes of description the pancreatic and intestinal juices and the bile may be discussed separately, it is to be remembered that in normal digestion they always act together. Cannon's observations showed that after a certain amount of food and digestive juices has accumulated, as just described, in the first loop of the small intestine, the mass all at once becomes segmented by constrictions of the intestinal walls, and the segmentation is repeated rhythmically for several minutes, so that the individual portions are subjected to relatively extensive and energetic to-and-fro movement, which is doubtless very important in facilitating the emulsification of fat. Other effects of the muscular constrictions which cause the segmentation are (1) a further mixing of food and digestive juices, (2) the bringing of the digested food into contact with the absorbing membrane, (3) the emptying of the venous and lymphatic radicles in the membrane, the material which they have absorbed being forced into the veins and lymph vessels by the compression of the intestinal wall. After a varying length of time the segmentation ceases and the small segments are carried forward individually by the peristaltic movement, or join and move on as a single body.

Alvarez has investigated the movement of material along the digestive tract, with special reference to the underlying basis of the phenomena. In general, he holds that the movement of the food mass or its intestinal residue is dependent upon differences in the muscular tone of the wall of the alimentary canal; and that such a difference is a normal inherent characteristic of the musculature at different " levels " of the digestive tract, so that if a segment of the intestine is by operation reversed in its position the direction of its peristalsis is thereby reversed and food will no longer pass through it. On the examination of sections at intervals from the duodenum to the ileum, Alvarez found a progressive decrease in the rate of rhythmic contraction,

in tonus, and in irritability. There was also a definite gradation in carbon dioxide production of equal weights of muscle from duodenum to colon. On the basis of these findings, Alvarez suggests that the progress of food through the intestine depends upon the relative rate at which metabolism takes place in the musculature of succeeding portions of the tract, a relation to which he gives the name " metabolic gradient."

The fluid food mass which the stomach pours into the duodenum contains a small amount of free hydrochloric acid besides a larger amount combined with protein and sometimes organic acids from the food as eaten, or from bacterial fermentation of carbohydrates in the stomach. The pylorus having closed, the alkalinity of the bile, the pancreatic juice, and the intestinal juice combine to neutralize the acids present.

In man the main duct of the pancreas and the bile duct unite and empty into the small intestine about 8 to 10 cm. (3 to 4 inches) below the pylorus. *The pancreatic juice* is a clear liquid having an alkalinity probably equivalent to a 0.5 per cent solution of sodium carbonate and containing three important enzymes or their zymogens — trypsin, amylopsin (amylase), and steapsin or lipase.

The outflow of the pancreatic juice begins at once when any of the acid stomach contents passes through the pylorus, and has been shown by Bayliss and Starling to be due to a definite chemical substance, *secretin*, a hormone produced as the result of the action of the acid upon some constituent of the intestinal mucous membrane, which is absorbed and carried by the blood to the pancreas and there stimulates the flow of pancreatic juice.

Human bile, which, as already stated, enters the intestine through the same duct with the pancreatic juice, is a slightly alkaline solution containing, in addition to water and salts, bile pigments, bile acids (as salts), cholesterol, lecithin, and a peculiar protein derived from the mucous membrane of the bile ducts and gall bladder. The presence of the bile in the intestinal contents

greatly increases the solubility of the fatty acids, while at the same time it diminishes the surface tension between watery and oily fluids, thus breaking up the oil droplets and permitting the lipase of the digestive juice to come into more effective contact with the fat of the food. Bile may also accelerate the action of pancreatic lipase in a more direct way. Thus bile aids both the digestion and the absorption of fats. The bile acids are themselves absorbed to a considerable extent and again secreted by the liver. The secretion of bile by the liver, although variable in amount, is continuous. Its ejection from the gall bladder into the intestine occurs, however, only during digestion, and appears to be excited by the passage of chyme through the pylorus, and to run parallel to the outpouring of the pancreatic juice. According to Starling, the rapid flow of bile during intestinal digestion is due not only to the pouring out of what was previously stored in the gall bladder, but also to an increased rate of secretion to which the liver is stimulated by the same chemical mechanism which stimulates the flow of pancreatic juice.

The intestinal juice is a distinctly alkaline liquid secreted by the tubular glands (crypts of Lieberkühn) with which the small intestine is lined. It contains at least five enzymes: enterokinase, by the action of which trypsinogen is converted into trypsin; erepsin, which produces further cleavage of the proteoses and peptones into amino acids; and the three enzymes, sucrase (or invertase), maltase, and lactase, which hydrolyze respectively the three disaccharides, sucrose, maltose, and lactose. The secretion of intestinal juice is probably stimulated by secretin, and possibly also by another hormone whose production is dependent upon the presence of pancreatic juice.

The acidity of the chyme is, of course, neutralized by the alkalinity of the pancreatic juice, the intestinal juice, and the bile. Without attempting any technical discussion of the matter it may be said that in general under normal conditions the reac-

tion (*i.e.* the range of hydrogen ion concentration) found in the intestinal contents is favorable to the activity of the pancreatic and intestinal enzymes. Under such conditions all three classes of foodstuffs are readily attacked by the digestive enzymes present, and brought into condition for absorption — the carbohydrates as monosaccharides; the fats as fatty acids and glycerol; the proteins (chiefly at least) as amino acids.

The rate of passage of different foodstuffs through the small intestine has been studied by Cannon with the aid of the Roentgen rays, according to the general method already described. Fat, carbohydrate, and protein foods, uniform in consistency and in amount (25 cc.), were fed to cats which had been fasted for 24 hours. At regular intervals for 7 hours after feeding, the shadows of the stomach and intestinal contents were observed by means of the Roentgen rays.

The process of rhythmic segmentation above described was seen with all three kinds of foodstuffs, and the frequency of its occurrence corresponded roughly to the amount of food present in the intestine.

Absorption takes place very readily in the small intestine — more readily and completely than can be explained by the purely mechanical laws of diffusion. On this account the process is sometimes called " resorption " to distinguish it from passive absorption such as takes place by diffusion through non-living membrane.

Observations have been made upon a patient having a fistula at the end of the small intestine. In this case it was found that 85 per cent of the protein matter of the food was absorbed before this point was reached, and the absorption of the other foodstuffs is probably equally complete. For this patient the food began to pass the ileocaecal valve in from 2 to $5\frac{1}{4}$ hours after eating, but the time required from the eating of the food until the last portions had passed into the large intestine was 9 to 23 hours.

Digestion in the large intestine. We have seen that in the small intestine the conditions are very favorable both for digestion and for absorption, and that very much the greater part of the available nutrients has been absorbed before the food mass reaches the ileocaecal valve. Hurst has observed, however, that often the ileum is still full at the end of four or five hours after the last trace of chyme has left the stomach. Consequently there may be an accumulation of incompletely digested food and active digestive enzymes in the last few inches of the ileum, where it remains and undergoes digestion for perhaps a longer period than in the stomach. During all this time there is active segmentation, but very little peristalsis.

Beginning at infrequent intervals some time after the chyme first reaches it, the ileocaecal valve relaxes each time a peristaltic wave passes along the last few inches of the ileum. Cannon finds that the ileocaecal valve is physiologically " competent " for food which passes through it normally from the small intestine. This means that the food which has reached the large intestine in the natural way is ordinarily never forced back into the small intestine again. This is important because in the anterior portion of the large intestine the waves which appear most frequently are those of antiperistalsis — *i.e.* tend to force the food back toward the small intestine. Since the ileocaecal valve prevents the food passing back, these antiperistaltic waves result in thoroughly mixing the food mass in this part of the large intestine and constantly bringing fresh portions in contact with the intestinal wall so that the conditions here are quite favorable for absorption. Moreover, the walls of the large intestine furnish an alkaline secretion which further aids the completion of the digestive changes already begun. So far as known the large intestine secretes no digestive enzyme of its own.

With the passage of material from the ileum into the caecum, the caecum and ascending colon become gradually filled. Ob-

servations show that this passive filling takes place very slowly except during and immediately after meals (Hurst). The material remains in the large intestine for a comparatively long time (generally about a day, often longer); for the peristaltic movements which carry the material onward, while stronger than the waves of antiperistalsis, are of less frequent occurrence, at least in the first part of the large intestine. During this time there is a marked absorption of water, along with the remaining products of digestion. The residual material gradually becomes more solid and takes on the character of feces.

Bacterial Action in the Digestive Tract

The digestive tract of an infant contains no bacteria at birth, but usually some gain access during the first day of life. In the average adult it is estimated that each day's food in its passage through the digestive tract is subjected to the action of over one hundred billion bacteria, chiefly in the large intestine.

Since bacteria are regularly present in the digestive tract in such large numbers, it has been questioned whether they may not perform some essential function in connection with the normal processes of digestion. Experiments to demonstrate whether animals are independent of such bacteria are beset with many difficulties.

On examination of the intestinal contents of Arctic animals in Spitzbergen, Levin found the digestive tracts free from bacteria, showing that the latter are not essential to normal processes of digestion and nutrition. Kendall, however, in citing the evidence presented by Levin, points out that Arctic mammals, as soon as they are brought to temperate regions, rapidly acquire intestinal bacteria which do not seem to interfere with the well-being of the host. It would seem fair to conclude from the observations of Levin that if it were possible to exclude absolutely all bacteria from the digestive tract, the well-being of the body would be in no wise impaired; yet under such con-

ditions as ordinarily exist, the bacteria which usually predominate in the digestive tract of the healthy man probably render an important service in helping to protect the body against occasional invasions of obnoxious species.

According to Herter, a few species, such as *B. lactis aërogenes*, *B. coli*, *B. bifidus*, have adapted themselves so well to the conditions existing in the human digestive tract that they are ordinarily not harmful to the host unless present in abnormally large numbers, and being able to hold their own against newcomers they may act beneficially in giving rise to conditions which check the development of other types of organisms, capable of doing injury, which under ordinary conditions man can hardly prevent from occasionally gaining ingress through food or drink.

" The presence in the colon of immense numbers of obligate micro-organisms of the *B. coli* type may be an important defense of the organism in the sense that they hinder the development of that putrefactive decomposition which, if prolonged, is so injurious to the organism as a whole. We have in this adaptation the most rational explanation of the meaning of the myriads of colon bacilli that inhabit the large intestine. This view is not inconsistent with the conception that under some conditions the colon bacilli multiply to such an extent as to prove harmful through the part they take in promoting fermentation and putrefaction."

More recently the *Bacillus acidophilus* has come to be regarded as the species best adapted to the function of maintaining a favorable condition in the human intestine. The taking of cultures of this organism and the liberal consumption of lactose and dextrin, the carbohydrates most favorable to it, often assist in the establishment and maintenance of a good condition of intestinal hygiene.

Coefficients of Digestibility of Food

The fecal matter passed per day varies considerably in health, but, on an ordinary mixed diet of reasonably digestible food materials, is usually between 100 and 200 grams of moist substance containing 25 to 50 grams of solids. The feces contain any indigestible substances swallowed with the food and any undigested residues of true food material; but ordinarily they appear to be largely composed of residues of the digestive juices, together with certain substances which have been formed in metabolism and excreted by way of the intestine; together with bacteria, living and dead.

Prausnitz studied the feces of several persons placed alternately on meat and on rice diets and found that, although the solids of the meat were about ten times as rich in nitrogen as the solids of the rice, the two diets yielded feces whose solids were of practically the same composition.

In view of such results Prausnitz considers that "normal" feces have essentially the same composition irrespective of the food, the quantity of food residues in such "normal" feces being negligible. From this point of view the feces show not so much the extent to which the food has been absorbed as whether it is a large or a small feces-former. On the other hand, so far as the nitrogen compounds of the feces are concerned, it is probably true, as generally assumed, that they represent material either lost or expended in the work of digestion, and therefore that the nitrogen of the feces is to be deducted from that of the food in estimating the amount available for actual tissue metabolism. This, however, is by no means equally true of the ash constituents, many of which after being metabolized in the body are eliminated mainly by way of the intestine rather than through the kidneys.

On a liberal diet consisting entirely of non-nitrogenous food the amount of nitrogen in the feces was 0.5 to 0.9 gram per day,

which is more than is sometimes found in feces from food furnishing enough protein to meet all the needs of the body. Thus the expenditure of nitrogenous material in the digestion of fats and carbohydrates may be larger than in the digestion of protein food.

The feces always contain fat (or at least substances soluble in ether) as well as protein. Fasting men have eliminated 0.6 to 1.3 grams of fat per day; and when the diet is very poor in fat, the feces may contain as much as was contained in the food. As the fat content of the food rises, the actual amounts in the feces increase, but the relative amounts decrease, so that up to a certain point the apparent percentage utilization of the fat becomes higher. The limit to the amount of fat which can be thus well digested varies with the individual and with the form in which the fat is given. Quantities up to 200 grams per day have been absorbed to within 2 to 3 per cent when given in the form of milk, cheese, or butter.

In addition to protein and fat the feces always contain various other forms of organic matter which in the routine proximate analyses usually made in connection with feeding experiments are collectively reported as "carbohydrates determined by difference."

With these facts in mind one may make use of the coefficients of digestibility without being misled by them. These coefficients show the relation between the constituents of the food consumed and the corresponding constituents of the feces. Thus if the feces from a given diet contain 5 per cent as much protein as was contained in the food, this proportion is assumed to have been lost or expended in digestion, and the coefficient of digestibility of the protein of the diet is stated to be 95 per cent. While as just shown this assumption is not entirely correct, yet it is approximately true of the organic nutriments that the difference between the amounts in the food and in the feces represents what is available to the tissues of the body, and thus these coeffi-

cients serve a useful purpose in the computation of the nutritive values of foods.

From the results of hundreds of digestion experiments Atwater computed the coefficients of digestibility of the organic nutrients of the main groups of food materials, when used by man as part of a mixed diet, to be as follows:

AVERAGE COEFFICIENTS OF DIGESTIBILITY OF FOODS WHEN USED IN MIXED DIET (ATWATER)

	PROTEIN PER CENT	FAT PER CENT	CARBOHYDRATES PER CENT
Animal foods	97	95	98
Cereals and breadstuffs	85	90	98
Dried legumes	78	90	97
Vegetables	83	90	95
Fruits	85	90	90
Total food of average mixed diet	92	95	98

In some cases these figures are higher than have been reported for similar foods by other observers, the differences being due mainly to the fact (not formerly recognized) that a food may be more perfectly utilized when fed as part of a simple mixed diet than when fed alone. Milk is an example of such a food, and has, when consumed as part of a mixed diet, a much higher coefficient of digestibility than is often assigned to it on the basis of earlier experiments.

It will be seen that the coefficients differ less for the different types of food than might be expected from popular impressions of " digestibility " and " indigestibility." It is also noteworthy that the coefficients of digestibility are less influenced by the conditions under which the food is eaten and vary less with individuals than is generally supposed. In explanation of this it may be noted that general impressions of digestibility relate mainly to *ease* of digestion and particularly to ease and rapidity

of gastric digestion, and that there is little direct relation between the ease with which a food is digested in the stomach and the extent to which it is ultimately digested in its passage through the entire digestive tract. Substances which are resistant to gastric digestion will tend to remain long in the stomach and will probably excite a greater flow of gastric juice. Thus a greater amount of acid chyme will enter the duodenum, and this will result in the secretion of a greater amount of pancreatic juice also.

Similarly an increase in the amount of food eaten may have little effect upon the coefficient of digestibility of the foodstuffs. In a series of experiments by the writer it was found that the doubling of a small diet decreased the coefficient of digestibility by less than 1 per cent. Snyder reports that as between medium and large amounts of oatmeal and milk, the protein was 7 per cent and the fat 6 per cent more completely absorbed in the case of the medium ration.

The coefficients of digestibility of individual articles of food are summarized and discussed in the writer's *Food Products, Revised Edition*, 1924.

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CHAPTER V

THE FATE OF THE FOODSTUFFS IN METABOLISM

Carbohydrates

The carbohydrate of the food, having been converted into monosaccharide in the intestine, is taken up by the capillary blood vessels of the intestinal wall and passes from them into the portal vein. After a meal rich in carbohydrate the blood of the portal vein is rich in glucose, sometimes reaching twice its normal glucose content; and may show levulose and galactose as well. In the blood of the general circulation, however, only glucose is found, and this remains small in quantity — about one tenth of one per cent — even after a meal rich in carbohydrates, so that a considerable part of the carbohydrate absorbed must be stored temporarily in the liver and given up gradually to the blood in the form of glucose, thus keeping nearly constant the glucose content of the blood of the general circulation. The carbohydrate thus stored in the liver cells is deposited in the form of glycogen, which, after an abundant meal, may reach 10 per cent of the weight of the liver (or, in rare cases, an even higher figure) and may fall to nearly nothing when no carbohydrate food has been taken for some time. To a less extent the muscles store glycogen in a similar way, their glycogen contents varying from traces to about 2 per cent.

The fact that the carbohydrate stored in the liver after a meal is so largely converted into glucose and passes into the blood current before the next meal, while the glucose content of the blood remains small and nearly constant, indicates that the glucose of the blood must be quite rapidly used, and from our

present standpoint the most important question of the carbohydrate metabolism is the fate of the glucose carried to the muscles and other tissues by the blood.

Oxidation of Carbohydrate

By comparison of the arterial and venous blood, it is plain that in its passage through the muscles the blood becomes poorer in glucose and oxygen and richer in carbon dioxide, and this change is more marked when the muscle is active than when it is at rest. The oxidation of glucose in the muscles and, in fact, the normal metabolism of glucose in the body as a whole, is dependent upon a substance secreted into the blood by the "islet cells" of the pancreas (islands of Langerhans). This appears to be a typical hormone. Its chemical nature is, at the time this is written (1925), under active investigation. It has been given the name *insulin* to indicate its source. The exact mode of action of insulin is still a subject of research; but the evidence is that it plays an important part at some early stage in the metabolism of glucose, possibly causing the rearrangement of the glucose molecule into a more reactive form, which in turn may either be stored as glycogen, converted into fat, or oxidized as fuel. (See also Chapter XIII.)

It is not to be supposed that the glucose molecule, even in its most active form, is burned as such directly to carbon dioxide and water.

There is much evidence that glucose first combines with phosphoric acid and is then broken to three-carbon compounds before actual oxidation occurs.

Some lactic acid is always produced by working muscle and this has long been regarded as a possible intermediate product in the metabolism of glucose.[1] Lactic acid appears to bear

[1] It should perhaps be noted here that lactic acid plays a part not only in the metabolism of carbohydrate but of other foodstuffs as well. It may be formed, for instance, from glycerol and from certain amino acids.

important relationships both to carbohydrate metabolism and to muscle contraction. The discussion of the significance and rôle of lactic acid cannot be attempted here. It may be said, however, that in recent years much experimental evidence has accumulated in support of the view that lactic acid is not formed directly from glucose, but rather through the intervention of other three-carbon compounds, probably glyceric aldehyde or methyl glyoxal (pyruvic aldehyde) or both.

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \longrightarrow 2\ \underset{\text{Glyceric aldehyde}}{CH_2OH \cdot CHOH \cdot CHO}$$

or

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \longrightarrow 2\ \underset{\text{Methyl glyoxal (Pyruvic aldehyde)}}{CH_3 \cdot CO \cdot CHO} + 2\ H_2O$$

Both glyceric aldehyde and methyl glyoxal have been shown to result from the cleavage of glucose under the influence of alkali *in vitro* and there are doubtless enzymes in the tissues which catalyze one or both of these reactions with the result that glucose readily undergoes such cleavage as a preliminary to oxidation in the body.

Opinion is at present divided as to whether glyceric aldehyde or pyruvic aldehyde (methyl glyoxal) is to be regarded as the usual first step in the actual breakdown of glucose. In either case it is probable that the bulk of the carbohydrate material passes through the form of pyruvic aldehyde (methyl glyoxal) on its way to oxidation.

According as we assume the process to go on with or without the intermediary formation of glyceric aldehyde, the production of lactic acid from glucose in the body may be represented in either of the following ways:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \longrightarrow \underset{\text{Glyceric aldehyde}}{CH_2OH \cdot CHOH \cdot CHO} \longrightarrow \underset{\text{Pyruvic aldehyde}}{CH_3 \cdot CO \cdot CHO}$$

$$\longrightarrow \underset{\text{Lactic acid}}{CH_3 \cdot CHOH \cdot COOH}$$

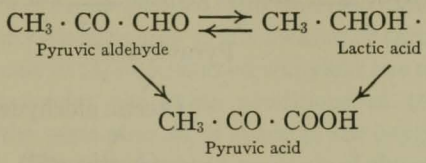
or

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \longrightarrow \underset{\text{Pyruvic aldehyde}}{CH_3 \cdot CO \cdot CHO} \longrightarrow \underset{\text{Lactic acid}}{CH_3 \cdot CHOH \cdot COOH}$$

Each of these reactions has been brought about in the laboratory by heating with alkali and at the lower alkalinity of the body the tissue enzymes are believed to catalyze the same or similar changes, the glucose probably entering into these reactions not in the free state but in combination with phosphoric acid. It has been shown that under suitable experimental conditions lactic acid is formed from glyceric aldehyde and from pyruvic aldehyde by the action of surviving liver tissue; and the further fact that in experimental diabetes glucose may be formed from glyceric or pyruvic aldehyde as well as from lactic acid tends also to confirm the belief that these aldehydes are intermediary products between glucose and lactic acid — both in normal metabolism and experimental diabetes. Glycerol also when perfused through liver tissue yields lactic acid, and since the first product of oxidation of glycerol is in all probability glyceric aldehyde, we have here a further reason for believing that the latter is a normal precursor of lactic acid. There has been no direct demonstration of the presence of glyceric aldehyde or of pyruvic aldehyde (methyl glyoxal) in the body; but this is probably due to their unstable or highly reactive nature. The view that glyceric aldehyde passes through pyruvic aldehyde in being transformed into lactic acid is not only probable on stereochemical grounds but is strongly supported by much recent evidence indicating that pyruvic aldehyde occupies a central position in the intermediary metabolism.

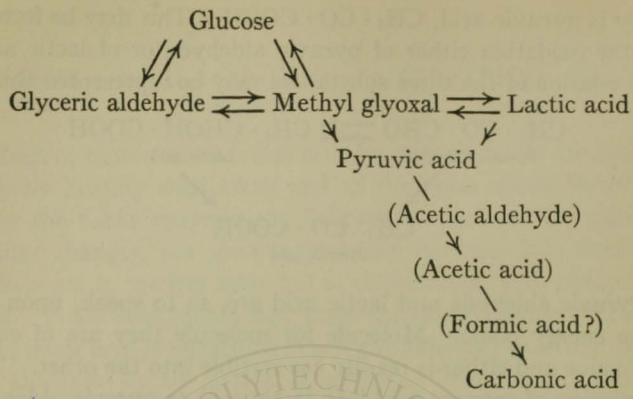
Thus far in our study of the catabolism of glucose we have considered no oxidative changes but only the cleavages and transformations which, from the standpoint of the use of glucose as fuel, may be regarded as preliminary to oxidation. Probably the first *oxidation* product to be formed from glucose in the

body is pyruvic acid, $CH_3 \cdot CO \cdot COOH$. This may be formed by the oxidation either of pyruvic aldehyde or of lactic acid. The relation of the three substances may be represented thus:



Pyruvic aldehyde and lactic acid are, so to speak, upon the same energy plane. Molecule for molecule they are of equal fuel value and either is readily convertible into the other. The conversion of pyruvic acid into lactic acid or pyruvic aldehyde probably takes place under certain conditions, but this involves reduction and so is not to be expected in the normal course of glucose oxidation. The fate of pyruvic acid under normal conditions is probably to undergo further oxidation through acetic acid to carbonic acid and water. It is possible that acetaldehyde or alcohol or both may intervene between pyruvic acid and acetic acid, and that formic acid may be produced as an intermediate step between acetic and carbonic acids.

To summarize what now appears to be the most promising theory of the intermediary metabolism of carbohydrate, we may say that the glucose is first combined with phosphoric acid and then transformed, either directly or through glyceric aldehyde, into pyruvic aldehyde (methyl glyoxal), which may either be changed to lactic acid or oxidized directly to pyruvic acid which in turn readily undergoes oxidation to carbon dioxide and water through steps not yet fully worked out. Lactic acid may also be converted into pyruvic acid and thus ultimately be completely oxidized. In case of excessive formation or inadequate oxidation, as in extreme muscular fatigue or asphyxial conditions, lactic acid may accumulate in the body or may be excreted unchanged.



Whatever the exact mechanism of the process, a large part of the glucose brought by the blood is oxidized in the muscles to furnish energy, which appears as external or internal work.

In general, the rate at which combustion takes place in the tissues depends upon the activity of the tissue cells, rather than upon the supply either of combustible matter or of oxygen. When a sufficient supply of oxygen is provided, any further increase has little effect upon the rate of combustion, and, as we have seen, a surplus of carbohydrate instead of being burned is stored as glycogen or changed into fat. But while the absorption of an abundance of carbohydrate does not greatly change the amount of combustion taking place in the body, it may result in the use of carbohydrate as fuel almost to the exclusion of fat for the time being, as is shown by observations upon the respiratory quotient.

The respiratory quotient is the quotient obtained by dividing the volume of carbon dioxide given off in respiration by the volume of oxygen consumed. That is —

$$\frac{\text{Volume of } CO_2 \text{ produced}}{\text{Volume of } O_2 \text{ consumed}} = \text{Respiratory quotient.}$$

The numerical value of this quotient will evidently depend upon the elementary composition of the materials burned. Carbohydrates will yield a quotient of 1.0 since they contain hydrogen and oxygen in proportions to form water, so that all oxygen used to burn carbohydrate goes to the making of carbon dioxide, and each molecule of O_2 so consumed will yield one molecule of CO_2, occupying (under the same conditions of temperature and pressure) the same amount of space as the oxygen consumed to produce it. Thus in burning a molecule of glucose, six molecules of oxygen are consumed and six molecules of carbon dioxide produced:

$$C_6H_{12}O_6 + 6\,O_2 \longrightarrow 6\,CO_2 + 6\,H_2O$$

Here the volumes of oxygen and of carbon dioxide are equal and the respiratory quotient is 1.0.

Fats contain much more hydrogen than can be oxidized by the oxygen present in the molecule, and therefore a part of the oxygen used to burn fat goes to form water, so that the volume of oxygen consumed is greater than the volume of carbon dioxide produced, which gives a respiratory quotient lower than 1.0. The common fats of the body and of the food give quotients approximating 0.7. Thus the oxidation of stearin is represented by the equation:

$$2\,C_{57}H_{110}O_6 + 163\,O_2 \longrightarrow 114\,CO_2 + 110\,H_2O$$

Since 163 volumes of oxygen are consumed and 114 volumes of carbon dioxide produced, the respiratory quotient is

$$\frac{114}{163} = 0.699$$

Proteins give quotients intermediate between those of carbohydrates and fats, but if the amount of protein used in the body be determined by other methods (see Chapter VIII) and allowed

for, one may then deduce from the respiratory quotient the proportions of carbohydrates and fats which are being burned in the body at any given time. The body will show a respiratory quotient of 1.0 when burning carbohydrate alone, of 0.7 when burning fat alone, and of an intermediate value when both fat and carbohydrate are being burned. If, now, the respiratory quotient rises soon after the eating of carbohydrate food, it is evident that the carbohydrate is being used more freely and fat less freely (either absolutely or relatively) than before.

In an experiment by Magnus-Levy the subject before taking food showed a quotient of 0.77. He then ate 155 grams of cane sugar, after which the quotient was determined at intervals of an hour for 7 hours with the following results: 1.01, 0.89, 0.89, 0.92, 0.82, 0.82, 0.79. The quotient here shows that within an hour after the sugar was eaten the body was making use of the carbohydrate to such an extent that fat either was not being used at all or was being formed from carbohydrate as fast as it was burned; and that for seven hours after the meal the body continued to use carbohydrate to a greater, and fat to a less, extent than was the case at the beginning of the experiment.

It has been pointed out that, when carbohydrate is absorbed in larger quantity than is required to meet the body's immediate needs for fuel, the surplus normally accumulates as glycogen, which is stored conspicuously in the liver, but also to a considerable extent in the muscles and other organs. The amount of carbohydrate which will be stored in the entire body after rest and liberal feeding is estimated at 300 to 400 grams. Thus the total amount of carbohydrate which can be stored as such in the body is no more than is frequently taken in one day's food.

When the supply of carbohydrate is so abundant that it continues in excess of the needs of the body and accumulates until the liver and muscles have no tendency to increase their store of glycogen, the further surplus of carbohydrate tends to be converted into fat.

Production of Fat from Carbohydrate

Experimental evidence of the transformation of carbohydrate into fat has been cited in Chapter II, where it was shown that animals which fatten readily on carbohydrate food may store more body fat than could possibly be derived from the fats and proteins eaten; that milch cows have yielded more fat in the milk than could be accounted for on any other assumption than that fat was formed from carbohydrate; and that there may be more carbon stored in the body from the carbohydrate food eaten by a fattening animal than can be accounted for in any other way than that a part of the carbon taken into the body as carbohydrate was retained as body fat.

Further proof of the ability of the animal body to change carbohydrate into fat is obtained from the respiratory quotient. As noted above, observations made after a fast tend to show quotients approaching that of fat, while after feeding carbohydrates the quotient may rise rapidly. If the quotient reaches 1.0, it shows that the body as a whole is using carbohydrate and not fat as fuel; and a quotient greater than 1.0 may be taken as evidence that the carbohydrate is itself supplying part of the oxygen which appears as carbon dioxide, or, in other words, that it is breaking down in such a way that a part is burned while another part goes to form in the body a substance more highly carbonaceous and having a lower respiratory quotient than the carbohydrate itself. In many cases it is certain that this substance must be mainly if not entirely fat. Respiratory quotients greater than 1.0 have been observed after liberal carbohydrate feeding in several species, including man. Each such observation furnishes evidence of a conversion of carbohydrate into fat.

The formation of fat from carbohydrate in the animal body is therefore established by four distinct lines of experimental evidence: (1) by determination of the amounts of body fat formed, (2) by determination of the milk fat produced, (3) by

observation of the amount of carbon stored, (4) by observations upon the respiratory quotient.

Chemical Steps in the Formation of Fat from Carbohydrate

While there is no doubt whatever of the ability of the animal to synthesize fat from carbohydrate, the mechanism of the process is far from clear. As expressed by Leathes, " the chemical changes involved are fascinating in their obscurity." Whatever the exact steps, the transformation of carbohydrate into fatty acid radicles must involve reduction of hydroxyl groups and condensations to form the long chains of the higher fatty acids.

We have already seen that in what we believe to be the normal course of carbohydrate catabolism there occurs, either along with or quickly following the breaking of the glucose molecule into three-carbon compounds, a reduction of certain hydroxyl groups with transfer of the oxygen so that substances such as methyl glyoxal, pyruvic acid, and lactic acid are formed. From pyruvic acid or lactic acid, acetaldehyde may be formed; two molecules of acetaldehyde may then undergo aldol condensation and the aldol be transformed (by simultaneous reduction and oxidation, or transfer of oxygen from the β to the terminal carbon) into butyric acid. Such an hypothesis is consistent with reactions observed *in vitro* and with the well-known production of butyric acid in certain bacterial fermentations of sugar and of lactic acid. Leathes favors this hypothesis and comments upon it (in part) as follows: " The biochemical significance of the synthesis of butyric acid from lactic acid and from sugar by bacteria becomes greater, however, when it is remembered that in this fermentation normal caproic acid is simultaneously formed, and as Raper showed also, though in still smaller amount, normal octoic or caprylic acid. . . . In butyric fermentation it seems that the reactions that lead to the synthesis of butyric acid may lead to the synthesis of acids of longer chains but still unbranched and containing an even number of carbon atoms, in other words,

that these acids may be produced by condensation of two, three, or four acetic aldehyde molecules. In higher organisms, plants or animals, this same condensation carried further would result, as Nencki suggested, in the formation of the series of acids with straight chains of even numbers of carbon atoms leading up to palmitic and stearic acid." Raper [1] has shown experimentally that condensation of two molecules of aldol in alkaline solution yields a straight chain product which on oxidation and reduction by laboratory methods yields normal octoic (caprylic) acid.

Smedley [2] has developed an alternative hypothesis regarding the mechanism of fatty acid synthesis from carbohydrate material, according to which the most probable starting point is a condensation of pyruvic acid with acetaldehyde followed by loss of CO_2 and molecular rearrangement.

In support of Smedley's hypothesis, it will be remembered that as an intermediary step in the metabolism of carbohydrate, pyruvic acid is probably formed in large quantities in the body, though its reactivity may prevent it from accumulating in measurable amounts. Pyruvic acid readily breaks down to acetaldehyde and carbon dioxide. It also condenses with aldehydes to form products which, under conditions similar to those existing in the body, undergo rearrangements (through simultaneous or successive oxidation and reduction) which result in the splitting out of carbon dioxide, leaving an acid of two more carbon atoms than were contained in the original aldehyde; or an aldehyde of two more carbon atoms than the original aldehyde may be formed, and this in turn react with another molecule of pyruvic acid, forming a fatty acid or aldehyde of two more carbon atoms.

Each of these hypotheses assumes as a starting point only substances which we have good reason to believe are regularly

[1] *Journal of the Chemical Society*, Vol. 91, pages 1831–1838 (1907). See also Leathes, *The Fats*, pages 106–109.

[2] *Journal of Physiology*, Vol. 45, *Proceedings*, page 26; *Biochemical Journal*, Vol. 7, page 364.

formed in carbohydrate metabolism, and both are consistent with the well-known fact that natural fats contain fatty acid radicles having all multiples of two carbon atoms from four to eighteen, but none having uneven numbers of carbon atoms in the molecule.

Fats

In digestion the fats are split into fatty acids and glycerol which, however, upon absorption are recombined into neutral fat. It is believed that this recombination occurs during the passage of these digestion products through the intestinal wall. The fat thus absorbed is taken up by the lymph vessels rather than the capillary blood vessels, and is poured with the lymph into the blood. The fat which renders the blood plasma turbid at the height of absorption will usually have passed from the blood into the tissues after a few hours.

The fat thus leaving the blood may be burned as fuel, or stored for use as fuel in the future, and a part may be transformed into tissue lipoid or enter into combination with proteins to form some of the chemically more complex substances of body tissue.

Oxidation of Fat in the Body

The fat burned as fuel serves as a source of energy for muscular work and other bodily activities. The mechanism of its oxidation is not the same as that of glucose and does not fit in so directly with current theories of the source of muscular energy; but in net result the potential energy of the fat seems to be used almost as well as that of carbohydrate. The average results of a long series of very thorough experiments by Atwater and associates indicated that, when both were fed as constituents of a normal mixed diet, the potential energy of fat was about 95 per cent as efficient as that of carbohydrate for the production of muscular work; while the corresponding estimate more recently reached by Krogh and Lindhard is 89 per cent.

The glycerol from fat is presumably oxidized to glyceric aldehyde which passes to methyl glyoxal, whose fate is doubtless the same in this case as when the same substance is formed in carbohydrate metabolism.

The fatty acid presents a separate problem. Through the work of Dakin and of Knoop and Embden the " beta-oxidation theory " has been developed and is now generally accepted. According to this theory the fatty acid is attacked by oxidation at the β-carbon atom with the probable formation first of β-hydroxy, and then of β-ketonic acids. Further oxidation at this point must then cause a separation of the α- and β-carbon atoms; thus two carbons of the original fatty acid break away, presumably to undergo complete oxidation, and there remains a fatty acid with two less carbon atoms than the original. By such a process stearic acid would yield palmitic; palmitic would yield myristic; myristic, lauric; and so on to butyric acid. Beta-oxidation of butyric acid would yield successively β-oxybutyric and acetoacetic acid. Normally the acetoacetic acid should yield two molecules of acetic which in turn should burn to carbon dioxide and water.

The sequence of changes from caproic acid to the final oxidation products would thus be as follows:

Caproic acid	β-oxy (hydroxy)	β-keto-caproic	Butyric	β-oxy-butyric	Aceto-acetic	Acetic	Carbonic
CH_3	CH_3	CH_3	CH_3	CH_3	CH_3	CH_3	CO_2
CH_2	CH_2	CH_2	CH_2	$CHOH$	CO	$COOH$	
CH_2	CH_2	CH_2	CH_2	CH_2	CH_2		
CH_2	$CHOH$	CO	$COOH$	$COOH$	$COOH$		
CH_2	CH_2	CH_2					
$COOH$	$COOH$	$COOH$					

When the normal process is interfered with or overtaxed, another reaction may occur with the formation from acetoacetic acid of carbon dioxide and acetone, which latter like acetoacetic acid and β-oxybutyric acid sometimes appears in the urine, especially in many cases of diabetes mellitus. The acidosis of diabetes is believed to be due to the β-oxybutyric acid and acetoacetic acid thus formed.

On the basis of the β-oxidation theory it has been anticipated that the diabetic should be able to utilize fatty acid radicles containing odd numbers of carbon atoms without the production of β-oxybutyric or acetoacetic acid or any corresponding residual acid and that thus a source of energy might be provided without danger of adding to the tendency toward acidosis. To what extent this will prove feasible in practice is still a matter of active investigation at the time of writing (1925). References to the work of Heft, Kahn and Gies, of Lundin, of Modern, and of Lyon, Robson and White, will be found at the end of this chapter.

Ketogenesis and Antiketogenesis. It is now generally believed that many animals, including man, are unable to oxidize the fatty acids completely to carbon dioxide and water unless there is an accompanying oxidation of glucose. It has been suggested that perhaps the four-carbon products of β-oxidation (β-hydroxybutyric acid or acetoacetic acid) may combine with a derivative of glucose to form a condensation product which is readily oxidized. It has long been known that the products of incomplete oxidation of the fatty acids, β-oxybutyric acid, acetoacetic acid, and acetone (sometimes spoken of collectively as "acetone bodies") may appear in the urine when carbohydrate is removed from the diet, during fasting or under-feeding when the glycogen stores have been depleted, and in severe cases of diabetes as a consequence of the inability of the body to oxidize glucose.

Because of their tendency to form "acetone bodies" under certain conditions, the fatty acids are said to have "ketoge-

netic" properties. Since certain amino-acids, leucine, phenylalanine, and tyrosine, have been found to produce "acetone bodies," proteins are likewise ketogenetic in the degree in which these amino acids are present in the protein molecule. On the other hand, carbohydrates, since they either contain glucose or yield it on digestion, tend to prevent the formation of "acetone bodies," and therefore are "antiketogenetic." It has been mentioned that the glycerol from fat may doubtless be converted to glucose in the course of metabolism; it will be seen later in this chapter that some of the amino acids may yield glucose in the body. Consequently both fat and protein possess antiketogenetic as well as ketogenetic properties. The relation between the total number of potential ketogenetic molecules from all sources in the diet and the total number of antiketogenetic molecules is known as the ketogenetic-antiketogenetic ratio $\left(\frac{K}{A}\right)$.

As yet, different investigators seem to attach very different degrees of importance to this ratio.

Bloor's view of the mechanism of oxidation of the fatty acid radicle contemplates its desaturation and conversion into phosphatid before β-oxidation occurs. He closes his recent (1925) review of the intermediary metabolism of fats as follows: "The mechanism of oxidation of the fatty acids as far as is known at the present time may be summed up as follows: The stable fatty acids of the food or stores are made less stable by desaturation, a process which takes place mainly if not entirely in the liver. Further reactivity is conferred by phosphorization of the glycerides. The long chains are then shortened two carbon atoms at a time by β-oxidation, probably with the aid of organic peroxides, at least to the four-carbon stage where a different type of oxidation involving the simultaneous oxidation of glucose appears to be required in most animals. The two carbon fragments are oxidized to carbon dioxide and water."

Storage of Food Fat in the Body

That fat derived from the food may be stored as body fat has already been shown (Chapter II) and need not be discussed further here. Mills[1] has found that fatty oils injected with antiseptic precautions into the subcutaneous tissue may under favorable conditions be absorbed therefrom and used in the body in the same way as if obtained by feeding. Whether fat once deposited in the tissues will remain and accumulate or be returned to the circulation and used as fuel, will depend upon the balance between the food consumption and the food requirements of the organism as a whole. In this respect, there is no difference between fat consumed and deposited as such and fat formed in the body from other food materials.

Can Carbohydrate Be Formed from Fat?

Glycerol is readily convertible into glucose in the body, probably passing through the form of glyceric aldehyde as an intermediate step; but the glycerol radicle represents only about one twentieth of the energy value of the fat molecule.

Whether carbohydrate is ever formed from fatty acid in the animal body is an open question.

As evidence of such formation of carbohydrate from fat, Hill cites observations upon hibernating animals showing increase of glycogen during sleep, accompanied by respiratory quotients lower than 0.7.

On the other hand, in severe experimental diabetes produced either by removal of the pancreas or by phlorizin poisoning,[2]

[1] *Archives of Internal Medicine*, Vol. 7, page 694 (1911).

[2] In clinical diabetes, or after removal of the pancreas in an experimental animal, the failure of the body to utilize glucose results in its accumulation in the blood in excess of the normal concentration. This condition of *hyperglycemia* in turn gives rise to the escape of the glucose through the kidneys. In these conditions, therefore, the appearance of glucose in the urine is in itself an evidence of a more than normal concentration of glucose in the blood. In phlorizin poisoning, on the other hand,

when it would seem that all material in the body capable of transformation into glucose is being thus changed, there does not appear to be a production of glucose from fat (fatty acid). As this latter type of experimentation has been extensively employed while relatively little evidence of the sort cited by Hill has been presented, the trend of opinion is rather away from the view that the animal body can form carbohydrate from fatty acid radicles, or transform fat into carbohydrate beyond the limited amount obtainable from the glyceryl radicles of the fat. It has been suggested that the low respiratory quotients above mentioned may be due to accidental fluctuations, since the blood does not always show the same carbon dioxide content. The question of actual transformation of fat into carbohydrate is not of great practical importance in normal nutrition, because under ordinary conditions fats may be used interchangeably with carbohydrates as source of energy to a very large, though not unlimited, extent.

Proteins

It is now believed that the hydrolysis of proteins to amino acids in the digestive tract is practically complete. The significance of this digestive cleavage lies not simply in the formation of more soluble and more readily diffusible substances but also in the resolution of the complex molecules of food protein into their simple amino acid "building stones" ("Bausteine") which may be very extensively rearranged by the body in the synthesis of its own tissue proteins.

the primary pathological effect is upon the kidney, rendering it abnormally permeable to glucose. As the result of this, glucose is drained away from the blood through the kidney and the appearance of glucose in the urine may be accompanied by a condition not of *hyper-* but of *hypo-glycemia*. Notwithstanding this difference, the two experimental methods of making an animal diabetic may both be used and to some extent interchangeably in studies of the intermediary metabolism, for both induce large losses of glucose from the body and thus tend to bring into glucose formation the various substances which are capable of being changed into glucose and which presumably exist in a state of approximate equilibrium with glucose in the body.

Absorption and Distribution of Protein Digestion Products

The work of the past few years, to be described in the paragraphs which follow, indicates that the amino acids, resulting from digestive hydrolysis of the food proteins, pass through the intestinal wall and into the blood of the portal vein unchanged, are carried through the liver into the blood of the general circulation and are thus distributed throughout the body, and are rapidly absorbed from the blood into the various tissues. Thus each tissue receives its protein material in the form of amino acids from which can be synthesized the particular kind of protein characteristic of the tissue in question. In other words, each tissue makes its own proteins from the amino acids brought by the blood. Amino acids not used in synthesizing protein (whether brought by the blood or formed by breakdown of tissue material) are broken down or deaminized in the manner described beyond.

A brief account of experimental work on the distribution and immediate fate of the amino acids may serve to give a more adequate impression of the modern view.

In 1906 Howell obtained a qualitative indication of amino acids in the blood, but conclusive evidence of the relation of these amino acids to metabolism required the development of better methods than were then available for the estimation of amino acid nitrogen in the fluids and tissues of the body. Such methods were developed and applied independently and almost simultaneously in 1912 by Folin and Denis and by Van Slyke and Meyer.

Folin and Denis distinguished between the nitrogen of proteins, non-proteins, ammonia, and urea. The non-protein nitrogen includes that of amino acids and they were able to show that this form of nitrogen increased in the blood and tissues when glycine or a mixture of amino acids resulting from tryptic digestion of protein was undergoing absorption from the small intestine. Moreover, the increase in the non-protein nitrogen

of the blood and muscles was nearly sufficient to account for the nitrogenous material absorbed from the intestine, from which it appeared that they had traced the absorbed amino acids and found them to be carried through the blood and to the muscles without being either built up into protein or broken down into ammonia or urea on the way. Urea formation was found to follow distinctly later than the absorption and distribution of the amino acids.

Van Slyke and Meyer estimated amino acids by quantitative determination of the nitrogen present as amino groups in the non-protein fraction of the blood or tissue. They found that, during the digestion of protein, amino acids pass through the intestinal wall and appear not only in the portal blood but also in the blood of the general circulation, showing that the amino acids, for the most part at least, pass both the intestinal wall and the liver unchanged.

Closely following the work of Folin and of Van Slyke, Rona (1912) demonstrated by experiments upon isolated segments of intestine that the amino acids pass unchanged through the intestinal wall; Abel (1913) dialyzed free amino acids from the circulating blood of living animals by means of his vivi-diffusion apparatus and actually separated alanine in crystalline form; and Abderhalden (1914) separated glycine, alanine, valine, leucine, aspartic acid, glutamic acid, lysine, arginine, histidine, and tryptophane from large quantities of shed blood. Soon afterward (1915) Henriques and Anderson showed that dogs and goats could be kept in a normal condition of nutrition and might even store nitrogen and gain weight when they were nourished exclusively by intravenous injection of a food solution containing nitrogen only in the form of completely digested protein — a strong confirmation both of the completeness of cleavage of protein in normal digestion and of the fact that the body is nourished by free amino acids carried by the blood without intervention of chemical changes in the intestinal wall.

Van Slyke (working upon dogs) continued his investigation of the fate of the amino acids and found that they are rapidly taken up from the blood by the tissues. The extent to which amino acids can be taken up by the muscles seems to have a fairly definite limit at about 75 milligrams of amino acid nitrogen per 100 grams of muscle. In the case of liver tissue this "saturation capacity" seems somewhat more elastic and the concentration may reach about twice the maximum observed in muscle, *i.e.* up to 150 milligrams of amino acid nitrogen per 100 grams of liver. In the muscles the amino acids taken up as just described disappear only very gradually and may not seem to be appreciably changed for several hours; in the liver they disappear rapidly; in the kidney, pancreas, and spleen they disappear less rapidly than in the liver.

The disappearance of the amino acids from the tissues may be due either to a building up into protein or a breaking down with the formation of ammonia and urea or both. It seems probable that in general both processes go on in all tissues, each tissue building its own proteins and each also taking part in the deaminization of amino acids with formation of ammonia or urea. The more rapid disappearance of amino acids from the liver tissue is probably due to the greater activity of the liver in deaminization and urea formation.

Van Slyke's experiments show also that the blood contains amino acids at all times and that the tissues are not freed from amino acids by fasting, while on the other hand high protein feeding does not result in any great accumulation of amino acids as such either in the blood or tissues. All these observations confirm the view that amino acids are the normal intermediary products in both the building up and breaking down of body protein and that any large storage of nitrogen in the body must be due to formation of body protein and not to mere accumulation of free amino acids.

Utilization of Protein in the Tissues

The proteins of the digested food, absorbed and distributed in the form of amino acids as described above, soon become available for nutrition; and among other functions they, like the carbohydrates and fats, may be oxidized [1] as fuel for muscular work. Pflüger proved that protein may serve as a source of muscular energy by feeding a dog for 7 months exclusively upon meat practically free from fat and carbohydrate, and requiring it throughout the experiment to do considerable amounts of work, the energy for which must in this particular case have been derived largely from the protein consumed.

The experimental facts and theoretical explanations regarding the breaking down of proteins (or of the amino acids arising from them) in the body tissues must now be considered. By experiment it has been found that if a meal extra rich in protein be eaten, an increased elimination of nitrogenous end products can be observed within 2 or 3 hours, and probably much the greater part of the surplus nitrogen will have been excreted within 24 hours of the time it was taken into the stomach. It does not follow, however, that the whole of the protein molecule is broken down and eliminated so quickly, and many experiments have shown that the carbon often does not leave the body so rapidly as does the nitrogen. Evidently, the nitrogenous radicles of the protein may be split off in such a way as to leave a non-nitrogenous residue in the body, and the study of protein metabolism involves a consideration of the fate of both the nitrogenous and the non-nitrogenous derivatives. The fate of the latter may conveniently be considered first on account of its relation to the metabolism of carbohydrates and fats. Of special interest is the problem to what extent the deaminized

[1] It will of course be understood that the protein is not supposed to be oxidized directly. Protein is split to amino acids, the amino acids deaminized, and the non-nitrogenous residues of the amino acids are oxidized. (See also Chapter XIII.)

cleavage products of protein may be actually transformed into carbohydrate or fat in the body.

Formation of Carbohydrate from Protein

As early as 1876 Wolffberg tested the formation of carbohydrate from protein by fasting fowls for two days in order to free them from glycogen and then feeding for two days with meat powder which had been washed free from carbohydrate. Two of the fowls were killed soon after this protein feeding and showed more glycogen in their livers and muscles than could be accounted for except as derived from the protein fed. Two similar fowls killed 17 and 24 hours after feeding showed much less glycogen. This formation of glycogen from protein was fully confirmed by Kulz in a long series of experiments in which the food consisted of chopped meat thoroughly extracted with warm water (Lusk).

Independent evidence of the production of carbohydrates from protein is found in the work of Seegen, who chopped and mixed the liver of a freshly killed animal and determined the amount of carbohydrate in it by analysis of a portion, while the remainder was kept at body temperature and sampled for analysis from time to time. The percentage of carbohydrate was found to increase, showing that the liver cells can form carbohydrate from their own protein substance.

The most striking evidence of the origin of carbohydrate from protein in the animal body is found in the many observations and experiments which have been made in cases of diabetes, and in experimental glycosuria produced either by administration of phlorizin or by removal of the pancreas. In such cases large amounts of carbohydrate may be given off in the form of glucose even when there is little body fat and no carbohydrate is fed. The glucose must therefore result from the metabolism of protein. In Lusk's exhaustive experiments upon dogs rendered diabetic by phlorizin, 58 per cent of the total weight of protein

broken down in the body (whether in fasting or on a meat diet) was eliminated in the form of glucose. According to Lusk: "After ingestion of protein in the normal organism this sugar becomes early available and may be burned before the nitrogen belonging to it is eliminated, or, if the sugar be formed in excess, it may be stored as glycogen in the liver and muscles for subsequent use. In this way it is obvious that at least half the energy in protein may be independent of the curve of nitrogen elimination, but may rather act as though it had been ingested in the form of carbohydrates."

The way in which the production of carbohydrate from protein may take place has received much attention. Lusk demonstrated experimentally that alanine, one of the cleavage products of all known proteins, may yield glucose abundantly in the body; and he suggested that the change might occur through the formation of lactic acid as an intermediary product, since he had already shown that lactic acid is convertible into glucose. The work of Dakin has thrown further light on the intermediate steps of this transformation. He has shown that glyoxals have been formed from α-amino and α-hydroxy acids *in vitro* — *e.g.* pyruvic aldehyde (methyl glyoxal) from alanine and lactic acid; and on the other hand α-hydroxy acids have been formed from glyoxals, both *in vivo* and *in vitro*.

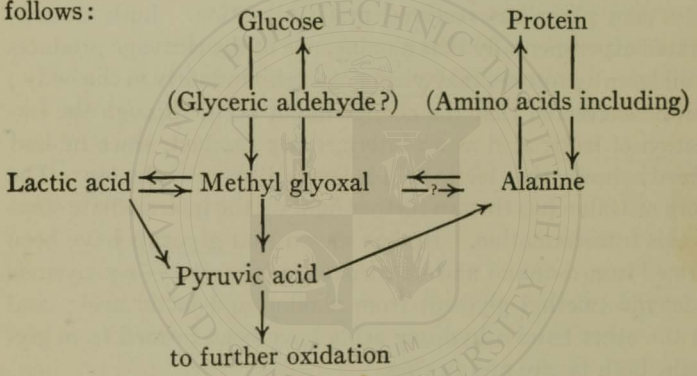
$$\underset{\text{Alanine}}{CH_3 \cdot CHNH_2 \cdot COOH} \longrightarrow \underset{\text{Methyl glyoxal}}{CH_3 \cdot CO \cdot CHO} + NH_3$$

$$\underset{\text{Lactic acid}}{CH_3 \cdot CHOH \cdot COOH} \rightleftarrows \underset{\text{Methyl glyoxal}}{CH_3 \cdot CO \cdot CHO} + H_2O$$

Attempts, however, to synthesize amino acids directly from glyoxals *in vitro* were not successful. There is some evidence of that synthesis *in vivo*, but it cannot be considered as fully established whether it takes place directly by the addition of ammonia to free glyoxals, or whether the α-amino acid is formed secondarily from the α-ketonic acid, resulting from the oxidation

of glyoxals. The work of Knoop and of Embden and Schmitz leaves no doubt of the ability of the liver cells to form amino acids from the ammonium salts of the corresponding α-ketonic acids. Alanine, phenylalanine, and tyrosine were produced in this way.[1] It is of course possible that there may have occurred, in these liver perfusion experiments, intermediate steps not recognized by the investigators, but this does not detract from the significance of the fact that the synthesis of amino acids from ammonium salts has now been repeatedly demonstrated by experiment.

The relations emphasized by Dakin may be represented as follows:



Attention may be called in passing to the possible importance of the interrelations of alanine, methyl glyoxal, and lactic acid to the regulation of neutrality, not only in the body as a whole (Chapter IX) but also in the particular cells in which deamination may be more active than oxidation. It will be noted that alanine (a nearly neutral substance) yields on deamination another neutral substance (methyl glyoxal) and a base (ammonia)

$$CH_3 \cdot CHNH_2 \cdot COOH \longrightarrow CH_3 \cdot CO \cdot CHO + NH_3$$

[1] Embden also obtained alanine after perfusion of ammonium lactate, but the lactate may have been first changed to pyruvate and the alanine formed from the latter.

And furthermore that the neutral substance methyl glyoxal may react with water to form lactic acid

$$CH_3 \cdot CO \cdot CHO + H_2O \longrightarrow CH_3 \cdot CHOH \cdot COOH$$

Experiments *in vitro* have shown that the production of lactic acid from methyl glyoxal is promptly checked unless the free acid is quickly neutralized; also that the conversion of alanine into methyl glyoxal and ammonia is accelerated by acids (Dakin).

Thus far the possible mechanism of formation of carbohydrate from protein cleavage products has been considered here chiefly in terms of alanine. To what extent is its behavior representative of that of the other amino acids?

Experiments *in vitro* show that the transformation of an α-amino acid into the corresponding α-ketonic aldehyde is a very general reaction. Dakin and Dudley demonstrated it for all the amino acids with which they worked — glycine, alanine, phenylalanine, valine, leucine, and aspartic acid.

Experiments *in vivo* (chiefly on dogs rendered diabetic by phlorizin poisoning) have shown that glycine, alanine, serine, cystine, aspartic acid, glutamic acid, arginine, and proline are all capable of yielding large amounts of glucose. Leucine, tyrosine, and phenylalanine when similarly administered to phlorizinized dogs increase the elimination of acetoacetic acid rather than glucose. Valine, lysine, and tryptophane yield neither glucose nor acetoacetic acid to any important extent (Dakin).

The amino acids which yield glucose are called glucogenetic, and the amount of glucose which a given protein can yield in the body will naturally depend upon the glucogenetic amino acid radicles which it contains. Since the amino acids resulting from protein hydrolysis cannot be quantitatively recovered by any laboratory method thus far developed, it is not yet possible to calculate just how much carbohydrate a given protein should theoretically yield. For meat protein and some others the yield has been determined experimentally as in Lusk's in-

vestigations cited above. For further discussion of this point see Lusk's *Science of Nutrition.*

We have therefore abundant evidence from the work of independent investigators, using different methods, that the animal body may form carbohydrates readily and in large proportion from the protein of the food; and the mechanism of the process is beginning to be fairly well understood.

Production of Fat from Protein

There has been much controversy regarding the formation of fat from protein in the animal body. A number of observations by Voit which were believed to demonstrate such a production of fat were subjected to vigorous criticism by Pflüger and apparently shown to be capable of other interpretations. Later experiments by Cremer in Voit's laboratory appear, however, to establish the formation of body fat from protein food beyond reasonable doubt.

Thus in one of these experiments a cat after a preliminary period of fasting was placed in a respiration apparatus and fed liberally with lean meat for eight days. The amount of protein broken down in the body was estimated from the nitrogen eliminated. The carbon eliminated was also measured, and it was found that 58.4 grams of carbon had been retained in the body. This would correspond to 130 grams of glycogen, but the total amount of glycogen in the body at the end of the experiment was only 35 grams, hence about three fourths of the carbon retained by the cat from the protein food must have been stored as body fat.

The evidence of formation of milk fat in part from protein, while perhaps not amounting to a mathematical demonstration, is very strong.

Since there is already abundant experimental evidence of the production of carbohydrate from protein and of the transformation of carbohydrate into fat, it is evident that protein food can

indirectly, if not directly, contribute to the formation of fat in the body.

The Fate of the Nitrogen in Protein Metabolism

It has already been shown that the nitrogen of the protein of food enters the circulation chiefly, if not wholly, as amino acids and is taken up as amino acids by the various body tissues. The amino acids thus obtained by the tissues from the food serve as material for the building up of body proteins; but in the breaking down of body proteins there is doubtless a liberation of amino acids of the same kinds. Amino acids from either source are subject to deaminization in the tissues, and in so far as α-amino groups are concerned the process doubtless consists chiefly in the splitting out of the nitrogen as ammonia, most of which is later changed to urea.

It is probable that the deaminization may occur, according to conditions existing at the place and time, in any of three ways as illustrated by the following reactions of alanine:

(1) $CH_3 \cdot CH(NH_2) \cdot COOH \longrightarrow NH_3 + CH_3 \cdot CO \cdot CHO$

Simple deaminization

(2) $CH_3 \cdot CH(NH_2) \cdot COOH + O \longrightarrow NH_3 + CH_3 \cdot CO \cdot COOH$

Oxidative deaminization

(3) $CH_3 \cdot CH(NH_2) \cdot COOH + H_2O \longrightarrow NH_3 + CH_3 \cdot CHOH \cdot COOH$

Hydrolytic deaminization

Nitrogen in other forms than α-amino groups may be expected to undergo a somewhat different metabolism, and it is well known that the urine always contains other nitrogen compounds in addition to ammonium salts and urea.

Much light has been thrown upon the chemistry of protein metabolism by the study of the quantitative relations existing among the different forms of nitrogen in the urine under different

conditions. For our present purpose it will be sufficient to consider only the more important of the nitrogen compounds of the urine and the relations which they are believed to bear to the processes of normal metabolism.

Urea. The proteins, on being metabolized in the body, yield varying amounts of arginine, which may undergo hydrolysis into ornithine and urea. In this way a small part of the nitrogen of protein may reach the urea stage through a series of direct cleavages. It is altogether probable, however, that much the greater part of the urea eliminated arises as follows: The protein is hydrolyzed to amino acids, which are deaminized, the nitrogen of the amino group being split out as ammonia, which with the carbonic acid constantly being produced in metabolism forms ammonium carbonate.[1] Loss of one molecule of water yields ammonium carbamate, which in turn on loss of one molecule of water yields urea.

$$(NH_4)_2CO_3 \longrightarrow NH_4CO_2NH_2 + H_2O.$$
$$NH_4CO_2NH_2 \longrightarrow CO(NH_2)_2 + H_2O.$$
urea

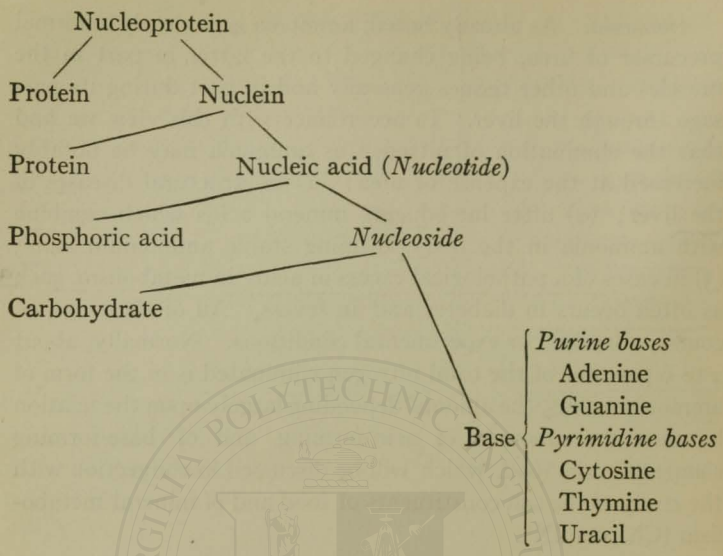
Ammonium chloride or sulphate evidently cannot be changed to urea in this way; and experiments show that if hydrochloric or sulphuric acid is introduced into the blood, it is eliminated by the kidneys largely as ammonium salt, and the quantity of urea is correspondingly decreased. In diseased conditions of the liver the organic salts of ammonia (which normally should be burned to carbonate and then converted as above) may also pass through and be eliminated without being changed to urea. In health and on a full protein diet (say about 100 grams protein per day) from 82 to 88 per cent of the total nitrogen excreted by the kidneys is usually in the form of urea. On a low protein diet this percentage is lower.

[1] If ammonium salts of organic acids are first formed, the complete oxidation of the organic acid radicle will bring this ammonia also into the form of carbonate.

Ammonia. As already noted, ammonia is evidently a normal precursor of urea, being changed to the latter in part in the muscles and other tissues generally and in part during its passage through the liver. In accordance with this view we find that the elimination of nitrogen as ammonia may be notably increased at the expense of urea: (1) in structural diseases of the liver; (2) after introducing mineral acids which combine with ammonia in the body, forming stable ammonium salts; (3) in cases of a pathological excess of acids in metabolism, such as often occurs in diabetes and in fevers. All of these are, of course, abnormal or experimental conditions. Normally, about 2 to 6 per cent of the total nitrogen eliminated is in the form of ammonium salts, the amount depending largely upon the relation between the amounts of acid-forming and of base-forming elements in the food, which will be discussed in connection with the study of the ash constituents of food and of mineral metabolism (Chapter IX).

Uric acid and the purine bases (nucleic acid metabolism). A part of the nitrogen of human urine is always in the form of uric acid and purine bases. These owe their origin either to the free purine substances of the food, such as the guanine and hypoxanthine of meat extract, or to the metabolism of nucleic acid derived from the nucleoproteins of the food or of the body tissues. The constituent groups of the nucleic acids and the order of their liberation on hydrolytic cleavage such as occurs in metabolism may be represented by the diagram on page 152 adapted from the works of Wells and of Jones.

Explanation of diagram. The distinction between nucleoproteins and nucleins is somewhat arbitrary and perhaps of doubtful value. Wells regards nucleoproteins simply as complexes containing a larger proportion of protein than is contained in nucleins. Jones prefers to discuss nuclein metabolism entirely in terms of nucleic acid in order to avoid the danger of unnecessary confusion with protein metabolism. The nucleic acids do



not contain any of the radicles found in simple proteins; they are compounds of phosphoric acid and carbohydrate with purine and pyrimidine bases in which the acid and base radicles are not linked to each other but both to the carbohydrate radicle. Phosphoric acid-carbohydrate-base chains of this sort are called *nucleotides*, and the nucleic acids containing four such chains in the molecule are, in this terminology, *tetranucleotides*. *Nucleotidases* are enzymes which split nucleic acids liberating the phosphoric acid and leaving compounds of carbohydrate with base which are collectively known as *nucleosides*. *Nucleosidases* are enzymes splitting nucleosides into their constituent carbohydrates and bases. In the case of plant nucleic acid the carbohydrate is a pentose (*d.* ribose) and the bases are adenine, guanine, cytosine, and uracil. In animal nucleic acid the carbohydrate is a hexose and the bases are adenine, guanine, cytosine, and thymine.

Lusk summarizes the hydrolysis of yeast nucleotides as follows:

Nucleotide − H_3PO_4	⟶ *Nucleoside* − *d.* ribose	⟶ *Base*
Adenylic acid	⟶ Adenosine	⟶ Adenine
Guanylic acid	⟶ Guanosine	⟶ Guanine
Cytodin-nucleotide	⟶ Cytodine	⟶ Cytosine
Uridin-nucleotide	⟶ Uridine	⟶ Uracil

And to show at a glance the characteristic cleavage products of the two types of nucleic acid:

Animal Nucleic Acid (Thymus)	Plant Nucleic Acid (Yeast)
Phosphoric acid	Phosphoric acid
Guanine	Guanine
Adenine	Adenine
Cytosine	Cytosine
Thymine	Uracil
Hexose	Pentose

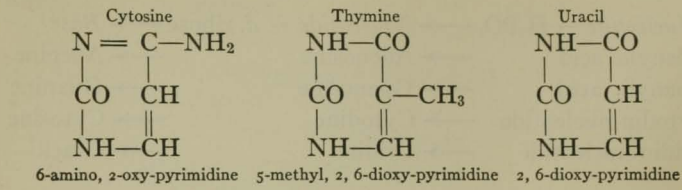
Formulae and relationships. The chemical relationships of the purine bases and uric acid so far as these are shown by empirical formulae are as follows:

Purine, $C_5H_4N_4$
Adenine, $C_5H_3N_4NH_2$, amino-purine
Guanine, $C_5H_3N_4ONH_2$, amino-oxy-purine
Hypoxanthine, $C_5H_4N_4O$, oxy-purine
Xanthine, $C_5H_4N_4O_2$, dioxy-purine
Uric acid, $C_5H_4N_4O_3$, trioxy-purine

Uric acid, the most highly oxidized of these purines, is the one chiefly found in the urine.

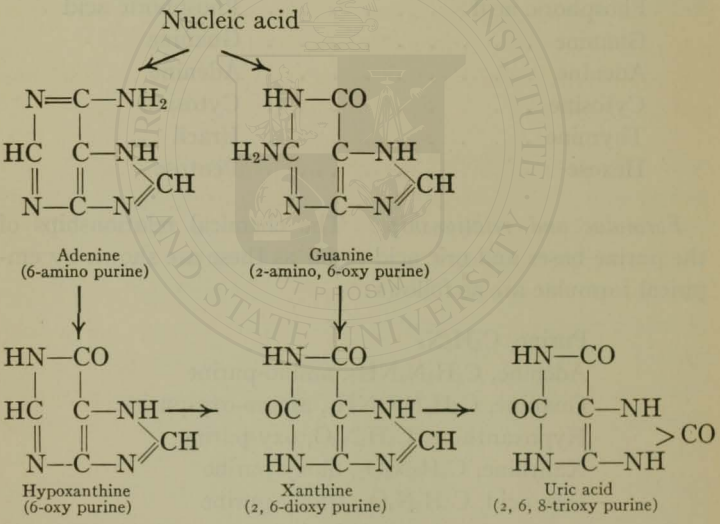
The chemical relations of these substances to each other are more fully shown by the structural formulae given on page 154.

The chemical structure of the pyrimidine bases is indicated by the following formulae:



Since these substances do not yield uric acid or purine bases their fate will not be discussed here.

The mode of origin of uric acid from nucleic acid through the purine bases is as follows:

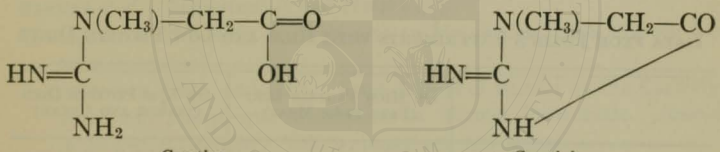


Purines undergoing metabolism in the body may be derived either (1) from the nucleoprotein of body tissue or (2) from the food which may contain both nucleoproteins and free purines. Sometimes the term " endogenous uric acid " is applied to that

fraction having the former origin, while " exogenous uric acid " indicates that fraction which is directly due to the food. The endogenous uric acid in the urine of a man of average size amounts usually to about 0.3 to 0.4 gram per day; the exogenous varies from mere traces to 2 grams or more according to the kind and amount of food consumed. On ordinary mixed diet the total urinary output of uric acid averages about 0.6 to 0.7 gram per man per day. The usual range is about 0.5 to 1.0 gram of uric acid per man per day, in which case the uric acid nitrogen constitutes about 1 to 3 per cent of the total nitrogen of the urine.

Investigations of Jones, of Levene, and others have greatly elaborated the theory of nucleic acid structure and purine metabolism outlined above. For full discussion the reader is referred to the works cited at the end of the chapter.

Creatine and creatinine. Chemically creatinine is the anhydride of creatine:



Creatine Creatinine

The biochemical relationships and physiological significance of these substances have been much discussed and the literature of the subject is far too extensive to be summarized satisfactorily here. The main facts with regard to their elimination as end products of metabolism are: that creatine appears in the urine of children normally and in that of adults during starvation, fevers, and other wasting diseases and when there is impaired functioning of the liver; that normal adults ordinarily excrete little or no creatine but a considerable amount of creatinine. The quantity of creatinine excreted is fairly constant for the individual, averaging about 0.02 gram per kilogram of body

weight per day. On ordinary mixed diet the creatinine nitrogen usually constitutes 3 to 7 per cent of the total nitrogen of the urine.

Distribution of excreted nitrogen as influenced by level of protein metabolism. The above statements regarding the distribution of the eliminated nitrogen among the different end products refer to results obtained upon an ordinary mixed diet containing an average amount of protein. Folin has shown by a careful and extended study of the urines of healthy men living first upon high and then upon low protein diets, that the distribution of the nitrogen between urea and the other nitrogenous end products depends very largely upon the absolute amount of nitrogen metabolized. In the case of a man who on one day consumed high protein diet free from meat, and a week later was living on a diet of starch and cream, which furnished in all about 6 grams of protein per day, the distribution of end products was changed as shown in the following table:

DATA FROM FOLIN'S EXPERIMENTS WITH HIGH AND LOW PROTEIN DIETS

	On High Protein Diet (Free from Meat)		On Low Protein Diet (Starch and Cream)	
	Grams	*Per cent*	*Grams*	*Per cent*
Total nitrogen	16.8		3.6	
Urea nitrogen	14.7	87.5	2.2	61.7
Ammonia nitrogen . . .	0.49	2.9	0.42	11.3
Uric acid nitrogen . . .	0.18	1.1	0.09	2.5
Creatinine nitrogen . . .	0.58	3.6	0.60	17.2
Undetermined nitrogen .	0.85	4.9	0.27	7.3

Thus, on passing from the high protein to the low protein diet (both being free from meat products) there was a marked decrease in both the absolute and the relative amounts of urea, and a decrease in the absolute, but increase in the relative, amount of uric acid, while the absolute amount of creatinine

remained unchanged, so that its relative amount was greatly increased.

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CHAPTER VI

THE FUEL VALUE OF FOOD AND THE ENERGY REQUIREMENT OF THE BODY

We have seen that, under normal conditions, carbohydrate after its absorption into the body may either be oxidized, or stored as glycogen, or transformed into fat; that fat may be oxidized or stored and that at least its glyceryl radicle may be converted into carbohydrate; and that protein absorbed as amino acids may either be built up into body protein, or deaminized and oxidized, or may yield carbohydrate, or may (either directly or indirectly) contribute to the production of fat. It has also been shown that any or all of these foodstuffs may be utilized as fuel for muscular work.

Thus the body is not restricted to the use of any one foodstuff for the support of any one kind of work, but on the contrary has very great power to convert one nutrient into, or use it in place of, another, and so to utilize its resources that the total potential energy of all of these nutrients is economically employed to support the work of all parts of the organism. The carbohydrates, fats, and proteins stand in such close mutual relations in their service to the body that for many purposes we may properly consider the food as a whole with reference to the total nutritive requirements, provided a common measure of values and requirements can be found. Since the most conspicuous nutritive requirement is that of energy for the work of the body, and since these organic nutrients all serve as fuel to yield this energy, the best basis of comparison is that of energy value.

The energy value of food is commonly and conveniently ex-

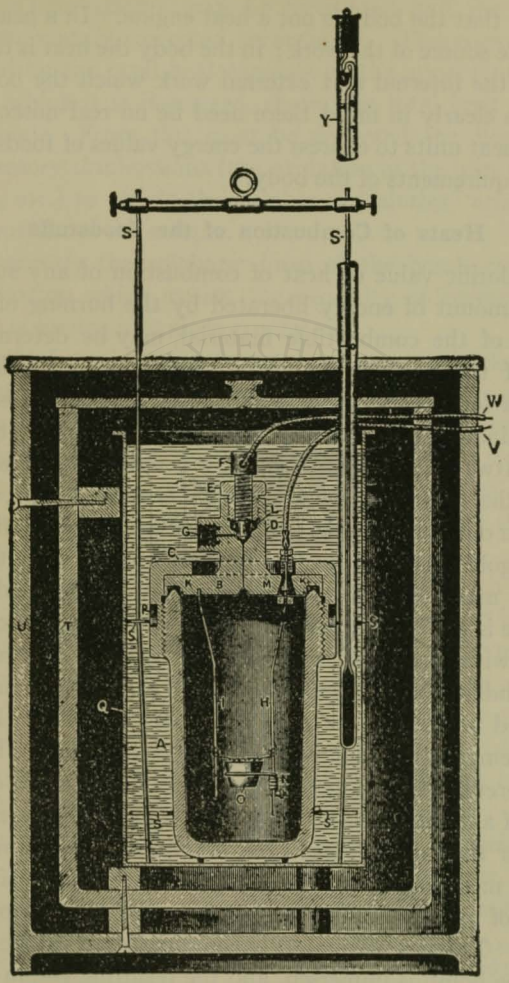

FIG. 7. — The Atwater bomb calorimeter.

pressed in terms of heat units (Calories); but it is important to realize that the body is not a heat engine. In a heat engine, heat is the source of the work; in the body the heat is rather the result of the internal and external work which the body does. With this clearly in mind there need be no real misconception in using heat units to express the energy values of foods and the energy requirements of the body.

Heats of Combustion of the Foodstuffs

The calorific value or heat of combustion of any substance, *i.e.* the amount of energy liberated by the burning of a given quantity of the combustible material, may be determined by means of the bomb calorimeter devised by Berthelot. The particular form of Berthelot bomb which has been most used in the examination of food materials and physiological products is that of Atwater and Blakeslee, fully described by Atwater and Snell in the *Journal of the American Chemical Society*, for July, 1903. In outline it consists of a heavy steel bomb with a platinum or gold-plated copper lining and a cover held tightly in place by means of a strong screw collar. A weighed amount of sample is placed in a capsule within the bomb, which is then charged with oxygen to a pressure of at least 20 atmospheres (300 pounds or more to the square inch), closed, and immersed in a weighed amount of water. The water is constantly stirred and its temperature taken at intervals of one minute by means of a differential thermometer capable of being read to one thousandth of a degree. After the rate at which the temperature of the water rises or falls has been determined, the sample is ignited by means of an electric fuse, and, on account of the large amount of oxygen present, undergoes rapid and complete combustion. The heat liberated is communicated to the water in which the bomb is immersed, and the resulting rise in temperature is accurately determined. The thermometer readings are also continued through an " after period," in order that the

" radiation correction " may be calculated and the observed rise of temperature corrected accordingly. This corrected rise, multiplied by the total heat capacity of the apparatus and the water in which it is immersed, shows the total heat liberated in the bomb. From this must be deducted the heat arising from accessory combustions (the oxidation of the iron wire used as a fuse, etc.) to obtain the number of Calories [1] arising from the combustion of the sample.

More recently the adiabatic form of the bomb calorimeter (a modification which avoids the necessity of corrections for heat loss) is coming into more general use.

The heat of combustion of organic substances is closely connected with their elementary composition. One gram of carbon burned to carbon dioxide yields 8.08 Calories and 1 gram of hydrogen burned to water yields 34.5 Calories. If a compound consisting of carbon and hydrogen only be burned, it gives nearly the amount of heat which these would give if burned separately.

On the other hand, in carbohydrates and fats, which are composed of carbon, hydrogen, and oxygen, the carbon and hydrogen are already partly oxidized by the oxygen present in the molecule; so that 100 grams of glucose, for example, containing 40 grams carbon, 6.7 grams hydrogen, and 53.3 grams oxygen, would yield considerably less heat than would be obtained by burning 40 grams of pure carbon and 6.7 grams of pure hydrogen to carbon dioxide and water respectively.

Proteins when burned in the calorimeter give off their carbon as carbon dioxide, their hydrogen as water, and their nitrogen as nitrogen gas.[2] Thus the nitrogen contributes nothing to

[1] When the term "Calorie" is used in this work it will be understood to mean the "greater calorie," or "kilogram calorie," *i.e.* the amount of heat required to raise the temperature of one kilogram of water one degree centigrade. This is very nearly the same as the heat required to raise four pounds of water one degree Fahrenheit.

[2] As a matter of fact a small part of the nitrogen is oxidized to nitric acid in the bomb calorimeter, but this is determined and its heat of formation subtracted, so that the final results are as stated above.

and takes nothing from the heat of combustion; and the latter is essentially dependent here, as in the case of carbohydrates and fats, upon the amounts of carbon and hydrogen present and the extent to which they are already combined with oxygen. A little additional heat is obtained by the burning of the small amount of sulphur present in the protein.

The relation between the elementary composition and heat of combustion will be made clearer by the following table, which includes a number of typical compounds found in the food or formed in the body.

HEATS OF COMBUSTION AND APPROXIMATE ELEMENTARY COMPOSITION OF TYPICAL COMPOUNDS

SUBSTANCE	HEAT OF COMBUSTION CALORIES PER GRAM	CARBON PER CENT	HYDROGEN PER CENT	OXYGEN PER CENT	NITROGEN PER CENT	SULPHUR PER CENT	PHOSPHORUS PER CENT
Glucose	3.75	40.0	6.7	53.3	—	—	—
Sucrose	3.96	42.1	6.4	51.5	—	—	—
Starch } Glycogen }	4.22	44.4	6.2	49.4	—	—	—
Body fat	9.60	76.5	12.0	11.5	—	—	—
Butter fat	9.30	75.0	11.7	13.3	—	—	—
Edestin	5.64	51.4	7.0	22.1	18.6	0.9	—
Legumin	5.62	51.7	7.0	22.9	18.0	0.4	—
Gliadin	5.74	52.7	6.9	21.7	17.7	1.0	—
Casein	5.85	53.1	7.0	22.5	15.8	0.8	0.8
Albumin	5.80	52.5	7.0	23.0	16.0	1.5	—
Gelatin	5.30	50.0	6.6	24.8	18.0	0.6	—
Creatinine	4.58	42.5	6.2	14.1	37.2	—	—
Urea	2.53	20.0	6.7	26.7	46.6	—	—

Since the energy used in the body is obtained from the oxidation of the same kinds of compounds which exist in food, *i.e.* from carbohydrates, fats, and proteins (or their cleavage products), we can estimate the amount of energy transformed in the body if we know the amount of each kind of foodstuff oxi-

dized. Account must, however, be taken of the completeness of the oxidation in each case.

When undergoing complete oxidation in the bomb calorimeter the foodstuffs yield the following average heats of combustion:

Carbohydrates	4.1 Calories per gram.[1]
Fats	9.45 Calories per gram.[1]
Proteins	5.65 Calories per gram.[1]

In the body, carbohydrates and fats are oxidized to the same products as in the calorimeter and so yield the same amounts of heat. Protein, however, which burns in the bomb to carbon dioxide, water, and nitrogen, yields in the body no free nitrogen, but urea and other organic nitrogen compounds which are eliminated as end products. These organic nitrogenous end products are combustible; they represent a less complete oxidation of protein in the body than takes place in the bomb. The loss of potential energy calculated on the assumption that all nitrogen left the body as urea would be about 0.9 Calories per gram of protein, but on account of the elimination of other substances of higher heat of combustion (creatinine, uric acid, etc.), the actual loss in the form of combustible end products is considerably greater and averages about 1.3 Calories for each gram of protein broken down in the body.

Hence, when the body burns material which it has previously absorbed it obtains:

From carbohydrates	4.1 Calories per gram.
From fats	9.45 Calories per gram.
From protein (5.65 − 1.30 =)	4.35 Calories per gram.

In calculating the fuel value of the food, however, allowance must be made for the fact that a part of each of the materials is lost in digestion.[2]

[1] These are weighted averages, taking account of relative amounts of different carbohydrates, different fats, and different proteins in ordinary food supplies.

[2] The expression "lost in digestion" is here used in the sense explained in Chapter IV.

The approximate averages on a mixed diet are:

Carbohydrates	2% lost, 98% absorbed.
Fats	5% lost, 95% absorbed.
Protein	8% lost, 92% absorbed.

The approximate *physiological fuel values* of the food constituents are then:

Carbohydrates	$4.1 \times 98\% = 4$.	Calories per gram.
Fats	$9.45 \times 95\% = 9$.	Calories per gram.
Protein	$4.35 \times 92\% = 4$.	Calories per gram.

The figures given by Rubner as representing the fuel values of food constituents are as follows:

Carbohydrates	4.1
Fats	9.3
Protein	4.1

These were derived from experiments with dogs fed on meat, starch, sugar, etc., and therefore do not allow for so much loss in digestion as has been found to occur with men living on ordinary mixed diet.

Fuel Value of Food in Nutrition

If the composition of a food is known, its approximate fuel value is easily computed by means of the above factors. Thus milk of about average composition contains:

Protein, 3.3 per cent; fat, 4.0 per cent; carbohydrate, 5.0 per cent.

One hundred grams of such milk will furnish in the form of protein, $(3.3 \times 4. =)$ 13.2 Calories; of fat, $(4.0 \times 9. =)$ 36.0 Calories; of carbohydrate, $(5.0 \times 4. =)$ 20.0 Calories; total for 100 grams of milk, 69.2 Calories.

Eggs contain[1] on the average, in the edible portion, 13.4 per cent protein, 10.5 per cent fat, and no appreciable amount

[1] These and all similar statements of average composition are based on Bull. 28, Office of Experiment Stations, U. S. Dept. Agriculture.

of carbohydrate. They would then furnish per 100 grams $(13.4 \times 4) + (10.5 \times 9) = 148.1$ Calories.

As sources of energy, the quantities of foods to be taken as equivalent or mutually replaceable are those which furnish equal fuel value, *e.g.* 100-Calorie portions, the weights of which may be calculated directly from the fuel values of 100 grams.

Thus, for milk — 100 grams furnish 69.2 Calories; then, if x be the number of grams which furnish 100 Calories:

$$100 : 69.2 :: x : 100;\ x = 145.^{1}$$

Similarly for eggs:

$$100 : 148 :: x : 100;\ x = 68.$$

And since the two extremes in the proportion are always the same, the weight in grams of the 100-Calorie portion may always be found by dividing 10,000 (the product of the extremes) by the number of Calories per 100 grams.

The fuel value of foods is often stated in Calories per pound. Thus in the same table (Bull. 28) from which the above figures for composition are taken, the fuel value of milk is given as 325 Calories per pound. Since 453.6 grams furnish 325 Calories,—

$$453.6 : 325 :: x : 100;\ x = 139.6,$$

the number of grams required to furnish 100 Calories. This figure is about 3 per cent less than the one found above because it is based on a fuel value computed by Rubner's factors, which are 2.5 to 3.3 per cent higher than the factors based on more recent work. (See above.)

The following figures for a few common food materials[2] are

[1] It is considered sufficiently accurate to state these quantities to the nearest whole number of grams.

[2] Arranged according to the classification used in the bulletins of the U. S. Department of Agriculture and in König's well-known reference work, *Die Chemie der Menschlichen Nahrungs- und Genussmittel*, viz. meats, fish, eggs, dairy products, grain products, sugars and starches, vegetables, fruits, nuts, oils.

based upon the more recent factors, and show the weight of the 100-Calorie portion in grams and ounces, and the distribution of the Calories between proteins, fats, and carbohydrates:

TABLE OF 100-CALORIE PORTIONS[1] OF FOOD MATERIAL BASED ON THE FACTORS — PROTEIN, 4; FAT, 9; CARBOHYDRATE, 4

FOOD MATERIAL (EDIBLE PORTION)	WEIGHT OF PORTION		DISTRIBUTION OF CALORIES		
	Grams	Ounces	In protein	In fat	In carbohydrates
Beef, free from visible fat	86	3.0	80.4	19.6	
Beef, round steak	64	2.3	54.5	45.5	
Beef, corned	33	1.3	20.9	79.1	
Ham, lean	37	1.2	29.7	70.3	
Ham, fat	19	0.7	11.1	88.9	
Bacon, smoked	16	0.6	6.7	93.3	
Codfish	143	5.0	95.0	5.0	
Salmon	49	1.7	43.3	56.7	
Eggs	67	2.3	36.1	63.9	
Milk	145	5.1	19.0	52.0	29.0
Butter	14	0.5	0.5	99.5	
Corn meal	27	1.0	9.0	11.4	79.6
Oatmeal	25	0.9	16.1	16.2	67.7
Rice	28	1.0	9.1	0.7	90.2
Wheat, "entire"	28	1.0	14.7	3.5	81.8
Wheat flour	28	1.0	11.8	2.8	85.4
Bread, white	38	1.3	14.1	4.5	81.4
Sugar	25	0.9			100.0
Asparagus	450	16.0	32.4	8.2	59.4
Beans, dried	29	1.0	26.1	4.7	69.2
Beans, string	240	8.4	22.2	6.5	71.3
Beets	216	7.4	13.8	2.0	84.2
Cabbage	317	11.1	20.3	8.6	71.1
Carrots	220	7.7	9.7	7.9	82.4
Celery	540	19.1	23.8	4.8	71.4
Corn, green or canned	99	3.2	12.2	9.8	78.0
Lettuce	523	18.4	25.2	14.1	60.7
Potatoes	120	4.2	10.5	1.2	88.3
Spinach	418	14.7	35.1	11.3	53.6
Tomatoes	438	15.5	15.7	15.7	68.6

[1] Table 1 of Appendix B shows 100-Calorie portions of a much larger number of food materials.

TABLE OF 100-CALORIE PORTIONS OF FOOD MATERIAL BASED ON THE FACTORS — PROTEIN, 4; FAT, 9; CARBOHYDRATE, 4 (*Continued*)

FOOD MATERIAL (EDIBLE PORTION)	WEIGHT OF PORTION		DISTRIBUTION OF CALORIES		
	Grams	Ounces	In protein	In fat	In carbohydrates
Turnips	253	8.9	13.2	4.6	82.2
Apples	159	5.6	2.5	7.2	90.3
Bananas	101	3.5	5.2	5.4	89.4
Currants, dried	31	1.1	3.0	4.7	92.3
Oranges	194	6.8	6.2	3.5	90.3
Peaches	242	8.5	6.8	2.2	91.0
Pineapple	232	8.2	3.7	6.3	90.0
Plums	118	4.1	4.7		95.3
Prunes, dried	33	1.2	2.8		97.2
Raisins	29	1.0	3.0	8.6	88.4
Almonds	15	0.5	13.0	76.4	10.6
Chestnuts	43	1.5	10.7	16.6	72.7
Peanuts	18	0.6	18.8	63.4	17.8
Olive Oil	11	0.4		100.0	

Since proteins and carbohydrates have the same average fuel value and the ash of food does not as a rule constitute a large percentage, the striking differences in the weights of the various foods required to furnish 100 Calories are usually referable to differences in water content or fat content or both. That beans have nearly 20 times the fuel value of celery is essentially due to the difference in moisture, while the difference in fuel value between lean beef and bacon, or between codfish and salmon, is chiefly a matter of fat content. Meat free from fat is about three fourths water and one fourth protein, and so has a fuel value of about one Calorie per gram, while clear fat has a fuel value about nine times as great.

Fuel values of meats as given in the standard tables are apt to be somewhat misleading, inasmuch as they allow for all the fat ordinarily found on the various cuts as taken from the animal, whereas in many cases a considerable part of this fat is trimmed off by the butcher and treated as a by-product; and often much of the remaining fat is removed either in the kitchen or at the

table. If a pound of steak consists of 14 ounces of clear lean, and 2 ounces of clear fat, and the fat is not eaten, at least half of the total fuel value of the pound of steak is lost.

Many vegetables are more watery than lean meats and so contrast even more strikingly with the fats. An ounce of clear fat pork is equal in fuel value to about two pounds of cabbage; an ounce of olive oil to over three pounds of lettuce.

In connection with such comparisons of fuel value, however, it should be emphasized that the fuel value of a food, while of primary importance, is not alone a complete measure of its nutritive value.

Energy Requirement in Metabolism — Methods of Study and Amounts Required for Maintenance at Rest

We know definitely from accurate experiments that the " physiological fuel values " which have been deduced represent the energy which is actually obtained by the body from the food and which appears as muscular work or as heat; and we have every reason to suppose that under ordinary conditions the carbohydrates, fats, and proteins each supply the body with the kinds of energy needed for its maintenance and for its work, approximately in proportion to their fuel values as calculated above. We do not now believe that any one nutrient is used to the exclusion of others as a source of energy for any particular function, nor indeed that the body makes any particular distinction between the foodstuffs as sources of energy. The fuel value of the diet as a whole is utilized to meet the energy requirements of the whole body. For the present, therefore, it is the fuel value of the day's dietary which we have to consider rather than the distribution of this as regards protein, fat, and carbohydrate.

The total food (or energy) requirement is best expressed in Calories per day, either for the whole body or per kilogram of body weight, and for convenience of discussion it is usually

assumed that the average body weight (without clothing) is for men 70 kilograms (154 pounds) and for women eight tenths as much, 56 kilograms (123 pounds).

There are four important methods of studying the food requirements of man:[1]

1. By observing the amount of food consumed (dietary studies).

2. By observing the amount of oxygen consumed — preferably also the respiratory quotient (respiration experiments).

3. By determining the balance of intake and output (carbon and nitrogen metabolism experiments).

4. By direct measurement of heat given off by the body (calorimeter experiments).

Dietary Studies. Most dietary studies give little more than a general indication of the food habits of the people studied; but in cases where persons have maintained for a long time the same dietary habits and other conditions of life, and the body weight has remained practically constant, it may be fairly safe to assume that the food has furnished just about the right amount of energy for the maintenance of the body under the observed conditions.

Great care must be taken in drawing inferences from the body weight because of the readiness with which the body gains or loses moisture. Athletes often lose 2 or 3 pounds in an hour of vigorous exercise and regain it in less than a day. Gain or loss of body weight during short periods, therefore, does not by any means necessarily imply a corresponding gain or loss of fat. The body may lose fat and at the same time maintain its weight through gaining water, or *vice versa*. When, however, the weight remains nearly the same for months at a time, it may usually be assumed that there is no important gain or loss of tissue and that

[1] For an account of the historical development of the principles which underlie the measurement of metabolism, see the introductory chapter of Lusk's *Elements of the Science of Nutrition*.

the body is receiving just about the proper amount of total food for its needs. Under these conditions an accurate observation of the food consumed may give valuable indications as to the actual food requirement. Of such dietary studies perhaps the most useful individual example is that of Neumann, who reduced his diet to what appeared to be just about sufficient for his needs and then recorded all food and drink taken during a period of 10 months in which the body weight remained nearly constant. The average daily food furnished:[1]

Nutrients	Factors	Calories	Total Calories per Day
Protein	66.1 grams × 4. =	264.4	2242
Fat	83.5 grams × 9. =	751.5	
Carbohydrate *	306.5 grams × 4. =	1226.0	

* Including some alcohol (taken in the form of beer), which is estimated as equivalent in fuel value to 1.75 times its weight of carbohydrates.

The 2242 Calories per day were evidently fully sufficient to meet the energy requirements of this man, whose weight was 66.5 to 67 kilograms (about 147 pounds) and who was engaged at his usual (mainly sedentary) professional work in the Hygienic Institute at Kiel.

Later, when his weight had increased to 71.5 kilograms (157 pounds) as the result of following for a time a more liberal diet (furnishing about 2600 Calories per day), he again observed his dietary while taking what was supposed to be an amount of food sufficient for the maintenance of the body and no more. This second dietary study was continued for 8 months, during which the average daily food consumption was found to be:

Nutrients	Factors	Calories	Total Calories per Day
Protein	76.2 grams × 4. =	304.8	2000
Fat	109.0 grams × 9. =	981.0	
Carbohydrates †	178.6 grams × 4. =	714.4	

† Including some alcohol (taken in the form of beer), which is estimated as equivalent in fuel value to 1.75 times its weight of carbohydrates.

The body weight remained nearly constant.

[1] The data are taken from Chittenden's *Nutrition of Man*, page 286.

These results indicate that this subject, a man of average size, living a normal professional life involving no manual labor in the ordinary sense, but not excluding such muscular movements as are naturally incidental to a sedentary occupation, found his

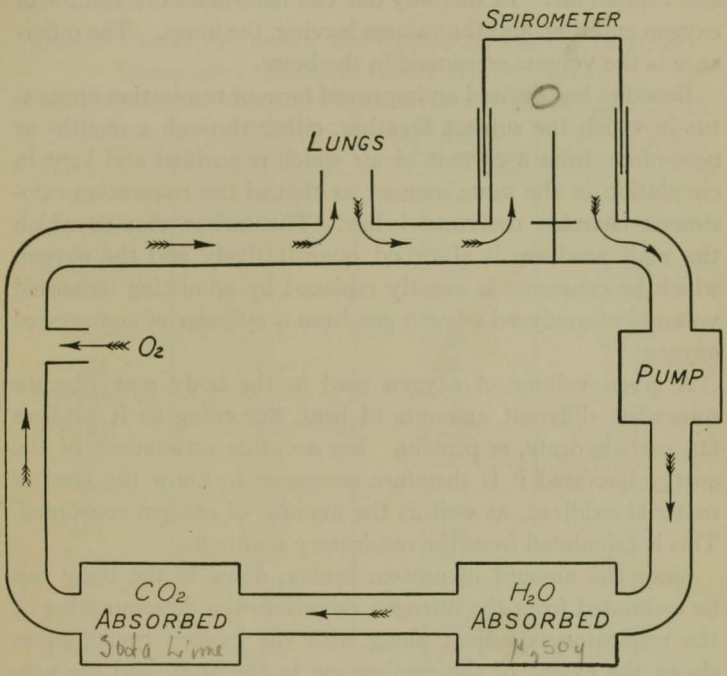

FIG. 8. — Diagram of Benedict respiration apparatus. Courtesy of Dr. F. G. Benedict.

energy requirements satisfied with food furnishing 2000 to 2250 Calories per day.

Respiration experiments. Since the foodstuffs yield their energy through being oxidized in the body, it is evident that a measure of the energy metabolism can be obtained by finding either the amount of foodstuffs oxidized or the amount of oxy-

gen which is consumed in the process. The apparatus devised and used by Zuntz for this purpose provides a mask, fitting air-tight over the mouth and nose and connected by means of valved pipes with apparatus for measuring and analyzing the inspired and expired air. In this way one can determine the volume of oxygen entering, and the volume leaving, the lungs. The difference is the volume consumed in the body.

Benedict has devised an improved form of respiration apparatus in which the subject breathes, either through a mouth- or nose-piece, from a current of air which is purified and kept in circulation in the same manner as that of the respiration calorimeter chamber described below. The carbon dioxide which the man produces is absorbed quantitatively and the oxygen which he consumes is exactly replaced by admitting measured volumes of analyzed oxygen gas from a cylinder of compressed oxygen.

A given volume of oxygen used in the body may liberate somewhat different amounts of heat, according as it oxidizes fat, carbohydrate, or protein. For accurate estimations of the energy liberated it is therefore necessary to know the kind of material oxidized, as well as the amount of oxygen consumed. This is calculated from the respiratory quotient.

Since the amount of protein broken down in the body can be estimated from the nitrogen excretion, the determination of the respiratory quotient along with the oxygen consumption shows the extent of the combustion in the body and the proportions of fat and carbohydrate burned.[1] From these data the energy can be calculated.

As a matter of fact it is not necessary to go through the actual calculation of the amounts of fat and carbohydrate burned

[1] Or, with very little error, it may be assumed that 15 per cent of the oxygen goes to burn protein and the rest is divided between fat and carbohydrate. The values given in the table herewith agree with this assumption. Attention should be called to the fact that estimates of energy metabolism based on carbon dioxide production alone involve larger errors than those based on oxygen consumption alone.

since the energy derived from a liter of oxygen when used to burn carbohydrate and fat in different proportions can be calculated once for all and expressed in relation to the respiratory quotient as shown in the accompanying table.

ENERGY VALUES OF OXYGEN AND CARBON DIOXIDE AT DIFFERENT RESPIRATORY QUOTIENTS (ZUNTZ AND SCHUMBERG)

RESPIRATORY QUOTIENT	CALORIES PER LITER OF OXYGEN	CALORIES PER LITER OF CARBON DIOXIDE	CALORIES PER GRAM OF CARBON DIOXIDE
0.70	4.686	6.694	3.408
0.71	4.690	6.606	3.363
0.72	4.702	6.531	3.325
0.73	4.714	6.458	3.288
0.74	4.727	6.388	3.252
0.75	4.739	6.319	3.217
0.76	4.752	6.253	3.183
0.77	4.764	6.187	3.150
0.78	4.776	6.123	3.117
0.79	4.789	6.062	3.086
0.80	4.801	6.001	3.055
0.81	4.813	5.942	3.025
0.82	4.825	5.884	2.996
0.83	4.838	5.829	2.967
0.84	4.850	5.774	2.939
0.85	4.863	5.721	2.912
0.86	4.875	5.669	2.886
0.87	4.887	5.617	2.860
0.88	4.900	5.568	2.835
0.89	4.912	5.519	2.810
0.90	4.924	5.471	2.785
0.91	4.936	5.424	2.761
0.92	4.948	5.378	2.738
0.93	4.960	5.333	2.715
0.94	4.973	5.290	2.693
0.95	4.985	5.247	2.671
0.96	4.997	5.205	2.650
0.97	5.010	5.165	2.629
0.98	5.022	5.124	2.609
0.99	5.034	5.085	2.589
1.00	5.047	5.047	2.569

It is then only necessary to determine the respiratory quotient and the volume of oxygen used in order to know the number of Calories of energy metabolized. This is sometimes called the *method of indirect calorimetry.*

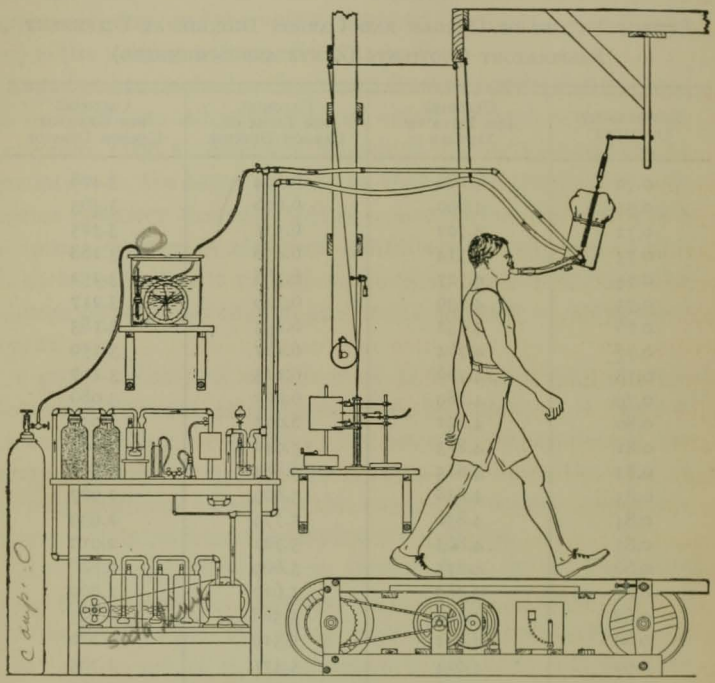

FIG. 9. — General view of apparatus used in walking experiments, with subject in position. Courtesy of Dr. F. G. Benedict, and the Carnegie Institution of Washington.

One form of apparatus is shown in Fig. 9. For descriptions of other, including simpler, forms of respiration apparatus. See DuBois's *Basal Metabolism in Health and Disease.*

This method of studying the total metabolism permits of experiments being carried out very quickly, and is therefore

especially useful for the direct investigation of conditions which affect metabolism promptly, such as muscular work or the eating of food. The periods of observation cannot be very long, but the probable results for the 24 hours' metabolism can be estimated by the data obtained during frequent short periods at different times of the day and night. For a critical comparison of this method with the Pettenkofer and Voit method of studying metabolism by the determination of the carbon balance, the reader is referred to the discussion by Magnus-Levy in Von Noorden's *Metabolism and Practical Medicine*, Vol. I, pages 186–198.

From the results of many observations by the Zuntz method Magnus-Levy estimates the minimum metabolism of a man of average size kept absolutely motionless and fasting at 1625 Calories per day. Food barely sufficient for maintenance would increase this by 175, and such incidental muscular movements as would ordinarily be made by a man at rest in bed would involve another 200, making a total of 2000 Calories as the estimated food requirement of a man at rest with a maintenance diet. Magnus-Levy further estimates that the man, if doing no work (in the ordinary sense), but allowed to move about the room instead of remaining in bed, would require 2230 Calories per day.

Carbon and nitrogen balance experiments. From a comparison of the constituents of the food consumed ("intake") and of the substances eliminated from the body ("output"), the material actually oxidized and the energy liberated in the oxidation may be determined.

The intake is found by weighing and analyzing all food eaten; the output by collecting and determining the end products eliminated through the lungs, the kidneys, the intestines, and sometimes (in very exact experiments) the skin. The time unit in experiments upon the intake and output is almost always 24 hours, the experimental day beginning preferably

just before breakfast. The feces belonging to the experimental days are marked, usually by giving a small amount of lampblack with the food as in ordinary digestion experiments, separated and analyzed. The end products given off by the lungs and kidneys during an experimental day are taken as measuring the material broken down in the body during the same period.

Some time is of course required for the elimination of the nitrogenous end products through the kidneys. This unavoidable " lag " in the elimination of nitrogen may introduce an error in determining the nitrogen balance unless the subject has been kept for a few days in advance upon the same diet which is to be used in the experiment.

Assuming that the total nitrogen and carbon of the absorbed food existed in the form of protein, fat, and carbohydrate, and that the amount of carbohydrates in the body is constant from day to day, it is only necessary to determine the carbon dioxide of the expired air and the carbon and nitrogen of the waste products, in order to calculate the amounts of material oxidized and of energy liberated in the body. Experiments of this sort have played a most important part in the development of our knowledge of nutrition. The calculations are usually based on the following average analyses of protein and body fat:

	Protein	Fat
Carbon	53	76.5
Nitrogen	16	—
Hydrogen	7	12
Oxygen	23	11.5
Sulphur	1	—
	100	100

The following data were obtained with a man on ordinary mixed diet.

CALCULATION OF ENERGY METABOLISM FROM CARBON AND NITROGEN BALANCE. MAN OF 64 KILOGRAMS AT REST IN ATWATER RESPIRATION APPARATUS

INTAKE	GRAMS PER DAY				
	Protein	Fat	Carbohydrate	Nitrogen	Carbon
Total in food . . .	94.4	82.5	289.8	15.1	239.0
Lost in digestion .	5.4	3.7	3.2	0.9	7.4
Absorbed	89.0	78.8	286.6	14.2	231.6
OUTPUT					
By lungs					207.3
By kidneys				16.2	12.2
Metabolized				16.2	219.5
Balance				− 2.0	+ 12.1

A loss of 2.0 grams body nitrogen indicates (2.0 × 6.25 =) 12.5 grams body protein burned. Also there were 89.0 grams absorbed from food, and, therefore, in all 101.5 grams total protein burned.

Since the respiratory quotient showed that the body was in carbohydrate equilibrium at the beginning and end of each experimental day, *i.e.* at seven o'clock each morning, it may be concluded that the amount of carbohydrate burned was the same as that absorbed from the food, viz. 286.6 grams per day.

From the carbon balance, therefore, we estimate the amount of fat burned as follows:

12.5 grams body protein yield (12.5 × 53 per cent =)	6.6 grams carbon
and there were in the absorbed food	231.6 grams carbon
∴ total available was	238.2 grams carbon
But total catabolized was only	219.5 grams carbon
∴ the body stored in the form of fat	18.7 grams carbon

Since fat contains 76.5 per cent carbon, 1 gram carbon ≏ 1.307 grams fat. ∴ 18.7 grams carbon = 24.4 grams fat.

The body therefore absorbed 78.8 grams fat
stored 24.4 grams fat
burned 54.4 grams fat

In all the body burned per day

101.5 grams protein, yielding	(101.5 × 4.35[1] =)	442 Calories
54.4 grams fat, yielding	(54.4 × 9.45[1] =)	515 Calories
286.6 grams carbohydrate, yielding	(286.6 × 4.1[1] =)	1175 Calories
		2132 Calories

By means of the carbon and nitrogen balance Sonden and Tigerstedt studied the energy metabolism of eight resting men between nineteen and forty-four years of age, with results which varied for the different subjects from 1853 to 2292 Calories per day. Many other experimenters have used the same method with similar results.

Calorimeter experiments. The most direct, and in some respects most convincing, way of ascertaining the energy metabolism is by the *method of direct calorimetry*. This consists in measuring the total energy expenditure of the body as heat or as heat and mechanical work by confining the subject in a chamber permitting of actual measurement of the heat produced. It was not until the development of the Atwater-Rosa-Benedict respiration calorimeter that complete and satisfactory data covering periods of one to several days were obtained. This apparatus consisted of an air-tight copper chamber, surrounded by zinc and wooden walls with air-spaces between, and was large enough for a man to live in without discomfort, being about 7 feet long, 4 feet wide, and 6½ feet high. An opening in the front of the apparatus, which was sealed during an experiment, served as both door and window, admitting sufficient light for reading and writing. A smaller opening, having tightly fitting caps on

[1] Here the factors for fuel value are not reduced to allow for loss in digestion, because this loss has already been deducted in computing the amount of each nutrient actually absorbed and rendered available.

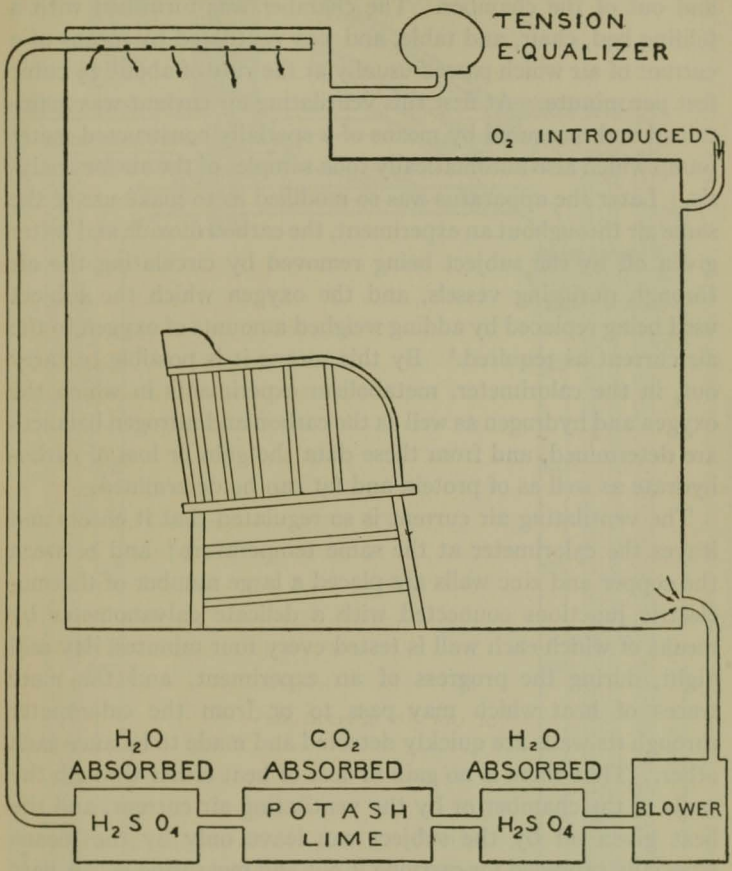

FIG. 10. — Diagram of ventilation of respiration calorimeter. The air is taken out at lower right-hand corner and forced by the blower through the apparatus for absorbing water and carbon dioxide. It returns to the calorimeter at the top. Oxygen can be introduced into the chamber itself as need is shown by the tension equalizer. Courtesy of Dr. F. G. Benedict and the Carnegie Institution of Washington.

both ends, was used for passing food, drink, excreta, etc., into and out of the chamber. The chamber was furnished with a folding bed, chair, and table, and was ventilated by means of a current of air which passed usually at the rate of about $2\frac{1}{2}$ cubic feet per minute. At first this ventilating air current was maintained and measured by means of a specially constructed meter pump which also automatically took samples of the air for analysis. Later the apparatus was so modified as to make use of the same air throughout an experiment, the carbon dioxide and water given off by the subject being removed by circulating the air through purifying vessels, and the oxygen which the subject used being replaced by adding weighed amounts of oxygen to the air current as required.[1] By this means it is possible to carry out, in the calorimeter, metabolism experiments in which the oxygen and hydrogen as well as the carbon and nitrogen balances are determined, and from these data the gain or loss of carbohydrate as well as of protein and fat can be determined.

The ventilating air current is so regulated that it enters and leaves the calorimeter at the same temperature; and between the copper and zinc walls are placed a large number of thermoelectric junctions connected with a delicate galvanometer by means of which each wall is tested every four minutes, day and night, during the progress of an experiment, and the mere traces of heat which may pass to or from the calorimeter through its walls are quickly detected and made to balance each other. Thus there is no gain or loss of heat either through the walls of the chamber or by the ventilating air current, and the heat given off by the subject can leave only by the means especially provided for carrying it out and measuring it. A part of the heat liberated is carried from the chamber in latent form by the water vapor in the outgoing air, which is accurately determined. The rest of the heat is brought away by means of

[1] Fig. 10 indicates diagrammatically the ventilating system as applied in one of the later forms of apparatus.

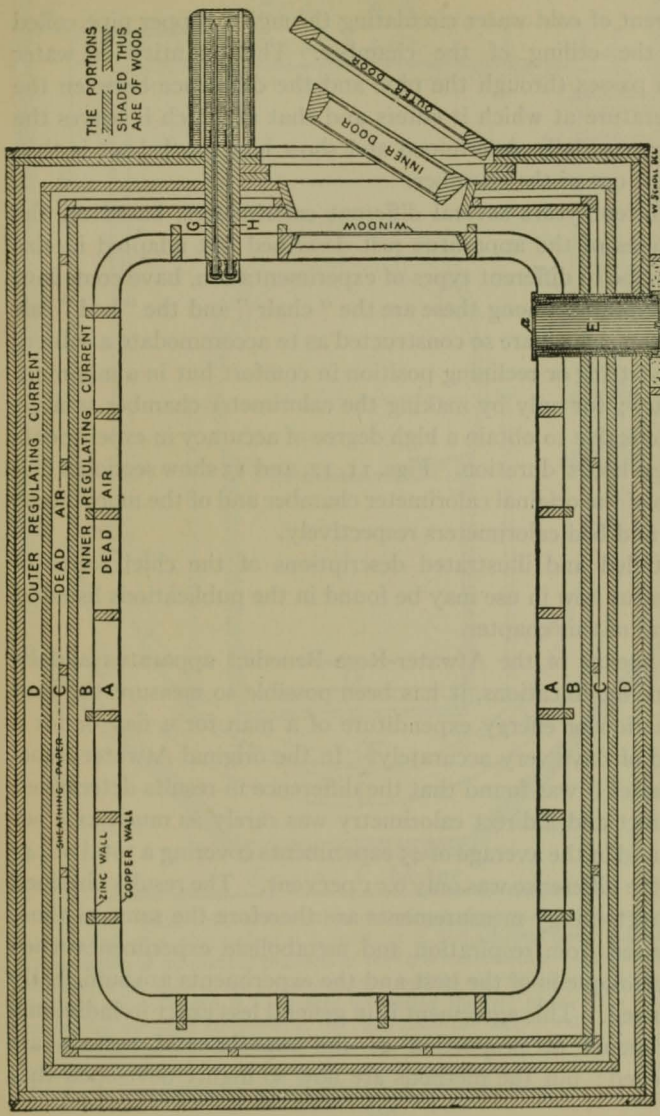

FIG. 11. — Horizontal section of the original Atwater-Rosa-Benedict respiration calorimeter. Courtesy of the United States Department of Agriculture.

a current of cold water circulating through a copper pipe coiled near the ceiling of the chamber. The quantity of water which passes through the pipe and the difference between the temperature at which it enters and that at which it leaves the coil are carefully determined and show how much heat is thus brought out of the chamber.

In recent years several different calorimeters, based on the principles of the apparatus just described but adapted in size and shape to different types of experimentation, have come into use. Notable among these are the " chair " and the " bed " calorimeters, which are so constructed as to accommodate a subject in the sitting or reclining position in comfort but in a minimum of space; for only by making the calorimeter chamber small is it practicable to obtain a high degree of accuracy in experiments of a few hours' duration. Figs. 11, 12, and 13 show sectional diagrams of the original calorimeter chamber and of the more recent chair and bed calorimeters respectively.

Detailed and illustrated descriptions of the chief forms of apparatus now in use may be found in the publications listed at the end of this chapter.

By means of the Atwater-Rosa-Benedict apparatus and its various modifications, it has been possible to measure the heat production or energy expenditure of a man for a day or for a period of days very accurately. In the original Atwater-Benedict series it was found that the difference in results determined by direct and indirect calorimetry was rarely as much as 2 per cent, and in the average of 45 experiments covering a total of 143 days the difference was only 0.01 per cent. The results obtained by direct energy measurements are therefore the same as those computed from respiration and metabolism experiments when the technique is of the best and the experiments are sufficiently prolonged. This agreement is in general less exact in individual experiments in proportion as the experimental periods are shortened; but the methods are now so highly developed that

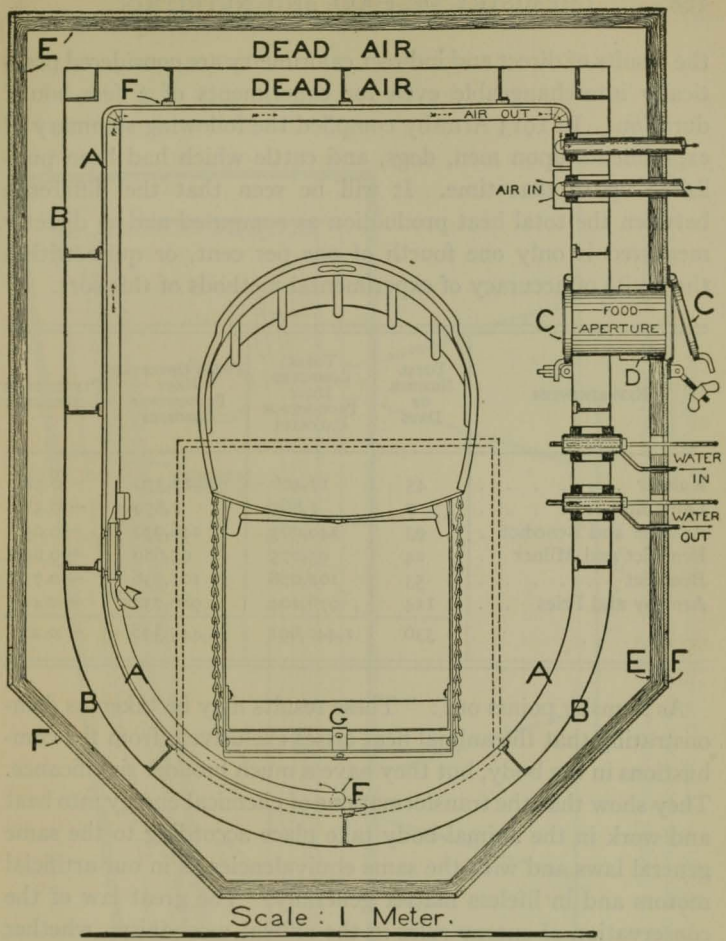

FIG. 12. — Horizontal cross-section of chair calorimeter, showing cross-section of copper wall at *A*, zinc wall at *B*, hair-felt at *E*, and asbestos outer wall at *F*; also cross-section of all upright channels in the steel-construction. At the right is the location of the ingoing and outgoing water and the thermometers. At *C* is shown the food aperture, and *D* is a gasket separating the two parts. The ingoing and outcoming air-pipes are shown at the right inside the copper wall. The telephone is shown at the left, and in the center of the drawing is the chair with its foot-rest, *G*. In dotted line is shown the opening where the man enters. Courtesy of Dr. F. G. Benedict and the Carnegie Institution of Washington.

the results of direct and indirect calorimetry are considered practically interchangeable even for experiments of a few hours' duration. In 1913 Armsby compiled the following summary of experiments upon men, dogs, and cattle which had been published up to that time. It will be seen that the difference between the total heat production as computed and as directly measured is only one fourth of one per cent, or quite within the limits of accuracy of experimental methods of this sort.

Experimenter	Total Number of Days	Total Computed Heat Production Calories	Total Observed Heat Production Calories	Percentage Difference
Rubner	45	17,406	17,350	− 0.32
Laulanié	7	1,865	1,859	− 0.31
Atwater and Benedict .	93	249,063	248,930	− 0.05
Benedict and Milner .	24	95,075	95,689	+ 0.65
Benedict	53	102,078	101,336	− 0.73
Armsby and Fries . .	114	976,204	980,234	+ 0.41
	336	1,441,691	1,445,398	+ 0.26

As Armsby points out: "These results may be taken as demonstrating that the animal heat arises exclusively from the combustions in the body, but they have a much broader significance. They show that the transformations of chemical energy into heat and work in the animal body take place according to the same general laws and with the same equivalencies as in our artificial motors and in lifeless matter generally. The great law of the conservation of energy rules in the animal mechanism, whether in man, carnivora, or herbivora, just as in the engine. The body neither manufactures nor destroys energy. All that it gives out it gets from its food, and all that is supplied in its food is sooner or later recovered in some form."

Since the time of Armsby's compilation the agreement between the observed and computed heat production has been

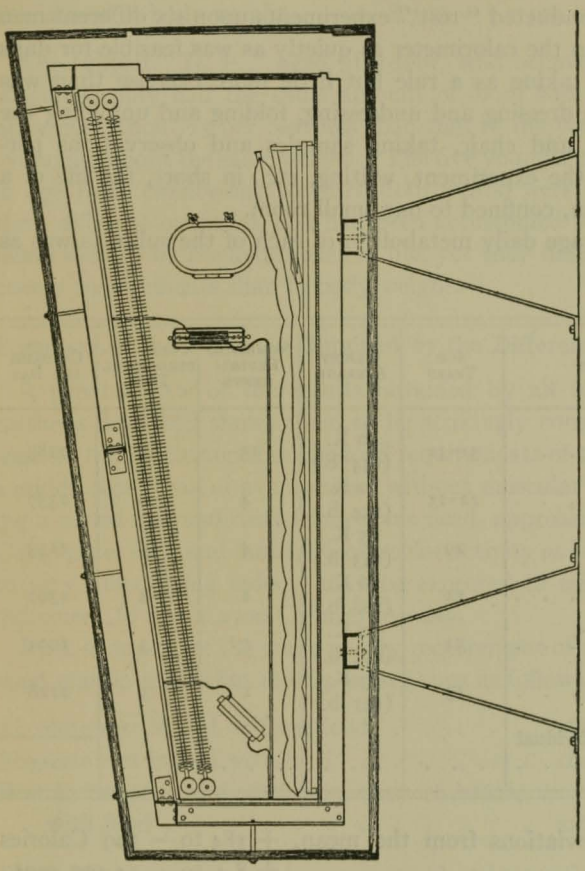

FIG. 13. — Vertical cross-section of bed calorimeter, showing parts of steel construction, also copper and zinc walls, food aperture, and wall and air-resistance thermometers, and heat absorbers. Courtesy of Dr. F. G. Benedict and the Carnegie Institution of Washington.

confirmed in many additional experiments, and both by the same and different experimenters.

Working with the original Atwater calorimeter, Atwater and Benedict conducted " rest " experiments upon six different men who lived in the calorimeter as quietly as was feasible for days at a time, taking as a rule but little more exercise than was involved in dressing and undressing, folding and unfolding the bed, table, and chair, taking samples and observations pertaining to the experiment, writing, etc., in short, the life of a healthy man, confined to one small room.

The average daily metabolism of each of the subjects was as follows:

Subject	Age Years	Weight Average	Number of Experiments	Total Experimental Days	Calories per Day
E. O.	31–34	70 K. (154 lb.)	13	42	2283
A. W. S.	22–25	70 K. (154 lb.)	4	9	2337
J. F. S.	29	65 K. (143 lb.)	4	12	2133
J. C. W.	21	76 K. (168 lb.)	1	4	2397
H. F.	54	70 K. (154 lb.)	1	3	1904
B. F. D.	23	67 K. (147 lb.)	1	3	2228
Mean of individual averages . . .	. . .	. . .	. . .	. . .	2213

Extreme deviations from the mean, + 184 to − 309 Calories
or + 8.4 to − 14 per cent.

Omitting the results obtained with the one subject who was considerably older than the others, the figures become as follows:

Mean of individual averages, 2277 Calories.
Extreme deviations from mean, + 120 to − 144 Calories,
or + 5.2 to − 6.3 per cent.
Deviations in body weight, + 8.7 to − 7.1 per cent.

The subject "H. F.," aged fifty-four, who believed that he consumed only half the usual amount of food, had a food requirement about 15 per cent less than that of the younger men averaging about the same weight. The five younger men varied in age from twenty-one to thirty-four years, were natives of three different countries, and had been accustomed to very different dietary habits and modes of life, yet they differed less in energy requirements than in body weight.

Summary of the Evidence Obtained by the Different Methods

A general view of the results obtained by all four of the methods described shows them to be strikingly consistent and leads to the conclusion that the food requirements of a young to a middle-aged man of average size, without muscular work, eating a mixed diet sufficient to meet his need, approximates 2000 Calories per day, and that such muscular activity as is incidental to very quiet living indoors may be expected to raise this requirement to about 2200 Calories per day.

Lusk summarizes the mean energy requirement of an average sized man in somewhat more precise terms as follows:

Absolute rest in bed without food . . . . . 1680 Calories
Absolute rest in bed with food . . . . . 1840 Calories
Rest in bed, 8 hours, sitting in chair 16 hours,
with food . . . . . 2168 Calories

The very close agreement in results reached by many independent investigators, using four distinct methods of study, must be taken as establishing the approximate average food requirement of a man at rest beyond any reasonable doubt.

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CHAPTER VII

CONDITIONS GOVERNING ENERGY METABOLISM AND TOTAL FOOD REQUIREMENT

ACTIVITY, age, and size are the most important factors affecting the rate of transformation of energy in the healthy body. The kind and amount of food consumed also measurably affect the energy metabolism.

The term " basal " metabolism is used to designate the rate of energy metabolism of the body when at complete rest in a room of ordinary comfortable temperature, and when the observations are made in the so-called " post-absorptive state," *i.e.* 12 to 18 hours after the last intake of food.

This basal rate of energy metabolism for a given age and size is much used as a starting point for the calculation of total energy requirements of people of different muscular activities, and also as a basis of comparison in the investigation of disease. " It is sometimes spoken of as the overhead charge or the cost in Calories of running the machine when no external work is being done. It is the amount of energy required for sheer maintenance of the organism." (MacLeod.)

Basal Energy Metabolism

More observations have been made upon persons from 20 to 40 years old than upon either younger or older subjects. Hence our knowledge of the fundamental energy expenditure is more accurate for adults than for children and therefore it will be best to consider the requirement of the adult first and the demands of growth later. Such analyses of the maintenance

requirement of the body with reference to its principal functions as have been made, indicate that in the healthy adult the basal expenditure of energy may be attributed in part, perhaps up to one fourth, to the functional activities of the various organs (heart action, kidney action, respiration, etc.). The greater part of the basal heat production is thought to be due to oxidations in the resting tissues, principally in maintaining the tone of the skeletal muscles. In the healthy adult this basal metabolism depends chiefly upon the size, shape, and composition of the body and the intensity of certain internal processes.

Influence of the size, shape, and composition of the body. — For different adults of the same species the energy metabolism (and therefore the total food requirement) as a rule increases with the size, but not to the same extent that the body weight increases; so that the requirement, though greater in absolute amount, is less per unit of body weight in the larger individual than in the smaller. The energy metabolism increases in proportion to the surface rather than the weight. Thus, Rubner collected the following data from experiments upon seven different dogs, all full grown but differing greatly in size.

No.	Body Weight Kilograms	Heat Production in Calories per Day		
		Total	Per Kilogram of Body Weight	Per Square Meter of Body Surface
I	3.10	273.6	88.25	1214
II	6.44	417.3	64.79	1120
III	9.51	619.7	65.16	1183
IV	17.70	817.7	46.20	1097
V	19.20	880.7	45.87	1207
VI	23.71	970.0	40.91	1112
VII	30.66	1124.0	36.66	1046

Here the heat production in Calories per kilogram was over twice as great in the smallest as in the largest dog, but the

total metabolism was nearly proportional to the surface area throughout.

That the relationship of energy metabolism to body surface is not due simply to loss of heat through the cooling effect of the environment will be apparent from the observations upon the regulation of body temperature.

Armsby, in his *Principles of Animal Nutrition*, cites the explanation offered by von Hösslin — that the internal work and the consequent heat production in the body are substantially proportional to the two thirds power of its volume, and since the external surface bears the same ratio to the volume, a proportionality necessarily exists between heat production and surface.

Largely as the result of Rubner's work it became customary to express energy requirements in terms of surface rather than of weight; but on account of the difficulties involved in actual measurements of the surface it has usually been computed from the weight. The earlier computations were made by Meeh's formula, $S = W^{\frac{2}{3}} \times C$ or $S = 12.3 \sqrt[3]{W^2}$, in which S represents surface, W the weight, and the constant 12.3 represents the average value found by Meeh in a series of measurements of men.

Lissauer's modification of Meeh's formula, based on measurements of infants, is used extensively for estimating the surface area of infants and young children:

$$S = 10.3 \sqrt[3]{W^2}$$

A new series of measurements of body surface made by DuBois and DuBois led them to the conclusion that Meeh's formula yields results higher than the true average, probably because his measurements were made on thin men.

DuBois and DuBois have devised two new methods by which the surface may be computed with much greater accuracy: (1) from a series of nineteen measurements of different parts of the body, the surface of each part being computed and the

results added together (" linear formula "), and (2) a " height-weight formula " which these authors have derived mathematically from the data of all available measurements of height, weight, and surface.

The height-weight formula may be written thus:

$$A = W^{0.425} \times H^{0.725} \times C$$

or in the form:

$$\text{Log } A = (\text{Log } W \times 0.425) + (\text{Log } H \times 0.725) + 1.8564$$

in either of which

A = Surface area in square centimeters
H = Height in centimeters
W = Weight in kilograms
C = A constant (71.84)

In connection with this formula the authors give also a chart from which the approximate surface area may be obtained at a glance if height and weight are known. The data given in the accompanying table have been taken from the accompanying DuBois chart.

Height in Centimeters	Weight in Kilograms																
	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	105
200							1.84	1.91	1.97	2.03	2.09	2.15	2.21	2.26	2.31	2.36	2.41
195						1.73	1.80	1.87	1.93	1.99	2.05	2.11	2.17	2.22	2.27	2.32	2.37
190				1.56	1.63	1.70	1.77	1.84	1.90	1.96	2.02	2.08	2.13	2.18	2.23	2.28	2.33
185				1.53	1.60	1.67	1.74	1.80	1.86	1.92	1.98	2.04	2.09	2.14	2.19	2.24	2.29
180				1.49	1.57	1.64	1.71	1.77	1.83	1.89	1.95	2.00	2.05	2.10	2.15	2.20	2.25
175	1.19	1.28	1.36	1.46	1.53	1.60	1.67	1.73	1.79	1.85	1.91	1.96	2.01	2.06	2.11	2.16	2.21
170	1.17	1.26	1.34	1.43	1.50	1.57	1.63	1.69	1.75	1.81	1.86	1.91	1.96	2.01	2.06	2.11	
165	1.14	1.23	1.31	1.40	1.47	1.54	1.60	1.66	1.72	1.78	1.83	1.88	1.93	1.98	2.03	2.07	
160	1.12	1.21	1.29	1.37	1.44	1.50	1.56	1.62	1.68	1.73	1.78	1.83	1.88	1.93	1.98		
155	1.09	1.18	1.26	1.33	1.40	1.46	1.52	1.58	1.64	1.69	1.74	1.79	1.84	1.89			
150	1.06	1.15	1.23	1.30	1.36	1.42	1.48	1.54	1.60	1.65	1.70	1.75	1.80				
145	1.03	1.12	1.20	1.27	1.33	1.39	1.45	1.51	1.56	1.61	1.66	1.71					
140	1.00	1.09	1.17	1.24	1.30	1.36	1.42	1.47	1.52	1.57							
135	0.97	1.06	1.14	1.20	1.26	1.32	1.38	1.43	1.48								
130	0.95	1.04	1.11	1.17	1.23	1.29	1.35	1.40									
125	0.93	1.01	1.08	1.14	1.20	1.26	1.31	1.36									
120	0.91	0.98	1.04	1.10	1.16	1.22	1.27										

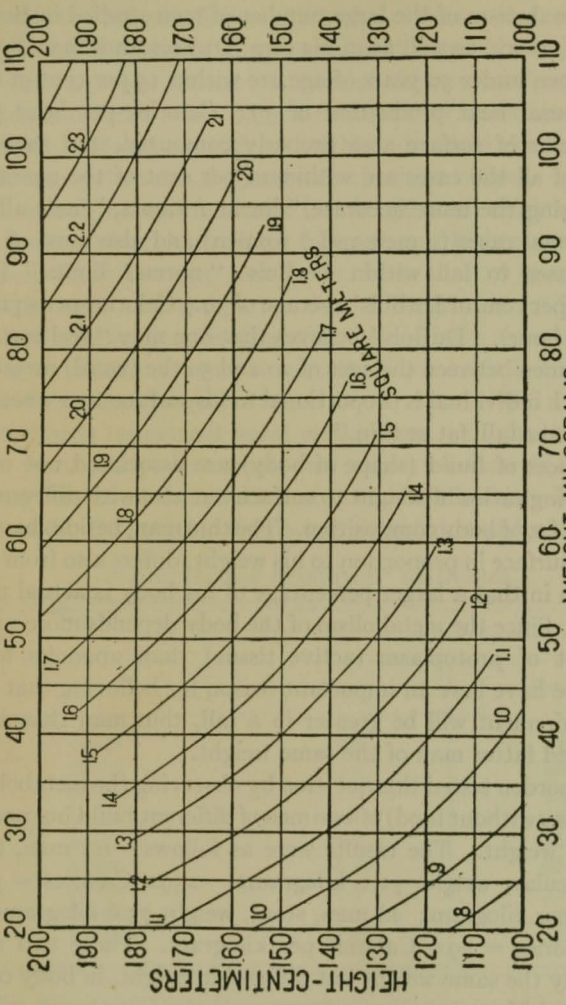

FIG. 14. — Chart for determining surface area of man from weight in kilograms and height in centimeters according to the Du Bois formula. Courtesy of Dr. E. F. DuBois.

On applying the " height-weight formula " to the recorded energy metabolism of the large number of men studied in Benedict's laboratory, as well as in his own, DuBois finds that all the data for men under 50 years of age are within 15 per cent of the average basal heat production of 39.7 Calories per hour per square meter of surface area properly computed, and that 86 per cent of all the cases are within 10 per cent of the average. Means, using the more accurate " linear formula," finds all of his 16 normal cases (9 men and 7 women) and also most of his obesity cases to fall within DuBois' " normal limits " (*i.e.* within 10 per cent of DuBois' average of 39.7 Calories per square meter per hour). DuBois [1] believes that one may " feel certain that with men between the ages of 20 and 50 the (basal) metabolism of each individual is proportional to his surface area whether he be short or tall, fat or thin."

Differences of build (shape of body) are associated not only with varying ratios of weight to surface but also with differences of fatness, *i.e.* of body composition. The thin man, besides having a greater surface in proportion to his weight, differs also from the stout man in that a larger percentage of his body is actual protoplasm. Since the metabolism of the body depends more upon its weight of protoplasm (active tissue) than upon its total weight, we have here an important reason for believing that the food requirement will be greater in a tall, thin man than in a shorter and fatter man of the same weight.

Von Noorden tested this question by observing the metabolism (for one day without food) of two men of different build but nearly the same weight. The results were as follows: 1st man, thin and muscular, weight 71.1 kilograms — 2392 Calories = 33.6 Calories per kilogram; 2d man, stout, weight 73.6 kilograms — 2136 Calories = 29.0 Calories per kilogram. These two men had nearly the same weight but differed in height, in body composition, and in energy expenditure.

[1] *American Journal of the Medical Sciences*, June, 1916, page 786.

Even with the same height and weight there may be differences in the composition of the body. Thus a man of average height and weight but large-boned and loosely built will be of less than average fatness; a man of the same height but less broad-shouldered must be somewhat fatter in order to weigh the same. Hence equality of height and weight does not necessarily imply the same shape and composition of body. Benedict finds among normal adults of like height and weight the basal metabolism of athletes about five per cent higher, and that of women about five per cent lower, than that of average non-athletic men. He attributes these divergencies to differences in body composition, holding that women have somewhat more fat, and athletes somewhat less, than non-athletic men of the same weight and height.

Standards for normal basal metabolism. On the basis of the data from many determinations, tables or formulae for predicting the basal metabolism of normal individuals have been evolved by DuBois, by Harris and Benedict, and by Dreyer.

"Sage Normal Standards" of DuBois

Age in Years	Cal. per Sq. M. of Body Surface per Hour	
	Males	Females
14–16	46.0	43.0
16–18	43.0	40.0
18–20	41.0	38.0
20–30	39.5	37.0
30–40	39.5	36.5
40–50	38.5	36.0
50–60	37.5	35.0
60–70	36.5	34.0
70–80	35.5	33.0

The figures of DuBois are most significant for the age range where the greatest number of studies have been made (males

from 20 to 40 years). The figures for women are not the averages of direct determinations but were calculated from an examination by Gephart and DuBois of the results of studies of the basal metabolism of women, by Benedict and Emmes and by Means.

Harris and Benedict made a biometric study of their findings on 136 men, 103 women, and 94 infants in an attempt to evolve an accurate method of calculating unknown basal metabolism. Their prediction formulae are based on the factors: height, weight, and age.

For males, $H = 66.473 + 13.752\,W + 5.003\,S - 6.755\,A$
For women, $H = 665.096 + 9.563\,W + 1.850\,S - 4.676\,A$
H = Total heat production in 24 hours
W = Weight in kilograms
S = Stature in centimeters
A = Age in years

Carpenter has compiled tables giving the weight factor and the height and age factor for both men and women.[1]

Krogh holds that the Harris and Benedict formula predicts accurately only for individuals within the range of weight, height, and age from which these measurements were taken. He believes that it does not hold good for individuals whose weight varies widely from the average for their height and age. The prediction formula for men can be applied to boys but the formula for women does not predict accurately for girls. The reason for this is not clearly understood.

Dreyer made a statistical study of the data of Benedict, DuBois, and other investigators with the object of evolving formulae which would predict basal metabolism more accurately than those of Aub and DuBois or Harris and Benedict.

[1] Carpenter, *Tables, Factors, and Formulas for Computing Respiratory Exchange and Biological Transformations of Energy*. Carnegie Institution of Washington, Publication No. 303 *A* (1924).

His formulae are based on those measurements which he considers most significant, namely trunk length, chest circumference, age, and weight. These formulae, which are applicable to males and females of the age of 5 years upward, are as follows:

$$\text{For males} \quad C = \frac{\sqrt{W}}{0.1015 \times A^{0.1333}}$$

$$\text{For females} \; C = \frac{\sqrt{W}}{0.1127 \times A^{0.1333}}$$

C = Calories per 24 hours
W = Weight in grams
A = Age in years

MacLeod and Rose have compared the results of their observations on 86 normal women between the ages of 20 and 50 with the values predicted by the formulae stated above. Their findings and those of Blunt indicate that the results of prediction by all these formulae for women are as much as 5 per cent too high.

Investigators have found variations of 10 to 15 per cent from the average basal metabolism in normal individuals of the same age group. Differences of similar magnitude are found in determinations made on the same individual over a prolonged period.

Influence of internal activities. The work of maintaining the respiration and circulation evidently involves a continual expenditure of energy. It is clear too that deep and rapid breathing or vigorous heart action must involve an increased activity of the muscles concerned. But it is not always clear to what extent increased respiratory and heart action are a cause and to what extent they are an effect of increased energy metabolism. Thus Murlin and Greer [1] emphasize the close relationship of the heart to the requirements of the tissues for energy in that the energy metabolism is immediately dependent upon oxygen supply. Since but little available oxygen can be stored in the living sub-

[1] *American Journal of Physiology*, Vol. 33, page 253.

stance, " the response of the heart to variations in the (energy) requirement must be immediate and, within very narrow limits of time, proportional to this requirement."

A large factor in basal metabolism is the maintenance of muscular tension or tone. That every living muscle is always in a state of tension is evident from the fact that it gapes open if cut. It is equally evident that the degree of tension (and therefore the expenditure of energy required to maintain it) varies greatly in different individuals under similar conditions and in the same individual under different conditions. The differences observed by Atwater and Benedict between the metabolism of the sleeping hours and that of the hours spent sitting up without muscular movement (65 and 100 Calories respectively) are largely due to the more complete relaxation of the muscles during sleep. Thus there is in the " resting " muscle a continual expenditure of energy which first takes the form of muscular tension, or tone, but ultimately appears as heat, so that the heat production, or energy metabolism, of the body at rest depends to a considerable extent upon the degree of tension which still persists in the muscles.

Benedict and Carpenter report the following figures in Calories per hour for the energy metabolism during sleep (1 A.M. to 7 A.M.) following different conditions of activity and showing the after effects of work upon muscular tension during rest:

ENERGY METABOLISM DURING SLEEP — CALORIES PER HOUR

SUBJECT	SLEEP AFTER REST	SLEEP AFTER MODERATE WORK	SLEEP AFTER SEVERE WORK	SLEEP AFTER VERY SEVERE WORK
E. O.	69.3	74.8	—	—
J. F. S.	60.4	65.3	—	—
J. C. W.	77.2	—	83.1	—
B. F. D.	69.8	—	83.3	—
A. L. L.	78.3	—	83.7	97.9

Does mental work influence energy metabolism? In any consideration of this question it is important to distinguish sharply between the nervous control of muscular conditions and the metabolism of the brain and nerve substance itself. As emphasized particularly by Mathews, the brain receives a copious blood supply, and the blood coming to the brain is arterial, while that leaving the brain is venous, indicating that considerable oxidative metabolism occurs in brain tissue. Tashiro has shown that the carbon dioxide production of nerve fiber is increased when the nerve is stimulated to activity. But since the entire weight of brain and nerve substance constitutes only about 2 per cent of the body weight, it remains questionable whether, even if its metabolism increases with "mental activity," the increase would be appreciable in measurements of the energy expenditure of the body as a whole. Probably the best-controlled experiments upon this problem, certainly the ones affording most accurate measurement of the energy expenditure, are those of Benedict and Carpenter, in which a number of college students were given course examinations in the respiration calorimeter and their energy metabolism during the three-hour period covered by the examination was compared with that during the same period on another day when the student sat in the calorimeter at rest. In some individuals the metabolism was higher during the examination period, while in others it was lower — results much more likely due to involuntary increase or decrease of muscular tension than to altered metabolism of the brain tissue. In the average of the entire series of experiments there appeared a slight increase of oxygen consumption, carbon dioxide output, and heat production during the examination, but the increase was so small and the exceptions so numerous that the investigators were not willing to conclude from their results that mental work has any positive effect upon the total metabolism, but rather infer the opposite.

Apparently we must conclude that such changes in energy

metabolism as may result from differences in activity of the brain and nerves involved in the performance of mental work are so small, in comparison with the energy exchanges always going on in the muscles, that the former are quite obscured by the unavoidable fluctuations of the latter, and so play no measurable part in determining the total food requirement of the body. This conclusion, however, does not exclude the probability of stimulation of the basal rate by strong emotions, acting directly to increase muscle tone or through the mechanism of the internal secretions in the ways suggested in the following paragraph.

Internal secretions and pathological conditions. Some internal secretions, notably that of the thyroid gland, influence energy metabolism, augmenting the heart action and respiration rate, probably also increasing oxidation in other ways. Lusk says: "With the possession of such a gland as the thyroid, whose suppression may diminish metabolism twenty per cent and whose stimulation may increase it 100 per cent, it is truly strange that the normal person should have a basal metabolism so regulated as to correspond to a definite heat loss per square meter of body surface." If, however, the thyroid gland is conspicuously over- or under-developed in size or activity the condition is regarded as a departure from health (goiter, myxedema).

Certain pathological conditions are accompanied by a characteristic alteration in the basal rate. DuBois reports an average increase in fevers of 13 per cent for each degree centigrade (or 7 per cent for each degree Fahrenheit) rise in temperature above the normal level. Basal metabolism is accelerated in exophthalmic goiter, erysipelas, leukemia, malarial and typhoid fevers, and in tuberculosis accompanied by fever; it is neither increased nor decreased materially in uncomplicated cases of obesity, arthritis, gout, nephritis, and cardiac and renal disorders. Hypothyroidism and myxedema are marked by a lowered heat output.[1]

[1] For a more extended discussion of the clinical significance of basal metabolism determinations see DuBois's *Basal Metabolism in Health and Disease.*

The influence of age and growth. From the fact that in animals of the same species, but of different size the heat production is proportional to the surface rather than to the weight, it would follow that children must have a greater maintenance requirement per unit of weight than adults.

From the data obtained in extensive studies of the basal metabolism of children, Benedict and Talbot have made charts

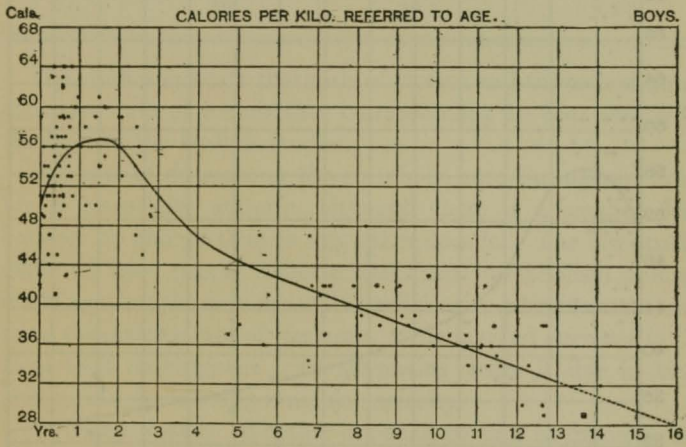

FIG. 15. — Basal heat production of boys per kilogram of body weight per 24 hours referred to age. Courtesy of Dr. F. G. Benedict and the Carnegie Institution of Washington.

showing the results in individual cases with curves indicating the averages.

From Figs. 15 and 16 it will be seen that the basal metabolic rate is highest between the ages of one and two years. The newborn infant has an average basal metabolic rate of 48 calories per kilogram per 24 hours, much lower than the rate for older children. Krogh attributes the regular increase in basal metabolism in infancy to " the development of the muscular system as such and perhaps simply the gradual development of muscle

tone." Since the rate is comparatively low during the first few months when growth is most rapid, the high metabolism of children does not seem to be due to a specific stimulus associated with growth.

There is some question whether the downward trend of the basal metabolic curve after the second year is temporarily

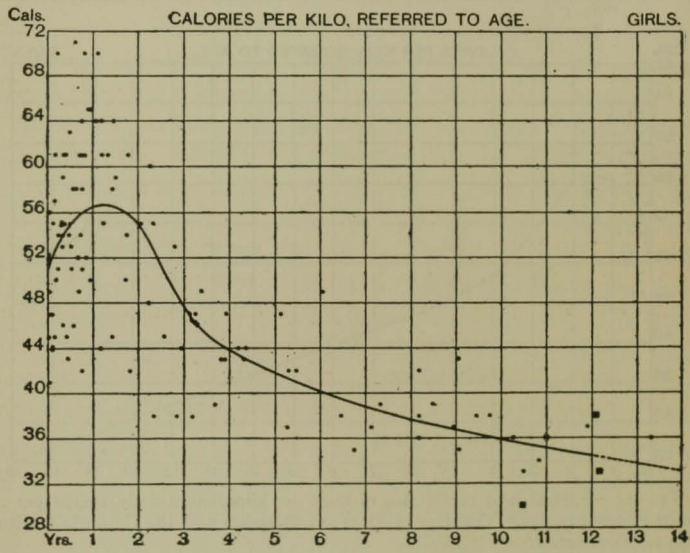

FIG. 16. — Basal heat production of girls per kilogram of body weight per 24 hours referred to age. Courtesy of Dr. F. G. Benedict and the Carnegie Institution of Washington.

broken during the prepubescent period. Comparative studies of boys made by DuBois before and after the onset of puberty indicate that there is a temporary rise in the prepubescent period. Benedict and Hendry, in group determinations on 105 girls, found no proof of the influence of prepubescence on the metabolic rate. The most extensive studies to date are those made by MacLeod in 362 experiments upon 43 girls between

eleven and fourteen years of age, the results of which are given in the accompanying table:

Age	Av. Cal. per Sq. M. per 24 Hours	Av. Cal. per Kg. per 24 Hours	Av. Calories per 24 Hours
11	900	29.2	1191
12	990	32.3	1295
13	985	31.1	1365
14	880	36.7	1308

The studies indicate that girls of twelve and thirteen show an increased rate of metabolism corresponding to that shown by the boys of the DuBois studies.

The rate of decrease in the metabolic rate throughout adult life is essentially uniform, although there is a smaller drop between 20 and 30 than in any other decade. The few studies made on aged people indicate that basal metabolism is lower in old age, but the data are still insufficient to permit a statement as to the definite rate of decrease. The marked decrease in the total food requirement with advancing years is due in large measure to diminishing muscular activity.

Influence of sex. Whether sex shall be said to influence the energy requirement will depend upon our use of terms. Benedict and Talbot found no difference in the basal metabolic rate of boys and girls of the same age until they reach a weight of 11 kilograms or a surface area of 0.48 sq. meters. Beyond a weight of 14 kilograms, boys maintain a persistently higher rate which is not apparently due at this early age (about 3 years) to greater muscular development.

Benedict and Emmes found, as previously noted, a slightly higher basal metabolism in men than in women of the same height and weight, but attribute this to a difference in the average composition of the body. While sex alone seems not to be a measurable factor in energy metabolism, the performance of

the reproductive functions may make large demands upon the maternal organism.

Root and Root made fortnightly determinations of the basal metabolism of a woman from the fourth month of pregnancy until the ninth week of lactation. Beginning with the sixth month, the metabolic rate rose steadily; on the eleventh day before delivery it was 7.6 per cent higher per kilogram of body weight than in the fourth month.

Sandiford and Wheeler made frequent determinations extending from a period previous to the establishment of pregnancy through the fourth month of lactation. Heat production just before delivery was 25 per cent higher than at the beginning of the study, while weight had increased 23 per cent. From an estimate of the surface area of the fetus, it was possible to calculate a quota of the total basal heat production of the pregnant woman which might be assigned to the metabolism of the fetus. The remainder might be considered to represent the heat production in the maternal body. This figure averaged 35.4 Calories per square meter per hour as compared with 34.7 Calories per square meter per hour before pregnancy, a variation well within the limits of experimental error. Equally close agreement was shown by the data of other observers when recalculated by this method. In a recent review, Harding concludes: "Pregnancy would thus appear to result in no alteration in the energy exchange beyond that produced by the growth within the maternal organism of a new mass of active protoplasmic tissue of a higher basal metabolic rate and comparable to that in infants."

Sandiford and Wheeler found no increase in basal heat production during lactation, and conclude that the conversion of the mother's food into milk does not involve a material loss of energy. But practical human experience as well as experiments upon farm animals supports the view that liberal increases in the food intake must be provided if optimum results in lactation are to be obtained.

Influence of race. Attempts to study the racial factor in metabolism have been largely unsuccessful due to the difficulty in eliminating other possible contributing factors, such as climate, habits of food intake, and exercise. Recent studies have been made by MacLeod, Crofts, and Benedict on Chinese and Japanese women students living in this country under conditions strictly comparable to those of the group of American subjects of an earlier study. Their results furnish evidence that Oriental women have a characteristically lower metabolism which survives, at least temporarily, a change to Western surroundings.

Influence of food. Benedict and his associates studied the effect of a prolonged reduction in diet on basal metabolism. In a group of 12 college students an average loss of weight of 12 per cent was accompanied by an average lowering of the basal metabolic rate of 18 per cent per unit of body weight or of body surface. This finding supports the view that chronic undernutrition in adults, or even simple restriction of food consumption in health, if continued sufficiently, may bring the organism to a lower level of energy metabolism than would be indicated by the weight or surface. Underweight school children, as studied by Blunt, showed an increase in the basal rate of 16 to 25 per cent per kilogram of body weight. The striking contrast between the effects of undernutrition in the child as compared with the adult has not been satisfactorily explained.

No significant difference has been found between the basal metabolic rate of vegetarians and non-vegetarians.

Habits of exercise. After severe muscular activity basal metabolism is maintained at a higher level for a period proportionate to the amount of work done. There is also some evidence of a relation between the degree of habitual exercise and the basal metabolic rate. A dog in Lusk's laboratory showed a remarkably uniform basal rate during the period when he was confined in a cage and a much higher metabolism on his return from an active vacation in the country.

Twelve white subjects living in Brazil had a basal metabolic rate 24 per cent lower than the accepted standards for a similar group in the United States. One of these men, all of whom led sedentary lives, entered upon a program of systematic exercise for a year and raised his basal metabolism from 22.7 to 32 Calories per square meter per hour.

Climate, season, housing, clothing, are all factors which may influence energy metabolism principally through their bearing upon the **regulation of body temperature.**[1] It is evident that the maintenance of the body at a temperature above that of its ordinary environment involves a continual output of heat. This output of heat may be regulated in either of two ways: (1) By variations in the quantity of blood brought to the skin, which tend to control the loss of heat by radiation, conduction, and sweating; this is called "physical regulation." (2) By an increase in the rate of oxidation in the body in response to the stimulus of external cold; such a change in the rate of oxidation is known as "chemical regulation." The extra heat production which follows the taking of food (the specific dynamic action of the foodstuffs) may take the place of the "chemical regulation" and so help to protect the body from the necessity of burning material simply for the maintenance of its temperature. Muscular work, by increasing the production of heat in the body, may also render chemical regulation unnecessary; but apparently the specific dynamic action does not furnish energy which can be utilized for muscular work.[2]

The presence of a layer of adipose tissue under the skin as well as the custom of covering the greater part of the external surface with clothing also tends to keep down the loss of heat to the point where "physical regulation" will suffice. Lusk cites experiments by Rubner upon a man whose metabolism was determined

[1] For full discussion of the influence of surrounding temperature upon metabolism and the relation of metabolism to the regulation of body temperature the reader is referred to Lusk's *Science of Nutrition*.

[2] See Lusk's *Science of Nutrition*, 3d Edition, pages 311–313.

when kept in the same cold room but with different amounts of clothing, and observes that when the man was sufficiently clothed to be comfortable the " chemical regulation " was eliminated (*Science of Nutrition*, 3d edition, page 149).

In general it seems probable that people warmly clothed and living in houses which are heated in winter are not called upon to exercise " chemical regulation " to any considerable extent; in other words, they probably do not burn any considerable amount of material merely for the production of heat, the heat required for the maintenance of body temperature being obtained in connection with the metabolism which is essential to the maintenance of the muscular tension and the various other forms of internal work. If, however, the body be exposed to cold, it may be forced to employ " chemical regulation " with a resulting increase of the food requirement, and this will occur more readily in a thin person than in one who is well protected by subcutaneous fat.

The extra heat required in cold weather is probably obtained for the most part through the activities of the muscles. It is a matter of general experience that one instinctively exercises the muscles more vigorously in cold weather than in warm, and if one attempts to endure much cold without muscular exercise there results shivering — a peculiar involuntary form of muscular activity whose function appears to be to increase heat production through increasing the internal work of the body.

To a large extent therefore the regulation of body temperature, in case of exposure to cold, is accomplished through the activity and tension of the muscles.

The foregoing discussion has reference primarily to adults. In the case of the infant whose surface is much greater in proportion to his weight and whose muscle tone is not yet fully developed, the loss of heat to the surroundings is not so readily checked by " physical " nor so easily made good by " chemical " regulation. Unless the infant is either warmly clothed or supplied with an

artificial source of heat in cold weather he may be forced to burn, for warmth, material that might better be employed for growth.

Summary. In a general review of the factors affecting normal basal metabolism Benedict [1] concludes " that the basal metabolism of an individual is a function, first, of the total mass of active protoplasmic tissue, and, second, of the stimulus to cellular activity existing at the time the measurement of the metabolism was made." And that: " Perhaps the most striking factors causing variations in the stimulus to cellular activity are age, sleep, prolonged fasting, character of the diet, and the after effect of severe muscular work."

The Specific Dynamic Action of Foodstuffs

Atwater and Benedict determined directly by means of the respiration calorimeter the heat production of the same man during five fasting experiments of one to two days each, and during a four-day experiment with food about sufficient for maintenance. The average total metabolism on the fasting days was about 9 per cent lower than on the days when food was taken.

In longer fasts there may be a somewhat greater decrease in heat production. Thus, Benedict found that a man who weighed at the start 59.5 kilograms (131 pounds) metabolized, on the successive days of a seven-day fast, 1765, 1768, 1797, 1775, 1649, 1553, and 1568 Calories respectively. Naturally in long fasts factors other than the simple sparing of the direct effect of food come into play.[2]

Tigerstedt studied by means of the carbon and nitrogen balance the metabolism of a man who fasted for five days and for the next two days took a very liberal diet. The data obtained are shown in the accompanying table.

[1] *Proceedings of the National Academy of Sciences*, Vol. 1, pages 105–109.

[2] For a detailed account of the results obtained in a fasting experiment of 31 days' duration, see Benedict, *A Study of Prolonged Fasting*, Publication No. 203 of the Carnegie Institution of Washington.

DATA OF TIGERSTEDT'S FASTING AND REFEEDING EXPERIMENT

	BODY WEIGHT KILOS	CALCULATED TOTAL METABOLISM CALORIES	CALORIES PER KILO
1st fast day	67.0	2220*	33.2*
2d fast day	65.7	2102*	32.0*
3d fast day	64.9	2024	31.2
4th fast day	64.0	1992	31.1
5th fast day	63.1	1970	31.2
Fed 4141 Calories	64.0	2437	38.1
Fed 4141 Calories (2d day)	65.6	2410	36.8

* These figures are slightly too high because the loss of carbon on these days was due in part to combustion of glycogen, but is calculated as if due simply to protein and fat.

These results show for man (as had previously been shown with dogs) that in fasting the total metabolism continues at a fairly constant rate in spite of the fact that the energy is obtained entirely at the expense of the body material. In this case, the diet given at the end of the fasting period (4141 Calories) was approximately double what would have been required for maintenance, but the increase in energy metabolism was only 22.5 per cent over that of fasting.

The results of fasting experiments thus make it evident that the body has but little power in the direction of adjusting its energy metabolism to the energy value of its food supply.

Rubner found that each type of food exerted a more or less specific influence upon the energy metabolism, so that when the foodstuffs were fed separately, somewhat different energy values were required for the maintenance of body equilibrium. Thus, if the total metabolism of a dog fasting at 33° C. be represented by 100 Calories, he must be fed, in order to prevent loss of body substance, about 106.5 Calories of sugar, or 114.5 Calories of fat, or 140 Calories of protein. A man observed by Rubner metabolized in fasting 2042 Calories; when fed 2450 Calories

in the form of sugar alone, he metabolized 2087 Calories; when fed 2450 Calories in the form of meat alone, he metabolized 2566 Calories.

Lusk and his coworkers have investigated the influence of the foodstuffs upon metabolism ("specific dynamic action") very extensively and have developed the subject to such an extent that for an adequate discussion of their results the original articles in the *Journal of Biological Chemistry* or Lusk's own summaries[1] should be consulted. It appears from this work that when the digestion products of carbohydrate or fat are carried by the blood to the tissues the energy metabolism (rate of oxidation) rises simply because of the increased concentration of oxidizable material; but that some of the products of the digestion and intermediary metabolism of protein increase metabolism not only to a greater extent, but also in a somewhat different manner, since they seem to act as stimuli rather than merely as fuel. On an ordinary mixed diet, however, this apparent loss of energy due to eating of protein is not a very large factor in the total metabolism, since the total specific dynamic action makes the metabolism of energy for the day probably less than one tenth higher on a full maintenance ration than when no food is eaten.

It is sometimes thought that superior preparation or very thorough mastication of food results in such improvement in its utilization that a material saving may be effected in the amount of food required. But it will be remembered that under average conditions only about 5 per cent of the energy value of the food is lost in digestion or expended upon the digestive process. Any improvement in those conditions through superior preparation or mastication of the food can therefore at most effect a saving of less than 5 per cent of the energy value. Thus the influence upon total food requirement is scarcely appreciable. The advantages of good preparation and thorough chewing of the

[1] Lusk. *Medicine*, Vol. 1, page 311 (1922). *Science of Nutrition*, Chapter VII.

food are very important, but they lie in other directions than reduction in the amount of food required.

Influence of Muscular Work upon Metabolism and Food Requirement

Muscular work is much the most important of the factors which raise the food requirements of adults above the basal rate necessary for mere maintenance.

Accurate measurements by means of the calorimeter have shown that the average total metabolism of a man sitting still is about 100 Calories per hour; while the same man working actively increases his metabolism up to about 300 Calories per hour; and a well-trained man working at about his maximum capacity metabolizes material enough to liberate 600 Calories per hour, *i.e.* his metabolism may be six times as active during the hours actually spent in such work as when he is at rest. If during 24 hours a man works as hard as this for 8 hours and spends 2 hours in such light exercise as going to and from work, his food requirement for the day will be somewhat over 6000 Calories, or three times the maintenance requirement. Thus, work may increase the day's metabolism as much as 200 per cent, whereas liberal feeding at the end of a fast was found to increase the metabolism only 22.5 per cent, or one ninth as much. Only a few exceptional occupations, such as that of lumbermen, for example, involve such heavy work as to cause a metabolism of 6000 Calories per day. More often the man who works eight hours a day at manual labor will increase his metabolism by 1000 to 2000 Calories above what is needed for maintenance at rest, making his total food requirement 3000 to 4000 Calories.

Voit estimated the food requirement of a " moderate worker " at about 3050 Calories; and Atwater, in adapting this standard to American conditions, increased the allowance to 3400–3500 Calories in the belief that the American works more rapidly and therefore with a greater expenditure of energy. The mistake is

often made of supposing that these estimates were intended for every one who leads an active life, whereas they really contemplate a long day of manual labor, for Voit's definition of " moderate worker " was a man laboring 9 or 10 hours a day at an occupation such as that of a carpenter, mason, or joiner.

The amount of energy spent during 24 hours by a sedentary worker will depend not only upon the number of hours which he devotes to exercise, but especially upon the kind of exercise chosen. Lusk estimates that an average-sized man sleeping 8 hours, sitting 14 hours, and walking 2 hours spends about 2500 Calories; whereas if he spends 2 hours in vigorous exercise instead of walking, his total energy output rises to about 3000 Calories.

The importance of muscular activity as the chief factor governing the energy expenditure and food requirement of healthy adults calls for a careful quantitative study of its effect upon metabolism.

Quantitative relation between work performed and total metabolism. Theoretically it is possible to determine the mechanical efficiency of a man by dividing the mechanical effect of his work by the increase of energy metabolism which the work involves. This gives the basis on which to ascertain how much extra food would be necessary to supply the energy required for the performance of any given task.

Zuntz and his associates in Berlin carried out a long series of experiments of this kind which are described by Magnus-Levy in Von Noorden's *Metabolism and Practical Medicine.*

Benedict and others have studied the mechanical efficiency of both men and women in level and grade walking. These studies indicate that in level walking the efficiency is highest at moderate speeds. Very fast walking (about $5\frac{1}{2}$ miles per hour) required a greater heat output than running at the same speed. In grade walking the expenditure for a given amount of work tended to be less at low speed on a high grade than at high speed

on a low grade. The greatest net efficiency in grade walking (approximately 30 per cent) was reached only when the work done was less than 500 kilogrammeters per minute.

From the data obtained from these and similar studies on walking it is possible to estimate roughly the food requirements of men who neither do active physical labor nor take vigorous exercise, yet move about more freely than in the so-called rest experiments already described. If, for example, it be assumed that a healthy man would require 2200 Calories per day when remaining in one room, and that the total additional muscular movements of a day at business and recreation were equivalent to walking five miles on level ground, his total food requirement for the day would become 2500 to 2700 Calories (36 to 39 Calories per kilogram), while activity equivalent to walking ten miles on level ground would bring the total daily requirements to 2800 to 3200 Calories (40 to 46 Calories per kilogram).

By means of the respiration calorimeter, Atwater and Benedict studied the question of mechanical efficiency with a different form of muscular work. They placed in the calorimeter chamber an ergometer, which consisted of a fixed bicycle frame having in place of the rear wheel a metal disk which revolved against a measured amount of electrical resistance, so that the mechanical effect of the muscular work was very accurately determined. The expenditure of energy involved in the performance of this work was estimated by comparing the total metabolism of a working day with that of the same man when living in the calorimeter chamber at rest. The average results obtained with three different men were as shown in the table on page 218.

With an improved ergometer of the same type as that used in the experiments just cited, Benedict and Carpenter working with J. C. W. (one of the 3 men above mentioned) found efficiencies ranging from 20.7 to 22.1 per cent and averaging 21.6 per cent; with other men studied, the efficiencies ranged from 18.1 to 21.2 per cent.

Benedict and Cathcart, in similar bicycle ergometer experiments in which the basis of comparison was complete rest on a couch, found efficiencies vary-

ATWATER AND BENEDICT DATA ON MECHANICAL EFFICIENCY OF MAN

SUBJECT AND NATURE OF EXPERIMENT	ENERGY TRANSFORMED		HEAT EQUIVALENT OF WORK PERFORMED	MECHANICAL EFFICIENCY
	Total per day	Excess over that at rest		
	Calories	*Calories*	*Calories*	*Per cent.*
Subject E. O.				
Average 13 rest experiments (42 days)	2279			
Average 3 work experiments (12 days)	3892	1613	214	13.3
Subject J. F. S.				
Average 4 rest experiments (12 days)	2119			
Average 6 work experiments (18 days)	3559	1440	233	16.2
Subject J. C. W.				
Average 1 rest experiment (4 days)	2357			
Average 14 work experiments (46 days)	5143	2786	546	19.6

ing from 10 to 25 per cent, depending on load, speed, and the familiarity of the subject with the work, the maxima for the six men studied being 23.1, 20.4, 21.6, 22.7, 20.8, and 25.2 per cent respectively.

In another series of experiments they subtracted from the expenditure of energy during work, the amount spent when the subject, instead of lying on a couch, sat on the ergometer and allowed the pedals to be turned under his feet. Using this method of estimation they were able by careful adjustment of speed and load to realize with a professional bicycle rider an efficiency of 33 per cent or as much as Zuntz and his associates had estimated from the walking experiments.

Only under the most favorable circumstances and with subjects fully accustomed to the kind of work being performed will the actual mechanical effect produced amount to as much as one fourth to one third of the extra energy expended during work over that during rest, *i.e.* to an efficiency of 25 to 33 per cent. Not only do most occupations involve kinds of work which in their nature must be done with less efficiency than walking (or riding a stationary ergometer) but the usual hours of

labor are longer than those in which the maximum mechanical efficiency is attained. The efficiency may begin to decline before any sensation of fatigue is felt.

Thus Leo Zuntz found, when he rode his bicycle for four successive hours at an average rate of 15 to 17 kilometers (about 9 miles) per hour, that he experienced no feeling of fatigue, but his determinations showed that the expenditure of energy necessary to produce a given effect had increased about 9, 13, 10, and 23 per cent at the end of 1, 2, 3, and 4 hours respectively. This is because if the same kind of work be performed for a series of hours, auxiliary muscles are gradually brought increasingly into action, partly for the performance of the work itself, partly for the fixation of the bodily framework (maintenance of posture). These auxiliary muscles work less economically than those which are used first and most naturally. For much the same reasons there is a lower efficiency in the case of work which is from the first of too fatiguing a nature because of being either excessive or unsuitably distributed. When Leo Zuntz increased his speed 2.4 times, he found his metabolism increased 4.3 times, implying a considerable loss of efficiency. Under the conditions of Benedict and Cathcart's experiments also, the efficiency was usually decreased upon increasing the speed; on the other hand a moderately heavy load was more economical than a light one.

Total Energy Requirement of Adults

It is now possible to estimate the approximate average expenditure of energy per hour under a considerable number of conditions of muscular activity. For convenience of comparison and application the original data have been reduced to a common basis of 70 kilograms (154 pounds), with the results shown in the table on page 220 compiled by Professor M. S. Rose.

By the use of these estimates the probable food requirement for a person of 70 kilograms (154 pounds), may be calculated very simply, as, for instance, in the following example:

8 hours of sleep at 65 Calories	=	520 Calories
2 hours' light exercise[1] at 170 Calories	=	340 Calories
8 hours' carpenter work at 240 Calories	=	1920 Calories
6 hours' sitting at rest at 100 Calories	=	600 Calories
Total food requirement for the day,		3380 Calories

[1] Going to and from work, for example.

ENERGY EXPENDITURE PER HOUR UNDER DIFFERENT CONDITIONS OF MUSCULAR ACTIVITY

FORM OF ACTIVITY	CALORIES PER HOUR		
	Per 70 Kilograms (Average man)	Per Kilogram	Per Pound
Sleeping	65	0.93	0.43
Awake lying still	77	1.10	0.50
Sitting at rest	100	1.43	0.65
Reading aloud	105	1.50	0.69
Standing relaxed	105	1.50	0.69
Hand sewing	111	1.59	0.72
Standing at attention	115	1.63	0.74
Knitting (23 stitches per minute on sweater)	116	1.66	0.75
Dressing and undressing	118	1.79	0.81
Singing	122	1.74	0.79
Tailoring	135	1.93	0.88
Typewriting rapidly	140	2.00	0.91
Ironing (with five-pound iron)	144	2.06	0.93
Dishwashing (plates, bowls, cups, and saucers)	144	2.06	0.93
Sweeping bare floor (38 strokes per minute)	169	2.41	1.09
Bookbinding	170	2.43	1.10
"Light exercise"	170	2.43	1.10
Shoe making	180	2.57	1.17
Walking slowly (2.6 miles per hour)	200	2.86	1.30
Carpentry, metal working, industrial painting	240	3.43	1.56
"Active exercise"	290	4.14	1.88
Walking moderately fast (3.75 miles per hour)	300	4.28	1.95
Stoneworking	400	5.71	2.60
"Severe exercise"	450	6.43	2.92
Sawing wood	480	6.86	3.12
Swimming	500	7.14	3.25
Running (5.3 miles per hour)	570	8.14	3.70
"Very severe exercise"	600	8.57	3.90
Walking very fast (5.3 miles per hour)	650	9.28	4.22

Tigerstedt, in his *Textbook of Physiology*, gives estimates of food requirements for different degrees of activity as indicated by means of typical occupations, which may be useful in checking results calculated as above.

According to Tigerstedt:

2000–2400 Calories per day suffice for a shoemaker.
2400–2700 Calories per day suffice for a weaver.
2700–3200 Calories per day suffice for a carpenter or mason.
3200–4100 Calories per day suffice for a farm laborer.
4100–5000 Calories per day suffice for an excavator.
Over 5000 Calories per day are required by a lumberman.

Lusk gives the following summary of energy requirements of women at work at typical occupations as investigated by Becker and Hamäläinen in Finland:

A seamstress sewing with a needle required 1800 Calories.

Two seamstresses, using a sewing machine, required 1900 and 2100 Calories, respectively.

Two bookbinders required 1900 and 2100 Calories.

Two household servants, employed in such occupations as cleaning windows and floors, scouring knives, forks, and spoons, scouring copper and iron pots, required 2300 to 2900 Calories.

Two washerwomen, the same servants as the last named, required 2600 and 3400 Calories in the fulfillment of their daily work.

Energy Requirement of Children

The relatively high rate of energy expenditure in children as compared with adults is due to a higher basal metabolism, vigorous muscular activity, and stimulation from their liberal food intake.

As between children and adults the energy metabolism is more nearly proportional to the surface than to the weight; but among children of about the same age the energy require-

ment may be computed on the basis of weight about as well as on the basis of surface.

Murlin and Bailey estimate from their own observations, and the earlier ones of Benedict and Talbot, that the energy requirement of the newborn baby *kept comfortably warm and sleeping quietly* may be placed tentatively between 1.7 and 2.0 Calories per kilogram per hour, the lower figure for a very fat (10 lb.) child and the higher for a thin (6 lb.) child. More recently Murlin, Conklin, and Marsh found the average basal metabolism of 38 normal newborn babies to be 2 Calories per kilogram per hour. During a period of crying, the energy output was increased 100 per cent over the basal level. Benedict and Talbot [1] give measurements of minimum heat production of 94 newborn infants (2 hours to 6 days old) which range from 1.33 to 2.17 Calories, averaging 1.75 Calories per kilogram per hour. Vigorous crying and kicking may increase this figure by 50 per cent or more.

With the development of the musculature and of muscle tonus, the energy expenditure of the normal infant increases for a time even more rapidly than his body weight, and averages 2.3 Calories per kilogram per hour at six months. During exercise the rate of expenditure is of course materially higher, and in calculating food requirements allowance must also be made for growth and for the possibility of losses through imperfect utilization of the food.

With normal adults the energy requirement is generally considered to be approximately equal to the energy expenditure. With children, on the other hand, the energy requirement includes an additional provision for growth. In studies of infants 7 to 9 months old, Rubner and Heubner found a storage of 12.2 per cent of the energy value of the food consumed, and Camerer found a storage of 15 per cent of the energy and 40 per cent of the protein of the diet.

[1] *Physiology of the New Born Infant*, Publication No. 233, Carnegie Institution of Washington, 1915.

In order to provide adequately for all contingencies and support the rapid growth which is normal at this age, it is estimated that a vigorous child will require during the greater part of the first year about 100 Calories of food per kilogram of his body weight per day. But in cases of artificial feeding, since the digestive tract must be gradually educated to handle the milk of a different species, it will often be necessary to feed much less than 100 Calories per kilogram per day at first, perhaps for several months, and only very gradually increase the food allowance.

From the end of the first year until growth is completed the food requirement increases, but not so rapidly as does the body weight, so that while the allowance of food becomes larger per day it becomes smaller per kilogram. On the latter basis the energy requirement at the different ages may be estimated approximately as follows:

Under 1 year	100 Calories per kilo	(45 Calories per lb.)
1– 2 years	100–90 Calories per kilo	(45–40 Calories per lb.)
2– 5 years	90–80 Calories per kilo	(40–36 Calories per lb.)
6– 9 years	80–70 Calories per kilo	(36–32 Calories per lb.)
10–13 years	75–65 Calories per kilo	(34–30 Calories per lb.)
14–17 years	65–50 Calories per kilo	(30–23 Calories per lb.)
18–25 years	55–40 Calories per kilo	(25–18 Calories per lb.)

Children who are very active or growing very rapidly may require even more food than the table just given suggests. Such cases are perhaps most frequently found among boys between 10 and 15 years of age. DuBois finds in boys 12 and 13 years old an average *basal* metabolism (complete rest and almost complete fasting) of 1.76 Calories per kilogram per hour, or about 75 per cent above that of healthy adults.[1] Assuming average activity for boys of this age the energy expenditure during 24 hours would probably amount to 60 to 70 Calories per

[1] Per unit of surface the basal energy metabolism of these boys was about 25 per cent higher than that of healthy men.

kilogram and as this is a period of rapid growth the *requirement* would be materially higher than the rate of expenditure.

Assuming average size at the different ages the allowances in Calories per day become about as follows:[1]

Children of 1–2 years	1000–1200 Calories per day
Children of 2–5 years	1200–1500 Calories per day
Children of 6–9 years	1400–2000 Calories per day
Girls of 10-13 years	1800–2400 Calories per day
Boys of 10–13 years	2300–3000 Calories per day
Girls of 14–17 years	2200–2600 Calories per day
Boys of 14–17 years	2800–4000 Calories per day

Energy Requirements for Pregnancy and Lactation

These factors have been discussed in relation to the basal metabolism earlier in the chapter.

Although the basal metabolism does not seem to be greatly increased per unit of weight in pregnancy, yet by virtue of her increase in weight the pregnant woman has a larger total energy requirement. The effect of increased weight is likely to be offset to some extent by the decrease in activity. During lactation, when the entire nutritive requirement of the nursing infant is being met through the mother, the energy needs of the latter are greatly increased. Production of milk involves an extra energy requirement beyond the actual energy value of the milk secreted, and the food allowance should provide for it accordingly. Liberal feeding of the nursing mother (*e.g.* up to 2800 to 3000 Calories for a woman with moderate muscular exercise) is not only important for the conservation of her own bodily resources but may prolong the period of lactation and thus be of great value to the child as well.[2]

[1] See also the more detailed table of energy allowances for children in Chapter XIX.

[2] For general discussion of the problem of maintaining breast feeding, see papers by Sedgwick, Abt, and Hoobler in the *Journal of the American Medical Association* for Aug. 11, 1917 (Vol. 69, pages 417–428).

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CHAPTER VIII

FACTORS DETERMINING THE PROTEIN REQUIREMENT

ANIMAL cells under all conditions of life are constantly breaking down proteins into simpler substances which the body eliminates. Since this breaking down or " catabolism " of protein does not stop either in fasting or under the most liberal feeding with fats and carbohydrates, it follows that there is always a need for protein whatever the supply of other food.

Protein metabolism differs widely from energy metabolism in the conditions which determine its amount, for protein metabolism is governed mainly by the kind and amount of food, and to only a slight extent if at all by muscular exercise; whereas energy metabolism is governed mainly by the muscular exercise, and to only a relatively small extent by the food. By giving food rich in fats and carbohydrates but poor in protein, the protein metabolism of a healthy man can easily be brought to less than 50 grams per day, and then by changing to a diet rich in protein, it may be increased to 150 or even 200 grams per day; *i.e.* the rate of protein metabolism can be increased 200 to 300 per cent in a few days by a change in diet alone, all other conditions remaining the same.

Protein Metabolism in Fasting

Since the diet has such a great influence upon the amount of protein metabolized, it might be expected that the basal protein metabolism could be observed best in fasting. But in fasting the energy metabolism of the body is only a little lower

than with food; the amount of combustion continues nearly the same although only body material is available; and since the body must consume so much of its own substance to obtain the energy needed, there is always a chance that in fasting some protein may be burned simply as fuel. Accordingly the protein metabolism in fasting may be greater than that which represents the needs of the body when properly fed, while on the other hand it may be abnormally low through the effort of the body to adjust itself to the abnormal condition.

The amount of protein broken down in fasting is much influenced (1) by the previous habit as regards protein consumption, and (2) by the metabolism of stored glycogen and stored fat.

The direct effect of the level of protein metabolism on the days preceding the fast is shown in the following data obtained by Voit in experiments upon a dog weighing 35 kilograms:

INFLUENCE OF PREVIOUS DIET ON NITROGEN ELIMINATION IN FASTING (VOIT)

	FOODS OF PRECEDING DAYS AND GRAMS OF UREA PER DAY		
	Meat 2500 grams	Meat 1500 grams	Bread
Last day with food . . .	180.8	110.8	24.7
First day of fasting . . .	60.1	29.7	19.6
Second day of fasting . .	24.9	18.2	15.6
Third day of fasting . .	19.1	17.5	14.9
Fourth day of fasting . .	17.3	14.9	13.2
Fifth day of fasting . . .	12.3	14.2	12.7
Sixth day of fasting . .	13.3	13.0	13.0

The influence of the metabolism of the previously stored glycogen upon the amount of protein metabolized in fasting is well illustrated by the following three experiments with one individual:[1]

[1] Benedict, *Influence of Inanition on Metabolism.* Carnegie Institution of Washington (1907).

Experiment	First Day of Fasting		Second Day of Fasting	
	Glycogen metabolized	Nitrogen eliminated	Glycogen metabolized	Nitrogen eliminated
	grams	*grams*	*grams*	*grams*
I	181.6	5.84	29.7	11.04
II	135.3	10.29	18.1	11.97
III	64.9	12.24	23.1	12.45

It will be seen that the nitrogen output was less when there was available for metabolism a considerable supply of previously stored glycogen. Since most of the stored glycogen is used up on the first day of fasting, its influence upon the protein metabolism is short-lived as compared with that of the stored fat.

The influence of the available supply of body fat upon the protein metabolism of fasting is shown by the following observations of Falck, on the protein metabolism of two fasting dogs — the one lean, the other fat:

Falck's Lean Dog		Falck's Fat Dog	
Fasting days	Grams protein catabolized per day	Fasting days	Grams protein catabolized per day
1–4	26.1	1–6	29.9
5–8	24.6	7–12	26.7
9–12	33.9	13–18	26.1
13–16	38.0	19–24	22.3
17–20	31.9	25–29	20.0
21–24	3.9	30–34	16.8
		35–38	15.7
		40–44	13.0
		45–50	13.6
		55–60	12.2
On the 25th day the dog died.		Dog still healthy after 60 days.	

A rise in protein metabolism of the lean dog after the 8th day showed that from this time he used protein largely as fuel — so largely that the results were fatal in 25 days of fasting. The fat dog, having plenty of other fuel in the form of fat, used protein to a much smaller extent, so that he was able gradually to accommodate himself to a lower level of protein metabolism and to endure a fast of 60 days' duration.

The professional faster, Succi, starting with a good store of body fat, fasted 30 days,[1] metabolizing protein at the following rates:

Five days on ordinary food . .	101.4 grams protein per day
1– 5th days fasting	80.4 grams protein per day
6–10th days fasting	53.1 grams protein per day
11–15th days fasting	36.2 grams protein per day
16–20th days fasting	33.1 grams protein per day
21–25th days fasting	29.3 grams protein per day
26–30th days fasting	33.3 grams protein per day

Since Succi's health remained good throughout his fast, it might be thought that the true protein requirement of his body was not greater than the smallest figure found for any period — in this case about 30 grams per day. On the other hand, it may well be supposed that, since the body increases its protein metabolism to an abnormally high rate under influence of excessive protein feeding, so under the influence of fasting the body may be able to adjust itself to an abnormally low rate of protein metabolism; and the fact that the protein metabolism continues to diminish for such a long time in fasting gives weight to the supposition that the body is here gradually adapting itself to an abnormal condition. One might assume that in some particular period of Succi's fast, the effect of previous feeding might no longer be apparent, yet the conditions might not have become abnormal as the result of the fasting; in which case the expend-

[1] The output of nitrogen and of several other elements during a 31-day fast recently described by Benedict may be found in Chapter IX.

iture of protein during one of these periods would represent his normal requirement. Any such assumption must, however, be more or less arbitrary. A much more definite idea of the normal dietary need is obtained by determining experimentally how much protein must be contained in the daily food in order to keep the body in protein (or nitrogen) equilibrium.

Nitrogen Balance Experiments and the Tendency toward Equilibrium at Different Levels of Protein Intake

The estimation of the nitrogen balance has already been referred to as one factor in the determination of the total food requirement by means of metabolism experiments; and it has been shown that the balance may be found either by comparing the total intake with the total output, or by comparing the amount absorbed with the amount catabolized and eliminated through the kidneys.[1] When intake exceeds output, there is a plus balance which indicates a storage of nitrogen and therefore of protein in the body; a minus balance (greater output than intake) indicates a loss of body protein. When the balance is 0, or so near 0 as to be within the limits of experimental error, the body is said to be *in nitrogen (or protein) equilibrium.*

The healthy full-grown body tends to establish nitrogen equilibrium by adjusting its rate of protein metabolism to its food supply within wide limits. The time required by the body for this adjustment depends mainly upon the extent to which the diet is changed.

The following observations by Von Noorden illustrate the establishment of equilibrium after only moderate changes in the diet:

[1] Theoretically the elimination through the skin should also be determined and included in the calculation; practically this is usually neglected unless on account of warm weather or vigorous exercise the subject has perspired profusely. For data on nitrogen in perspiration see Benedict, *Journal of Biological Chemistry*, Vol. 1, page 263 (1906), and *A Study of Prolonged Fasting*, Publication No. 203 of the Carnegie Institution of Washington, pages 233–235.

A young woman weighing 58 kilograms (128 pounds) at rest in bed was given food furnishing 1860 Calories per day.

EXAMPLE OF ADJUSTMENT TO DIMINISHED INTAKE

Total nitrogen of food	16.96 grams
Lost in digestion (nitrogen in feces)	.94 gram
"Absorbed"	16.02 grams

	NITROGEN CATABOLIZED AND ELIMINATED THROUGH KIDNEYS	NITROGEN BALANCE
	grams	*grams*
1st day	18.2	− 2.18
2d day	17.0	− 0.98
3d day	15.8	+ 0.22
4th day	16.0	+ 0.02
5th day	15.7	+ 0.32

Here there was practical equilibrium after the second day. The small amount of nitrogen represented as stored on the third, fourth, and fifth days was very likely lost through the skin. This was a case of adjustment to a lowered protein intake, for the food previously taken was known to have been rich in protein.

Another experiment was made by Von Noorden with the same patient to show the time required to reach equilibrium after increasing the intake of protein. In this case the food furnished 2030 Calories per day and the nitrogen balance was as follows:

EXAMPLE OF ADJUSTMENT TO INCREASED INTAKE

DAY	NITROGEN IN FOOD	NITROGEN IN FECES	NITROGEN "ABSORBED"	NITROGEN CATABOLIZED	NITROGEN BALANCE
	grams	*gram*	*grams*	*grams*	*grams*
1	14.40	0.70	13.70	13.60	+ 0.10
2	14.40	0.70	13.70	13.80	− 0.10
3	14.40	0.70	13.70	13.60	+ 0.10
4	20.96	0.82	20.14	16.80	+ 3.34
5	20.96	0.82	20.14	18.20	+ 1.94
6	20.96	0.82	20.14	19.50	+ 0.64
7	20.96	0.82	20.14	20.00	+ 0.14

Here where the amount of protein fed was increased from 90 to 130 grams without change in the total fuel value of the diet, the body reached equilibrium on the fourth day after the increase.

It is apparent therefore:

(1) That the body tends to adjust its protein metabolism to its protein supply.

(2) That when the body is accustomed to a certain rate of protein metabolism, it requires an appreciable length of time to adjust itself to a materially higher or lower rate.

Hence the rate of protein metabolism on any given day will depend in part upon the rate of metabolism to which the body has been accustomed and in part upon the protein intake for the day. When the protein supply varies from day to day, the metabolism for each day is influenced by both the factors, with the net result that the elimination equals the intake when averaged for a sufficiently long period, although the data for any particular day might show a distinct gain or loss. When the protein supply is constant for a few days, the effect of previous habit usually disappears and equilibrium is established as in the above cases.

A transitory loss of nitrogen from the body is apt to be due simply to the taking of less than the usual amount of protein food, but a persistent loss indicates that the diet is insufficient, either in total food (calories) or in protein, to enable the usual adjustment to take place.

A transitory storage of nitrogen in the body may occur as the result of an increase either of the protein or of the total fuel value of the food; but a persistent storage occurs, as Von Noorden has pointed out, only under the following conditions:

(1) In the growing body (or in pregnancy) where new tissue is being constructed.

(2) In cases where increased muscular exercise results in enlargement of the muscles.

(3) In cases where, owing to previous insufficient feeding or to wasting disease, the protein content of the body has been more or less diminished and consequently any surplus available is utilized to make good the loss.

Protein-sparing Action of Carbohydrates and Fats

It has been shown above that, in fasting experiments, the amount of stored glycogen and fat in the body exerts a " sparing " influence upon protein metabolism, the amount of protein catabolized being smaller when the supplies of glycogen and fat are more abundant. Similarly the amounts of carbohydrates and fats in the food influence the rate of protein metabolism as indicated by the nitrogen excretion. The loss of protein which occurs on an insufficient diet may be diminished or even stopped by adding carbohydrates or fat to the food; and if carbohydrate or fat be added to the diet of a man in nitrogen equilibrium, there results a temporary decrease in nitrogen output with a corresponding storage of protein in the body. The former observation could be interpreted as meaning simply that the body draws upon its stored protein for energy so long, and only so long, as the fuel value of the food is insufficient; but the fact that addition of carbohydrate or fat to a diet already sufficient may cause an actual storage of protein indicates that the " protein-sparing action " or " protein-protecting power " of carbohydrates and fats involves something more than merely the question whether the body " needs " to burn its stored protein as fuel.

As this is a matter of great importance, it may be well to consider somewhat carefully (1) the experimental evidence, and (2) the theoretical explanations, regarding the protein-sparing action of the carbohydrates and fats. For an account of the earlier experiments on this subject, especially those of Voit and Rubner upon dogs, the reader is referred to Lusk's *Science of Nutrition*. Only some of the more important of the experiments upon men can be described here.

Lusk,[1] experimenting upon himself, showed the susceptibility of the protein metabolism to the sudden withdrawal of carbohydrate food. In one experiment a liberal mixed diet containing 20.55 grams of nitrogen was taken until the body was nearly in nitrogen equilibrium, and then, without any other change, 350 grams of carbohydrate were withdrawn from the daily food. On the first day the body protein was largely protected by the carbohydrate previously stored in the body in the form of glycogen, but on the second day the nitrogen metabolism had risen from 19.84 to 27.00 grams per day. In another experiment, upon a diet containing less protein, withdrawal of carbohydrate increased the nitrogen excretion from 11.44 to 17.18 grams per day.

In these cases, as in the fasting experiments, the loss of body protein was less in those subjects having a good store of body fat than in those which were thin.

Kayser compared the efficiency of carbohydrates and fats as sparers of protein by observing the effect upon the nitrogen balance of replacing the carbohydrates of the food by such an amount of fat as would furnish the same number of calories, and then after three days resuming the original diet. This experiment and that of Tallquist which follows are given somewhat fully, as they illustrate well the methods and results of investigations based mainly upon the question of nitrogen equilibrium. The observer, who served as his own subject, was twenty-three years old, of good physique, with a small store of body fat, and weighed 67 kilograms. In the first and third periods he ate meat, rice, butter, cakes, sugar, oil, vinegar, and salad. In the second period the diet was changed so as to consist of meat, eggs, oil, vinegar, and salad, so that practically all the carbohydrate was withdrawn and replaced by fat. The two diets had practically the same fuel value and protein content. The results of this experiment are shown in the following table:

[1] *Zeitschrift für Biologie*, Vol. 27, page 459 (1890).

NITROGEN BALANCE WHEN FEEDING ISODYNAMIC QUANTITIES OF CARBOHYDRATE AND FAT (KAYSER)

DAY	INTAKE				OUTPUT	NITROGEN BALANCE
	Total nitrogen	Fat	Carbohydrates	Fuel value	Total nitrogen	
	grams	*grams*	*grams*	*Calories*	*grams*	*grams*
1	21.15	71.1	338.2	2590	18.66	+ 2.46
2	21.15	71.8	338.2	2596	20.04	+ 1.11
3	21.15	71.8	338.2	2596	20.59	+ 0.56
4	21.31	71.8	338.2	2600	21.31	± 0.00
5	21.51	221.1	000.0	2607	23.28	− 1.77
6	21.55	217.0	000.0	2570	24.03	− 2.48
7	21.55	215.5	000.0	2556	26.53	− 4.98
8	21.10	70.4	338.2	2581	21.65	− 0.55
9	21.10	70.4	338.2	2581	19.20	+ 1.89
10	21.10	70.4	338.2	2581	19.65	+ 1.45

It is evident from the nitrogen balance of the first period that the amount of protein in the food was here greater than necessary, but that equilibrium was fully established in four days. On substituting fat for carbohydrate there is a marked increase of protein catabolism with corresponding loss of nitrogen from the body, and what is especially noteworthy, there was no evidence of any tendency to regain equilibrium during this period, but on the contrary the loss of nitrogen became greater each day the fat diet was continued; whereas, upon returning to the mixed diet, not only was the loss of protein stopped, but the body almost at once began replacing the protein it had lost, although the nitrogen and calories of the food were practically unchanged.

Tallquist [1] compared the protein-protecting powers of isodynamic amounts (amounts having equal energy value) of car-

[1] *Archiv für Hygiene*, Vol. 41, page 177.

bohydrates and fats when only a part of either was replaced by the other. The subject was Tallquist himself, a man twenty-eight years old, in good health, and weighing about 80 kilograms. The experiment was performed in Rubner's laboratory, and the diet contained such an amount of total food as was estimated by Rubner to be just about sufficient to supply the energy requirements of the body, viz., 36 Calories per kilogram per day. The experiment covered 8 days divided into two equal periods. In the first four-day period the diet was rich in carbohydrates, in the second period it was rich in fats. An excellent feature of this experiment is that there was no change in the nature of the protein fed. All foods furnishing any significant amount of nitrogen were the same in the two periods of the experiment.

The food of the first period consisted of meat, milk, butter, bread, sugar, coffee, beer. That of the second period contained the same amounts of meat, milk, bread, coffee, and beer, but less sugar, more butter, and some bacon. The same amount of salt was taken in each case. The principal data of the experiment may be summarized as follows:

NITROGEN BALANCE WHEN FEEDING ISODYNAMIC QUANTITIES OF CARBOHYDRATE AND FAT (TALLQUIST)

DAY	INTAKE					OUTPUT	NITROGEN BALANCE
	Total nitrogen	Fat	Carbohydrates	Alcohol	Fuel value	Nitrogen	
	grams	*grams*	*grams*	*grams*	*Calories*	*grams*	*grams*
1	16.27	44.0	466	18.5	2867	17.11	− 0.84
2	16.27	44.0	466	18.5	2867	14.40	+ 1.86
3	16.27	44.0	466	18.5	2867	14.65	+ 1.62
4	16.27	44.0	466	18.5	2867	15.58	+ 0.69
5	16.08	140.0	250	19.0	2873	17.66	− 1.58
6	16.08	140.0	250	19.0	2873	17.32	− 1.24
7	16.08	140.0	250	19.0	2873	15.94	+ 0.14
8	16.08	140.0	250	19.0	2873	16.22	− 0.14

Here only a part of the carbohydrate, about half of that present, and an amount representing about one third of the total fuel value of the diet, was replaced by fat. The change evidently had an unfavorable influence upon the nitrogen balance but the loss of body protein was relatively small and continued only 2 days.

Atwater[1] compared the protein-sparing action of carbohydrate and fat in experiments in which the subject, an athletic young man of 76 kilos, performed a considerable amount of work. The experiments were carried out in the respiration calorimeter and covered in all 15 experimental days upon a diet rich in carbohydrates, arranged in four periods which were alternated with four equal periods in which the diet was rich in fats. The change from carbohydrate to fat and *vice versa* involved about 2000 Calories or nearly half the fuel value of the diet. The average results per day for the entire series of experiments were as follows:

	On Diet Rich in Carbohydrate	On Diet Rich in Fat
Available Calories in food . .	4532	4524
Heat equivalent of work performed, Calories	558	554
Nitrogen in food, grams . . .	17.5	17.1
Nitrogen in feces, grams . . .	2.5	1.7
Nitrogen in urine, grams . . .	16.6	18.1
Nitrogen balance, grams . . .	− 1.6	− 2.7

Here again there is a difference in favor of the carbohydrate, but one which is so small as to be of almost no practical significance.

It appears that the carbohydrate of the food cannot be entirely replaced by an equal number of calories in the form of fat

[1] *Ergebnisse der Physiologie*, Vol. 3, Part I, page 497.

without an unfavorable effect upon the nitrogen balance; but that when the replacement is such as to affect not over one half of the total calories, the difference in protein-sparing action is but slight.

Landergren [1] also found that it is only when the carbohydrate of the diet is entirely replaced by fat that the comparison is so strikingly against the fat as it seemed to be in Kayser's experiment. In Landergren's experiments the condition studied was not one of approximate equilibrium, but rather of nitrogen hunger. He fed men diets of adequate fuel value but containing only about one gram of nitrogen daily, and found that by four days of such feeding the urinary nitrogen may be reduced to about 4 grams per day. In one experiment in which the daily food contained 750 grams of carbohydrates the urine of the fourth day showed 3.76 grams of nitrogen. The carbohydrate was then entirely replaced by fat, with the result that the following days' urines contained respectively 4.28, 8.86, and 9.64 grams of nitrogen. Evidently in the case of a man accustomed to feeding largely upon carbohydrates the complete replacement of carbohydrate by fat leads to a loss (or an increased loss) of body protein. But by subsequent experiments of the same series it was found that a diet containing nearly half its calories in carbohydrate, and nearly half in fat, had apparently the same protein-sparing power as one made up almost exclusively of carbohydrates.

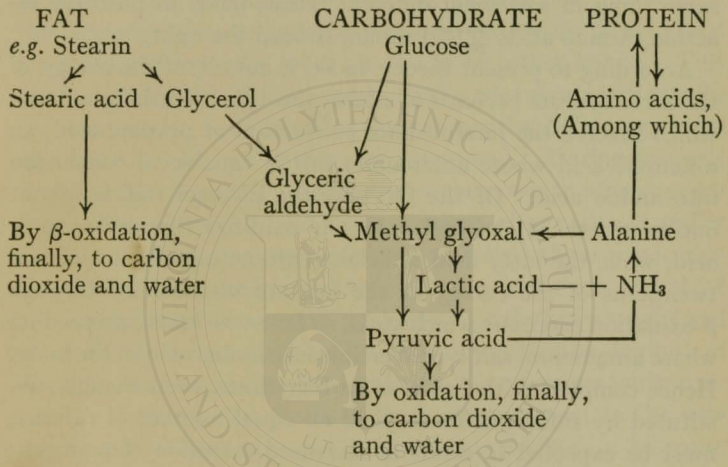
The explanation offered by Landergren is that when the diet supplies no carbohydrate, the glycogen of the body soon becomes exhausted, and the carbohydrate needed to keep up the constant glucose content of the blood is obtained largely by the breaking down of proteins.

This might suffice to explain the difference in effect of carbohydrate and fat, but not the fact that addition of a non-nitrog-

[1] *Skandinavisches Archiv für Physiologie*, Vol. 14, page 112 (1903); Abstract Experiment Station Record, Vol. 14, page 1099.

enous nutrient to a diet already sufficient may cause storage of nitrogen in the body.[1]

A satisfactory explanation of both sets of facts appears to be afforded by the advances in our knowledge of the fate of foodstuffs in metabolism which were outlined in Chapter V. The outstanding relationships of the three groups of foodstuffs in the intermediary metabolism may be indicated schematically as follows:



Since ammonia is always being formed in protein catabolism (by deaminization of amino acids), and since the ammonium salts of α-ketonic acids, such as pyruvic acid, are convertible into amino acids which are building materials for body protein, we have here a mechanism by which an intermediary product of carbohydrate metabolism (pyruvic acid) takes up a " waste product " of protein metabolism (ammonia) and turns it back into amino acid again. Thus carbohydrate, in undergoing metabolism, " spares " protein, not only by serving as fuel so that

[1] Furthermore Lusk points out that Landergren's explanation is hardly adequate to cover the results obtained in gelatin-feeding experiments.

protein need not be drawn upon for this purpose, but also by furnishing material which in combination with ammonia (otherwise a waste product) can actually be converted in the body into some of the amino acids of which body proteins are composed and with which they are in equilibrium. This explains how an increased intake of carbohydrate, with resulting increase of pyruvic acid, naturally leads to increased synthesis of amino acids and thus to a tendency toward protein storage, or, to express the same thing in somewhat different terms, tends to push the reaction, Amino acids $\rightleftharpoons$ Protein, toward the right.

According to present theory, most, if not all, of the energy of the carbohydrate becomes available through oxidation processes which involve the intermediate production of pyruvic acid, an α-ketonic acid whose ammonium salt is capable of conversion into amino acid. Of the fat, only the glyceryl radicle (about one twentieth of the fuel value) is oxidized through pyruvic acid, while the fatty acid radicles, representing about nineteen twentieths of the energy of the fat, are metabolized through β-oxidation processes which yield, so far as we know, no product whose ammonium salt is convertible into amino acid in the body. Hence complete withdrawal of carbohydrate, even though substituted by sufficient fat to yield an equal number of calories, must be expected to result in increased excretion of nitrogen; but when no more than half of the carbohydrate is replaced by fat there seems to be enough pyruvic acid produced to meet the practical requirements of economical metabolism of protein.

Protein Requirement of Adult Maintenance

From what has been said above it will be apparent that, within rather wide limits, the greater the amounts of carbohydrates and fats eaten, the smaller will be the amount of protein required to maintain nitrogen equilibrium.

For practical purposes, however, we may eliminate the question of the extent to which protein metabolism can be restricted

by the use of excessive amounts of other food and reduce the problem to this: When the total food is properly adjusted to the size and activity of the subject so that there is sufficient but not excessive fuel to meet all the energy requirements, how much protein must the daily food contain in order to keep the body in nitrogen equilibrium?

The most extended investigation on the protein requirement of man is that of Chittenden.[1] The general plan followed in this investigation was to have each man reduce his protein food gradually without any great change in his other habits. This gradual reduction of the protein intake was continued usually for some weeks, sometimes for several months, before any comparison of intake and output was attempted. During this preliminary period upon a restricted diet there was in almost every case a loss of weight, and from previous observations[2] under similar conditions we may safely assume that there was a considerable loss of body protein. After a sufficient period of adjustment there was usually a tendency for the body weight and the rate of protein metabolism (measured by the amount of nitrogen eliminated through the kidneys) to become fairly constant, indicating that the body had adapted itself to the new conditions. When this point had been reached, a nitrogen balance experiment was made, the intake and output being determined by weighing and analyzing for nitrogen all food consumed and all nitrogenous material given off from the body except that in the perspiration. The fuel value of the food consumed during the same period was calculated by means of figures taken from standard tables. From these calculated fuel values it would appear that the energy of food consumed by Chittenden's subjects was in general about equal to the usual estimates of the energy requirements for similar occupations,

[1] See Chittenden's *Physiological Economy in Nutrition* and *Nutrition of Man*.

[2] Neumann, for example, in 35 days on insufficient diet lost 96 grams of nitrogen corresponding to 600 grams of protein, equivalent to about 2.5 kilograms (5.5 pounds) of muscle tissue.

though in several specific instances the subject may have unduly restricted his total food intake and thus created an energy deficit and a tendency toward negative nitrogen balance.

Chittenden bases his estimate of the protein requirement, not only upon the nitrogen balances, but also upon the amounts of nitrogen observed to be eliminated daily through the kidneys over long periods in which the body may or may not have been in complete equilibrium, but in which health and efficiency were certainly maintained. The first men to serve as subjects in this investigation were Chittenden himself and his associates, who all continued their professional work and either reported no effect or felt benefited by the change to the low protein diet. Similar experiments were then made upon a squad of soldiers, who during the test were quartered near the laboratory and were given regular exercise in the gymnasium in addition to light duties about their quarters. These men showed marked improvement in physical condition during the test, probably due in part to their more regular habits of life and their gymnastic exercises. In order to eliminate this latter factor while still applying the low protein diet to young and physically active men, the investigation was extended to cover a group of university athletes who were already well-trained and in prime physical condition at the beginning of their dietary experiment. These athletes not only maintained, but in many cases improved, their gymnastic records while on the low protein diet, one of them winning an all-round gymnastic championship during the time. Chittenden states[1] that his data "are seemingly harmonious in indicating that the physiological needs of the body are fully met by a metabolism of protein matter equal to an exchange of 0.10 to 0.12 grams of nitrogen per kilogram of body weight per day, provided a sufficient amount of non-nitrogenous foods is taken to meet the energy requirements of the body." This would correspond to 44 to 53 grams of pro-

[1] *Nutrition of Man*, pages 226, 272

tein per day for a man of average weight (70 kilograms, 154 pounds, without clothing).

In a recent examination of the available literature upon this subject [1] there were found 109 experiments upon adults showing no abnormality of digestion or health, in which the diet was sufficiently well adjusted to the probable requirement and the nitrogen balance showed sufficient approach to equilibrium to make it appear that the total output of nitrogen might be taken as an indication of the protein requirement. These experiments are taken from 25 independent investigations in which 47 different individuals (39 men and 8 women) served as subjects. For purposes of comparison the daily output of total nitrogen in each experiment was calculated to protein and this to a basis of 70 kilograms of body weight. Reckoned in this way, the apparent protein requirement as indicated by the data of individual experiments ranged between the extremes of 21 and 65 grams, averaging 44.4 grams of protein per 70 kilograms of body weight per day.

Average results for men and for women were practically identical when calculated to the same basis of body weight (for women 44.6 grams, and for men 44.3 grams, per 70 kilograms).

Examination of the data recorded in the original papers indicates that the differences in amounts of protein catabolized in the different experiments cannot be attributed primarily to the kind of protein consumed nor to the use of diets of fuel values widely different from the energy requirements. Apparently the most influential factor was the extent to which the subject had become accustomed to a low protein diet.

In general the results obtained in the more careful investigations, which were usually longer and thus allowed more time for adjustment to the low-protein diet, have been lower than the average of all as just summarized. This average figure, 44.4 grams per 70 kilograms or 0.288 gram per pound of body

[1] *Journal of Biological Chemistry*, Vol. 41, page 97 (1920).

weight per day, is therefore undoubtedly somewhat above the actual need for maintenance in the average man or woman.

Difference between Minimum Requirement and Standard Allowance of Protein

It may be well to point out here the distinction between the amount of protein actually required on the one hand, and, on the other hand, the amount which it may be thought best to allow in the planning of dietaries. The term " requirement " should preferably be applied only to the former; the latter may better be called the protein allowance or the standard for protein. The difference between the amount actually required and the amount which would ordinarily be allowed in planning a dietary is much greater with protein than with fuel value. Surplus fuel is stored as fat, and if excessive fatness is to be avoided, the fuel value of the food must not greatly exceed the energy requirements of the body; but surplus nitrogen is rapidly eliminated from the body and, so long as no injury to health results, leaves no evidence of having been taken in excess of body needs. The eating of a considerable surplus of protein in the food is habitual, and such a surplus of protein in the food is believed by many people to constitute a desirable " factor of safety," if not indeed to exert a directly beneficial effect upon health and stamina. Hence there is a tendency to set the protein allowance or standard for protein considerably higher than the actual requirement. See discussion of standards for protein in the chapter on dietary standards beyond (Chapter XIX).

Influence of the Choice of Food

When isolated proteins are fed singly, striking differences in nutritive value appear, as has been shown in Chapter III. In view of this fact it may seem strange that in the experiments hitherto conducted to determine the protein requirement of

man the kind of protein fed has not exerted a more striking influence upon the results obtained. There is, however, no real discrepancy between the two sets of findings. The experiments described in Chapter III were for the purpose of comparing individual proteins isolated even from the other proteins which always accompany them in natural or commercial food materials, and were conducted largely upon rapidly growing young animals, in which there is an active synthesis and retention of protein, so that a deficiency in the supply of any amino acid which is required in the construction of body protein is apt to be quickly and plainly reflected in a diminution or cessation of growth. On the other hand, in experiments like those described in the preceding section, where the purpose is not to compare proteins but to measure the normal protein requirement, the diet is naturally made up, not of isolated proteins or even of single or unusual foods, but (ordinarily at least) of such combinations of staple foods as are believed to represent a normal diet, so that even a relatively simple ration arranged for the purposes of such an experiment would probably contain a number of different proteins among which any peculiarities of amino acid make-up would be apt to offset each other. Moreover, the experiments of the latter group have been made entirely upon adults whose protein requirement was limited to that of maintenance. The amount of an essential amino acid required for the so-called repair processes of maintenance may be relatively less than is needed in the construction of wholly new tissue in growth. Osborne and Mendel have suggested that in the protein metabolism of maintenance the need for a particular amino acid may be not so much for repair of tissue as to serve as the precursor of some essential hormone. McCollum suggested that the repair processes of maintenance may not involve the disruption and resynthesis of entire protein molecules, or that some of the amino acids may be used over again in the repair processes. It may also be helpful to think of the protein metabolism not only in

terms of building and repair, but also of maintaining the equilibrium which exists between proteins and amino acids in the cells of the animal tissues. In such a cell there is a constant tendency toward removal of amino acids by deaminization, offset by the inflow of amino acids derived from food protein and brought to the tissue cell by the blood stream. Concentration of any of the amino acids into which tissue proteins tend to be hydrolyzed may therefore be expected to help in pushing the reaction, amino acids $\rightleftarrows$ protein, toward the right; in other words, *any* of these amino acids will function in the *maintenance* of body protein, whereas for the synthesis of new protein as in *growth*, *all* the amino acids would be needed. Hence it is quite reasonable that proteins of very different efficiency for growth may show much more nearly equal efficiency in the normal maintenance nutrition of adults.

It is well known that most of the proteins of the cereal grains are less rich in certain amino acids essential to animal tissue, notably lysine and tryptophane, than are several other of the food proteins; and it is reasonable to expect that such differences in chemical structure among proteins imply corresponding differences in nutritive efficiency. But deficiencies established by experiments with isolated proteins do not necessarily imply corresponding deficiencies in the nutritive value of the natural mixtures of proteins found in our ordinary articles of food. Most experiments designed to compare the nutritive efficiencies of the proteins of different foods have been performed upon laboratory animals during growth, since the nutritive requirements of growth naturally tend to accentuate the differences of food value among proteins which the experiments are designed to discover or demonstrate. Largely for the same reason, however, there is danger that generalizations from such experiments may lead to exaggerated impressions. On the other hand, we would avoid giving an exaggerated impression in the other direction when we point out that any amino acid such as results from

hydrolysis of body protein may be expected to function in the maintenance metabolism. It is not probable that a molecule of tissue protein is hydrolyzed into a great number of molecules of amino acids of, for example, fifteen different kinds, in a single step. Autolysis experiments indicate rather that there are successive splittings more or less similar to those which occur in digestive proteolysis, with liberation of amino acids throughout the process as well as at the end. Any one amino acid, then, could be expected to check the process only at the point at which that amino acid would be liberated from the catabolizing protein molecule. If, however, we feed a protein which furnishes considerable amounts of, for example, twelve out of fifteen of the amino acids in question, then even though the three which are lacking are strictly essential there will still be twelve chances in fifteen of checking the catabolism of the body protein at an early stage, with correspondingly better efficiency of the "incomplete" food protein in the maintenance metabolism than in growth.

Numerous and carefully conducted experiments have now shown the possibility of healthy adult maintenance with nitrogen equilibrium upon dietaries furnishing not over 0.5 gram of protein per kilogram of body weight per day.

This was true, for example, in the experiments of Rose and Cooper with potato and of Sherman with bread as the sources of protein, in both of which nitrogen equilibrium was maintained on 0.5 gram of protein per kilogram of body weight per day; and in those of Sherman and Winters in which a young woman showed similar results upon a diet in which about nine tenths of the protein was from corn (maize) meal and about one tenth from milk.

Rose, MacLeod, and Bisbey experimented with the same subject upon diets in which the protein was furnished (1) almost entirely by meat, (2) almost entirely by milk, (3) almost entirely by a mixture of bread and milk in such proportions that the protein came in practically equal amounts from these two foods.

The food furnished 0.08 gram of nitrogen per kilogram of body weight per day. Nitrogen was stored in the body in all three cases as follows: (1) 0.06 gram per day; (2) 0.55 gram; (3) 0.41 gram.

These authors make the following comparison:

" Were we to assume a food to be biologically efficient for maintenance when nitrogen equilibrium is obtained on 0.08 gram of nitrogen or 0.5 gram of protein per kilo, per day, and assign it a value of 100 per cent, the proteins studied would have the following values: Meat, 106 per cent; milk, 155 per cent; bread and milk, 141 per cent."

A more elaborate plan of calculating the " biological values " of proteins was proposed by Karl Thomas and recently modified by H. H. Mitchell. This plan aims to express the relative nutritional values of proteins or protein mixtures for maintenance in terms of the percentage extent to which they replace or protect the protein of the body in metabolism. The data are, however, considerably influenced by the plane of protein feeding, *e.g.* the percentage of protein in the food mixture fed.

Hart and Steenbock secured satisfactory growth in young swine when about one third of the total protein of the food was furnished by milk and about two thirds by maize, whereas if milk alone or grain alone were the sole source of protein, the proteins of milk showed at least twice the nutritive efficiency of the grain proteins.

As in growth, so in lactation, the demand for material for the construction of new protein creates a condition in which differences of value in the protein fed may readily become more apparent than when only maintenance is involved. Hart and Humphreys find that in meeting the protein requirements of milch cows, milk protein and the protein of flaxseed, " oil meal," are about 50 per cent more efficient than the proteins of the corn (maize) or of the wheat kernel; and Hoobler has shown that milk is the best form of food protein for the production

of human milk and the protection of the body protein of the nursing mother.

Since estimates of protein requirement, in order to be of general application, should provide for the needs of growth, reproduction, and lactation, as well as for maintenance, it will be well to consider more fully the results obtained in feeding experimental animals upon known rations throughout the period of growth or the entire life cycle.

It will be remembered that Osborne and Mendel, feeding isolated proteins in liberal proportion (18 per cent) in diets adequate and well balanced as regards all other factors, found that edestin, a typical vegetable globulin, was able to supply all the protein requirements of maintenance, reproduction, and growth, even through three generations of rats. With gliadin as the sole protein, maintenance was satisfactory but growth was inhibited; but an addition of lysine to this diet caused an immediate resumption of growth. When the supply of lysine was cut off, growth again ceased. A ration containing zein as the sole protein did not suffice even for maintenance; but when tryptophane or gliadin, which contains tryptophane, was added to it, it served to maintain body weight, and on further addition of lysine, growth ensued.[1]

In order to emphasize such differences as these it is sometimes thought advantageous to classify proteins as:

A. Complete: Maintaining adults and providing for normal growth of the young when used as a sole protein food. Casein and lactalbumin of milk; ovalbumin and ovovitellin of egg; glycinin of soy bean; excelsin of Brazil nut; edestin, glutenin, and maize-glutelin of the cereal grains are examples.

[1] As explained in Chapter III, gliadin is now known to contain a small amount of lysine; and an additional small (and presumably constant) amount was introduced into both the gliadin and zein rations with the material used to supply the water-soluble vitamin. These facts were not appreciated in the earlier interpretation of the results; but, when properly understood, do not materially detract from the value of the experiments, at least for the purpose of this discussion.

B. Partially Incomplete: Maintaining life but not supporting normal growth. Gliadin of wheat is a well-demonstrated example of this class.

C. Incomplete: Incapable either of maintaining life or of supporting growth, when fed as the sole protein. Zein of corn (maize) and gelatin are the conspicuous examples.

Any such grouping of the proteins, however, must be used with much discrimination, and with great care to insure an understanding of the quantitative aspects of the experimental data, if misconceptions are to be avoided. Edestin is a conspicuous example of a "complete" protein, having served as above noted as the sole protein food of a family of rats for three generations; but when the percentage of edestin in the food mixture was considerably reduced, results like those above described for gliadin were obtained — the diet did not support a normal rate of growth, but this could be secured by adding lysine to the food mixture. Similarly casein when fed in reduced proportion to the total food mixture did not support normal growth; but growth became normal when cystine was added. Thus "complete" proteins may behave as "partially incomplete" when fed in reduced proportion. It is also to be remembered that varying rates of growth in different species (not to mention other differences) make inadmissible any broad generalizations as to the proportion in which any protein should be fed to species other than that with which its "completeness" or "incompleteness" has been demonstrated.

In 1916, Osborne and Mendel published quantitative measurements of the relative efficiency (for support of growth in young rats) of some of the "complete" proteins. The rate of gain obtained with 8 per cent of lactalbumin required 12 per cent of casein or 15 per cent of edestin; or, as they also state the results, "to produce the same gain in body weight 50 per cent more casein than lactalbumin was required, and of edestin nearly 90 per cent more."

Influence of Muscular Exercise

At one time it was supposed that muscular power was generated at the expense of muscle substance and this, of course, necessitated the belief that muscular work always increased protein metabolism. Since we now know that the muscles work quite as well at the expense of carbohydrates and fats as of protein, the conclusion that muscular work necessarily increases the metabolism of protein is far from inevitable. It is only necessary to observe the effects of regular muscular exercise, either in athletic training or in manual labor, to see that the muscles do not waste away when thus used, but rather tend to become larger. Such a growth of the muscles tends toward a storage rather than a loss of protein. Usually, however, muscular work also results in increased appetite, and it is difficult to separate the effects of the exercise from those of the extra food.

Whether muscular work acts directly to increase the amount of protein metabolized in the body can only be determined by experiments in which sufficient extra fats and carbohydrates are fed to furnish the extra fuel required on the working days. But since fats and carbohydrates spare protein, the feeding of these in any excess over just what is necessary to provide for the increased energy requirement would tend to decrease the metabolism of protein and counteract any effect which the muscular work might otherwise have in increasing protein metabolism. Hence, in order to show conclusively whether muscular work of itself has any influence upon the protein metabolism, it would be necessary to determine the mechanical efficiency of the man, then to bring him into equilibrium with an amount of food just sufficient for his needs, and finally to have him perform a measured amount of work at the same time adding to his diet an amount of fats and carbohydrates just sufficient to furnish the extra energy required for the work performed. Such elaborate experiments have not yet been made, but we have suffi-

cient data to show that they are not necessary for practical purposes. Many experiments have shown conclusively that increased work, when accompanied by a sufficient increase in the amount of fats and carbohydrates fed, does not necessarily increase the metabolism of protein.

The following data from Atwater (*Report of the Storrs, Connecticut, Agricultural Experiment Station for* 1902–1903, page 127) show the average results of a long series of rest and work experiments with men in the respiration calorimeter:

MUSCULAR WORK AND PROTEIN METABOLISM (ATWATER)

NATURE OF EXPERIMENT	AVERAGE METABOLISM PER DAY					
	PER PERSON		PER KILOGRAM BODY WEIGHT		PER SQUARE METER SURFACE	
	Energy, Calories	Protein, Grams	Energy, Calories	Protein, Grams	Energy, Calories	Protein, Grams
Rest: Food generally sufficient for equilibrium; 5 subjects, 27 experiments, covering 82 days	2310	103.8	33.5	1.51	1116	50.1
Work: 8 hours per day. Food generally not quite sufficient for equilibrium; 3 subjects, 24 experiments, covering 76 days	4556	108.1	62.9	1.49	2129	50.5

Comparing the figures either per unit of weight or of surface, it will be seen that muscular work sufficient to nearly double the energy metabolism had no appreciable effect upon the amount of protein metabolized. Considering the large amount of exceptionally accurate research represented in these figures, they seem to justify the conclusion that if muscular work has

any tendency to increase the " wear and tear " of muscle substance, such effect is normally balanced by the tendency of the muscles to grow (and therefore store protein) when exercised.

Moreover, it is certain that any effect which muscular work might possibly have in increasing protein metabolism would be incomparably less than its effect in increasing the total metabolism. If, then, starting with a diet which maintains protein equilibrium at rest, the total food is increased sufficiently to provide for the muscular work, and the increase in the diet is accomplished by adding any reasonable combination of food materials, we may feel sure that these will supply plenty of protein to meet any possible increase in the protein requirement. Hence, in planning the diet of a man at hard muscular work, any reasonable combination of foodstuffs given in sufficient abundance to meet the energy requirement will almost certainly supply an ample amount of protein.

Shaffer has studied the output of ammonia, creatinine, and uric acid as well as of total nitrogen during rest and work and finds no significant change in any of these. According to Lusk it may be considered fully proved that neither the amount nor the character of protein metabolism is changed by muscular activity.

Cathcart, however, in a recent review argues that if the effect of muscular work could be sufficiently investigated in the absence of interfering factors it would be found to have a slight influence in the direction of increasing the exchange of protein material in the muscular tissues.

Protein Requirement in Relation to Age and Growth

If a man at moderately active work takes a diet which furnishes 3000 Calories and 75 grams of protein, he is taking 10 per cent of his calories in the form of protein. Of course the protein requirement cannot bear a fixed relation to the calorie requirement, since the latter is largely influenced by activity, while

the former is not. Most men, when at complete rest, would require about 10 per cent of their calories in the form of protein because the lack of exercise would not reduce the protein requirement to the same extent as the energy requirement. On the other hand, most Americans are accustomed to take more than 10 per cent of their calories as protein regardless of whether they require it or not. If, then, the active man's need for protein is met by supplying him with 10 per cent of his needed calories in the form of protein, this will serve as a convenient starting point in considering the requirements of a child. Let this be compared with the normal dietary of an infant. Human milk averages about 1.6 per cent protein, 4.0 per cent fat, 7.0 per cent carbohydrate. Here about 9 per cent of the calories are taken in the form of protein, or about the same proportion as has been allowed for the full-grown active man. Furthermore, Hoobler has shown experimentally that this is as high a proportion of protein as the infant is likely to utilize with the highest efficiency in growth of body tissue. During the suckling period the growth is relatively more rapid than at any other age. Mendel [1] gives the following figures:

THE RELATIVE DAILY GAIN IN BODY WEIGHT OF CHILDREN

In the first month is about	1.00 per cent
At the middle of the first year	0.30 per cent
At the end of the first year	0.15 per cent
At the fifth year	0.03 per cent
Maximum in later years	
for boys	0.07 per cent
for girls	0.04 per cent

If, then, the full-grown man and the child at the time of most rapid growth each requires but 10 per cent of his calories in the form of protein, it seems probable that this proportion is also sufficient for any intermediate age, if the diet is of ample fuel

[1] *Childhood and Growth*, p. 18.

value, and the protein is of the right kind. But the proper selection of the protein is of very great importance in the feeding of children, who differ from most other young mammals in that their period of growth is so many times longer than the suckling period. Even the child that is nursed for a year and attains three times his birth-weight before weaning will still have much the greater part (probably five sixths) of his growth to make on other food. By the time growth is complete he will probably have about twenty times the body weight and more than twenty times the body protein with which he was born.

Growth at the normal rapid rate of early childhood involves the conversion of a very considerable part, sometimes as much as one third, of the protein of the food into body protein. This can be accomplished to the best advantage only when (1) the protein of the food is largely of the kind most efficient in supporting growth, *i.e.* milk protein; (2) the protein is well "protected" by the protein-sparing action of liberal amounts of carbohydrate and fat.

That the child needs a diet of high fuel value to meet the requirements of his energy metabolism has already been pointed out (Chapter VII). It is because the high protein requirement of childhood (for young children more than twice as much per unit of weight as for adults) is paralleled by an equally high energy requirement that the diet of the child need not contain a higher percentage of its calories in the form of protein than does the ordinary diet of the adult, if the protein for the child is well chosen.

Usually, however, a well planned dietary for a child will show a somewhat more than average proportion of its calories in the form of protein because after weaning the main feature of the child's diet should be cows' milk which furnishes about 19 per cent of its calories in the form of protein. A child, fed mainly upon cows' milk and taking enough food to cover amply his energy requirement, will therefore receive a safe surplus of pro-

tein in the best available form. With a full quart of milk in the daily dietary of the growing child the other foods may be selected chiefly with reference to other qualities than their protein content; without a liberal use of milk the proper feeding of a growing child becomes a very difficult problem.

Having discussed the protein requirements of ordinary adult maintenance and of growth, the requirements of the aged should also be considered. This does not require extended discussion since advancing age involves no new features, but only a gradual modification of those pertaining to middle life.

In general, elderly people show a somewhat diminished protein requirement and likewise a diminished power of dealing with excess. Surplus protein taken in the food is not so rapidly absorbed and catabolized, and, while there appears to be no essential difference in the form in which the nitrogen is finally excreted, the susceptibility to excessive putrefaction of protein appears to be increased. It would seem that in the dietary of the aged the protein should be reduced to at least as great an extent as are the calories.

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CHAPTER IX

INORGANIC FOODSTUFFS AND THE MINERAL METABOLISM

The Elementary Composition of the Body

FROM various estimates by different writers the average elementary composition of the human body may be presumed to be approximately as follows:

ELEMENT	APPROXIMATE PER CENT IN BODY
Oxygen, about	65.
Carbon, about	18.
Hydrogen, about	10.
Nitrogen, about	3.0
Calcium, about	1.5
Phosphorus, about	1.0
Potassium, about	0.35
Sulphur, about	0.25
Sodium, about	0.15
Chlorine, about	0.15
Magnesium, about	0.05
Iron, about	0.004
Iodine, about	0.00004
Fluorine, Silicon, Perhaps other elements	Very minute amounts

Traces of some other elements such as manganese, zinc, copper, and aluminium may perhaps be normal constituents of the body also, and even arsenic has been discussed as being possibly an essential element.

No attempt is made to discuss these latter elements in this book; but the reader who desires may get in touch with the

literature regarding them through the references at the end of the chapter.

Since all of the substances in the body are continually undergoing disintegration and renewal, it follows that there must be a constant metabolism or exchange of every element which enters into body structure. More or less of each element must each day be metabolized and eliminated; and, if equilibrium is to be maintained, an equal amount must be supplied.

Simple proteins furnish only five of the fifteen chemical elements which are known to be essential to human nutrition, while fats and carbohydrates are composed of but three of these five. Ten of the fifteen essential elements, or eight of the thirteen which are essential in amounts sufficiently large to be measurable by present methods, must therefore be furnished by some ingredients of the intake other than simple proteins, fat, and carbohydrates. These same elements are found to remain either wholly or largely in the ash of food materials when the latter are burned in the air; and when the food is metabolized in the body they are excreted chiefly in the form of mineral matter. These elements are therefore grouped as "ash constituents," or "minerals," "mineral salts," "inorganic elements," or "the inorganic foodstuffs"; and their metabolism is commonly designated as "the mineral metabolism." None of these terms is entirely appropriate. To designate the elements which remain in the ash when food is burned as ash constituents is accurate but not very instructive, since the materials of which a food ash is composed may have existed in quite different forms of combination in the food before it was burned. The terms "mineral" and "inorganic" are likely to be somewhat misleading. Some of the elements (as sodium and chlorine) do exist in the food and enter and leave the body in inorganic forms; others (as iron and sulphur) exist in the food and function in nutrition as essential constituents of organic matter and become inorganic only as the organic matter is

oxidized, *i.e.* only in the late stages of their metabolism; still others (as phosphorus) are supplied to the body by the food in both organic and inorganic forms.

General Functions of Mineral Elements

The elements concerned in " the mineral metabolism " may exist in the body and take part in its functions in at least three kinds of ways:

(1) As bone constituents, giving rigidity and relative permanence to the skeletal tissues.

(2) As essential elements of the organic compounds which are the chief solid constituents of the soft tissues (muscles, blood cells, etc.).

(3) As soluble salts (electrolytes) held in solution in the fluids of the body, giving these fluids their characteristic influence upon the elasticity and irritability of muscle and nerve, supplying the material for the acidity or alkalinity of the digestive juices and other secretions, and yet maintaining the neutrality or slight alkalescence of the internal fluids as well as their osmotic pressure and solvent power.

The unequal distribution of some ions in the body, as, for example, the greater concentration of sodium salts in the plasma and of potassium salts in the cells of the blood, may now be accounted for on the principles of membrane equilibria as formulated by Donnan, rather than in the older terms of " selective permeability " or " selective absorption." These principles also afford the best explanation of the secretion of a gastric juice containing free hydrochloric acid from cells bathed with lymph which is probably always more or less (though only faintly) alkaline.

The importance of right concentrations of the inorganic salts and ions in the tissues and fluids of the body is very great. Any considerable departure from the normal is incompatible with life.

Orr says:[1]

"The brilliant researches of Ringer, Hardy, Moore, Loeb, Höber, and others have shown that those inorganic elements play a vitally important part in all physiological processes, and that the hidden mysteries of cell life which are slowly being unraveled are intimately connected with their activities. The fundamental facts revealed by these workers throw new light on the nature of the vital processes, which are shown to be phenomena capable of explanation in terms of the comparatively simple laws which obtain in "inorganic" systems. It is impossible here, however, to do more than refer briefly to the nature of the functions of the inorganic salts and ions.

"As has been already indicated, these functions are intimately connected with colloidal activities. The visible phenomena of life are the resultants of an enormous number of chemical and physical changes in the colloids of protoplasm and of exchanges between masses of protoplasm separated by membranes or interfaces. But these changes in the physical state of colloids are determined by the association and dissociation of colloids and inorganic ions. These ions also affect the permeability of membranes and the tensions at interfaces. Hence, in a real sense, protoplasmic activity is regulated by the action of the mineral elements in solution in the protoplasm or attached to its colloids. Thus, in the contraction of muscle, though the ultimate source of energy is the oxidation of organic compounds, the initiation of the process, the mechanism by which it is carried through and the factors by which it is controlled, depend on the action of the ions and salts present which involve changes in osmotic pressure and other physical forces.

"The foregoing considerations suggest that definite degrees of concentration of the various inorganic ions in the cell fluids are necessary for the maintenance of normal protoplasmic activity. This has been fully demonstrated by work done to determine the effect of changes in the normal concentration of the different ions. The results of experiments with unicellular organisms and with isolated organs such as the perfused heart, have shown that slight alterations either in the absolute or relative concentrations of any of the inorganic ions may accelerate, retard, or even reverse, processes being carried out by means of the colloidal mechanism.

"In the animal body these changes in the concentration of the inorganic ions can be correlated with changes in the functions of the organs. Thus, all the organs regulated by the central nervous system depend for the integrity of their functions upon the maintenance of definite ratios of calcium, potassium, and sodium in the fluids within the nerve tissues. Changes in the relative proportions of these are accompanied by alteration in the excitability of nerve and in the irritability of muscle. The classical experiments of

[1] J. B. Orr: The Mineral Elements in Animal Nutrition. *Journal of the Society of Chemical Industry*, Vol. 44, No. 40, page 964 (1925).

Ringer on the perfused heart show that minute changes in the concentrations of calcium or potassium in the perfusing fluids have a profound effect on the activity of the heart. These examples are merely illustrations of the general law that any disturbance of the normal physiological balance of the salt solution of the body is accompanied by a correlated impairment of function."

Moss of Birmingham, England, has recently reported that miners working in warm air become exhausted less rapidly if supplied with drinking water containing even one fifth of one per cent common salt; which Haldane explains as due to the fact that the salt thus received through the drinking water helps replace that lost by perspiration.

Excretion of salts is constantly going on also through the kidneys and through the intestinal wall.

A man under average conditions of diet, activity, and health usually excretes daily from 20 to 30 grams of mineral salts, consisting essentially of chlorides, sulphates, and phosphates of sodium, potassium, magnesium, and calcium (as well as ammonium salts from the protein metabolism).

In a fast of 31 days recorded by Benedict, the elimination of several elements was determined quantitatively from day to day or in periods of two to three days with the results shown in the accompanying table.

It will be noted that the nitrogen output and the output of chlorine run entirely different courses, especially in the early days of the fast. Each of the other elements seems to run its own course except that the sulphur tends to remain relatively constant like the nitrogen (both being derived from protein metabolism), and the output of sodium tends to run parallel with that of chlorine, since these two elements are excreted mainly in combination with each other as common salt.

In order to support permanently normal nutrition, the intake of each essential element must of course be sufficient to cover the output; and, in the case of the growing body, to provide an additional amount for daily storage as a constituent of the new-

URINARY EXCRETION OF DIFFERENT ELEMENTS DURING A 31-DAY FAST (BENEDICT)

DAY	NITROGEN gms.	CHLORINE gms.	PHOSPHORUS gms.	SULPHUR gms.	CALCIUM gms.	MAGNESIUM gms.	POTASSIUM gms	SODIUM gms.
1	7.10	3.77	0.73	0.46	0.217	0.046	1.630	2.070
2	8.40	1.02	1.08	0.61	.243	.106	1.368	.926
3	11.34	0.79	1.10	0.68	.243	.106	1.368	.926
4	11.87	0.59	1.27	0.67	.243	.106	1.368	.926
5	10.41	0.41	1.15	0.65	.274	.098	1.445	.276
6	10.18	0.40	1.02	0.65	.274	.098	1.445	.276
7	9.79	0.55	0.80	0.62	.253	.070	.883	.154
8	10.27	0.32	0.80	0.64	.253	.070	.883	.154
9	10.74	0.31	0.93	0.66	.253	.070	.883	.154
10	10.05	0.28	0.86	0.61	.220	.072	1.006	.100
11	10.25	0.36	0.85	0.62	.220	.072	1.006	.100
12	10.13	0.31	0.74	0.62	.216	.065	—	—
13	10.35	0.32	0.85	0.62	.216	.065	—	—
14	10.43	0.26	0.81	0.60	.236	.071	.814	.109
15	8.46	0.16	0.64	0.50	.236	.071	.814	.109
16	9.58	0.14	0.89	0.59	.214	.078	—	—
17	8.81	0.12	0.87	0.53	.214	.078	—	—
18	8.27	0.15	0.81	0.54	.251	.059	.676	.051
19	8.37	0.16	0.77	0.55	.251	.059	.676	.051
20	7.69	0.15	0.64	0.51	.237	.053	.644	.066
21	7.93	0.18	0.70	0.51	.237	.053	.644	.066
22	7.75	0.21	0.69	0.50	.179	.050	.643	.083
23	7.31	0.18	0.71	0.51	.179	.050	.643	.083
24	8.15	0.10	0.68	0.49	.167	.056	.787	.065
25	7.81	0.18	0.67	0.49	.167	.056	.787	.065
26	7.88	0.16	0.65	0.54	.153	.051	.656	.055
27	8.07	0.16	0.62	0.52	.153	.051	.656	.055
28	7.62	0.14	0.59	0.53	.131	.047	.585	.036
29	7.54	0.12	0.64	0.52	.131	.047	.585	.036
30	7.83	0.14	0.61	0.52	.138	.052	.606	.053
31	6.94	0.13	0.58	0.49	.138	.052	.606	.053

formed body substances. The common assumption that all this can safely be left to chance is not correct. Not only do the different food materials differ greatly in the absolute and relative abundance of the different elements, but the same is also true of the total food intake of different groups of people. Studies of

150 freely chosen American dietaries each covering the food of a group of people for a week or more show the following range and average intake, per man per day and per 3000 Calories.

INORGANIC ELEMENTS IN 150 AMERICAN DIETARIES

ELEMENTS	PER MAN PER DAY			PER 3000 CALORIES		
	Minimum	Maximum	Average	Minimum	Maximum	Average
	gms.	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*
Calcium . . .	0.24	1.87	0.73	0.35	1.47	0.73
Magnesium . .	0.14	0.67	0.34	0.17	0.53	0.34
Potassium . .	1.43	6.54	3.39	1.63	5.27	3.40
Sodium . . .	0.19	4.61	1.94	0.22	4.83	1.95
Phosphorus . .	0.60	2.79	1.58	0.72	2.30	1.59
Chlorine . . .	0.88	5.83	2.83	0.83	7.26	2.88
Sulphur . . .	0.51	2.82	1.28	0.80	2.35	1.30
Iron	0.0080	0.0307	0.0173	0.0090	0.0234	0.0174

Since these dietary records did not show the quantities of table salt used, the figures for sodium and chlorine in the table cover only the amounts in the food as purchased and are greatly below the actual intake of these elements. It will be seen that the intake of any given element may be widely different in the different dietaries, even though each represents the daily average for at least a week. To some extent this is due to the variable amounts of total food consumed, but even when the data are reduced to a uniform basis of 3000 Calories the differences between minimum and maximum are still quite wide.

When, having in mind the great importance of right relationships among the mineral elements, we find, as in the accompanying table, that a day's food even when averaged over a period of a week or more, may contain anywhere from 0.24 to 1.87 grams of calcium; 0.14 to 0.67 gram of magnesium; 1.43 to 6.54 grams of potassium, it is evident that the mineral elements of food and their functions in nutrition are subjects calling for the most careful and quantitative study.

The purpose of this chapter is to sketch briefly the metabolism of such of these so-called mineral elements as will account for the chief bulk of the salts excreted from the body. The more detailed and quantitative study of the four elements (calcium, phosphorus, iron, and iodine) which assume special prominence in the practical problems of nutrition will be taken up in the chapters which follow.

Metabolism of Chlorides — Use of Common Salt

Except for the hydrochloric acid of the gastric juice, practically all the chlorine involved in metabolism enters, exists in, and leaves the body in the form of chlorides — much the greater part as sodium chloride. The amount of sodium chloride which is ordinarily added to food as a condiment is so large that the amounts of sodium and chlorine present in the various foods in the fresh state become of little practical consequence. Among animals, the herbivora require salt while the carnivora do not, the latter obtaining sufficient salt for their needs from the flesh, and more especially from the blood, of their prey.

Sodium salts occur abundantly in the blood and other fluids of the animal body and in much lower concentration in the tissues. Potassium salts, on the other hand, occur to a greater extent in the soft solid tissues — in the corpuscles of the blood, the protoplasm of the muscles, and other organs, and also in the highly specialized fluids which some of the glandular organs secrete, notably in milk. Since the cells are in constant contact with the circulating fluids, the abundance of potassium salts in the cells and of sodium salts in the fluids makes it evident that the taking up of salts by the cells is an active or " selective " process. This, as has been pointed out, may now be accounted for in terms of Donnan's theory of membrane equilibrium. A conspicuous function of the salts in the tissues is the maintenance of the normal osmotic pressure, but solutions of different salts of equal osmotic pressure are by no means inter-

changeable, and it is not possible to replace successfully the potassium in the cell by an equivalent amount of sodium.

There seems to be a relation between the taking up of salt and the retention of water in the tissues. The effect of decreasing the salt in the diet is to decrease the quantity of salt in the tissues, and at the same time their water content. An explanation of this lies in the fact that, since body tissues and fluids must maintain a constant concentration of sodium chloride, a reduction in the absolute quantity of salt must result in a corresponding reduction in the quantity of water present.

Attention is frequently called to the fact that sodium chloride is the only salt which we seem to crave in greater quantities than occur naturally in our food, and that we share this appetite with the herbivorous animals. Bunge held that this is because a high intake of potassium (as furnished by most vegetable foods) tends to increase sodium elimination. Bunge tested this theory upon his own person by taking potassium phosphate and citrate which was found to increase the elimination of sodium chloride.

In his *Physiological and Pathological Chemistry* (Chapter VII), Bunge records extended and interesting observations and discussion upon the relation of diet to the craving for salt, and concludes that while one might live without the addition of salt to the food even on a diet largely vegetarian, yet without salt we should have a strong disinclination to eat much of the vegetables rich in potassium, such as potatoes. "The use of salt enables us to employ a greater variety of the earth's products as food than we could do without it." But also, according to Bunge: "We are accustomed to take far too much salt with our viands. Salt is not only an aliment, it is also a condiment, and easily lends itself, as all such things do, to abuse." While Bunge's explanations may not be entirely adequate in detail, there seems to be little doubt as to the correctness of his main deductions.

Since the sodium chloride taken with the food passes through the body and is excreted by the kidneys without undergoing any chemical change, the rate of excretion quickly adapts itself to the rate of intake within wide variations.

When no chloride is taken, the rate of excretion falls rapidly to a point where the daily loss is only a very small fraction of the amount ordinarily consumed and excreted. Thus in an experiment by Goodall and Joslin [1] in which a healthy man was placed upon a diet adequate in protein and energy value but practically free from salt, the excretion of chlorine on each of 13 successive days was respectively: 4.60, 2.52, 1.88, 0.87, 0.69, 0.48, 0.46, 0.40, 0.26, 0.22, 0.22, 0.17, 0.17 grams.

Cetti in ten days of fasting excreted altogether 13.13 grams, and Belli in ten days on a diet poor in salt lost 11.8 grams of sodium chloride. In Benedict's study of prolonged fasting [2] his subject lost 8.44 grams of chlorine (equivalent to 13.93 grams sodium chloride) during the first ten days, 2.13 grams chlorine during the second ten days, and 1.57 grams chlorine during the third ten days of the fast. (The detailed data may be found on a previous page.) Since the body is supposed to contain about 100 grams of sodium chloride, it will be seen that even when there was complete deprivation of salt for ten to thirty days, the total losses did not exceed 10 to 20 per cent of the amount estimated as usually present in the body. The salt thus readily given off by the body has been regarded by some as a measure of the excess which the body has been forced to carry in consequence of the extravagant amounts of salt which are commonly taken with the food. Magnus-Levy, however, thinks that the reduced amount of sodium chloride left in the body after such a loss is "not a physiological optimum, but rather a physiological minimum."

[1] Goodall and Joslin, *Transactions of the Association of American Physicians*, Vol. 23, page 92 (1908).

[2] Benedict, Publication No. 203, Carnegie Institution of Washington.

Moderate variations in the amount of salt taken have no immediate significant effect upon metabolism. Large amounts increase the quantity of protein catabolized, and, through overstimulating the digestive tract, may also interfere with the absorption and utilization of the food. Current medical opinion favors diminished intake of sodium chloride after the age of forty or thereabouts, as it is believed to contribute to the tendency to increased blood pressure.

Metabolism of Sulphur

Plants absorb sulphates from the soil and use the sulphur in the synthesis of proteins. Minute quantities of inorganic sulphates may be taken by man in food and drink, but by far the greater part of the sulphur concerned in metabolism enters the body in organic combination and, so far as known, chiefly as protein. The metabolism of sulphur is therefore a part of the protein metabolism, and in many respects the metabolism of sulphur tends to run parallel with that of nitrogen. In a series of ten experiments (each of 3 to 5 days' duration) upon man,[1] in which the food consisted of bread and milk in varying amounts and proportions, the percentage absorption from the digestive tract was nearly the same for the sulphur as for the nitrogen of the food, and the excretion of the end products ran so closely parallel that in every case in which the body stored nitrogen it also stored sulphur, and *vice versa*.[2]

It is well known that individual proteins show relatively much greater differences in sulphur than in nitrogen content, so the ratio of nitrogen to sulphur varies widely, as is shown by the following examples selected from the data for pure proteins compiled by Osborne:

[1] Bulletin 121, Office of Experiment Stations, U. S. Department of Agriculture.

[2] Exceptions to such parallelism of nitrogen and sulphur balances have, however, been reported in certain pathological conditions.

Kind of Protein	Nitrogen Per Cent	Sulphur Per Cent	Ratio of Nitrogen to Sulphur
Legumin	18.04	0.385	46.9 : 1
Zein	16.13	0.600	26.9 : 1
Edestin	18.69	0.88	21.2 : 1
Gliadin	17.66	1.027	17.2 : 1
Leucosin	16.80	1.280	13.1 : 1
Casein	15.78	0.80	19.7 : 1
Myosin	16.67	1.27	13.1 : 1
Serum globulin	15.85	1.11	14.3 : 1
Egg albumin	15.51	1.616	9.6 : 1

Thus, while many proteins approximate the usually assumed average of 16 per cent nitrogen and 1 per cent sulphur, there are considerable deviations from this ratio in both directions.

Under ordinary conditions, however, no protein is eaten in a pure state, but only as the material containing it is used as an article of food. It is therefore the proportion of sulphur to the total protein of the food which determines the ratio of sulphur to nitrogen available for nutrition.

The proportion of sulphur to total protein has been determined in most staple foods, of which the following are representative: [1]

Food Material	Sulphur in Percentage of Total Protein
Lean beef	0.95–1.00
Eggs	1.4
Milk	0.95–1.09
Wheat flour, crackers	1.15–1.29
Entire wheat	1.30
Oatmeal	1.55
Beans	0.69–1.00
Peas	0.80–0.94
Potatoes	1.07

[1] In the data here given, nitrogen and sulphur were determined in the same specimens. Average percentages of protein and sulphur in nearly all important food materials may be found in Tables I and II, respectively, of the Appendix.

Taking these figures as typical, it would appear that in those staple foods which contribute the greater part of the protein of the diet, the ratio of protein to sulphur does not differ greatly, and that in most cases of ordinary mixed diet there would be consumed not far from 1 gram of sulphur in each 100 grams of protein. We may therefore expect that in health and on an ordinary diet the sulphur requirement will usually be covered when the protein supply is adequate.

When proteins (or their cleavage products) are oxidized in the body, the sulphur becomes converted for the most part into sulphuric acid, which, of course, must be neutralized as rapidly as it is formed. The greater part of the sulphuric acid formed in metabolism appears in the urine as inorganic sulphates; a smaller part is found combined with organic radicles in the form commonly known as "ethereal" or "conjugated" sulphates. The amount of ethereal sulphate or the ratio of ethereal to inorganic sulphate is quite variable, depending mainly upon the amount and character of the intestinal putrefaction, which in turn is apt to be considerably influenced by the food. On ordinary mixed diet about one tenth or one twelfth of the sulphate sulphur in the urine ordinarily appears as ethereal sulphates; but when the meat in the diet is replaced by milk, the putrefaction is usually lessened and the proportion of ethereal sulphates lowered. In one case of a healthy man who had been on a bread and milk diet for a week, only one thirtieth of the sulphate sulphur was in the form of ethereal sulphates.

Not all of the metabolized sulphur is eliminated as mineral or as "ethereal" sulphate; a part is given off in less completely oxidized forms. This "unoxidized" or "neutral" sulphur usually constitutes in healthy persons on full diet from 5 to 15 per cent of the total sulphur eliminated. In Folin's experiment upon very low protein diet, although the total sulphur metabolism was markedly decreased, the quantity of neutral sulphur

excreted remained about constant, so that the relative proportion of sulphur appearing in this form was increased.

Metabolism of Phosphorus

Phosphorus compounds are as widely distributed in the body and as strictly essential to every living cell as are proteins.

Phosphates are constantly excreted from the body even after long fasting. During a fast the rate of excretion of phosphates does not fall off rapidly like that of chlorides, but tends to run more nearly parallel with the nitrogen excretion, as would be expected in view of the fact that the phosphates of the urine represent not only an excretion of preëxistent salts, but also the result of the metabolism of body tissue.

While the phosphorus compounds of the body and of the food are very numerous and might be classified differently according to the standpoint from which they are being considered, it will be convenient for our present purposes to divide them into four main groups:

1. Inorganic phosphates, of which potassium phosphate is probably the most abundant in food and in the fluids and soft tissues of the body, while calcium phosphate is the chief inorganic constituent of bones.

2. Phosphorus-containing proteins, including the nucleo-proteins of cell nuclei, the lecitho-proteins, and the true phospho-proteins such as casein or caseinogen of milk and ovovitellin of egg yolk.

3. Phosphatids, phospholipins or phosphorized fats — including lecithins, lecithans, kephalins, etc. — which occur in large quantity in brain and nerve tissue and in smaller concentration (but probably as essential components) in all the cells and tissues of the body, not only of man, but of plants and animals generally. The phosphatids are therefore widely distributed in food materials, but are found in extremely varying

proportions in foods of different types. Egg yolks are conspicuously rich in phosphatids, about two thirds of the phosphorus of the egg being present in this form.

4. Phosphoric acid esters of carbohydrates and related substances such as inositol ("inosite") and the natural salts of such esters. The calcium, magnesium, and potassium salts of "phytic acid," [1] collectively known as phytates, phytins, or phytin have for some years been regarded as the most abundant phosphorus compounds of the wheat kernel and probably of the grains and legumes generally, if not of all vegetable foods. Investigations indicate, however, that not all the phosphorus compounds which were supposed to be phytins are really salts of phytic acid. As has been explained in Chapter I, starch contains phosphorus as an essential constituent, and there are other indications of phosphorus-containing carbohydrates or carbohydrate-phosphoric acid esters in food materials and also of the formation of hexose-phosphoric acid esters in the body in the course of the carbohydrate metabolism.

Thus we may think of the phosphorus with which we have to deal in food and nutrition as being partly in the form of inorganic phosphates and partly in combination with (or present as a constituent of) each of the three groups of organic foodstuffs — proteins, fats, and carbohydrates, or closely related substances.

In the course of digestion and metabolism the phosphoric acid radicles are split off from the organic radicles and ultimately nearly all of the phosphorus leaves the body as inorganic phosphate. To what extent the cleavage of the organic phosphorus compounds occurs in the digestive tract under ordinary conditions and to what extent, if at all, the phosphorus of phosphoproteins or phosphatids, for example, is absorbed in organic form is still a subject of investigation.

[1] Phytic acid is probably inositol-hexa-orthophosphoric acid, $C_6H_{24}O_{24}P_6$ (Robinson and Mueller).

Interrelations of Phosphates, Phosphoproteins, and Phosphatids

Phosphates, phosphorus-containing proteins, and phosphatids are all prominent as body constituents.

The insoluble phosphates constitute the chief mineral matter of bone; while soluble phosphates are essential constituents of the blood and protoplasm. It is largely to the presence of the phosphates that the blood plasma and protoplasm owe their ability to remain neutral or faintly alkaline, notwithstanding the constant production of acid in metabolism, as will be seen in connection with the discussion of the maintenance of neutrality below.

The nucleoproteins, as constituents of cell nuclei, and the phosphatids, as prominent constituents of brain and nerve tissue, and as less prominent but doubtless essential components of the tissues generally, have functions distinct from each other and from the phosphates. On the assumption of a more active metabolism in the cell nuclei or in the brain and nerve tissue than in the bones, there has sometimes been a tendency to regard fluctuations of phosphorus output as indicative of increased or decreased metabolism of nucleoproteins or phosphatids. It is probable, however, that the eliminated phosphorus represents more largely material which has functioned as phosphate. One reason for this is that the bones contain so large a share of the total phosphorus of the body. According to Voit's estimate, a man's skeleton contains about 600 grams of phosphorus; his muscles, about 56 grams; his brain and nerves, about 5 grams. With the bones in possession of such a predominant share of the body phosphorus, it would seem that the metabolism of bone tissue, even though relatively inactive, must exert a considerable influence upon the phosphorus output. Moreover, the soluble phosphates of the blood and protoplasm are constantly tending to be eliminated from the body (through the kidneys or the intestinal walls or both) and perhaps increasingly so in pro-

portion as they become changed into acid phosphates in the performance of their function of maintaining neutrality by reacting with the acids produced in metabolism. Before taking up the quantitative study of the phosphorus requirement we must consider the nutritive relations of the different types of phosphorus compounds, and whether these are sufficiently interchangeable in nutritive function so that one may properly speak of phosphorus requirement, simply, without discriminating between phosphates, phytates, phosphoproteins, and phosphatids.

Such experimental evidence as is cited here will be given in general in chronological order, to indicate, if possible, how present views have actually developed, and to suggest that they may at any time require modification as a result of further research.

Meischer studied the formation of complex from simpler phosphorus compounds in the adult animal body by observations upon the Rhine salmon, which during the breeding season remain a long time in fresh water, taking no food, but developing large masses of roe and milt at the expense of muscular tissue. This process evidently involves the formation of considerable amounts of nucleoproteins and phosphatids from simpler proteins, fats, and phosphorus compounds of the muscles. Paton [1] has studied the salmon of Scotland with similar results.

Marcuse,[2] followed by Steinitz,[3] Zadik,[4] and Leipziger,[5] studied, by metabolism experiments on dogs, the nutritive value of phosphoproteins, when fed to the exclusion of phosphates and when contrasted with equivalent amounts of phosphorus and nitrogen fed in the form of mixtures of inorganic phosphates

[1] *Journal of Physiology*, Vol. 22, page 333.
[2] *Archiv für die gesammte Physiologie* (Pflüger), Vol. 67, page 373.
[3] *Ibid.*, Vol. 72, page 75.
[4] *Ibid.*, Vol. 77, page 1.
[5] *Ibid.*, Vol. 78, page 402.

and simple proteins. Casein and ovovitellin were taken as typical phosphoproteins and compared with either myosin or edestin fed with inorganic phosphates. Rohmann [1] summarized the results as a whole and found a striking difference in the phosphorus balances in favor of the phosphoproteins as against the mixtures of simple proteins with inorganic phosphates. The storage of nitrogen was also more pronounced in the periods in which the phosphorized proteins were fed. The results appear to justify Rohmann's conclusion that the nutritive values of phosphorized and phosphorus-free proteins are not entirely the same, the former being especially adapted to furnish the material for tissue growth.

In experiments upon men, Ehrstrom [2] and Gumpert [3] have found that a smaller amount of phosphorus will maintain phosphorus equilibrium when taken in the form of casein than when taken largely as dicalcium phosphate or as meat, the phosphorus of which is largely in the form of potassium phosphate. On the other hand Keller [4] in a study of the phosphorus metabolism of young children found evidence that storage of phosphorus was favored by food (like milk) which contained a liberal supply of phosphates in addition to the organic phosphorus compounds; and Von Wendt found that the loss of phosphorus occurring on a diet very poor in ash could be greatly reduced by the addition of dicalcium phosphate to the food.

In cow's milk the greater part of the phosphorus appears to exist as phosphate, but there can be no doubt that the milk phosphorus as a whole is available for the needs of the young of the species, especially in view of the parallelism pointed out by Bunge and Abderhalden between the phosphorus and calcium content of milk and the rate of growth of the young. (See accompanying table.)

[1] *Berlin klinische Wochenschrift*, Vol. 35, page 789.
[2] *Skandinavisches Archiv für Physiologie*, Vol. 14, page 82.
[3] *Medische Klinik*, Vol. 1, page 1037.
[4] *Archiv für Kinderheilkunde*, Vol. 29, page 1.

Species	No. of Days Required to Double the Birth Weight	Percentage Composition of Milk (Partial)			
		Protein	Ash	Calcium	Phosphorus
Human . . .	180	1.6	0.2	0.02	0.02
Horse	60	2.0	0.4	0.09	0.06
Cow	47	3.5	0.7	0.12	0.09
Goat	22	3.7	0.78	0.14	0.18
Sheep	15	4.9	0.84	0.18	0.11
Swine	14	5.2	0.80	0.18	0.14
Dog	9	7.4	1.33	0.32	0.22
Rabbit . . .	6	14.4	2.50	0.65	0.43

It is, however, not without possible significance that the phosphorus of human milk is mainly in organic forms (Söldner) and that, notwithstanding its much lower content of total phosphorus, human milk contains as high a percentage of lecithin as does cow's milk (Stoklasa). An infant fed on diluted cow's milk must therefore receive less lecithin than the breast-fed infant while it may receive more total phosphorus.

In general, however, the more recent investigations favor the view that the body can use inorganic phosphates to meet all its phosphorus requirements.

Hart, McCollum, and Fuller showed in 1909 that with young pigs on a ration too poor in phosphorus to support normal growth the deficit could be made good by feeding phosphates as well as by feeding foods containing organic phosphorus compounds.

As young rats eat unpalatable food more readily than do adults, McCollum fed a ration containing phosphate as sole source of phosphorus to young growing rats, one of which ate the ration for 127 days, during which time he doubled in weight. At the end of this experiment the rat was killed and analyzed and found to be of normal composition. There was therefore no reason to doubt that the rat synthesized the nucleoproteins

and phosphatids of his growing tissues from the inorganic phosphorus of his food.

Subsequent experiments by McCollum and Davis, as well as those of Osborne and Mendel described in connection with the discussion of proteins (Chapter III), afford many instances of long-continued growth of rats on rations made up of isolated foodstuffs in which all or nearly all of the phosphorus was in the form of simple phosphates.

In order to determine whether the synthesis of lecithin in the animal body can be demonstrated experimentally, McCollum, Halpin, and Drescher (1912) fed 3 hens for 10 weeks a ration consisting of 30 per cent skim milk powder and 70 per cent polished rice, both of which were freed from phosphatids. This diet it will be noted contained phosphoprotein as well as phosphate, but very little fat, and it was believed no phosphatid. The hens produced eggs in normal number and of normal composition. The phosphatid in the eggs produced was 27.65 grams per hen, and this was believed to have been synthesized rather than to have come from material previously stored.

The evidence seems sufficient to warrant the statement that animal organisms are able to synthesize nucleoproteins, phosphoproteins, and phosphatids from inorganic phosphate. It may, however, be questioned whether the nutritive conditions are as favorable when the body receives its phosphorus wholly in the form of phosphates.

Forbes holds that even though the phosphorus be absorbed as inorganic phosphate there is advantage in having it supplied largely in organic forms since " much larger amounts of phosphorus may be utilized in a normal manner if they are gradually liberated in the usual way by the digestive cleavage of the organic complexes with which they are combined." [1]

The above-mentioned experiments of Rohmann and his pupils on dogs and of Ehrstrom and Gumpert on men seemed

[1] Ohio Agricultural Experiment Station, Technical Bulletin No. 5, page 357.

to demonstrate that the phosphoproteins have a higher food value than a corresponding mixture of simple proteins and simple phosphates; and the recent feeding experiments, while showing the efficiency of phosphates in meeting the phosphorus requirement, do not show conclusively that the phosphates are of fully equal value with the organic phosphorus compounds. Feeding experiments of long duration are well fitted to give convincing evidence on the former point, but are not so well suited for the purposes of exact quantitative comparisons because the very fact of their long duration gives opportunity for other factors to enter, such as differences in vitality among the experimental animals. Masslow, as the result of investigation of phosphorus metabolism during growth, holds that for the best results a considerable part of the phosphorus should preferably be supplied in organic forms.

On the other hand Marshall[1] considers the evidence fully sufficient to warrant the conclusion that organic phosphorus compounds are of no more value as food than are the inorganic phosphates; and Mendel writes[2] "... the once debated question of the superiority of calcium, phosphorus, and iron when furnished in some 'organic' form or combination no longer excites interest, since it has been observed that inorganic sources of these elements can apparently serve the requirements of animals adequately."

In the present state of our knowledge there is at least no quantitative measure of differences in nutritive value as between different forms of phosphorus. If differences in nutritive value between the different groups of phosphorus compounds exist, they are doubtless in favor of the phosphoproteins and phosphatids and are more significant for the growing than for the full-grown organism. At present it seems justifiable to assume that, if the food is properly selected, one may compute its

[1] *Journal of the American Medical Association*, Vol. 64, page 573 (1915).
[2] *Nutrition: the Chemistry of Life*, page 51 (1923).

total phosphorus content and compare it with the total phosphorus requirement of the body without separate computation of the different forms of phosphorus.

The quantitative consideration of the phosphorus requirement will be taken up in connection with that of calcium in the next chapter.

Sodium, Potassium, Calcium, Magnesium

The distribution of sodium and potassium in the body and some of their mutual relations in metabolism have been referred to in the section on the chlorides. The distribution and functions of calcium have been studied in greater detail than those of magnesium. It is estimated that about 85 per cent of the mineral matter of bone, or at least three fourths of the entire ash of the body, consists of calcium phosphate. Probably over 99 per cent of the calcium in the body belongs to the bones, the remainder occurring as an essential constituent of the soft tissues and body fluids. Of the magnesium in the body about 71 per cent is contained in the bones (Lusk). The muscles contain considerably more magnesium than calcium; the blood contains more calcium than magnesium.

That calcium salts are necessary to the coagulation of the blood has long been known and frequently cited as an example of the great importance of calcium salts to the animal economy. Equally striking is the function of these salts in regulating the action of heart muscle.

It is well known that heart muscle may be kept beating normally for hours after removal from the body when supplied, under proper conditions, with an artificial circulation of blood or lymph or a water solution of blood ash. Howell, Loeb, and others have studied the parts played by the several ash constituents. The sodium salts take the chief part in the maintenance of normal osmotic pressure and have also a specific influence. Contractility and irritability disappear if they are

absent, but when present alone they produce relaxation of the muscle tissue. Calcium salts also, although occurring in blood in very much smaller quantity, are absolutely necessary to the normal action of the heart muscle; while if present in quantities above normal, they cause a condition of tonic contraction ("calcium rigor"). There is a balance which must be maintained between calcium on the one hand and sodium (and potassium) on the other. Thus it is found that the alternate contractions and relaxations which constitute the normal beating of the heart are dependent in part upon the presence of a sufficient but not excessive concentration of calcium salts, and in part upon the quantitative relationship of calcium to sodium and potassium, in the fluid which bathes the heart muscle. Other active tissues of the body doubtless have analogous requirements as to inorganic salts.

Osborne and Mendel have shown that healthy growing animals (rats) can adapt themselves to shortage of chlorine, sodium, potassium, or magnesium to a rather striking degree; but that calcium and phosphorus are always needed in relatively liberal amounts.

Regarding the adequacy of the ordinary intake to meet the specific requirements for sodium, potassium, calcium, and magnesium, it would seem that only in the case of calcium is it ordinarily necessary to take thought in the selection of food materials or in the arrangement of dietaries. The amount of sodium chloride usually added to food is much more than sufficient to meet the sodium requirement of the body, even if the natural sodium content of the food be entirely disregarded. Potassium and magnesium are relatively abundant in meat (muscle) and also in most plant tissues, so that an ordinary mixed diet, unless it consists too largely of highly refined food materials, will usually furnish a safe surplus of these elements. Dietaries entirely adequate in energy value and protein content may, however, contain too little calcium. Calcium requirement is

therefore a question of much practical importance in human nutrition, and requires quantitative study, which will be taken up in the next chapter.

Relations of the Inorganic Elements to Each Other

It is evident from what has already been seen that the custom which has been more or less prevalent of referring to the ash or mineral matter of a food as if it were *a substance* is wholly illogical and incorrect. Food ash is always a mixture of the compounds of several different elements, and each element has its own functions and significance in nutrition. Even elements so closely related chemically as are sodium and potassium, or calcium and magnesium, are not only not interchangeable, but are, in some of their functions, directly antagonistic in their action in the body. Bunge's experiment showing the effect of potassium upon sodium excretion has already been noted. Meltzer and his associates have shown that the injection of magnesium salts has a marked general inhibitory effect, and that this can be quickly overcome by the subsequent injection of calcium salt. Summarizing the results of extended series of investigations by himself and others, Meltzer stated, in the *Transactions of the Association of the American Physicians* for 1908:

"Calcium is capable of correcting the disturbances of the inorganic equilibrium in the animal body, whatever the directions of the deviations from the normal may be. Any abnormal effect which sodium, potassium, or magnesium may produce, whether the abnormality be in the direction of increased irritability or of decreased irritability, calcium is capable of reëstablishing the normal equilibrium."

More recently Hart and Steenbock have found that the addition of magnesium salts to an otherwise well-balanced ration tends to cause a loss of calcium from the body. Several other observers have reported similar unfavorable effects of magnesium upon the metabolism of calcium, and some are inclined to

regard this as a matter of much importance to the well-being of the body. On the other hand, calcium seems to exert a favorable influence upon the economy of iron in metabolism, inasmuch as it appears to be possible to maintain equilibrium upon a smaller amount of iron when the food contains an abundance of calcium.

It would thus appear that an adequate study of the subject should take account of the relative, as well as the absolute, amounts of the different inorganic elements of the food. Tables are included in the Appendix at the back of this book.

The Maintenance of Neutrality in the Body

One of the interesting relationships among the ash constituents of foods is that between the acid-forming and the base-forming elements, since this has a direct bearing upon the important problem of the maintenance of neutrality in the body.

Although the reaction of normal human blood is alkaline to litmus, the actual excess of hydroxyl over hydrogen ions is found by modern methods to be so slight that blood as well as protoplasm is commonly spoken of as neutral. Thus Henderson writes: "Neutrality is a definite, fundamental, and important characteristic of the organism."

Actual measurements show that the normal reaction of the blood varies within narrow limits. The average normal hydrogen ion concentration of blood plasma has been found to lie at or near the slightly alkaline point of pH 7.4 and the range of reaction of blood compatible with life appears to be from about pH 7.0 to about pH 7.8.

The normal processes of metabolism involve a continual production in the cells of acids both "volatile" and "fixed" which must be promptly disposed of or neutralized. Much the largest in amount is carbonic acid, an exceedingly weak acid, which, as is well known, is eliminated through the lungs as carbon dioxide. In many of the earlier discussions of the subject

little attention was paid to the carbon dioxide as it was thought of as escaping so readily through the lungs; but, as will be brought out later, it has very important functions in maintaining the neutrality of the body. In contrast with carbonic acid are the so-called "fixed" acids typified by sulphuric acid, produced through the oxidation of the sulphur of the proteins. Sulphuric acid is a very much stronger acid than carbonic acid and would soon injure the cells which produce it, unless neutralized promptly. This neutralization is in fact effected, partly by the amphoteric proteins abundant in all body cells, partly by ammonia formed from the deaminization of proteins and amino acids, and notably by the "buffer" action of the mixtures of phosphates and carbonates together with hemoglobin as explained more fully below.

Neutrality and Buffer Action

As preliminary to even a brief consideration of the function of these different mechanisms for maintaining the normal, constant reaction of the blood it may be well to review briefly some of the fundamental conceptions involved.

In any solution at equilibrium, the product of hydrogen ions and hydroxyl ions at a constant temperature is approximately constant. This constant, kw, for pure water at 25° C. has been found to be about 1×10^{-14}, or, using brackets to denote molar concentrations, we have,

$$(1)\quad [H^+] \times [OH^-] = 10^{-14} \text{ at } 25^\circ \text{ C.}$$

In a neutral solution the concentrations of hydroxyl ions and of hydrogen ions are equal.

$$(2)\quad [H^+] = [OH^-]$$

then (1) may be written

$$(3)\quad [H^+]^2 = [OH^-]^2 = 10^{-14}$$

or

$$(4)\quad [H^+] = [OH^-] = 10^{-7}$$

Thus in terms of pH a neutral solution at 25° C. has a pH of 7.0.

A solution which contains a weak acid together with its salt in suitable proportions and concentrations has the property of maintaining itself at a practically constant hydrogen ion concentration even though relatively large quantities of acid or base be added to it. This is known as buffer action. The hydrogen ion concentration of such a solution depends on the ionization constant of the acid and the proportion (ratio) of the acid and its salt present. Mixtures of monosodium and disodium phosphates in suitable proportions form such buffer mixtures. The monosodium phosphate, NaH_2PO_4, gives rise to the ion, $H_2PO_4^-$, and acts as a weak acid, while the disodium phosphate, Na_2HPO_4, acts as the salt of this acid. A mixture of these salts in the proportion of approximately twice as much disodium phosphate as monosodium phosphate is neutral (pH = 7.0 at 25° C.) and, in suitable concentrations, is capable of receiving relatively large amounts of even strong acid or base with very little change of hydrogen ion concentration. Hence the presence of these phosphates in the blood and tissues is an important factor in their ability to remain nearly neutral while receiving the acid products of metabolism. Similarly the weak acid, carbonic acid, H_2CO_3, and its salt, sodium bicarbonate, $NaHCO_3$, in suitable proportions, constitute a buffer mixture and this represents an important function of these compounds in the body.

Buffers of Protoplasm and Blood

There are several factors which are generally recognized as concerned in the maintenance of the neutrality of the body. They act in part as actual means of elimination of acid and in part as buffers. Most of them function in the tissues where the active metabolism is taking place, as well as in the blood.

The blood possesses a number of buffers of which four require consideration from the point of view of this study: (1) the

plasma proteins, which, like proteins generally, are amphoteric; (2) carbonic acid and the carbonates, existing chiefly in the plasma; (3) the phosphates, in both the plasma and the corpuscles; (4) hemoglobin and its compounds in the red corpuscles of the blood. All of these except the last are as abundant and as important in the protoplasm as in the blood.

Carbonic acid produced in metabolism and eliminated as carbon dioxide through the lungs is the chief excretory product of the body. It has, in the process of excretion, a very important rôle in regulating the reaction of the body. There is a tendency for the respiratory process to hold the tension of carbon dioxide in the blood nearly constant. It has been found that a tendency to acidity in the tissues and blood is accompanied by a lowering of the tension of carbon dioxide in the lungs and alveolar air. The resulting diminution in the concentration of carbonic acid in the blood helps to restore it to its normal hydrogen ion concentration.

The fact that such large quantities of carbonic acid are produced in the body daily makes it in one sense the chief regulator of hydrogen ion concentration of the body tissues and fluids. From the more usual point of view, however, special interest attaches to the acids and salts which must be disposed of by way of the kidneys.

Phosphates are regularly present in blood and urine in notable amounts. From what has already been seen regarding the reaction of the blood, it may be inferred that in it the primary and secondary phosphates are normally present in such concentrations and proportions as to produce a practically neutral buffer mixture. In urine, on the other hand, acid phosphate predominates, because the kidney usually removes from the blood a larger proportion of primary than of secondary phosphate. Thus by virtue of this ability of the kidney to secrete an acid urine from a neutral blood, a considerable part of the excess acid produced in metabolism is readily disposed of.

The blood is capable of receiving relatively large concentrations of sulphuric acid produced in the metabolism of protein without an appreciable change in its reaction. This is accounted for largely by the buffer action of the mixtures of phosphates and of carbonic acid and bicarbonate present in the blood. The buffer action of these substances has already been discussed. When a strong acid, such as sulphuric acid which is highly ionized and which would increase the hydrogen ion concentration of the solution markedly, enters a neutral or slightly alkaline solution of phosphates and carbonates such as that found in the blood, it reacts with the salt, bicarbonate or disodium phosphate, to form the weak acid, carbonic acid or monosodium phosphate, and thus, by the formation of slightly ionized acids, the extra hydrogen ions from a highly ionized acid are taken care of. The tendency of the disodium phosphate to be hydrolyzed and thus to form hydroxyl ions also accounts in part for its ability to neutralize acids.

Moreover, there are more complicated interrelationships among the various factors in maintaining the neutrality of the body. As shown above, an increase in acid with its tendency to change bicarbonate into carbonic acid is accompanied by a decreased tension (concentration) of carbon dioxide in the blood and alveolar air. This aids in maintaining the normal proportions of the acid and its salt in the blood and tissues. Simultaneously a production of ammonia occurs which takes the place in the urine of alkali existing as salt in the blood. On the other hand the kidneys remove less alkali in combination with phosphates than exist in this state in the blood. Thus there is a tendency to spare the alkali needed as salt in the blood.

The blood has also another important means of regulating its neutrality.

Hemoglobin has a very important property and function in relation to the transfer of oxygen and carbonic acid in (and to and from) the lungs and tissues.

Blood, in passing through the lungs, takes up oxygen until the partial pressure of oxygen dissolved in the liquid is nearly the same as that in the alveolar air, and the hemoglobin has been changed almost entirely to oxyhemoglobin. The oxyhemoglobin, which is a stronger acid than hemoglobin, then reacts with the bicarbonate of the blood, liberating carbonic acid which escapes through the lungs, and forming the salt of oxyhemoglobin (commonly represented by the symbol, $BHbO_2$). Then when this oxygenated or arterial blood passes through the tissue capillaries it gives up oxygen which diffuses into the tissue cells (in which the oxygen tension is less because of the consumption of oxygen in them) leaving the salt of reduced hemoglobin, BHb. At the same time carbon dioxide diffuses from the tissues into the blood. This would tend to reduce the alkalinity of the blood; but since reduced hemoglobin is a weaker acid than oxyhemoglobin the passage of oxygen from blood to tissue tends to make the blood more alkaline.

Simultaneously the carbon dioxide entering the blood from the tissues tends to make it more acid, and thus to increase the dissociation of oxyhemoglobin into oxygen and (met)hemoglobin, raising the oxygen tension, and thereby facilitating the diffusion of oxygen from the blood to the tissues. The (met)hemoglobin is less highly ionized than the oxyhemoglobin and this change therefore "releases base for combination with carbonic acid." Thus hemoglobin while serving as a carrier of oxygen to the tissues is also taking a leading part in facilitating the removal of carbonic acid produced in the tissues.

The normal fluctuations of *fixed* acid production in healthy man on ordinary mixed diet are apparently in the main taken care of in part by neutralization with ammonia and in part by the formation and excretion of acid phosphate. In an experiment upon man by Gettler and the writer it was found that, of the extra acid formed in metabolism as the result of replacing the potato of a mixed diet by rice, about 33 per cent was ac-

counted for by the increased ammonia and about 40 per cent by the increased acidity of the urine. The remainder may have been eliminated, in part at least, through the skin, since no attempt was made to measure the amount or acidity of the perspiration, or may have been neutralized by sodium or potassium carbonate in the blood or other fixed alkali from the body with a corresponding increase in the carbon dioxide output and decrease of the reserve alkalinity of the body. In this experiment the intake and output of phosphorus was approximately the same on both diets. The increased acidity of the urine, therefore, implied an increased ratio of primary to secondary phosphate in the urine but not necessarily any increase in the amount of fixed base leaving the body. In the neutralization of sulphuric acid by means of phosphate, each molecule of the acid (representing one atom of sulphur oxidized in protein metabolism) changes two molecules of secondary into primary phosphate. In order that the original condition of equilibrium may continue, the surplus acid phosphate thus formed must be excreted. Whether or not this results in an increased excretion of phosphates and therefore of sodium or potassium (or only, as in the experiment just cited, in an altered ratio of primary and secondary phosphates in the urine), apparently depends not only upon the balance of acid-forming and base-forming elements in the food, but also upon the quantities of fixed bases and of phosphates which are being metabolized and of ammonia available from the protein metabolism. It would seem that in any case in which sulphuric acid produced in metabolism is neutralized by the sodium or potassium carbonate of the blood, the resulting sulphate must be eliminated with corresponding loss of sodium or potassium and decrease of the capacity of the blood for combining with carbon dioxide. This is an important feature of acidosis. It is diagnosed by determining the carbon-dioxide-holding capacity of a sample of blood serum and the result is expressed as the " alkali reserve " or " reserve alkalinity " of the blood.

Thus while the phosphates and carbonates of the blood and tissues serve for the immediate neutralization of acid without appreciable change in the normal reaction of the blood or tissue itself, yet when much strong acid such as the sulphuric acid from protein metabolism is neutralized in this way, there is apt to result an increased output of the base-forming elements, which if not made good by the intake must tend to diminish the " reserve alkalinity " or " alkali reserve " of the body.

That an excess of acid-forming elements in food, even if long continued, does not necessarily lead to any apparent injury is shown by experiments of McCollum, in which rats were maintained throughout a large part of their adult lives and produced healthy young on a diet of egg-yolk, in which there is a great predominance of acid-forming over base-forming elements. Yet in man an increase in the ammonia content and acidity of the urine is usually regarded (if pronounced and persistent) as indicating an unfavorable tendency. In this connection the decreased uric acid solvent power of the more acid urine is to be considered, especially in view of the present belief that the human organism does not destroy uric acid but must transport and excrete all that is produced in the body. Hindhede [1] found that the eating of vegetables, particularly potatoes, increases the capacity of the urine for dissolving uric acid. Furthermore, Hasselbalch [2] showed that the carbon dioxide tension of the alveolar (expired) air, which is indicative of the carbon-dioxide-carrying capacity and therefore of the reserve alkalinity of the blood, is influenced in a similar way by the food. On a diet rich in meat, he found a tension of 37.8 mm.; on an ordinary mixed diet, 38.3 mm.; on a vegetarian diet, 43.3 mm.

In an extended series of experiments, Blatherwick [3] likewise finds that foods which have a preponderance of base-forming

[1] *Skandinavisches Archiv für Physiologie*, Vol. 26, pages 87, 384 (1912).
[2] *Biochemische Zeitschrift*, Vol. 46, page 403 (1912).
[3] *Archives of Internal Medicine*, Vol. 14, pages 409–50 (1914).

elements lead to the formation of a urine which is less acid, both as regards hydrogen ion concentration and titration acidity, and which has an increased capacity for dissolving uric acid, while the ammonia content of the urine is diminished and the carbon dioxide tension of the alveolar air, indicative of reserve alkalinity, is increased. Conversely, foods with a predominance of acid-forming elements increase the urinary acidity and urinary ammonia, decrease the uric acid solvent power, and show, through lowered carbon dioxide tension of the alveolar air, a tendency toward depletion of the reserve alkalinity of the blood.

The benefit to health which so generally results from a free use of milk, vegetables, and fruits in the diet may be attributable in part to the fact that these foods yield alkaline residues when oxidized in the body; but this point should not be too greatly emphasized, for there are several other respects in which the eating of liberal amounts of milk, vegetables, and fruits is certainly beneficial, notably in supplying calcium, iron, and vitamins, and in improving the intestinal conditions.

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CHAPTER X

CALCIUM AND PHOSPHORUS REQUIREMENTS

As explained in Chapter IX, less emphasis than formerly is now laid upon the form of chemical combination in which calcium and phosphorus are furnished in the food. Thus it becomes logical to discuss the amounts of these elements required in nutrition, and to compare these with the amounts furnished by food materials, without necessarily giving great weight to the form or forms in which the element occurs in the food.

The present chapter will deal chiefly but not exclusively with this quantitative aspect of the calcium and phosphorus needs and supplies.

Method of Determining the Amounts Required

The method which permits of the most direct approach to the question of the amount of calcium or phosphorus needed in human nutrition, is that of the *balance experiment* conducted upon the same general plan as in the case of the nitrogen balance experiments described in Chapter VIII in connection with the study of the quantitative aspects of the protein metabolism and protein requirement.

Since both calcium and phosphorus after being metabolized in the body are excreted very largely by way of the intestine, and since the proportions of eliminated calcium or phosphorus leaving the body by way of the two chief paths of output (the kidneys and the intestine) vary widely, no conclusions whatever regarding bodily need can be drawn except from *complete* balance experiments which include quantitative determinations

of the element studied in both urine and feces. The fecal elimination is relatively much more important in studies of phosphorus than of nitrogen (protein) metabolism; and in the metabolism of calcium the excretion by way of the intestine is a more prominent factor still. Usually the greater part of the metabolized calcium, and sometimes the greater part of the metabolized phosphorus, is returned to the intestine for elimination.

Hence, also, it would be entirely misleading to consider the amount of calcium or phosphorus found in the urine alone as a measure of the amount which the body had absorbed; or the amount in the feces as a measure, or even as an indication, of what had escaped utilization. It must be kept clearly in mind that *after utilization* a large part of the phosphorus and a still larger part of the calcium is likely to be eliminated through the intestine instead of through the kidneys.

Maintenance requirements of adults are studied by determining the balance of intake and output on diets of normal character but low calcium or phosphorus content until it is found what is the minimum amount of calcium or phosphorus which will just permit of the maintenance of an equilibrium of intake and output of the element in question. (If instead of exact equilibrium there is a very small negative balance, the total output may be taken as indicating approximately the maintenance requirement.)

In studying the calcium and phosphorus requirements of growth, balance experiments are also made, but here the plan is to find the intake which will support an optimum rate of storage of the element in question in the growing body.

In pregnancy also the estimate of requirement must be based upon optimum storage rather than mere maintenance of equilibrium of intake and output.

In lactation, the output in milk must be considered in striking the balance, and what is sought is that intake which will support

if possible both a liberal milk flow and maintenance of body equilibrium during the lactation period. If this does not prove feasible, the problem is thus extended to embrace a consideration of the restoration to the body between lactation periods of an amount equal to what has been lost during lactation.

Growth experiments have been made with laboratory animals, followed by the analysis of the entire body of the experimental animal, to compare its store of calcium or phosphorus with that found in the average of undoubtedly normal cases.

Data from all of these methods will be made use of in the course of this chapter and the references at the end of the chapter will direct the reader who desires to study the original literature of the different types of investigation.

The Calcium Requirement

Calcium constitutes a larger proportion of the body weight than does any other of the " inorganic " elements. It is very unevenly distributed in the body, over 99 per cent of the total amount being in the bones. It is also very irregularly distributed among the staple articles of food, many of which are extremely poor in calcium, while milk contains it in abundance. The " ordinary mixed diet " of Americans and Europeans, at least among dwellers in cities and towns, is probably more often deficient in calcium than in any other chemical element.

In studying the effects of insufficient calcium, Voit kept a pigeon for a year on calcium-poor food without observing any effects attributable to the diet until the bird was killed and dissected, when it appeared that, although the bones concerned in locomotion were still sound, there was a marked wasting of calcium salts from other bones such as the skull and sternum, which in places were even perforated. Thus in adults there may be a continued loss of calcium without the appearance of any distinct symptoms because the losses from the blood and soft tissues may be replaced by calcium withdrawn from the

bones. The injurious effect of an insufficient intake of calcium is of course more noticeable with growing than with full-grown animals. Abnormal weakness and flexibility of the bones (resembling the condition of rickets in children) has been produced experimentally by feeding puppies with lean and fat meat only, while others of the same litter, receiving the same food, but with the addition of bones to gnaw, developed normally. In this connection it should be remembered that no animal is literally carnivorous in nature, that is, none lives on flesh alone; the animals called carnivora always eat more or less of the bones of their prey.

According to Herter [1] many cases of arrested development in infancy may be due to an insufficient assimilation of calcium from the food. Such a deficiency in the amount assimilated may be due to defective digestion or to a diet inadequate in calcium content.

Many medical writers have attributed different diseases to inadequate calcium supply or disturbance of calcium metabolism. Conclusive proof or disproof of such theories would, however, require more detailed and exact quantitative studies of the intake and output of calcium in health, and the amounts required in normal nutrition at different ages and under different conditions, than have yet been made.

The fact that normal urine has a low calcium content while the feces usually contain much the greater part of the calcium which has been taken in the food has often been interpreted as meaning that the absorption of food calcium is poor or that the calcium requirement of the body is low. It is now known, however, from experimental evidence, that most of the calcium which has been absorbed and carried through the metabolic processes is normally excreted through the intestinal wall and thus leaves the body in the feces instead of the urine. When the diet is very poor in calcium and the output of this element mate-

[1] *On Infantilism from Chronic Intestinal Infection*, New York, 1908.

rially exceeds the intake, the feces often contain a larger amount of calcium than was present in the food.

Observations upon Breithaupt and Cetti showed a considerable elimination of calcium in the feces during fasting. On the other hand, Benedict reports the result of a 31-day fast during which no feces were passed, but considerable quantities of calcium continued to be lost through the urine throughout the entire period.

Quantitative Study of the Maintenance Requirement

On account of the fluctuating distribution of the calcium between urine and feces, conclusions regarding the calcium requirement can properly be drawn only from those experiments in which the amounts of this element in the food, in the feces, and in the urine have been directly determined. A compilation of such experiments has been made, and the reported results calculated to a uniform basis of 70 kilograms of body weight. On this basis, 97 experiments show calcium outputs ranging from 0.27 to 0.82 gram and averaging 0.45 gram of calcium " per man per day." [1]

The experiments included about equal numbers of men and women subjects.

The average is that of all available data which appear reliable as indicating the actual (minimum) requirement in that the food did not furnish an excess of calcium over the needs of the subject, and the calcium balance showed a reasonable approach toward equilibrium. It will be noted that this average of 0.45 gram calcium (equivalent to 0.63 gram CaO) represents the expenditure under conditions of closely restricted calcium intake. It corresponds to the average of 44.4 grams of protein per man per day reached in Chapter VIII, and approxi-

[1] The data of, and references to, the individual experiments upon which this average is based are summarized in the *Journal of Biological Chemistry*, Vol. 44, pages 21–27 (October, 1920).

mates the minimum of actual need rather than a normal allowance. The margin for safety should probably be larger for calcium than for protein because of the likelihood of relatively greater losses in cooking and in digestion, while there is much less danger of any injurious result from surplus calcium than from surplus protein.

Moreover, one should always be liberal in making allowance for the added calcium requirements of pregnancy, lactation, and the growth of the young.

Requirements of Pregnancy and Lactation

The calcium requirements of women are greatly increased by maternity. The need of an abundance of calcium for the rapidly growing skeleton of an infant is obvious. Before birth, and normally for several months after, this demand of the child is satisfied through the mother, whose calcium requirement is thus greatly increased. The weakening of the bones and teeth which is said to be a common accompaniment of pregnancy and lactation is held to be largely due to a withdrawal of calcium from these structures to meet the nutritive requirement of the embryo or the nursling.

Lusk also emphasizes the importance of a diet rich in calcium for pregnant women, especially during the last ten weeks of pregnancy, when the fetus is storing calcium at a rapid rate. He cites [1] the data of Hoffström,[2] who computed in considerable detail the demands of the fetus upon the mother for nitrogen, phosphorus, calcium, and magnesium at different stages of intrauterine life.

Strong confirmation of this has recently been obtained from investigation of farm animals. The experiments of Steenbock and Hart show that the production of milk in cows and goats causes a heavy drain upon the calcium of the skeleton unless

[1] Lusk. *Science of Nutrition*, 3d edition, pages 389–390.
[2] Hoffström. *Skandinavisches Archiv für Physiologie*, Vol. 23, page 326 (1910).

the amount of calcium contained in the food be very abundant. They also point out that the mammary glands likewise make large demands upon the phosphorus supply and suggest that if the food is not rich in phosphorus, the destruction of bone tissue to furnish phosphorus for milk production may result in still further loss of calcium from the body.

Forbes and Beegle in studying the mineral metabolism of the milch cow found a heavy loss of body calcium notwithstanding the fact that the food was believed to supply liberal amounts of all essential elements and was eaten in sufficient quantity to induce storage of nitrogen. That calcium may be lost from the body while nitrogen is being stored has also been emphasized by several other investigators (Steenbock and Hart, Weiser, and others). According to Forbes it may be necessary to continue high calcium feeding for some time after the cessation of lactation, in order to replace the calcium which the maternal organism has lost.

Requirements of Normal Growth

In children after weaning and throughout early childhood there are apt to be frequent disturbances of the absorption and metabolism of calcium, in some cases due to distinct disorders of digestion, in other cases to more obscure irregularities in nutrition. In order that these fluctuations shall not interfere with the steady growth of the child, it is obvious that the food must furnish a fairly liberal surplus of calcium. Even under the most favorable conditions, a rapidly growing child will presumably need more bone-making material in proportion to its total food than do adults, who alone have served as subjects for the metabolism experiments upon which our usual estimate of calcium requirement is based. Camerer, in summarizing a long series of investigations upon the food requirements of children at different ages, concluded that the amount of calcium received by the average nursling is just about sufficient to main-

tain a normal rate of growth, leaving little if any " margin of safety "; and Bunge, from a comparison of the calcium contents of different staple foods, points out that calcium more than any other inorganic element is likely to be deficient as the result of the change of diet from mother's milk to other forms of food.

Herter [1] estimates that in order to support normal growth of the skeleton there must be an average storage of about 37 grams of calcium (51.6 grams of calcium oxide) annually throughout the period from the third to the sixteenth year. This means an average daily storage of somewhat more than 0.10 gram of calcium during this thirteen-year period. In order to accomplish such a storage it is plain that the daily food of the child must contain a surplus of more than 0.10 gram of calcium per day beyond the amount required for maintenance, which latter amount should provide for the frequent failures of complete utilization which have already been mentioned.

Herbst [2] studied the calcium metabolism of 6 boys between the ages of 6 and 14 years and found that they were storing from 0.010 to 0.016 gram of calcium per kilogram per day, or 0.21 to 0.39 gram per capita per day. If normal growth of boys of these ages involves such a large storage of calcium, it is plain that the food of such boys must be rich in calcium if they are to develop advantageously. These boys consumed about 3 to 4 times as much calcium in proportion to their weight as is required for the maintenance of men.

Recently an extended series of quantitative studies of the balance of intake and output of calcium in children of from 3 to 13 years of age has been completed by Sherman and Hawley.[3]

" These experiments were carried out in four series. The purpose of the first series was to study the relation of calcium retention to age. Twelve children from 3 to 13 years old were

[1] *Infantilism.*

[2] *Jahrb. Kinderheilkunde*, Vol. 76. *Ergänzungsheft*, pages 40–130.

[3] *Journal of Biological Chemistry*, August, 1922; *Journal of Home Economics*, September, 1922; and Sherman, *Proceedings of the World's Dairy Congress*, 1923.

studied as to balance of intake and output of calcium and phosphorus during a period of 9 days. All the children were normal and received a normal mixed diet including a fixed allowance of 750 gm. of milk per child per day. This resulted in a nearly uniform calcium intake of about 1 gm. per child per day. The calcium retention varied from 0.15 to 0.62 gm. per day, increasing with the age and size of the child. Calculated to the basis of size the results show fair uniformity and indicate an average daily storage of 0.01 gm. of calcium and 0.008 gm. of phosphorus per kilo of body weight per day in normally growing children of 3 to 13 years of age. In the second series of experiments three of the children who had served as subjects during the first series were kept under continuous control and observation with quantitative determination of intake and output of calcium and phosphorus for 48 days — a series of eight experiments of 6 days each — the calcium intake being varied from period to period by systematic changes in the amount of milk in the diet, in order to determine what daily allowance of milk would induce optimum storage of calcium in the growing organism of the child. The three children studied in this series were 4, 5, and 12 years of age, and in each case it was found that optimum storage of calcium required an allowance of about a quart (750 or 1000 gm.) of milk per child per day. Combining the data of both series it is found that the average storage of calcium was 70 per cent higher (0.017 gm. as against 0.010 gm. per kilo of body weight per day) when the daily allowance of milk was increased from 750 to 1000 gm. per child per day.

"When the food intake included 1000 gm. of milk per day, the other foods of the mixed diet being taken *ad libitum*, the daily intake of calcium by these normally growing children of 4 to 12 years averaged 0.053 gm. of calcium per kilo of body weight, and of this intake approximately one third was retained in the growing body.

"The experiments of the third and fourth series were designed

to determine whether children utilize the calcium of vegetables as well as they do that of milk. In Series III the same three children were used as in Series II and calcium and phosphorus balances were determined continuously for 27 days divided into three experiments of 9 days each. During the first and third of these periods each child received 500 gm. of milk per day as the only calcium-rich food of a mixed diet, while during the second period there were added to the diet such amounts of carrots and spinach as would furnish the quantity of calcium which would have been supplied by a second 500 gm. of milk. Had the calcium of the vegetables been utilized as efficiently as that of milk, this would have been equivalent to increasing the daily allowance of milk from 500 to 1000 gm. and a distinct increase of calcium storage would have resulted as shown by the experiments of Series II. This was found not to be the case. In the final series of experiments three different children were used and the method of comparing the utilization of the calcium of the vegetables with that of milk was modified by using 750 gm. of milk per day in the first and third periods while during the second period one half of this milk was replaced by enough of the vegetables to furnish the same amount of calcium. Here again it was found that the children utilized the calcium of milk to better advantage than they did the calcium of the vegetables. The writers entertain no doubt as to the desirability of a liberal use of vegetables in the feeding of children, but the vegetables should be used in addition to a liberal allowance of milk and should not be allowed to reduce the amount of milk consumed."

Expressed merely in terms of quantity of calcium in the intake, these experiments show that for children of all ages from 3 to 13 years, inclusive, an average intake of not less than one gram of calcium per day (about twice as much as the maintenance requirement of an average man) is needed to support an optimum rate of storage in the normally growing child. But the experiments also show that better storage results when the

calcium is furnished mainly in the form of milk, than when even one half of the milk was replaced by vegetables of equal calcium content, even though the vegetables were selected and prepared with the greatest care to make them as suitable and as acceptable to the children as possible. Hence it seems better to state the optimum intake not as such a weight of calcium, merely, but as a diet containing a full quart of milk per day together with other foods suitable to the age of the child. Such a dietary will practically always contain 1.0 gram or more of calcium and a proportionately liberal amount of phosphorus, as well as an excellent protein and vitamin content.

As pointed out editorially in the *Journal of the American Medical Association* in the discussion of the experiments above described: "The dietary rule of a quart of milk each day for every child is much more than a precept based on individual opinions or drawn by analogy from the results of feeding experiments with lower animals; it now rests on scientific evidence obtained by extensive and intensive experiments directly upon the children themselves."

The Phosphorus Requirement.
Quantitative Study of the Maintenance Requirement

Since the excretion of metabolized phosphorus through the intestine is in man too large to be neglected and too variable to be allowed for by calculation, we can expect reliable data on phosphorus requirements from those experiments only in which the amounts of phosphorus are actually determined in food, in feces, and in urine. In such experiments it is found (as in the case of nitrogen) that the output obtained upon the experimental days is influenced not only by the food taken at the time, but also by the rate of metabolism to which the body had been accustomed on the preceding days. This is shown by the following results obtained in a 12-day series of experiments upon a healthy man:

PHOSPHORUS METABOLISM WITH DIFFERENT AMOUNTS OF PHOSPHORUS IN THE FOOD

EXPERIMENTAL PERIOD		PHOSPHORUS PER DAY				
No.	Duration	In Food	In Feces	In Urine	Output	Balance
		Grams	*Grams*	*Grams*	*Grams*	*Grams*
I	3 days	0.40	0.45	0.70	1.15	− 0.75
II	6 days	0.77	0.19	0.72	0.91	− 0.14
III	3 days	1.51	0.50	0.99	1.49	+ 0.02

Here the output of phosphorus was greater in the first period with 0.40 gram in the food than in the second when the food furnished 0.77 gram, probably because the first period followed and was influenced by a preceding diet fairly rich in phosphorus, whereas the output in Period II was influenced by the low-phosphorus diet of Period I. For the same reason Period II offered favorable conditions for the establishment of equilibrium on a minimum diet, but the results show that in this case the subject was unable to reach equilibrium on 0.77 gram per day, the output averaging 0.91 gram. When the intake was increased to 1.51 grams, the output rose rapidly and averaged 1.49 grams. In this case the amount which would have been just sufficient for equilibrium evidently lay between 0.91 and 1.49 grams per day. By means of well-planned experiments or series of experiments it is possible to fix for a given individual much narrower limits within which the exact amount required for equilibrium must lie, and when it is known that the intake approximates this required amount, it is justifiable to regard the output as an indication of the normal maintenance requirement.

Study of the data of 95 such phosphorus balance experiments upon 21 subjects, 14 men and 7 women, has shown a range of 0.52 to 1.20 grams with an average of 0.88 gram phosphorus (2.02 grams P_2O_5) per 70 kilograms of body weight per day. This corresponds with the average requirement of 44.4 grams of

protein and of 0.45 gram of calcium per 70 kilograms of body weight per day. In each case the figure is an average of findings as to actual requirements and should not be confused with dietary allowances or so-called dietary standards. These latter will be discussed in Chapter XIX.

Requirements of Normal Growth

In the experiments of Sherman and Hawley described in connection with the discussion of calcium requirements for growth above, it was found that optimum storage of phosphorus in the growing children of 3 to 13 years of age was not obtained until the intake reached from 1.16 to 1.46 grams of phosphorus per child per day, thus indicating that the child needs for optimum growth about one and one half times as much phosphorus as is needed by a full-grown man for maintenance.

Here, as in the case of the calcium, it seems better not to state the child's requirement in terms of the adult's, nor necessarily in terms of grams per day of intake, but rather to put the emphasis upon the actual central fact of the experiments which is that the calcium and phosphorus requirements of growth were best met by including 1000 grams (or one quart) of milk in the daily diet.

References to other studies of the balance of intake and output of calcium and phosphorus in children will be found in the paper by Sherman and Hawley (*Journal of Biological Chemistry*, Vol. 53, pages 375–399; August, 1922).

Interpretation and Application of the Findings on Calcium and Phosphorus Requirements

The Requirements of Adult Maintenance. Having now on record about 100 cases each of reasonably comparable experiments designed to measure the maintenance requirements for protein, phosphorus, and calcium, respectively, in normal human nutrition, it is of interest to compare the variability of the in-

dividual observations in the three series of experiments (each representing the work of several different investigators) and the probable errors of the three mean results as computed by accepted statistical methods.[1]

The 109 experiments upon protein requirement show a mean of 44.4 gm., a standard deviation of 9.07 gm., a coefficient of variation of 21, and a probable error of the mean of ± 0.58 gm.

In 95 experiments upon phosphorus requirement the mean with its probable error is 0.88 ± 0.01 gm., the standard deviation is 0.15 gm., and the coefficient of variation is 17.

The 97 experiments upon calcium requirement mentioned above give a mean result of 0.45 gm. with a probable error of ± 0.008 gm. and show a standard deviation of 0.12 gm. and a coefficient of variation of 27.

In each of the three cases the data are now sufficiently numerous and consistent so that the probable error of the mean is less than two per cent of its value, making it probable that all of these averages will remain nearly unchanged upon accumulation of more experimental evidence of the same kind.[2]

These mean results express the general average requirements and will lose nothing of their value as such if it be found, as it probably will be, that under special conditions the requirement becomes higher or lower than the general average figure here given. The papers of Bogert and associates cited at the end of this chapter deal with some of the special conditions which may influence the balance of intake and output of calcium and phosphorus. The apparent requirement would seem to be correspondingly affected, but only for these special conditions.

In later chapters it will also appear that one of the fat-soluble vitamins may markedly influence the economy of calcium and phosphorus in the body, but no justification has been found for

[1] A summary of such methods in so far as required for the simple statistical interpretation of experimental data such as here given, may be found in the Appendix.

[2] That a more rigorous selection of the data would result in a somewhat lower estimate of the protein requirement has been pointed out in Chapter VIII.

such reliance upon vitamin intake as would lead to neglect of an adequate normal intake of the mineral elements.

It is evident that much remains to be done to work out the detailed conditions which may influence the calcium balance; but the methods for such work are now well defined and the orientation of future efforts in this direction should be greatly helped by our present knowledge of the vitamin values of foods and especially by the fact that the average calcium requirement for the maintenance of equilibrium in normal men and women is now well established.

The calcium requirement is a little over one hundredth of the protein requirement, and the need for a margin over actual requirement as a " factor of safety " would seem to be at least as great in the case of calcium as of protein. Hence whatever the factor of safety adopted or margin allowed for individual variations, it would follow that a food supply, in order to furnish these essential nutrients in relative proportions corresponding to the needs of the body should contain at least one hundredth as much calcium as protein. This is not usually the case in American dietaries, from the study of which it appears that most Americans carry a much larger insurance as regards protein than as regards calcium.

This is clearly shown by a study of the data of over 200 American dietaries, each representing the customary food consumption of a family or larger group for a period of a week or more.

It appears from these data that both city and country dietaries in the United States are more often deficient in calcium than in any other chemical element so far investigated. The *average* of these dietaries shows a margin above actual need of about 140 per cent in the case of protein, about 80 per cent in the case of phosphorus, and about 60 per cent in the case of calcium. But in such studies the average tells us only the lesser part of what we wish to know. The high intakes of some families raise the average but do not confer any benefit upon the families whose

intake is low. In this case the most important question is the frequency with which dietary deficiencies occur. Taking the requirements of normal maintenance as the basis of comparison, it appears that among the dietaries here considered about 1 per cent were deficient in amount of protein, about 4 per cent in amount of phosphorus, and about 16 per cent were deficient in the amount of calcium which they contained. Some of the deficiencies are plainly due, at least in part, to the consumption of an insufficient amount of food. If the data of all such dietaries be increased in such ratio as to make their total food value equal to 3000 Calories per man per day, none of the dietaries would then be deficient in the amount of protein which they contain, only about 1 per cent would be deficient in phosphorus, but about 9 per cent would still be deficient in their calcium content. And if deficient from the point of view of the calcium requirement of the adult, they would be still more so when considered from the standpoint of the needs of growing children.

Thus the careful study of over two hundred dietaries representing the ordinary food habits of the people of the United States leads to the same view as that so frequently emphasized by McCollum in discussing the results of his laboratory experiments with rats, namely, that American dietaries tend to be too poor in calcium, and that for this as well as for other reasons more prominence should be given, in American food supplies, to milk in its various forms and to the leaf vegetables.

The Requirements of Children. The interpretation and practical dietary application of the data obtained from calcium and phosphorus balance experiments upon children have already been discussed. Further light upon the subject is, however, afforded by the results obtained in recent extended studies (with rats) of the influence of age, growth, and food upon the calcium and phosphorus content of the body by Sherman and MacLeod and Sherman and Quinn respectively. Here it was

found that a diet adequate according to ordinary standards but somewhat low in calcium content resulted in a retarded gain of calcium by the body which could be brought up to the normal average by addition of simple calcium salt, but not by addition of codliver oil to the diet. Since this was largely a matter of the deposition of calcium phosphate in the developing bones, the phosphorus content of the body was found low when the calcium was low and rose to normal with the calcium when calcium was added to the food, although this involved no increased intake of phosphorus. That the better retention of both calcium and phosphorus should depend thus directly upon the calcium intake and be so little affected by the " antirachitic " or " calcium conserving " vitamin of the codliver oil is striking evidence of the fundamental importance of an adequate intake of calcium and phosphorus in the food, especially throughout the period of growth and development.

What happens when the intake of calcium is below the requirement? Usually growth is retarded. Sometimes growth in size occurs while the normal development of the bone is interfered with.

McCollum noticed that in his rats whose growth was retarded by diets consisting too largely of seeds, there was a tendency for the skeleton to lose its long lithe form and become more or less abnormally stocky with frequent gross appearances suggestive of rickets.

Sherman and Pappenheimer found that experimental rickets in rats could be produced or prevented at will by a simple change in the phosphate content of the diet, all other conditions being exactly the same. The " rickets-producing " diet in this case consisted of patent flour to which had been added certain salts including a salt of calcium. Without a corresponding supply of phosphorus, rickets developed; with it, the rickets was prevented. This observation was quickly confirmed and extended by McCollum, Shipley, and Park and their associates in Balti-

more, and by Hess, Pappenheimer, and Zucker and their collaborators in New York.

The results obtained by these two groups of investigators are for the most part in good agreement. Both recognize two types of rickets which may be designated as low-phosphorus and low-calcium rickets respectively. Both recognize rickets as essentially a perversion of the mineral metabolism; and that, while there is no substance holding the same all-controlling relation to rickets that the antineuritic vitamin holds to beriberi and the antiscorbutic vitamin to scurvy, yet the rickets which would otherwise result, or which has resulted, from the abnormal proportions of phosphorus and calcium presented to the developing bone (for this does not always depend upon abnormal proportions in the food) may be prevented, or cured, by sunlight or by codliver oil.

Further discussion of the rickets problem will be deferred until the consideration of the " antirachitic vitamin " is reached (Chapters XVI and XVII).

Influence of pregnancy and lactation. The calcium and phosphorus content of the body of the young mammal at the end of the suckling period represents a very important added nutritional demand which the mother has been called upon to meet, partly during the period of pregnancy and partly during lactation. In connection with the studies of the influence of age, growth, and food upon the calcium and phosphorus content of the body (in rats), referred to above, it was found that the bearing and suckling of young resulted in a very appreciable lowering of the mother's body store of calcium and phosphorus, even though she was supplied *ad libitum* with a diet rich enough in both these elements to meet all the requirements of even the most rapid periods of growth. Similar observations have been made upon farm animals. These findings indicate that the calcium requirements of pregnancy and lactation are relatively greater not only than those of maintenance, but also than those

of growth. When, as doubtless often happens, these requirements are not fully covered by the intake, the deficit is covered by drafts upon the stored calcium of the body, much the greatest part of which is in the bones. The calcium phosphate content of the bones is thus brought below normal and can be subsequently restored to the normal only by sustaining for a very considerable time after the cessation of lactation an intake of calcium and phosphorus more liberal than would be required for the mere maintenance of equilibrium of these elements, *i.e.* an intake materially higher than the average maintenance requirement.

Calcium and Phosphorus Contents of Typical Foods

Since it is now apparent that a freely chosen food supply may not always furnish the needed amounts of calcium and phosphorus, it becomes important to know the calcium and phosphorus contents of foods and to keep this consciously in mind in the choice of foods and in the planning of dietaries so that the intake of these elements may always be maintained at a safe margin above the actual nutritive requirement. Apparently the American dietary is more often deficient in calcium than in any other element; certainly more attention should be paid to the choice of such foods as will increase the calcium content of the dietary. The use of more milk and vegetables with less meat and sugar will accomplish this and usually improve the diet in other directions as well.

The tables on the following pages show the comparative richness in calcium and phosphorus of a number of staple articles of food.

It will be seen that there are enormous differences in the calcium content of different foods, whether expressed in percentage of the food material or in relation to its protein content or energy value. Meat is exceedingly poor in calcium and is therefore, notwithstanding its high protein and phosphorus

content, a very one-sided and inadequate source of "building material." Milk is nearly as rich in phosphorus as lean meat (richer than meat of average fatness) and many times richer than any of the meats in calcium. The difference in calcium

APPROXIMATE AMOUNTS OF CALCIUM IN FOOD MATERIALS

FOOD	CALCIUM PER 100 GRAMS EDIBLE SUBSTANCE	CALCIUM PER 100 GRAMS PROTEIN	CALCIUM PER 3000 CALORIES
	Grams	*Grams*	*Grams*
Beef, all lean	0.007	0.03	0.18
Eggs	0.067	0.5	1.35
Egg yolk	0.137	0.9	1.1
Milk	0.120	3.7	5.2
Cheese	0.931	3.5	6.4
Wheat, entire grain	0.045	0.33	0.40
White flour	0.020	0.18	0.18
Rice, polished	0.009	0.06	0.04
Oatmeal	0.069	0.4	0.5
Beans, dried	0.160	0.7	1.4
Beets	0.029	1.9	1.9
Cabbage	0.045	2.8	4.3
Carrots	0.056	5.1	3.7
Potatoes	0.014	0.6	0.5
Turnips	0.064	5.0	4.8
Apples	0.007	1.9	0.36
Bananas	0.009	0.7	0.27
Oranges	0.045	5.7	2.6
Prunes, dried	0.054	2.6	0.5
Almonds	0.239	1.2	1.1
Peanuts	0.071	0.3	0.4
Walnuts	0.089	0.5	0.4

content between the whole grains and the "fine" mill products, while not so great as in the case of iron or phosphorus, is still considerable. Polished rice and new process corn meal are even poorer in calcium than white flour. In general the milling removes more than half of the calcium and at least as large a proportion of the phosphorus. The fruits and vegetables in

general are fairly rich in calcium, while some of the green vegetables are strikingly so; but in most cases the intake of calcium depends mainly upon the extent to which milk (and its products other than butter) enters into the dietary. By far the most

Approximate Amounts of Phosphorus in Food Materials

Food	Phosphorus Per 100 Grams Edible Substance	Phosphorus Per 100 grams Protein	Phosphorus Per 3000 Calories
	Grams	*Grams*	*Grams*
Beef, all lean	0.218	0.96	5.2
Eggs	.180	1.35	3.66
Egg yolk	.524	2.73	3.54
Milk	.093	2.82	4.02
Cheese	.683	2.58	4.68
Wheat, entire grain	.423	3.25	3.54
White flour	.092	0.81	0.78
Rice, polished	.096	1.19	0.81
Oatmeal	.392	2.36	2.97
Beans, dried	.471	2.20	4.11
Beets	.039	2.42	2.52
Carrots	.046	4.17	3.03
Potatoes	.058	2.60	2.07
Turnips	.046	3.55	3.51
Apples	.012	3.15	0.60
Bananas	.031	2.35	0.93
Oranges	.021	2.58	1.20
Prunes, dried	.105	5.00	1.05
Almonds	.465	2.25	2.16
Peanuts	.399	1.55	2.19
Walnuts	.357	1.96	1.53

practical means of insuring an abundance of calcium in the dietary is to use milk freely as a food.

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CHAPTER XI

IRON IN FOOD AND ITS FUNCTIONS IN NUTRITION

THE amount of iron contained in the body is small — rather less than 3 grams or hardly one tenth of an ounce in the entire body of a healthy full-grown person, or about 0.004 per cent, or 1 part in 25,000 by weight — yet its functions are very important.

Iron stands in the closest possible relation to the fundamental processes of nutrition, being an essential element both of the oxygen-carrying hemoglobin of the blood and of the chromatin substances which appear to control (in some degree at least) the most important and " vital " activities within the cells. Recent work indicates also that small amounts of iron in other forms may play an important part in catalyzing the oxidation-reduction reactions through which the potential energy of the oxidizable foodstuffs is made kinetic for the support of the work of the active tissues of the body.

It is therefore of the highest importance that the food shall supply sufficient amounts not only of iron, in forms which can be absorbed, but also of all other elements and substances which may be needed in connection with the assimilation of the iron and its transformation into such complex body substances as hemoglobin and the chromatins.

Much the greater part of the iron present in the body at any given time is believed to exist in the form of actively functioning substances; and if this is true it must follow that the body does not possess any such large reserve store of iron in any or all of its tissues as would correspond to the reserve of calcium and phosphorus which it normally carries in the fully developed bones.

Since the iron in the normal body exists so largely in the form of hemoglobin and any reserve store of iron must be relatively small, it follows that if the intake of iron fails to equal the output there must soon result some diminution of hemoglobin which, if allowed to continue, must lead to a greater or less degree of anemia. Hence studies of the occurrence and absorption of food iron and its assimilation in the body have been very largely interwoven with consideration of the causes and prevention of anemia and the mechanism of formation of hemoglobin and red blood cells in the body. Since the scope of this book does not permit of much consideration of the abnormalities of nutrition, the present chapter will deal chiefly with the iron requirements of normal nutrition, the occurrence and relative amounts of iron in staple articles or types of food, and the assimilation of food iron and its use in the formation of hemoglobin, including some consideration of the problem of the supply of organic radicles which are needed along with the iron as essential constituents of the hemoglobin molecule.

Development of Modern Views [1]

It has long been known that iron is essential to the nutrition of both plants and animals, and that small amounts of the oxide or phosphate of iron occur in the ash of all natural food materials. A few decades ago it was assumed that the iron exists in the food as oxide or phosphate, and that hemoglobin is formed in the body by the combination of protein with inorganic iron. This view was hardly consistent with the ideas of animal metabolism taught by Leibig and generally held at the time, but appeared to be supported by the successful use of inorganic iron in the treatment of anemia.

The results obtained in a number of investigations published

[1] A fuller account of the development of the subject up to 1907 has been given by the writer in Bulletin No. 185 of the Office of Experiment Stations, U. S. Department of Agriculture.

between 1854 and 1884 threw doubt upon the utilization of inorganic iron for the production of hemoglobin.

In the attempt to harmonize these results with clinical experience it was suggested that the inorganic iron might act by absorbing the hydrogen sulphide formed in the intestine, thus protecting the food iron from waste.

The view that medicinal iron acts by stimulation of the absorbing membrane was also advocated at about this time. It was held that the amount of iron in the ordinary food is sufficient for the needs of the body, but that sometimes the intestinal mucous membrane becomes so bloodless that it cannot properly perform its functions of absorption. Under such conditions inorganic iron was believed to stimulate and tone up the membrane so that in a short time the increased absorption of food iron makes good the deficiency in the blood.

A very suggestive discussion of the metabolism of iron, the effects of a lack of iron in the food, and the amounts of iron required for the maintenance of the body in health was published by Von Hösslin in 1882, and long before this some attention had been given to the iron content of food materials by Boussingault. Boussingault's figures, however, are not sufficiently accurate to be of value at the present time, and little attention was given to the subject discussed by Von Hösslin until it was reopened by Bunge about two years later.

Bunge, in 1884, doubting the ability of the animal body to form hemoglobin from inorganic iron, undertook the study of the iron compounds of food materials in order to find in what form iron is normally absorbed and from what sort of iron compounds the growing organism ordinarily forms its hemoglobin. Practically all of the iron of eggs was found to be in the yolk. Yolk of egg does not contain any hemoglobin, but it must contain substances from which hemoglobin can be formed, since the incubation of the egg results in the development of hemoglobin without the introduction of anything from without. Bunge

found no inorganic iron in egg yolk, but isolated considerable amounts of the precursor of hemoglobin, which he called " hematogen," and which exhibited the properties of a phosphoprotein containing about 0.3 per cent of iron in such firm " organic " combination that it gives none of the ordinary reactions of iron salts. In milk, cereals, and legumes similar organic compounds of iron and only traces of inorganic iron were found. At this time Bunge distinctly stated that iron occurs in food solely in the form of complicated organic compounds which have been built up by the life processes of plants. In this form, said Bunge, is the iron absorbed and assimilated, and from these compounds hemoglobin is produced.

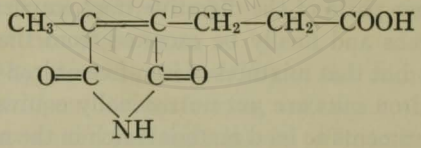
In 1890 and subsequently, the absorption and assimilation of iron was studied by several experimenters, usually with particular reference to the question whether inorganic or synthetic organic compounds of iron are absorbed and assimilated, and especially whether such preparations contribute directly to the formation of hemoglobin.

The chief net result of these investigations (of 1890 to 1905) was to show that both " organic " iron such as occurs in ordinary foods and " inorganic " iron such as is supplied by iron-containing medicines and medicinal waters may be absorbed from the same parts of the digestive tract, carried to the same internal organs and finally be excreted from the body by the same paths; but that mixtures of iron-free or iron-poor food materials with iron salts are not nutritionally equivalent in actual feeding experiments to food materials rich in the natural organic compounds of iron such as were shown to occur, for example, in green vegetables and in egg yolk and to which, as isolated from such food materials, the name hematogen was given.

These feeding experiments, many of which were very comprehensive and appear to have been very carefully performed, now require reinterpretation in the light of more recently developed knowledge.

The points which seem to need special consideration are whether the diets consisting of iron-poor food plus iron may not have been deficient in vitamin content or in their content of those organic radicles other than the ordinary amino acids which are needed with these and with iron for the formation of hemoglobin in the body. The problem of vitamin supply may be left for consideration in later chapters. What especially concerns us here is the question whether the undoubted superiority of the diets containing foods naturally rich in organic compounds of iron is really due, as believed at the time the experiments were made, to the fact that they contained iron in an organic form, or to the fact that they furnished along with the iron a good supply of organic radicles needed with iron and ordinary amino acids for the synthesis of hemoglobin.

The formation of hemoglobin in the body calls not only for iron and for the ordinary amino acids but also for the characteristic organic radicles of the hematin group. These radicles or some of them are known to have much in common with chlorophyll and with the related pigments such as the yellow substance of egg yolk. If any radicle common to chlorophyll and hemoglobin, such for example as hematoporphyrin, $C_{33}H_{38}N_4O_6$, or even the much simpler hematic acid,

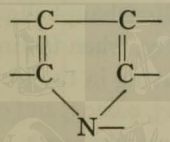


is not synthesized (or not readily synthesized) in the body, then we have in green leaves (and presumably also in egg yolks and in less degree in many other foods) sources of a necessary building stone for hemoglobin formation which would explain in part the favorable results obtained from such foods in the recent studies of blood regeneration by Whipple and in the blood formation of growing animals in the feeding experiments car-

ried out by Bunge, Hausermann, and Abderhalden during the period from 1890 to 1905 as mentioned above.

Strong support for the view that some such organic radicle or radicles may constitute an important factor in the problem of hemoglobin formation, at least as it presents itself in normal growth and very likely also in blood regeneration, may be found in the recent work of Hart, Steenbock, and their associates. They found that an anemia induced by iron-poor food was not cured by the addition of inorganic iron alone but was cured by feeding the same inorganic iron along with fresh cabbage, or an alcoholic extract of dried cabbage or of yellow corn meal, or an iron-free preparation of hemoglobin.

In this connection they quote from Robertson's *Principles of Biochemistry:* "There is every reason for supposing that the pyrrole group



cannot be synthesized by animals but must be obtained by them preformed; that is to say from the tissues of plants or from the tissues of animals which acquired it from plants. This pyrrole grouping is contained in small amounts in the majority of proteins and it forms a very important component of chlorophyll, the green coloring matter of plants, which . . . is very closely related to hemoglobin."

Thus it appears probable that the pigment-forming radicles are as essential to hemoglobin formation as are iron and protein, and that they are derived chiefly from chlorophyll and related coloring matters such as those of green and yellow vegetables, egg yolk, and milk fat.

Anson and Mirsky have used the term *haem* or *hem* for "the non-protein part of hemoglobin, containing pyrrole nuclei

and iron." [1] This they hold to be widely distributed in nature and to enter into combination with a wide variety of proteins and other nitrogen compounds. Hill has emphasized the importance of the supply of such pigment-substances in the diet of infants (*Boston Medical and Surgical Journal*, August 21, 1924).

Still more recently (M. S. Rose, 1926) it has been shown that systematic feeding of egg favored hemoglobin formation in growing children even when the iron content of the diet appeared already sufficient.

Quantitative Requirement for Iron in Nutrition

Comparatively few experiments upon the amount of food iron required for the maintenance of equilibrium in man have been made. Cetti and Breithaupt eliminated 0.0073 and 0.0077 gram per day, respectively, when fasting. Three men observed by Stockman while receiving in the food about 0.006 gram each per day eliminated 0.0063, 0.0093, and 0.0115 gram, respectively. Von Wendt found his requirements to range in a number of experiments on different diets, from 0.008 to 0.016 gram per day, the largest amount being required in a case where the diet was deficient in calcium. In three experiments by Sherman, in which the food contained 0.0057 to 0.0071 gram of iron, there was metabolized 0.0055, 0.0087, and 0.0126 gram per day, respectively, and here also the amount of iron which sufficed for equilibrium when taken in the form of bread and milk (a diet rich in calcium) was insufficient when taken in the form of a diet poor in calcium, consisting of bread and egg white, or bread alone. In this case, however, the difference in the economy of the metabolism of the iron may have been due not simply to the change in the calcium content of the food, but also to a

[1] Abstracted in *Chemical Abstracts*, Vol. 19, page 3098, from *Journal of Physiology*, Vol. 60, page 50 (1925).

superior nutritive value of the iron compounds of milk over those of bread and to the fact that the general conditions of digestion and nutrition were better when milk was included in the diet than when it was excluded. The nitrogen, phosphorus, calcium, and iron balances for two of these experiments performed upon the same man and with diets practically alike in energy value and protein content, are shown in the accompanying table:

COMPARISON OF BALANCES OF IRON AND OTHER ELEMENTS ON DIFFERENT DIETS

NATURE OF DIET	ELEMENT	AMOUNT IN GRAMS PER DAY			
		In Food	In Feces	In Urine	Balance
Bread and milk . .	Nitrogen	10.10	0.46	13.09	− 3.45
Bread and egg white .	Nitrogen	10.69	0.75	13.21	− 3.27
Bread and milk . .	Phosphorus	1.55	0.57	1.03	− 0.05
Bread and egg white .	Phosphorus	0.38	0.22	0.75	− 0.59
Bread and milk . .	Calcium	1.89	1.34	0.15	+ 0.40
Bread and egg white .	Calcium	0.10	0.34	0.07	− 0.31
Bread and milk . .	Iron	0.0057	0.0053	0.0002	+ 0.0002
Bread and egg white .	Iron	0.0065	0.0085	0.0002	− 0.0022

Here, although the nitrogen balance was practically alike on the two diets, there was on the bread and milk diet practical equilibrium of phosphorus and iron and a storage of calcium, while on the diet of bread and egg white there were noteworthy losses of all three of these elements.

Returning to the problem of the quantitative determination of the iron requirement, it will be seen that in the cases in which the intake and output of iron have been determined, the requirement appears to have varied with individuals and with

the nature of the diet from 0.006 to 0.016 gram (6 to 16 milligrams) of iron per man per day.

We might conclude from these results that a daily allowance of 10 to 12 milligrams of food iron should suffice for the maintenance of iron equilibrium in an average man under favorable conditions, but until the conditions which determine a larger metabolism of iron are more clearly defined, it would seem desirable to set a higher standard, perhaps 15 milligrams of food iron per man per day.

In calculating the iron requirement for a family dietary, it is well to make the allowance for women and children more liberal than would be indicated by their total food requirement. A woman requiring eight tenths as much food as a man will probably require more than eight tenths as much iron, and a child requiring half as much food may easily require more than half as much iron; for the influence of menstruation, pregnancy, and lactation in women and of growth and development in children may reasonably be expected to affect the demand for iron to an even greater extent than they affect the requirement for total food. It is probable that pregnancy and lactation increase the iron requirement of the mother by at least 3 milligrams per day, and at other times the losses of blood in menstruation must call for a greater intake of iron than would be needed by a healthy man of equal energy and protein requirement.

Since milk is the sole food of young mammals during a considerable period of rapid growth, Bunge was surprised to find only small amounts of iron in milk ash. Comparing the composition of the ash of milk with that of the newborn animals of the same species, it was found that, while other constituents occurred in nearly the same relative proportions, the iron was six times as abundant in the ash of the young animal as in that of the milk on which it was nourished. That the suckling animal grows rapidly and increases its blood supply in spite of this apparent paucity of iron in its food is doubtless largely due to

the fact that the body contains a reserve supply of iron at birth. In support of this view Bunge and his pupils have stated that the percentage of iron in the entire organism is highest at birth, and that during the suckling period the amount of iron in the body remains about constant, notwithstanding the increase in body weight.

In all cases in which the young depend entirely upon the milk of the mother during the suckling period the body constituents of the young must evidently be derived entirely from the maternal organism either before birth through the placenta or after birth through the milk glands of the mother and the digestive tract of the young. Since disordered digestion may readily lead to defective absorption of the iron of the food, "nature apparently takes the precaution of conveying the necessary iron from mother to offspring mainly by the safer method," *i.e.* through the placenta. Hence in the case of animals which feed solely upon milk for some time after birth, a relatively large amount of iron is stored before birth for use in the formation of hemoglobin during the suckling period. According to Bunge, this has been shown by analysis to be true of puppies, kittens, and rabbits; while on the other hand, guinea pigs, which feed on green leaves or other food rich in iron from the first day of life, are born without this reserve store of iron. The percentage of iron in the human body is believed to be about three times as high at birth as at maturity. If it be assumed, as indicated by Bunge's work, that during the milk feeding of infancy the amount of iron in the body remains about constant, it would follow that the percentage of iron in the child's body would be reduced to that in the adult when the body weight becomes about three times what it was at birth — usually when a little over one year old, — and that from this time on throughout the period of growth, care should be taken that the food be sufficiently rich in iron to provide not only for equilibrium, but also for the constantly increasing blood supply.

Iron in Foods

Little weight can be attached to such statements regarding the iron content of foods as are based upon the data obtainable from the ordinary tables of ash analyses, as these have usually been obtained by methods which are likely to greatly overestimate the amount of iron. In the table on the opposite page are shown the approximate amounts of iron now believed to be present in the average edible portion of typical food materials expressed (1) in milligrams per 100 grams of edible material, (2) in milligrams per 100 grams of protein, (3) in milligrams per 3000 Calories.

Percentages of iron in some other foods will be found in the tables of ash constituents in the Appendix. Using these data for iron in food materials, approximate estimates of the amounts of iron contained in 150 American dietaries have been made. The majority of these were found to furnish 14 to 20 milligrams of iron per man per day. Apparently, therefore, the typical American dietary does not contain any such surplus of iron as would justify the practice of leaving the supply of this element entirely to chance. The available data rather indicate that foods should be selected with some reference to the kinds and amounts of iron compounds which they contain.

Meats. In ordinary muscle meats the iron exists chiefly as hemoglobin, belonging in part to the muscle cells and in part to retained blood. Since fatty tissue contains much less iron, the iron content of fat meat is much lower than that of lean, and in order to establish any useful estimate of the amount of iron in meat it is practically necessary to consider the lean tissue alone or to refer the iron to the protein content rather than to the gross weight of the meat. The results will still be influenced by the extent to which the blood has been either accidentally or intentionally removed from the muscle.

For fresh lean beef (containing the usual proportion of blood),

IRON IN TYPICAL MATERIALS

FOOD	IRON PER 100 GRAMS FRESH SUBSTANCE	IRON PER 100 GRAMS PROTEIN	IRON PER 3000 CALORIES
	Milligrams	*Milligrams*	*Milligrams*
Beef, all lean	3.0 *	13	80 *
Beefsteak, medium fat	2.0 *	13	43 *
Eggs	3.0	22	57
Egg yolk	8.6	53	69
Milk, whole	0.24	7	10
Milk, skimmed	0.25	7	20
Cheese	1.3	5	9
Oatmeal	3.8	22	26
Rice, polished	0.9	11	7
White flour	1.0	7	7
Wheat, entire grain	5.0	37	42
Beans, dried	7.0	40	60
Beans, string, fresh	1.1	48	80
Beets	0.6	38	39
Cabbage	1.1	69	104
Carrots	0.6	55	40
Corn, sweet	0.8	26	23
Peas, dried	5.7	23	46
Potatoes	1.3	55	42
Spinach	3.6	135	450
Turnips	0.5	39	38
Apples	0.3	78	15
Bananas	0.6	47	18
Oranges	0.2	25	12
Prunes, dried	3.0	143	30
Almonds	3.9	19	18
Peanuts	2.0	8	11
Walnuts	2.1	11	9

* Figures for meats can be only rough approximations because of variations in fatness, as well as differences between different cuts. Forbes and Swift report that organs contain more iron than muscle meats, while pork and lamb contain much less than beef.

the results collected by the writer averaged 0.00375 per cent iron, but Forbes and Swift have very recently (1926) reported considerably lower results, *viz.* 0.0024 to 0.0025 per cent. Hence

in computing the data for the accompanying table a value intermediate between the averages of the two sets of findings has been used.

Some years ago, in lack of adequate investigations upon different kinds of meats, and chiefly as a means of avoiding the serious discrepancies which might otherwise arise from the great variability of meat in fatness, the writer suggested that a rough estimate of the amount of iron furnished by the meat of a dietary might be made by assuming that with every 100 grams of protein the meat would furnish about 0.015 gram (15 milligrams) of iron. This estimate of the amount of iron furnished by meats is now (1926) found by Forbes and Swift to be " a little high for beef and veal, and much too high for lamb and pork, while it does not apply at all closely in relation to heart, brain, liver, spleen, kidney, and blood." All of these latter are such minor products in comparison with ordinary muscle meats that even if completely utilized as human food their effect would be to raise but slightly the percentage of iron in the meat supply as a whole.

Hence it appears from the work of Forbes and Swift that the custom of assuming in dietary calculations that meats furnish about 15 milligrams of iron per 100 grams of protein has somewhat overestimated the value of beef and veal, and much overestimated that of lamb and pork, as sources of iron; but that the use of this factor will become more nearly correct if products such as liver, spleen, kidney, and blood are in future more largely utilized as human food. It should, however, always be kept in mind that any such single factor can serve merely for the discussion of meats as a whole and not for the comparison of one meat with another.

Eggs. The edible portion of hens' eggs has shown as the average of several analyses 0.00303 per cent of iron. Whether the iron content of eggs can be increased by giving to poultry food rich in iron, is a disputed question.

There can be no doubt regarding the assimilation and utilization of the iron compounds of eggs, since they serve for the production of all the iron-holding substances of the blood and tissues of the chick, there being no possibility of the introduction of iron from without during incubation.

Milk. Analyses of samples of cows' milk of various origin have given results varying from 0.0002 to 0.0003 per cent, and averaging 0.00024 per cent of iron in the fresh substance.

The iron of milk is readily absorbed and assimilated. Moreover, metabolism experiments indicate that the iron of milk is likely to be utilized to especially good advantage, perhaps on account of its association with a high proportion of calcium.

To what extent the vitamins of milk may also exert a favorable influence upon the economy of iron in the body remains to be determined.

Grain Products. Iron in combination with protein matter is found in considerable quantity in the cereal grains, but the greater part of it is in the germ and outer layers, and so is rejected in the making of the "finer" mill products, such as patent flour, polished rice, and new-process corn meal. In view of the part which the iron of the germ takes in the sprouting of the seed and the nutrition of the young plant, there is little room for doubt that it is of value also in the animal economy. To test the value of the iron in the outer layers of the grain Bunge [1] carried out the following experiment:

A litter of eight rats was divided into two groups of four each, one group fed upon bread from fine flour, the other upon bread made from flour including the bran. At the end of the fifth, sixth, eighth, and ninth weeks, respectively, one rat of each group was killed, and the gain in weight, the total amount of hemoglobin, and the percentage of hemoglobin in the entire body were determined. The average results were as follows:

[1] Bunge. *Zeitschrift für physiologische Chemie*, Vol. 25, page 36 (1898).

EFFECT OF FEEDING DIFFERENT KINDS OF BREAD ON GROWTH AND IRON CONTENT OF BODY IN EXPERIMENTS WITH RATS

KIND OF RATION	GAIN IN WEIGHT OF BODY	TOTAL HEMOGLOBIN IN BODY	PROPORTION OF HEMOGLOBIN IN BODY
	Grams	*Grams*	*Per cent*
White bread	4.81	0.2395	0.613
Bran bread	20.76	0.3492	0.714

Here the bran-fed rats not only made a much greater general growth, but developed both a greater amount and a higher percentage of hemoglobin. There can be no doubt that the iron and other ash constituents of the outer layers of the wheat were well utilized in these cases.

Vegetables and Fruits. Not many direct studies upon the iron compounds of the fruits and vegetables have been made, but Stoklasa has separated from onions an iron-protein compound very similar to the hematogen obtained by Bunge from egg yolk, but containing a considerably higher proportion of iron. Preparations similar in properties were also obtained from peas and from mushrooms.

In view of the fact that the herbivorous animals, which are less liable to anemia than the carnivora, obtain their normal food iron entirely from vegetable sources, there is every reason to suppose that man makes good use of the iron of the fruits and vegetables in his diet. Moreover, since (as Herter has shown) anemic conditions and excessive intestinal putrefaction often go together, the bulkiness and laxative tendency of fruits and vegetables, along with their relatively high iron content, is advantageous in combating the conditions which give rise to excessive putrefaction, and at the same time increasing the supply of food iron.

Among typical food materials omitted from the above table because of containing but little iron, may be mentioned fat

pork, bacon, lard and suet, butter, salad oil, sugars, starches, and confectionery. All of these foods have high fuel value, and many are economical and highly important factors in a normal dietary. Excessive use of these foods, however, would tend to satisfy the appetite and supply the body with the needed fuel without furnishing the desirable amount of iron. On the other hand, the fruits and fresh vegetables are often regarded as of low nutritive value because of their high water content and low proportions of protein and fat. But it is largely this property which makes them especially important as sources of food iron, because they can be added to the diet without replacing the staple foods of high calorific and protein value, and without making the total food consumption excessive. Thus the above table shows plainly that the ratio of iron both to protein and to fuel value is high in nearly all of the typical fruits and vegetables, so that in most cases it would be necessary to increase only slightly the amount of protein and fuel value derived from these sources, in order to effect a material increase in the iron content of the dietary. The iron content of eggs is also high, but the cost of these is often such as to restrict their use in families of limited means, while transportation facilities and methods of drying and preserving tend to equalize the cost and increase the available variety of fruits and vegetables throughout the year. The ratio of iron to fuel value is also high in lean meat, but here, as has already been pointed out, the iron exists largely in the form of hemoglobin, which appears to be of rather lower nutritive value than the iron compounds of milk, eggs, and foods of vegetable origin. Especially in families where there are young children it would be a mistake to rely too largely upon meat as a source of iron. Von Noorden, who is one of the strongest advocates of a liberal use of meat in the adult dietary, says in regard to the feeding of children:

"The necessity of a generous supply of vegetables and fruits must be particularly emphasized. They are of the greatest

importance for the normal development of the body and of all its functions. As far as children are concerned, we believe we could do better by following the dietary of the most rigid vegetarians than by feeding the children as though they were carnivora, according to the bad custom which is still quite prevalent. . . . If we limit the most important sources of iron, — the vegetables and the fruits, — we cause a certain sluggishness of blood formation and an entire lack of reserve iron, such as is normally found in the liver, spleen, and bone marrow of healthy, well-nourished individuals."

In an experimental dietary study made in New York City it was found that a free use of vegetables, whole wheat bread, and the cheaper sorts of fruits, with milk but without meat, resulted in a gain of 30 per cent in the iron content of the diet, while the protein, fuel value, and cost remained practically the same as in the ordinary mixed diet obtained under the same market conditions.

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CHAPTER XII

IODINE IN NUTRITION: SIMPLE GOITER AS A NUTRITIONAL DISEASE

IODINE is one of the essential chemical elements of the human body, although it constitutes only about one part in three million parts of the body weight. Sea salt, and therefore the spray which evaporates in the air at the seashore, is relatively rich in iodine; and as this sea-salt dust is carried inland by the wind it gives iodine to the rain-water, the soil water, the soils, and the crops of nearby regions. But regions which, because of too great distance or because of intervening mountains, receive practically none of the airborne sea-spray may contain in their waters and in the crops grown on their soils too little iodine to meet the needs of normal nutrition. The people who grow up in such regions show a high proportion of simple goiter, which is an enlargement of the thyroid gland resulting from its being obliged to function without an adequate iodine supply. When ordinary foods are too poor in this element, the small amount of iodine needed to prevent goiter can be supplied by adding iodide to the drinking water or the table salt, by incorporating this or some other form of iodine compound into tablets with food or confectionery, or by consuming in or with one's food a small amount of any of the sea plants which have been found to be rich in iodine. With increasing knowledge of the iodine contents of foods it may also become possible to increase materially the daily intake of iodine by proper selection and emphasis among ordinary articles of food.

Iodine and the Thyroid Gland

In 1895, Baumann discovered iodine in the thyroid gland. Subsequent work showed that this iodine is intimately connected with the activity of the thyroid. The characteristic physiological effects of administration of thyroid gland appear to be directly proportional to the iodine content of the gland administered.

In 1914, Kendall isolated from thyroid a pure crystalline substance containing sixty-five per cent of iodine, which substance he named " thyroxin " and showed to be capable of exerting the characteristic effects of thyroid upon metabolism. Whether or not the thyroxin thus obtained by Kendall in the laboratory is absolutely identical in every respect with the thyroid hormone as secreted by the gland, there is no doubt that this (the thyroid hormone) does contain iodine as an essential element. The thyroid utilizes inorganic iodine in the preparation of this hormone. Normally the small amounts of iodine contained in food and drink furnish sufficient iodide for the proper functioning of the thyroid gland and the production by it of the hormone needed to regulate metabolism throughout the body.

When the amounts of iodine furnished by the food and drink are insufficient, the thyroid gland becomes enlarged. It is now generally agreed that this is the cause of most simple goiter, and that simple goiter is, therefore, in its origin an iodine-deficiency disease.

Seaweeds and sea water have long been the familiar sources of iodine. In regions where the food comes largely from the sea, and the atmosphere and drinking water are constantly receiving the iodine-containing salt spray blown in from the sea, the intake of iodine is presumably almost always adequate and in such regions goiter is very rare; but in regions too remote from the sea or too mountainous to receive significant amounts either directly or indirectly from the sea, goiter is much more

common, as in the Great Lakes region and much of the Northwest of the United States and in parts of Switzerland.

Marine and Kimball, reasoning that if the prevalence of goiter in such regions is due to a lack of iodine it should be preventable by giving iodide to children at the age at which goiter ordinarily begins to develop, obtained permission to try their now classical experiment in the public schools of Akron, Ohio. Here as many as volunteered of the pupils of the ages known to be most susceptible to goiter were given small doses of sodium iodide dissolved in drinking water twice weekly over a period of a month and repeated twice yearly. As is well known this experiment was strikingly successful. In only 5 cases among over 2000 treated was there any enlargement of the thyroid gland when iodide was taken, while in a similar number of children of the same age in the same region not taking iodide about 500 showed enlargement during the same time. Hence it would appear that 99 per cent of the simple goiter of this region could be prevented by iodide, if means for its universal administration could be found. This work of Marine and Kimball published in 1917 and subsequently [1] has focussed attention upon the relation of iodine supply to goiter both in this country and abroad.

In three Swiss cantons where an attempt was made to give iodide to all school children during the three years 1918–1922, the incidence of goiter was diminished during the three years from 87 per cent to 13 per cent.

Iodine Requirement of the Body

Evidently the body has a more or less definite nutritive requirement for iodine. Qualitatively this requirement might be

[1] Marine and Kimball. *Journal of Laboratory and Clinical Medicine*, Vol. III, page 40 (1917), First Paper. Kimball and Marine. *Archives of Internal Medicine*, Vol. 22, page 41 (1918), Second Paper. Kimball, Rogoff, and Marine. *Journal of the American Medical Association*, Vol. 73, page 1873 (1919), Third Paper. Marine and Kimball. *Archives of Internal Medicine*, Vol. 25, page 661 (1920), Fourth Paper.

stated as a sufficient amount of iodine to meet the daily losses from the body and maintain within the body such store as is needed to provide for the manufacture within the thyroid gland and the distribution throughout the body of sufficient amounts of thyroxin (thyroid hormone) to support a normal rate of physiological activity.

Attempts to measure the actual quantities involved have now begun to appear. According to Marine, if the iodine content of the thyroid is maintained above 0.1 per cent no goiter can develop. This appears to be a suggestion providing a wide margin of safety. Under conditions of ordinary diet without administration of iodine, the percentage is likely to be lower than 0.1 per cent even in entirely normal glands. In the thyroids of healthy soldiers killed by war wounds Zunz found an average of about 0.05 per cent iodine, which, as the glands weighed about 26 to 30 grams, amounts to about 15 milligrams of iodine in the thyroid gland of a full grown healthy man. According to the estimates of Kendall and Plummer [1] the rest of the body may be expected to contain about 10 milligrams more of iodine, probably chiefly in the form of thyroxin which has been distributed by the thyroid and is serving to control metabolism in all the active tissues of the body.

Thus the full grown healthy man is estimated to contain in his 70 kilograms of body a total of about 25 milligrams of iodine, equivalent to 1 part in 2,800,000 of body substance or less than 0.00004 of one per cent of the body weight. Hence it appears that the body contains only about one hundredth as much of iodine as of iron, which hitherto has been regarded as the essential element occurring in smallest measurable amount, and both the iron and the iodine undoubtedly play indispensable parts in the processes of nutrition and must be supplied in sufficient amounts if the body is to be adequately nourished.

The difficulty of quantitative determination of iodine in the

[1] *Journal of the American Medical Association*, Vol. 77, pages 243, 1574 (1921).

extremely minute amounts in which it occurs in most plant and animal tissues must be expected to render somewhat slow and uncertain the working out of satisfactory data on the iodine values of foods and the quantitative requirements of the body for iodine at different ages and under different conditions. Something of this has been attempted by Fellenberg who estimates that the normal human adult requires about 0.000014 gram of iodine daily and that, when larger amounts are furnished by the food and drink, an easily mobilized reserve store of iodine is built up in the body. It is doubtless through the building of such a body store of iodine that the administration of iodide for two weeks to one month twice yearly by Marine and Kimball proved so effective in preventing the development of goiter throughout the year as above noted.

Iodine Content of Foods

In the case of iron the great difficulty of quantitative studies of nutritive requirements lies in the extreme smallness of the amounts concerned in the body's daily intake and output. In the case of iodine this difficulty appears to be increased many-fold, as the amounts involved are only about one-thousandth to one-hundredth as great. As a matter of fact, the quantities of iodine in most foods are so small that the analytical methods used in examining foods for iodine have not usually been very conclusive even as to whether iodine is present or absent. Doubtless in many cases iodine has been reported absent in foods which really contained it, but in quantity too small to be found by the analytical method employed.

The data in the accompanying table by McClendon will serve to illustrate both the wide variation of iodine content in foods and the tendency of foods from goiterous regions to show less of this element than food of the same sort from non-goiterous regions. Note that the figures are only *milligrams* of iodine per *metric ton* of dry matter in the food, or parts *per billion*.

IN MILLIGRAMS PER METRIC TON, OR PARTS PER BILLION, OF DRY FOOD

From Non-goiterous Regions

	IODINE CONTENT	LOCALITY
Wheat	4	Storrs, Conn.
Wheat	9.3	Edgecomb, Me.
Oats	23	Storrs, Conn.
Oats	175	Wiscasset, Me.
Corn	52	Wiscasset, Me.
Barley	73	Storrs, Conn.
Rye	3.5	Storrs, Conn.
Carrots	170	California Coast
Salmon	45	Alaska
Salmon	75	Oregon
Salmon	115	Alaska
Salmon	324	Alaska
Goat's milk	400	California Coast (Salinas)

From Goiterous Regions

	IODINE CONTENT	LOCALITY
Cereals:		
Oats	10	Minnesota
Wheat	1	Minnesota
Wheat	6.6	Minnesota
Straight flour	3.5	Minnesota
Bran	15.5	Minnesota
Shorts	9.6	Minnesota
Red Dog	3.7	Minnesota
Pot Herbs:		
Spinach	19.5	Oregon
String Beans	29	Oregon
Carrots	2.3	Oregon
Soup Vegetables	13.5	Oregon
Fruits:		
Apples (peeled and cored)	3	Oregon
Pears (peeled and cored)	15	Oregon
Prunes	4.8	Oregon
Bing Cherries	33	Oregon
Peaches	11.1	Oregon
Loganberries	160	Oregon
Animal Foods:		
Skim Milk	12	Minnesota
Butter	140	Minnesota

Fellenberg also found more iodine in the food of the nongoiterous regions of Switzerland than in the food of the Swiss regions where goiter is prevalent. In general Fellenberg finds the iodine content of grains and legume seeds to range from 8 to 64 parts per billion; of fruits from 6 to 120; of nuts up to 200; of vegetable oils 30 to 95; of codliver oil about 5000 parts per billion. Chili saltpeter contained in a fresh sample 192,000 and in an old sample 49,000 parts — such large quantities that they might be expected to have some effect upon the iodine content of the soils and ground waters where this nitrate is liberally used as fertilizer. Mineral waters examined by Fellenberg showed results ranging from 11 to 6000 parts of iodine per billion.

Fellenberg and his associates, in the series of papers to which references are given at the end of this chapter, have also discussed, much more fully than space permits here, the distribution and circulation of iodine in organic and inorganic nature.

As appears from the table, McClendon has shown that milk, and especially milk fat, furnishes iodine in significant quantities, and that other notable sources are found among the fruits and vegetables. From present indications, therefore, it appears probable that milk and green vegetables, classified by McCollum as " protective foods " because of their calcium and vitamin content, will also prove of value as dietary sources of iodine.

By this we do not intend to recommend nor even to suggest an exclusive dependence upon any food or foods for protection from goiter in highly goiterous regions. In such regions the deficiency of iodine in soil and water may be such that no locally grown food will suffice to meet the iodine requirement. There are, however, also wide regions which are neither highly goiterous nor wholly immune, in which the amount of iodine supplied by ordinary food and drink is near the border-line of adequacy; and in such regions it might well be that the choice of food with reference to its iodine content would suffice to turn the scale

from danger to safety, or would to a significant degree improve the margin of safety with respect to iodine as well as other nutritional needs.

Supplementary Sources of Iodine: Iodized Salt.

The prevention of goiter is a nutritional problem whose immediate solution depends not so much upon detailed knowledge of iodine contents of foods as upon the use of methods which lie largely in the province of the health officer.

The success of Marine and Kimball in preventing the development of goiter through the giving to the school child, by the teacher, of a little iodide in an occasional glass of drinking water naturally suggested the addition of iodide to public water supplies, and this has been done in several communities.

Two other methods are also receiving much favorable attention: the administration of iodine compounds in chocolate tablets or some equivalent palatable form and the addition of a small proportion of sodium iodide (0.02 per cent) to table salt intended for household use.

Dr. Olin in an interesting account [1] of the state-wide survey of thyroid enlargement and the preventive measures undertaken in Michigan writes in part as follows:

"The survey showed to us the extent of our problem and it suggested the state-wide remedy. The unvarying relationship between the percentage of thyroid enlargement and the iodine content of the water supplies gave added proof that simple goiter was a nutritional rather than a medical problem. Making good the iodine deficiency in the state's food supply would solve the problem. The question was how to do this.

"Treatment of water supplies was promptly ruled out. This method has been tried and found not entirely effective in several cities. Added to that, it would affect only the cities at best, and our problem was as much rural as urban.

"Chocolate iodine tablets are already in use in the school systems of

[1] Olin. Thyroid Enlargement in Michigan. *Child Health Magazine*, Vol. 5, page 295 (July, 1924).

several cities of Michigan. Their effectiveness is beyond question, but again, this way of solving the problem applies best to cities with well organized schools and continued educational propaganda among parents. It has the added disadvantage of failing to reach two important groups — the expectant mother and the pre-school child.

"It was clear to us that a condition that was the result of a state-wide food deficiency could best be remedied by supplying that deficiency through an inexpensive and universally used food stuff. Salt was, of course, the logical medium to be chosen, since crude salt commonly contains iodine. Representatives of all Michigan salt manufacturers were therefore invited to Lansing and at an enthusiastic conference the agreement was reached whereby all companies would put on the Michigan market, on May 1, 1924, a new "iodized" table salt. This salt differs from that ordinarily used in only one important particular — it contains 0.02 of one per cent of sodium iodide, the sure protection against simple goiter.

"The question then became one of popular education, since the use of the salt is entirely voluntary, and the most intensive, state-wide publicity campaign we have ever attempted was promptly launched. More than 500,000 letters to parents have been sent through the school system of the state. Practically all organized groups were circularized. Our lecturers and traveling representatives were especially instructed to spread the news. In short, every avenue of approach to the public that we can possibly utilize we are bringing into service.

"The outcome, of course, only time can determine. Whether simple goiter will become a thing of the past in Michigan depends upon the people of the state. We believe that it will."

The three methods above noted may not constitute the only practicable alternatives. Turrentine of the United States Department of Agriculture has recently emphasized the possibility that it may prove more advantageous to give iodine in a form less readily soluble and less rapidly absorbed into the circulation than is a simple iodide solution, and has advocated the use of kelp and particularly preparations of the species *Macrocystis pyrifera* which he finds to be particularly rich in iodine.

We have here discussed the relation of iodine supply to the occurrence and prevention of simple goiter. The cure of highly developed goiter, *i.e.* of cases in which the thyroid gland is already decidedly enlarged, is a medical problem. Adequate

iodine supply through salt or otherwise may cure incipient cases as well as preventing many that would otherwise occur; but for goiters already seriously developed the simple administration of iodide must not be regarded as a cure-all. In some cases of severe goiter it may even be undesirable to give iodide for fear of over-stimulating the already enlarged thyroid gland; and in some cases surgical operation upon the gland may be called for. Nutrition cannot always cure what it could almost certainly have prevented.

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CHAPTER XIII

CHEMICAL NATURE AND REGULATION OF OXIDATION PROCESSES IN THE BODY

The Purpose of this Chapter

HOPKINS has well said, " Among the most fundamental of the dynamic chemical events related to life are the oxidations which yield energy to the cell."

In previous chapters we have seen how important to an adequate grasp of the chemistry of food and nutrition are the conceptions of the energy requirements in nutrition, the energy values of the organic foodstuffs, and how these values have been derived from the radiant energy of sunlight as it impinges upon, and is utilized by, the green leaves of plants.

The energy thus caught from the sun's rays by green plants, and " fixed " or " stored " through being utilized to " drive " reduction processes in which organic foodstuffs are synthesized from carbon dioxide and water (photosynthesis), is the sole source of the energy later manifested as muscular work and as warmth (heat) in the human or other animal body.

We have reviewed the bare outlines: of the photosynthetic process as now understood (Chapter I); of the better-known chemical steps of the intermediary metabolism through which the organic foodstuffs are brought into the form of easily oxidizable derivatives which we conceive to be the substances actually entering into the oxidation reactions from which the body energy is obtained (Chapter V); of the methods and results of experimental measurement of the rates of energy transformation in the body under various physiological and nutri-

tional conditions (Chapters VI and VII); and have just seen (Chapter XII) that an iodine compound formed in the thyroid gland has such a profound influence upon the rates of oxidation and energy transformation in the tissues as to cause it to be regarded as a regulator of oxidation for the body as a whole, and furthermore this substance (thyroxin) is in all probability derived from an amino acid (tryptophane) and illustrates the functioning of a nutritionally essential (Chapters III and VIII) amino acid not only as a tissue-building constituent but also as the precursor of an essential body hormone.

It should be plain to the reader who has reached this point in the study of the chemistry of nutrition that a fuller understanding of the exact chemical processes of oxidation (and reduction) in the body may do much to unify our conception of the chemistry of nutrition as a whole and bring into clearer view some of the underlying relationships of the energy, protein, and mineral metabolism — and probably, in time, of some of the phenomena of vitamin action as well.

At the time of writing (1925) our knowledge in this field appears to be in a formative stage. It is too early for a safe formulation of all the relationships of which we see indications; or even for the answering in entirely unqualified terms of such seemingly simple and fundamental questions as whether the oxidations occurring in body tissues are reactions involving molecular oxygen or not. But if it is too early to give our discussion of oxidation in the body an entirely authoritative and definitive form, it seems also too late to be satisfied with a view of the subject presented only in the terms which have been used in previous chapters, and therefore appropriate to sketch here (1) the present general conception of the fundamental chemical nature of the processes of oxidation, and (2) a brief introduction to the chemistry of some of the substances which in the body are apparently intimately concerned in and with the oxidation process. The investigations of Hopkins and of Kendall in

isolating glutathione and thyroxin, respectively, and of Clark and others in developing quantitative methods of studying oxidation-reduction reactions, have given the subject new impetus in recent years. Before discussing their work and its relation to the chemistry of food and nutrition it may be well to review some of the terms and underlying principles involved.

Oxidation-Reduction

Oxidation was a term originally applied to the chemical union of a substance with oxygen, and in the progressive action of oxygen the products were known as higher and higher states of oxidation. The reverse process was looked upon as leading to lower states, reduced states, and was known as *reduction*. The latter term was soon broadened to include union with hydrogen as well as the removal of oxygen, and now both terms have been further expanded. This is perhaps best illustrated by an example taken from inorganic chemistry. The element iron is known in aqueous solutions of its simple salts in two states, that of lower oxidation being called the ferrous state, and that of higher oxidation, the ferric state. Each is recognized by specific reactions and may be converted into the other by means of oxidizing or reducing agents, which while they include oxygen and hydrogen are not confined to them. Thus the oxidation of ferrous iron may be brought about by potassium permanganate or by chlorine, for example, as well as by oxygen, and the reduction of ferric iron may be accomplished by the use of such reagents as hydrogen sulphide, iodides, and stannous salts as well as by hydrogen (with catalyst, if necessary).

As the best experimental evidence indicates that iron in solutions of its simple salts is in the ionic condition, we may write the equations for the above reactions in the ionic form. The reactions with sulphur and chlorine then become,

$$2Fe^{+++} + S^{--} \longrightarrow 2Fe^{++} + S^{\circ}$$

and

$$2Fe^{++} + Cl_2 \longrightarrow 2Fe^{+++} + 2Cl^-$$

According to the electron theory of matter *a unit negative charge is an electron* and a unit positive charge is probably the charge left on an atom when it has lost an electron. The ferric ion then is an iron atom which has lost three electrons. In terms of the electron theory, the ferric ion gains an electron on being reduced and the ferrous ion loses an electron on being oxidized. Thus any agent which contributes electrons to the ferric ion is a reducing agent and any agent which withdraws electrons to itself is an oxidizing agent. The reversible reaction may be written,

$$Fe^{+++} + e \rightleftharpoons Fe^{++}$$

where e represents an electron. In general, then, addition of electrons results in the reduction of a substance and withdrawal of electrons in its oxidation. Clark has pointed out recently, in discussing the subject, that the above equation should be regarded as a convenient mode of expression not necessarily descriptive of the actual mechanism. He defines oxidation and reduction broadly as follows: "Oxidation may be regarded as the withdrawal of electrons from a substance with or without the addition of oxygen or elements analogous to oxygen; or as the withdrawal of electrons with or without the withdrawal of hydrogen or elements analogous to hydrogen. Reduction is the reverse of oxidation as defined above."

The close connection between electrical charges and oxidation-reduction reactions in solutions may readily be shown by suitably arranged experiments. If an electric current is passed through a solution of an iron salt, it will cause the simultaneous oxidation of the ferrous ions and the reduction of the ferric ion at the positive and negative poles respectively; the current will act as an oxidizing-reducing agent. On the other hand, the actual

transfer of electricity in a simultaneous oxidation-reduction reaction may readily be shown by placing the two solutions to be studied, such as ferric chloride and stannous chloride, in separate containers and connecting them by a salt bridge and a delicate voltmeter to indicate the passage of a current. Whether an appreciable transfer of electrons occurs in such an arrangement depends upon the quantitative relations of the ions involved. This tendency to take on or give up electrons is a general one in oxidation-reduction systems and may be made use of, under suitable conditions, for quantitative electrometric measurements of the oxidation or reduction potentials of such systems. Thus ferrous ions have a tendency to give up electrons and to become oxidized to the ferric state. A concentrated solution of a reducing agent has, under given conditions, a greater tendency to give up electrons than a more dilute solution, that is, it has a greater *reduction potential.* The difference in potential between two such solutions may be measured electrometrically with suitable electrodes and under properly arranged conditions.[1]

The electrometric measurement of the difference in oxidation or reduction potentials of solutions has been made use of with satisfactory results in studies of more complicated inorganic and organic oxidation-reduction systems. Its direct application to the quantitative study of biological oxidation-reduction reactions, while accompanied by diffculties of manipulation and interpretation and still in the experimental stage, offers great promise.

The recent work of Clark and his coworkers and of others upon the oxidation-reduction potentials of various dyes used

[1] For further examples, detailed descriptions of the methods of making such measurements, methods of calculation of results and discussions of the principles involved in them, the reader is referred to standard textbooks and original papers on the subject, several of which are listed in the references at the end of this chapter. Those by Clark, by Eucken, Jette, and La Mer, by Fales, and by Stieglitz will be found especially helpful.

as indicators in oxidation-reduction reactions has made possible an important supplementary method of studying these biological systems quantitatively. Various dyes which change in color with changes in the state of oxidation or reduction have long been extensively used in attempts to follow the oxidation-reduction processes in tissues and other biological systems. Two well known examples of such dyes are methylene blue and indigo, both of which exist in a colored, oxidized, and a colorless, *leuco*, or reduced condition. Clark has made a careful study of the properties of these dyes among others. He has studied their oxidation-reduction potentials under conditions similar to those likely to occur in biological systems and has pointed out limitations and errors involved in their use as indicators in quantitative measurements of oxidation-reduction reactions. The hydrogen-ion concentration of the system is one of the important factors influencing their accuracy. A series of dyes better suited under many conditions to serve as indicators in such reactions has been prepared and studied and it will now be possible for the investigator to choose a suitable oxidation-reduction indicator in much the same manner as he would choose a suitable indicator for an acid-base titration.

Glutathione

Glutathione is a dipeptide of cysteïne and glutamic acid. It was isolated by Hopkins in 1921 from extracts of plant (yeast) and animal tissues. This substance is undoubtedly an important factor in the oxidation-reduction reactions of active plant and animal tissues and was isolated as the result of experiments undertaken in an attempt to throw light upon these processes. It is readily soluble in water and gives an intense nitroprusside reaction which is characteristic of all active plant and animal tissues. This test consists, in general, of treating a suspension of tissue or an extract with potassium nitroprusside in the presence of ammonia. When the test is positive a color resembling

that of potassium permanganate is developed.[1] It is probable that a reduction of the nitroprusside molecule takes place. Cysteïne which contains the sulphydryl group (—S—H) gives this test whereas its oxidation product cystine (R—S—S—R) does not. Because of this the reducing property of tissues was, even before the isolation of glutathione, more or less commonly ascribed to the sulphydryl group but until the work of Hopkins no experimental proof of this was available as cysteïne was known to be too reactive to exist to an appreciable extent as such in the tissues. Now this property is known to be due largely to the sulphydryl groups of the glutathione molecule.

Although all active tissues contain glutathione, it is present in very small amounts and the process of isolation which consists of fractional precipitation by means of the salts of heavy metals is laborious.

Glutathione is hydrolyzed by mineral acids yielding cysteïne and glutamic acid, but appears to be entirely resistant to the proteolytic enzymes of the tissues.

Unless special precautions are taken to avoid oxidation, the method of isolation of glutathione yields the product in its oxidized form. The oxidation of the dipeptide involves the change from the sulphydryl to the disulphide form with the union of two molecules.

$$2\ G^{*}—S—H \longrightarrow G—S—S—G.$$

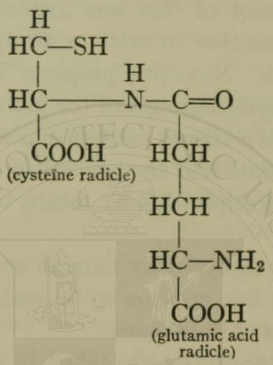
As the oxidized form does not give the nitroprusside reaction, the oxidation and reduction of glutathione may be followed by means of this test.

Oxidized glutathione is readily hydrolyzed by mineral acids yielding *l* cystine and *d* glutamic acid which are the same forms in which these amino acids are yielded by the proteins of food and of body tissues.

[1] Various modifications, errors, and methods of intensifying the color are described in the literature.

* G is used to represent the rest of the glutathione molecule.

Glutathione has been synthesized and the synthetic product has been found to be identical with the natural substance. The synthesis confirms the constitution which had been assigned to the compound based upon the results of analyses, hydrolyses, and various chemical reactions. In its molecule the α amino group of the cysteïne and the carboxyl group γ to the amino group of the glutamic acid are involved in the union thus:



Glutathione is readily oxidized and its oxidation product is readily reduced in neutral or slightly alkaline solutions in the presence of traces of iron, the conditions which prevail in the tissues. This ease in its oxidation together with the easily reversible nature of the oxidation-reduction process and the widespread distribution of the dipeptide in active tissues, point to the importance of glutathione in biological oxidation-reduction reactions. This has been confirmed experimentally by the work of Hopkins and his coworkers. Some examples of the types of experiments carried out and what they show are of interest.

Fresh tissues bring about the reversible oxidation and reduction of glutathione and its oxidation product. Thus a suspension of fresh tissues to which oxidized glutathione has been added and which is kept under aseptic conditions will give a nitroprusside reaction of increasing intensity due to

the reduction of the oxidized to the reduced glutathione, the reduction of the disulphide to sulphydryl groups. On the other hand, fresh tissue suspensions, which give a positive nitroprusside test, will, if held under anaerobic conditions for a sufficient time, cease to give the nitroprusside reaction owing to the anaerobic oxidation of the reduced glutathione to the oxidized product.

Hopkins, in commenting upon these and similar experiments, points out that, "Equilibrium in the living cell would seem to be such that the greater part of the substance (glutathione) present exists in the reduced condition; but oxidation and reduction of the constituent sulphur groups are reversible processes in the tissues, and both forms may at any moment be present." " . . . factors are present in the tissues which promptly reduce the oxidized product whenever its concentration is raised above an equilibrium value."

Glutathione aids in the oxidation of substances present in fresh tissues. The oxidation of fresh tissues by methylene blue in properly buffered solutions and under anaerobic conditions takes place very slowly and is greatly accelerated by the presence of the oxidized form of glutathione. This was measured by the velocity of reduction (disappearance in color) of the methylene blue. The hydrogen ion concentration of the system was found to be an important factor in this type of reaction.

As glutathione is already present in fresh tissues and their suspensions, its relations to other substances present in the tissues are more or less masked in experiments with them. Experiments were therefore undertaken with residues obtained after thoroughly extracting fresh tissues with water. Such treatment should remove the glutathione which is readily soluble in water. The extractions were carried out under aseptic and usually under anaerobic conditions so as to cause as little change as possible in the other substances present.

It was found that suspensions of such residues in buffered solutions, tested under anaerobic conditions, no longer reduced methylene blue to any appreciable extent, but reduced oxidized glutathione and in conjunction with it would reduce the dye. In discussing this Hopkins said, "The tissue residue first reduces the sulphur group and a system is thus established which under anaerobic conditions continuously reduces methylene blue until an equilibrium is reached."

Experiments with tissue residues gave additional evidence that glutathione aids in the oxidation processes in the tissues. This was shown by the type of experiment cited above in which methylene blue was the oxidizing agent and again in experiments with molecular oxygen.

A tissue suspension in a buffered solution which does not appreciably react with oxygen will, under suitable conditions and if supplied with glutathione, take up oxygen and yield carbon dioxide. The addition of gluta-

thione in relatively small amounts restores to some extent to washed tissue its ability to reduce and to "respire."

Fats containing unsaturated fatty acids are actively catalyzed in their oxidation by reduced glutathione and by oxidized glutathione in the presence of tissue residues or of proteins containing the sulphydryl group. These probably act by reducing the oxidized glutathione. Hopkins points out that such interdependence of oxidation-reduction reactions may indicate lines on which we can come to understand how in the body the oxidation of fats may depend on the oxidation of carbohydrates.

Glutathione has been identified as a constituent of the red blood corpuscles of sheep's blood and apparently exists in the reduced form in the corpuscles of many animals. Both cysteïne and reduced glutathione have the power of converting methemoglobin (the oxidation product of hemoglobin) into oxyhemoglobin, and oxyhemoglobin into hemoglobin. This may afford an explanation of the rapid conversion of methemoglobin into oxyhemoglobin in the animal body.

The reducing system in the washed tissues is very thermostable. The residues may be heated to 100° C. or extracted with boiling water without losing their ability to react with oxidized glutathione or with methylene blue or oxygen in its presence.

The oxidized form of glutathione aids in the oxidation of the reduced form and also in the oxidation of cysteïne and of thioglycollic acid, two other typical sulphydryl compounds. Dithioglycollic acid, the disulfide, oxidation product of thioglycollic acid, also aids in the oxidation of thioglycollic acid and of glutathione. Experiments of this kind have been interpreted to mean that oxidized glutathione and the disulphide group in general may aid in the oxidation of a compound containing the sulphydryl group such as glutathione perhaps by the formation of a more reactive addition compound.

There is considerable experimental evidence to show that the oxidation of glutathione and of cysteïne is catalyzed by the presence of traces of iron. When these compounds are prepared with special precautions to avoid their contamination with iron it is found that they are oxidized much more slowly than the products obtained by the usual methods. The addition of traces of iron to the purified compounds has been found to increase the rate of their oxidation. It has been suggested that iron aids in the oxidation of these substances by the formation of a highly reactive intermediate addition compound with them. The presence of hydrocyanic acid on the other hand was found to inhibit the oxidation of crude cysteïne and of glutathione. This is explained as being due to the formation of a complex compound of the hydrocyanic acid with the catalytic iron present and its removal from the reaction.

The mechanism by which glutathione acts in the tissues is not as yet fully understood, but enough is known to establish its undoubted importance in the oxidation-reduction processes of the body. While it may act in various ways, perhaps including the formation of addition compounds, such as have been suggested, with the oxidized form or with iron, the underlying reactions will undoubtedly be found to center around the sulphur groupings, and to involve the reversible change of the sulphydryl to the disulphide forms. Many of the findings which have been interpreted in terms of the transfer of hydrogen or of oxygen either directly or by the agency of water may now be reinterpreted perhaps more satisfactorily in terms of oxidation or reduction potentials and the transfer of electrons. The reversible change may be written thus:

$$2\,GSH - 2\,e \rightleftharpoons G{-}S{-}S{-}G + 2\,H^{+}.$$

Some electrometric measurements of the reduction potentials of this system have already been made in attempts to throw more light upon its mechanism, but the experimental details have not been sufficiently established to justify the drawing of conclusions based upon them (1925).

It is interesting to note that, while cysteïne is too unstable and reactive to exist, to an appreciable extent, as such in the tissues, it is protected in the dipeptide, glutathione, and thus furnishes the reactive sulphydryl group of this important compound. The dipeptide appears to be resistant to the proteolytic enzymes of the tissues and exists to an appreciable extent in all active tissues. In addition to protecting the cysteïne from metabolic processes, the dipeptide furnishes an oxidation-reduction system which is readily soluble. Both glutathione and its oxidized product are exceedingly soluble in water and in neutral or slightly alkaline solutions so that both are present in readily available forms in the tissues.

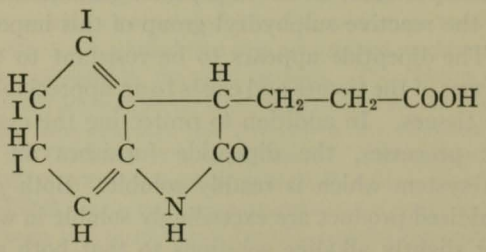
Thyroxin [1]

Thyroxin increases the basal metabolic rate in the animal organism and, within limits, this increase is proportional to the amount administered. Thus it is actively involved in the oxidation processes of the body.

It behaves as a very powerful catalytic agent in these processes, as it functions for a long time after a single injection and the increase in oxidation which follows its administration is enormous compared with the amount of thyroxin administered.

Thyroxin has not as yet (1925) been synthesized, but a structural formula based upon its composition and chemical reactions and those of related compounds has been assigned to it. While not yet confirmed by synthesis of the actual substance, all experimental data so far obtained point to its being correct. The synthesis of a series of closely related compounds recently by Kendall and a study of their chemical and physiological properties gives added evidence that the assigned formula is correct and has thrown further light upon the properties of thyroxin itself.

The structure assigned to thyroxin (Kendall, 1925) is given here. It is closely related to that of tryptophane and contains the indole ring.



[1] See also discussion of thyroxin in Chapters VII and XII.

Independent experimental proof of the relationships between tryptophane, indole, and thyroxin have recently been obtained in an interesting manner.

Hicks studied the ultra-violet absorption spectra of thyroxin and of several related compounds including tryptophane, and reached the conclusion that there is a definite group resemblance in the spectra of these compounds and that the absorption curves are sufficiently related to warrant the conclusion that the indole nucleus is present in thyroxin.

Experiments showing the direct nutritional relationship between the thyroid gland, thyroxin, and tryptophane are also of interest. It has been found that atrophy of the thyroid gland follows the feeding of diets otherwise adequate but deficient in tryptophane. Rats have been fed on diets which were planned to show the effects of tryptophane deficiency and the extent to which these effects are relieved by thyroxin. The experimental diets differed in their content of tryptophane, thyroxin, and protein. The conclusion from such experiments is that tryptophane is essential to the normal functioning of the thyroid and that a large amount of the needed tryptophane is available for the thyroid from the tissues of the body; the thyroid taking up tryptophane from the blood in quantities approximating its needs even when the total amount is so small that other tissues suffer from its lack.

A study of the chemical properties of thyroxin and of closely related compounds has led Kendall to formulate an hypothesis to explain the mechanism by which thyroxin may produce its marked catalytic effect upon the oxidation processes of the body. For detailed data and explanations the reader is referred to the original article.[1] In general, however, the hypothesis rests upon an easily reversible oxidation and reduction of the thyroxin molecule.

[1] Kendall. Influence of the Thyroid Gland on Oxidation in the Animal Organism. *Industrial and Engineering Chemistry*, Vol. 17, pages 525–534 (1925).

A brief summary of Kendall's view may best be given in his own words:

" As a working hypothesis thyroxin may be pictured as a compound that can be acted on in its open-ring form by mild oxidizing agents, among which is molecular oxygen. The result of this oxidation is the formation of an imine grouping. By an intramolecular rearrangement — that is, closure of the pyrrolidone ring — the activity of this grouping is much increased and the oxidizing potential of the molecule is raised to such an intensity that it can enter into those chemical processes involved in oxidation. The result of this reaction is to reduce the imine group, replacing it with two atoms of hydrogen. The pyrrolidone ring again opens and the same cycle is repeated.

" Two of the essential steps in this cycle are the opening and closing of the pyrrolidone ring, and in both these reactions the iodine in the benzene ring exerts a dominating influence. The iodine produces the finely balanced state of the molecule which acts as the governor of the rate at which it can react.

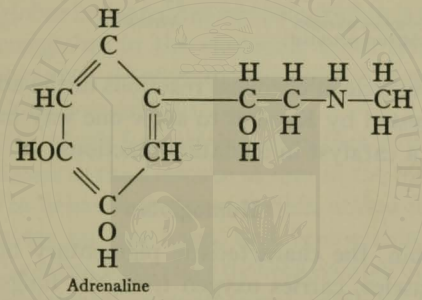
" Accompanying each oxidation there must be the coincident opening and closure of the pyrrolidone ring."

Adrenaline (Epinephrine)

Adrenaline (epinephrine), the active principle of the suprarenal glands, also has among other properties that of causing an increase in the rate of oxidation, especially of glucose, in the animal body. This is shown by a rise in the respiratory quotient directly following its administration. Its influence upon oxidation in the body is less marked than that of thyroxin and also more rapid and transitory. Following an injection of adrenaline there is an almost instantaneous increase in the basal metabolic rate which rapidly reaches a maximum and then soon drops back to normal. The entire effect on the basal metabolic rate of administration of adrenaline in the normal adult is over in a few hours. With thyroxin, on the other hand,

there is a well defined delay, six to eight hours, before the basal metabolic rate is increased and the effects of a single injection upon the basal metabolic rate may still be observable for five or six weeks after the injection. Adrenaline causes a rapid mobilization of blood sugar which then serves as fuel. Boothby has found that the extent of the increase in the rate of oxidation following the administration of adrenaline is dependent upon its concentration in the tissues. The other functions of adrenaline in the body will not be discussed here.

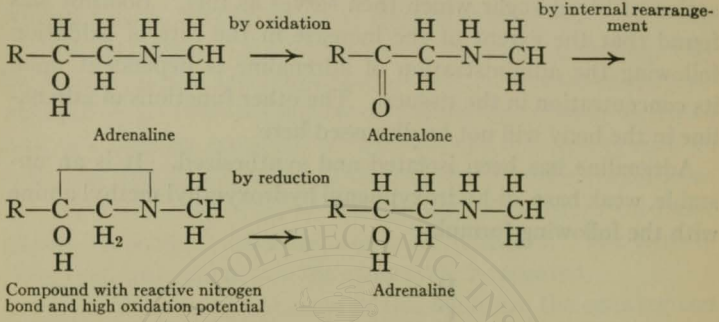
Adrenaline has been isolated and synthesized. It is an unstable, weak base, di-hydroxyphenyl hydroxyethyl methyl amine with the following formula:



Adrenaline

It is related in structure to tyrosine and is probably a derivative of this amino acid. Adrenaline is readily oxidized. It reacts with oxygen, and Kendall has obtained some experimental evidence which indicates that the mechanism by which adrenaline catalyzes oxidation processes may be similar to that which he has suggested for the action of thyroxin, due to its alternate oxidation and reduction. He found that the injection of adrenalone, the ketone oxidation product of adrenaline, causes an increase in the basal metabolic rate of a dog proportionate to that produced by adrenaline itself while the injection of the monoethyl ether or of the anhydride of adrenaline in which the

formation of the ketone is prevented did not cause an increase in the basal metabolic rate. He suggests the following mechanism which, like that suggested for thyroxin, involves the formation of a reactive nitrogen bond.



The above cycle of chemical reactions in the adrenaline molecule is suggested by Kendall to show one way in which it may function as a catalyst in oxidation reactions.

Hemoglobin

Hemoglobin, the characteristic pigmentary body of the red blood corpuscles, carries oxygen from the lungs to the tissues (and, in a sense, carbon dioxide from the tissues to the lungs) and is essential to the oxidation processes of the body. It is a conjugated protein, a chromoprotein, which yields a simple protein, globin, and a colored iron-containing complex hemochromogen which is readily oxidized to hematin. Globin is a histone-like substance containing more nitrogen than most proteins, about 17 per cent, and characterized by a large proportion of basic amino acids. Hematin is an acid, an iron pyrrole complex containing four pyrrol rings and probably one atom of iron to the molecule. The pyrrole nucleus is found in tryptophane and proline, and these amino acids probably serve as material from which the body constructs its hematin. Hemo-

globin readily unites with oxygen to form oxyhemoglobin. This change is easily reversible. As oxyhemoglobin is a stronger acid and is more highly ionized in the blood than hemoglobin the change from one to the other is intimately connected with the regulation of neutrality in the blood as well as with oxidation processes. The interdependence of these two functions of hemoglobin has already been discussed in Chapter IX.

As the change from oxyhemoglobin to hemoglobin is favored by increased acidity, the presence of lactic acid and other acid products of metabolism may aid in the liberation of oxygen and in the oxidation processes of the tissues.

Studies of the oxidation and reduction potentials of systems containing hemoglobin, methemoglobin, its oxidation product, and oxyhemoglobin indicate that methemoglobin is the oxidation product of hemoglobin in the electronic sense of involving an exchange of electrons, whereas oxyhemoglobin contains oxygen in some more loosely combined manner.

The ease with which hemoglobin combines with oxygen appears to be related to the presence of iron in the molecule.

Similarities in behavior between certain amino ferro-cyanides and ferri-cyanides on one hand, and hemoglobin and methemoglobin on the other, have recently been pointed out by Conant.

There is experimental evidence which indicates that the iron in the hemoglobin molecule or some derivative of it may be of importance in catalyzing oxidations in the body. Miss Robinson, working in Hopkins' laboratory, has recently found that hemoglobin and its iron-containing derivatives, methemoglobin and hemin, accelerate the oxidation of unsaturated fatty acids such as those present in linseed oil while an iron-free derivative of hemoglobin does not. The reaction did not appear to depend upon an easily reversible combination with oxygen as it occurred equally well in the presence of hemoglobin saturated with carbon monoxide and in the presence of hemoglobin itself. In this connection it is interesting to note that Hill in

England and Whipple in this country are inclined to emphasize the fact that not all of the hemoglobin of the body belongs to the red blood cells but that the tissues themselves possess a significant amount of hemoglobin (tissue-hemoglobin or muscle-hemoglobin). Its function in the tissues may be that of an oxidative catalyst.

Insulin

Insulin is sometimes spoken of as being concerned in the burning of glucose in the body. This appears to be true only in the sense that insulin helps to prepare glucose for the intermediary metabolism which may lead to oxidation; not in any sense which would imply that insulin is an oxidizing agent. Nevertheless, the preparation of glucose for the chemical changes leading to oxidation is in a broad view a phenomenon related to the oxidation processes; and, since insulin was but briefly referred to in Chapter V, a brief additional consideration of it at this point seems appropriate.

It is to be regretted that space does not permit the inclusion here of the interesting and inspiring story of the discovery of insulin; but the reader may be all the more interested to read this through the original publications with which one can get into touch through the references given at the end of this chapter and of Chapter V.

There is not yet (1925) a consensus of opinion as to just what chemical change insulin brings about or catalyzes. It is certainly an early step in the metabolism of glucose, for experiments have shown that insulin aids not only the utilization of glucose as fuel but also its transformation into glycogen or into body fat.

The most direct chemical evidence on the subject of the action of insulin is that it causes a lowering of the concentration of glucose in the blood; and this is observed both in the diabetic and in the normal body. This is attributable to the transforma-

tion of ordinary glucose into a more reactive form or derivative. It is sometimes spoken of as a change of glucose into gamma-glucose or "new-glucose." Whether through such a transformation or in a more direct way, insulin probably accelerates the formation of the glucose-phosphate or glucose-phosphoric acid ester which as we have already seen (Chapter V) is now regarded as an important early step in the metabolism of the glucose. One theory is that insulin activates the enzyme which catalyzes the formation of the glucose-phosphoric acid derivative. This derivative then presumably is rapidly transformed into substances more readily oxidizable (*e.g.* glyceric aldehyde, methyl glyoxal, or lactic acid) or into glycogen or fat; and its removal in any of these ways favors its continued formation from glucose, and thus the lowering of the concentration of glucose.

While this lowering of the glucose concentration is observed in the blood, it may actually take place either in the blood or the tissues or both. Hawley and Murlin find that total oxidation in the body increases after administration of insulin.

It is of interest to note that insulin or a substance or substances of similar action upon glucose can be demonstrated to occur widely in both plant and animal tissues. For this reason Collip has proposed the broader name *glucokinin.*

During the short time that has elapsed between the discovery of insulin and the date of writing (1925) investigations into its chemical nature have been so active and numerous that it would be impracticable to review such work at all fully here. Special mention may, however, be made of the fact that simultaneously Abel and Geiling and Taylor, Braun and Scott have adduced strong experimental evidence in support of the view that insulin is either a peculiar protein or a protein-like substance with a similar nitrogen content to that of ordinary proteins, and that it is free from phosphorus but contains sulphur as an essential constituent. Abel and Geiling emphasize the presence of unstable sulphur-containing radicles and the fact

that boiling with even weak alkali causes an alteration of sulphur linkage along with the inactivation of the insulin. Abel, in a paper published (1926) since the foregoing text was written, describes the isolation of insulin as a crystalline substance giving typical biuret, Millon, and ninhydrin reactions.

Possible Interrelations

We have here reviewed sketchily the chemistry of glutathione, thyroxin, adrenaline (epinephrine), hemoglobin, and insulin, and the relations of these substances to the oxidation processes by which the organic foodstuffs or their derivatives are burned as fuel in the body.

This, of course, is far from being a complete list of the substances concerned in the problem, but it will be noted that several phases of the general process are touched upon: Insulin in some sense prepares glucose, the chief body fuel, for its oxidation; hemoglobin brings oxygen to the tissues by serving as the oxygen-carrying constituent of the blood, and it is possible that a part of the hemoglobin may also function as an oxidation catalyst in the tissues or some of them; glutathione undoubtedly is a tissue constituent of wide distribution and of important function as a normal oxidation-reduction catalyst in cell processes; adrenaline (epinephrine) and thyroxin are typical hormones formed in glands of internal secretion and carried thence by the blood to the various active tissues throughout the body where they act to increase the rate of oxidative metabolism — adrenaline quickly and for a short time, thyroxin more slowly and for a much longer time.

Kendall has recently written: "Thyroxin, epinephrine, and glutathione are members of a group of substances which furnish the animal organism an active chemical grouping. They establish the intensity of the oxidation-reduction process occurring within the cells, and produce a poising effect maintaining the intensity of oxidation and reduction within narrow limits."

. . . "The fully oxidized and reduced forms of these substances are not in equilibrium with each other but they are in equilibrium with the partially oxidized or free radicle forms of the substances."

While investigations are only just now beginning to disclose the intimate chemistry of the mode of action of these substances, it is interesting to note that in their own chemical nature they appear to be more or less related to each other and to the typical body enzymes in that they all appear to be derived from proteins or amino acids. We have hypotheses which suggest a dependence of the characteristic catalytic property upon the presence and lability of peculiar groupings in the molecule. While such suggestions are still tentative it may not be out of place to mention here that it also seems not unlikely that one or more of the water-soluble vitamins may also be found to belong to this same broad group of substances which owe their important nutritional properties to the presence of easily transformable chemical linkages, and to be involved in the general system of chemical mechanisms through which the oxidation processes in the body are catalyzed and regulated.

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CHAPTER XIV

VITAMIN B, WITH BRIEF INTRODUCTION TO THE VITAMINS AS A GROUP

Introductory

UNTIL within recent years food chemistry was generally considered as having to do chiefly with "pure food" problems — with methods and criteria for the determination of the freedom of foods from adulteration. Food values were believed to be shown by analyses which demonstrated that objectionable substances were absent and that the known nutrients were present in normal proportions.

But good nutrition had never been obtained by feeding mixtures of all the substances which analyses of foods had revealed as essential constituents, even with all these substances in the purest possible forms. In fact, other things being equal, the purer the chemical substances fed the sooner the experimental animal would die.

Evidently the available methods of analyzing foods did not tell the whole story of food values, and the most promising way of extending our knowledge was through experimentation upon the nutrition of laboratory animals (See Introduction).

Hopkins of Cambridge University was the first to show clearly and convincingly that normal nutrition requires some substance or substances other than pure proteins, fats and carbohydrates, and the mineral elements, even when all of these are carefully selected and fed in favorable quantitative proportions. When, for example, he fed young rats with mixtures of protein, fat, carbohydrate, and mineral salts made up in imi-

tation of the natural mixture of known food substances occurring in milk, the animals soon ceased growing; but if a little genuine milk was fed, growth continued. This was true whether the milk was fresh or dried, and the alcohol-soluble fraction of dried milk (and also of certain vegetables which he tested) showed the same growth-promoting property, while milk ash did not.

Evidently, then, milk and some other natural foods contained some alcohol-soluble organic substance or substances essential to normal nutrition. This discovery was announced briefly by Hopkins in 1906, but the full account of the work was not published until 1912, at which time Osborne and Mendel were obtaining similar results in the feeding experiments with isolated food substances which they had begun with the primary purpose of studying the nutritive values of individual proteins as explained in Chapter III.

The work of Osborne and Mendel, and of McCollum and Davis, soon showed that in such experiments upon growing rats there are two unidentified substances involved: one soluble in fat, which McCollum called "fat-soluble A"; and the other soluble in water, which he called "water-soluble B." Since both of these are soluble in alcohol and both occur abundantly in milk and some vegetables, both had been supplied by the alcohol-extracts of dried milk and dried vegetable which Hopkins had fed.

Also practically simultaneous with the work of Hopkins was the discovery that the Oriental disease beriberi, a nerve disease, a neuritis, was due to the deficiency in the diet of a previously unknown substance, hence called the antineuritic substance; and that similarly the cause of scurvy was the deficiency in the food of an analogous antiscorbutic substance.

The antineuritic substance was believed to be identical with water-soluble B and the antiscorbutic substance was called "water-soluble C."

Meantime Funk had proposed the term *vitamine*, first as a designation for the antineuritic substance and then as a group name for all previously unknown substances the lack of which might be found to be responsible for any " deficiency disease."

Other designations, notably " accessory substances " and " food hormones " were also proposed, but were not so readily taken up and popularized as were the names given by Funk and by McCollum.

In 1920, Drummond suggested that the designations in most common use be combined and simplified by calling the three substances Vitamin A, Vitamin B, and Vitamin C respectively. (The omission of the final " e " frees the group-name from the original implication that these substances are amines in their chemical nature. By convention, only nitrogenous bases, among organic compounds, should be given names ending in " ine "; but names with " in " endings may be given to substances of various or of unknown chemical constitution, *e.g.* insulin and the proteins).

The terminology thus suggested by Drummond came rapidly into general use and since 1920 no qualified student has failed to recognize the existence of at least the three vitamins A, B, and C.

Nor has there been any serious confusion in the use of any of these three names, although perhaps not everyone has recognized with equal clearness that any one of these terms as originally introduced might with the progress of knowledge be found to have covered more than one substance. Vitamins A, B, and C are certainly different from each other; but it does not necessarily follow that each of these three designations stands for a single chemical individual.

As a matter of fact the subsequent additions to the " vitamin family " or " vitamin alphabet " have arisen not so much as wholly new nutritional conceptions as by differentiation from vitamin A or vitamin B.

Unfortunately the term vitamin D has been suggested for two entirely different and unrelated substances, one differentiated from vitamin A, the other from vitamin B. The former is the fat-soluble *antirachitic* vitamin; the latter is the water-soluble substance chiefly notable for its effect in stimulating the growth of yeast, and which is probably identical with the substance previously described by Wildiers under the name of *bios*. Bios has recently been isolated in what appears to be pure form by Eddy, Williams, and Kerr as described in the papers cited at the end of this chapter. Since the term *bios* has priority as the name of this substance, and since it is not known to be essential to animal nutrition, present usage seems to favor the employment of the term vitamin D for the antirachitic substance. If the terms *bios* and *antirachitic vitamin* are used as names for the two substances in question, the possible ambiguity involved in the use of the term vitamin D can be avoided entirely.

Vitamin E is the term now used to designate the fat-soluble substance, whose existence was discovered by Evans and Bishop and whose chemical nature is being investigated by Evans and Burr, which, in addition to other vitamins, is required for the nutritional processes of reproduction.

Each of the vitamins instead of being designated by a letter may be given a name based upon its nutritional function — or a nutritional function which is more or less characteristic of it. Thus vitamin A, since it prevents a characteristic eye disease which develops in its absence may be and sometimes is called "antiophthalmic" vitamin. Vitamin B, on the assumption that McCollum's "water-soluble B" and Funk's "antiberiberi vitamine" are identical, has frequently been called the antineuritic vitamin. Vitamin C and antiscorbutic vitamin are accepted as synonymous terms. The two substances which have been called vitamin D may be called respectively the yeast-growth-promoting substance (bios) and the antirachitic vitamin. Vitamin E is correspondingly known as the antisterility vitamin.

The significance of these physiological designations will be clearer after the functions of the vitamins have been studied. It may, however, also be said that the function of a vitamin may be much broader than the prevention of a single characteristic deficiency disease. Thus vitamins A, B, and C have functions in normal nutrition as well as in the prevention of ophthalmia, neuritis, and scurvy respectively. And it is largely for this reason that the alphabetical designations are apt to be used in nutritional discussions.

In studying the vitamins it seems much better to consider them one by one because there is little evidence of any true relationship, either in chemical nature or in physiological properties, running throughout all the substances to which the group name vitamins is applied. The fat-soluble vitamins appear likely to belong to one chemical group, while the water-soluble vitamins show no suggestion of chemical relationship to the fat-soluble, nor is it at all certain that vitamins B and C bear any special chemical relationship to each other. Nor is there any advantage in studying the vitamins in alphabetical sequence.

As the term vitamin was first applied to our present vitamin B and as this is the one which seems most probably related in its chemical nature to the hormones studied in the last chapter, we shall here study vitamin B first; then the other water-soluble vitamin (vitamin C); then the fat-soluble vitamins in later chapters.

Vitamin B

The substance to which the name vitamin was first applied, and which is perhaps most often referred to when the word vitamin is used alone, is that which prevents the disease beriberi. This is a nerve disease, a neuritis, and so the substance which prevents it was designated as antineuritic. Its existence was discovered through observations that beriberi in man and a corresponding neuritis in fowls often resulted from too great

dependence upon white rice as a food and could be prevented by the use of unmilled rice, or of the parts of the rice which had been removed in the milling, particularly the embryo. To this antineuritic substance Funk in 1912 gave the name " vitamine." At about the same time Hopkins of Cambridge and Osborne and Mendel of New Haven showed that milk and some other foods contain an organic substance, different from the known foodstuffs, which is essential to growth, and very shortly McCollum announced that the water-soluble growth-promoting substance was identical with the antineuritic substance. While this view has never been universally accepted, yet because of the apparent close parallelism of the two properties in most foods in which they are found and the fact that in most cases it has seemed neither possible nor practically important to determine whether the effects in question are attributable to one or more substances, the term vitamin B is employed to cover the chemical individual or group whose presence in food enables it to meet the needs of preventing neuritis and supplying that growth essential which McCollum called " Water-soluble B."

Evidence of the Existence of an Antineuritic Substance and Attempts at its Chemical Identification

For centuries and until within our own generation the nerve disease beriberi was very common in the Orient, particularly in the Malay States, Siam, parts of Japan, and the Philippines. Thus in 1878–1883 the entire enlisted force of the Japanese navy numbered about 5000 men, and of these from 1000 to 2000 each year were sick with beriberi. This enormous morbidity of 20 to 40 per cent from one disease led to an investigation by Takaki, a high medical officer of the Japanese navy, who worked upon the theory that the fault was most likely to be found in the food since climate was found to be without influence and the sanitary conditions on the Japanese ships were as good as those in the European navies which were not troubled with the disease.

A Japanese naval vessel with 276 men on a 9 months' cruise from Japan to New Zealand, Valparaiso, and Honolulu had 169 cases with 25 deaths. Another vessel with a similar crew was sent by Takaki over the same route with a ration in which the rice was decreased, the barley increased, and vegetables, meat, and condensed milk added. In this case only 14 men had beriberi and each of these had failed to eat his full allowance of the new foods. As the result of this experiment Takaki secured the adoption of his new ration for the entire Japanese navy with the result that the number of cases of beriberi soon became practically negligible.

From his government, Takaki received full recognition for this really great achievement; and yet it failed to accomplish for the world what might reasonably have been expected of it. Opinion continued to be divided, as it had been before, as to whether beriberi were a nutritional or an infectious disease, with the majority of the medical men of the Orient (where the disease continued to be common) inclining to the theory of an infection because the disease usually occurs epidemically and because (through the rapid and brilliant development of the science of bacteriology) epidemics had come to be so generally regarded as always due to infections either through known or unknown organisms.

Takaki as we now know was right in his belief that the disease was nutritional and he was entirely explicit in saying so, but his work did not carry conviction because he offered no adequate theoretical explanation of it. He discussed his improvement of the ration only in terms of protein, which others rightly regarded as unconvincing. Forty years ago was a time of great activity in the sanitary applications of the then new science of bacteriology. Sanitary improvements had naturally been made in the Japanese navy during the same years in which Takaki was succeeding in getting the ration changed, and the sanitary improvements were generally given credit for the eradi-

cation of the disease because the germ theory appeared adequate even with the germ unidentified, whereas Takaki offered no satisfactory scientific explanation of what he had accomplished.

Here as so often happens in work which is both scientific and practical, too great absorption in the practical aspect largely defeated its own aim.

Had Takaki pursued the scientific explanation of his work far enough to make it convincing, he might have removed the burden of beriberi not only from the little group of 5000 men which then constituted the Japanese navy, but from his country and the Orient as a whole; whereas in fact his work made little general impression and millions of his countrymen as well as other millions in nearby countries continued to suffer from beriberi, the physicians believing that it was an infectious disease due to an undiscovered germ.

Such was the view of our own Army Medical Officers in the Philippines until after their striking experience with beriberi in the Bilibid prison at Manila, where a serious outbreak of the disease resulted from the substitution of a commercially higher grade of rice (white, polished) for the cheap brown rice which had constituted the mainstay of the prisoners' diet.

At about the same time Fletcher, by experimenting with the diet in a lunatic asylum, showed that when 28 ounces of rice were fed daily with only small amounts of other food, the use of polished or unpolished rice was alone sufficient to determine the occurrence or non-occurrence of beriberi.

During 1907–1908 Fraser and Stanton took 300 laborers from Java into new and sanitary quarters in a virgin jungle and demonstrated in striking fashion that with rice as the main part of the diet, beriberi followed the use of polished but not of unpolished rice. Many other observations to the same effect were also published at about this time.

In 1909, convinced that beriberi was related to diet, the United States Army Medical Commission in the Philippines initiated

changes in the rations of the " Philippine Scouts " and in 1911 Chamberlain was able to announce the eradication of the disease from these troops by the substitution of unpolished rice (and a small quantity of beans) for the polished rice previously used. Until the year 1910, the number of hospital cases of beriberi ranged from 115 to 618 (the force numbering about 5000 men). During 1910 changes in the dietary were begun and that year the cases dropped to 50. In 1911 there were 3; in 1912, 2; in 1913, none; in 1914 up to June 30 there was 1.

In 1908–1909, when beriberi was at its worst among the Scouts, the diet consisted essentially of 12 oz. of beef, 8 oz. of white flour, 8 oz. of potatoes, and 20 oz. of rice (ordinarily polished). The change in the ration, as finally decided upon after some months of experimentation, consisted in giving, in place of the 20 oz. of polished rice, 16 oz. of unpolished rice and 1.6 oz. of dried beans. Experiments, made largely upon fowls as explained below, have shown that while beef has some effect in preventing beriberi, an equal weight of beans, peas, or peanuts is much more efficacious.

Chamberlain states, in fact, that the disease had disappeared as the result of adding the beans to the ration, before the substitution of unpolished for polished rice had been completed. He believes " that the consumption of beans to the daily amount of 1.6 ounces would, unaided, have prevented a recurrence of beriberi, but it would obviously be difficult to make sure that all the men ate their share of this article over long periods, and it is therefore much safer that the largest component of the diet, the rice, should be of the unpolished variety and by itself sufficient to prevent neuritis."

Several other investigations gave similar results. These repeated demonstrations of a close connection between a diet consisting too largely of polished rice and the occurrence of beriberi naturally gave a great impetus to experiments designed to find what constituents of the rice are directly concerned in the disease.

Such experiments were greatly facilitated by the fact that fowls or pigeons develop beriberi when fed upon polished rice for about 3 weeks, and that this experimental beriberi can be prevented or cured by feeding foods which prevent beriberi in man.

The English investigators Fraser and Stanton working in the Malay States and the American investigators Chamberlain, Vedder, and Williams, in the Philippines, together showed that the antineuritic constituent of foods was an organic substance more stable in acid than in alkaline solution, probably a nitrogenous base but not an alkaloid.

Funk's experiments attracted special attention since he was the first to announce (December, 1911) the isolation of a definite chemical substance possessing the antineuritic property. Pigeons paralyzed by neuritis induced by a polished rice diet were able to run and fly within a few hours after administration of 2 to 8 milligrams of this substance, which appeared to be an organic nitrogenous base related to the pyrimidines. He described the preparation of such substances from rice bran and from yeast, and inferred the existence of the same or a similar vitamin in all foods which have antineuritic properties.

Funk called this *beriberi vitamine*. It constituted only 0.05 per cent of the rice polishings corresponding to about 0.01 per cent in the whole grain.

In March, 1912, Edie, Moore, Simpson, and Webster (working independently of Funk) described the isolation from yeast of a base which promptly cured pigeons suffering from polyneuritis. This base they described as having composition corresponding to the formula $C_7H_{17}N_2O_5$. They called it *toruline*.

Schaumann (June, 1912) reported the preparation of a phosphorus-free nitrogenous crystallizable base corresponding in general to the description given by Funk and exerting a marked restorative action upon polyneuritic pigeons. This base he considers the "activator" in the cure of polyneuritis, holding that it "mobilizes" the phosphatid substances which must be rebuilt into the degenerated nerve tissue in order to effect a permanent cure.

In July, 1912, Suzuki, Shimamura, and Odake reported an extended investigation of experimental beriberi in which they had prepared from rice

polishings by an independent method a base of high curative power which they called *oryzanine*. In preparing oryzanine they precipitated an alcoholic extract of rice polishings with tannin, decomposed the tannate by baryta, removed the barium by sulphuric acid, and precipitated the base as a picrate. Only 0.005 to 0.01 gram of oryzanine was required to make the daily diet of polished rice adequate for a pigeon. Since the pigeons ate 25 to 30 grams of rice per day this means that the oryzanine was only $\frac{1}{2500}$ to $\frac{1}{5000}$ of the (dry) weight of the food eaten. Feeding 0.3 gram cured a dog that was already paralyzed by experimental beriberi.[1]

It will be seen that these independent investigations all indicate that the antineuritic property shown by rice polishings, yeast, and other natural food materials is due to some basic nitrogenous substance or substances. Much work published since 1912 confirms the general view that the antineuritic vitamin is probably a nitrogenous base, but the early work has not been satisfactorily confirmed in detail and no consensus of opinion has been reached as to the precise chemical nature of the substance.

Williams has attacked this problem by synthesizing substances of known structure and testing them for curative action upon polyneuritic pigeons. Since such chemical examinations as had been made in connection with previous work upon active preparations from natural foods had suggested the presence of pyridine-like substances and also of hydroxyl groups in a benzene ring, Williams began by synthesizing a series of hydroxy pyridines and other pyridine derivatives. Of these α-hydroxy pyridine; 2-, 4-, 6-trihydroxy; and 2-, 3-, 4-trihydroxy pyridine were found to have curative power when tested upon polyneuritic pigeons.

Continuing his work on the relation of chemical structure to antineuritic activity, Williams finds that β-hydroxy pyridine, nicotinic acid, trigonelline, and betaine are also capable of exis-

[1] Apropos of the small quantities of vitamin or oryzanine necessary for pronounced effects, Lusk calls attention to the fact that epinephrine (adrenaline), an essential of life, is present in the blood to the extent of only 1 part in 100,000,000.

tence in forms which are curative in the sense of being " able promptly to dissipate the acute symptoms of polyneuritis gallinarum." " On the basis of these results it may be concluded with reasonable certainty that the relief of the paralysis by such substances is intimately connected with a betaine-like ring."

In 1921 Williams presented further evidence in support of this hypothesis and concluded with the statement of his belief " that vitamin B eventually will be found to be a cyclic nitrogen compound with an oxygen substitution in the ring and capable of existence in a betaine configuration."

The extensive work of Seidell upon the concentration of the antineuritic substance as a picrate also confirms the view that it is probably a nitrogenous base. In this work Seidell takes the protection of pigeons from neuritis as the test for the active substance and considers the concentration of the vitamin in one of his picrate preparations as inversely proportional to the daily dose of the preparation required to protect a standard pigeon.

Investigation of Vitamin B as a Growth-promoting Substance

Parallel with the studies upon the antineuritic substance, as outlined above, have gone investigations upon the concentration of the growth-promoting vitamin B (which may or may not be the same substance), aiming at its ultimate isolation and the determination of its chemical nature.

After Osborne and Mendel had demonstrated the presence of a water-soluble substance important to the growth of rats in protein-free milk and in yeast, Osborne and Wakeman studied the concentration of the substance from yeast and described the preparation of a fraction from yeast which showed a greatly increased concentration of this growth-promoting property. As little as 13 milligrams of this concentrate met the daily needs of the growing rat for vitamin B.

Levene and van der Hoeven have very recently (1925) re-

ported the isolation of an extremely potent preparation from the Osborne-Wakeman fraction. By successive precipitation with lead acetate and barium hydroxide, adsorption with silica gel, and final purification, a product was obtained which was "potent in daily doses of 0.600 milligrams." (*Journal of Biological Chemistry*, Vol. 61, page 429 (1924); Vol. 65, page 483 (1925).)

As the work is being continued actively an attempt to summarize the findings at this time would be premature.

Are the Antineuritic and Growth-promoting Effects Due to the Same Chemical Individual?

That these two physiological effects are due to one chemical substance is implied in the use of the term vitamin B as a designation of the active substance in both cases; but it should be regarded as a tentative assumption rather than as a definite conclusion. This problem has been fully discussed by Mitchell in a paper in the *Journal of Biological Chemistry* in 1919 (Vol. 40, page 399).

According to Mitchell: "The conclusion that the two vitamins are identical seems to be based upon the following grounds. (1) The distribution of the two substances in natural food products is very similar and the correlation between the actual amounts (in so far as these have been measured by biological tests) found in different products appears to be close. (2) The lack of known sources of water-soluble B in the diet of various species of experimental animals seems generally, if not always, to result in symptoms of nerve degeneration and paralysis. (3) Extracts of natural food products possessing growth-promoting properties are said to contain very probably only one indispensable vitamin, though supplementing satisfactorily a ration containing no other possible source of antineuritic vitamin. (4) The solubilities of the two vitamins in the common solvents are said to be identical. (5) Attempts at isolating the

two vitamins have shown that they possess identical precipitants and adsorbents. (6) The stabilities of the two substances, especially to acids, alkalies, and elevated temperatures, seem to be similar if not identical."

After discussion of the evidence on these points Mitchell concludes that there is good reason for doubting the identity, and that what is chiefly needed for a definite solution of the problem is work which shall measure quantitatively the antineuritic and growth-promoting properties of the same preparations.

Such quantitative methods appear to be more fully developed for the testing of vitamin B as an agent for the support of growth in young rats than as an antineuritic agent.

The rat-growth method and its application to the study of some of the properties of vitamin B will next be described.

Quantitative Determination

The method used in the writer's laboratory is, in outline, as follows:

Healthy young rats 28 to 29 days of age [1] and weighing usually 40 to 55 grams are placed in individual metal cages with wire screen bottoms and given *ad libitum* distilled water and a basal diet adequate for the nutrition of rats in all other respects but devoid of vitamin B.

The basal ration chiefly used for this purpose in the writer's laboratory consists of: Casein (carefully purified), 18 per cent; Osborne and Mendel salt mixture,[2] 4 per cent; butterfat, 8 per cent; codliver oil, 2 per cent; starch, 68 per cent.

Some of the rats (called " negative controls ") are left upon this diet alone. After a few days they begin to lose weight, and on the average they die after about four weeks on this vitamin-B-free diet, and after losing about 15 grams of body weight.

[1] All the young animals should come from mothers of the same stock and which are kept on a uniform stock diet.

[2] *Journal of Biological Chemistry*, Vol. 37, page 572 (1919).

Other rats of the same initial age, of as nearly as possible the same initial size, and so far as possible from the same litters as the negative controls, are given in addition to the basal ration graduated daily allowances of the food to be tested as their sole source of vitamin B. Any advantage which they show over the negative controls should (if the experiment is properly planned and conducted) be attributable to the vitamin B which they obtain from the weighed portions of the food under examination. If the amount of vitamin B thus obtained is very small it results simply in a prolongation of the survival period. If a little larger, it may suffice to keep the animal alive for a long time but not permit of appreciable growth. As the allowance is increased a better rate of growth is obtained.

The experimental period is usually continued for 8 weeks. Each animal is weighed at least once each week.

If the food which is being tested for vitamin B is well relished and well digested by the experimental animals and a good source of other nutrients as well as the vitamin, the rats receiving the larger allowances may make a normal average growth. Often, however, it is difficult to feed enough of the food to induce normal growth, without displacing too much of the basal diet, hence what is sought is that allowance of the food under test which results in net maintenance of weight, *i.e.* that on which the test animal weighs the same at the end of the 8-weeks experimental period as at the beginning.

The unit of vitamin B, as used here, is that amount which when fed as a daily allowance results in net maintenance of a standard rat over an 8-weeks period under conditions such as have just been described.

The direct results of the feeding test show what weight of the food under test contains one unit of the vitamin; and from this the number of such units of vitamin B contained in a gram, a kilogram, a pound, an ounce, or a 100-Calorie portion of the food can readily be calculated.

The accompanying table [1] (page 408) shows the vitamin B values of some typical foods as thus determined quantitatively and expressed in the units defined above.

The Relative Thermostability of Vitamin B and the Influence of Hydrogen Ion Concentration (Acidity or Alkalinity)

Quantitative studies of the rate of destruction of vitamin B and of the influence of certain factors have recently been published.[2]

In one series it was found that there was no measurable diminution of vitamin B in milk powder when heated dry with free access of air at 100° C. even when this heating was continued for 48 hours. When the milk was heated in the fluid state for 6 hours at 100° there was a small diminution in its vitamin B content; probably about one fourth of the vitamin was thus destroyed. Vitamin B in the form in which it exists in milk is, therefore, comparatively stable to heating at 100°.

In another series of experiments the effects of heating vitamin B in tomato juice at various temperatures at the natural acidity of the juice, about pH 4.3, was studied. The influence of different amounts of vitamin B upon the weight curves of young rats was quantitatively measured, and the percentage of the vitamin destroyed by heat at each temperature was computed from the experimental determination of the quantity of heated juice which showed the same vitamin B value as a given standard quantity of the unheated juice. The average figures for destruc-

[1] The data given in this table are compiled from various sources. Those determined in the writer's laboratory are chiefly the work of Dr. H. E. Munsell and Miss M. P. Burtis, who have successively served as research assistant under grant from the Milbank Memorial Fund through the New York Association for Improving the Condition of the Poor. For permission to use representative results of this work the writer is indebted to the courtesy of Mr. Bailey B. Burritt, General Director of that Association.

[2] Sherman and Spohn. *Journal of the American Chemical Society*, Vol. 45, page 2719 (1923), and Sherman and Grose, *Journal of the American Chemical Society*, Vol. 45, page 2728 (1923).

RESULTS OF QUANTITATIVE EXPERIMENTS UPON VITAMIN B CONTENTS OF FOODS

FOOD	UNITS OF VITAMIN B * (APPROXIMATE)	
	PER POUND	PER OUNCE
Apple, raw	100–160	6–10
Beans, green, string, raw	150	10
Beans, navy, canned baked	300–400	20–24
Bananas	100–160	6–10
Beef, clear lean steak	about 160	about 10
Cabbage	150–300	9–18
Cantaloupe	about 130	about 8
Carrot	120–140	7–9
Cereals (whole grain, including embryo)	800–1200	50–75
Egg	about 260	about 16
Egg yolk	about 800	about 50
Flour, white	Too little to be measured by present methods	
Grapes	120–140	7–9
Lemon juice	about 150	about 10
Lettuce	150–200	10–12
Milk	about 150	about 9
Milk, dry	about 1200	about 70
Onion	about 100	about 6
Orange juice	about 150	about 9
Peaches, raw	100–160	6–10
Peas, raw	about 1000	about 60
Potatoes	about 130	about 8
Prunes, dry	about 600	about 38
Spinach	300–400	20–24
Tomato	130–250	8–16
Turnip	about 130	about 8

* For definition of these units see page 406.

tion of vitamin B in canned tomato juice at its natural acidity were found for a period of four hours' heating to be as follows: at 100°, 20 per cent; at 110°, 33 per cent; at 120°, 47 per cent; and at 130°, 55 per cent.

These figures establish a low temperature coefficient of the

destruction as one of the characteristics of vitamin B, a property also possessed by vitamin C. For an increase of 10° in the temperature range of 100° to 130°, the rate of the destruction of vitamin B in solutions such as canned tomato juice, which have a hydrogen ion concentration of about pH = 4.3, is increased only 1.3- to 1.4-fold as compared with a 2-fold increase in most chemical reactions. There was no indication of an increased temperature coefficient of the destruction at temperatures around 120° (as reported by some earlier workers) or of any departure from the orderly course of a chemical reaction under the accelerating influence of heat.

There has been some experimental evidence to show that the hydrogen ion concentration of solutions of vitamin B influence the rate of destruction of this vitamin by heat. This is an important question both from the standpoint of its application to the practical problems of nutrition and cookery and from the purely chemical standpoint of the possibility of throwing more light upon the nature of the vitamin. Much of the early evidence of the influence of changes of hydrogen ion concentration upon the destruction of this vitamin by heat was based upon experiments which were not sufficiently controlled to exclude the influence of other factors and in which the estimations of the presence of the vitamin were not always quantitative nor comparable. This problem has recently been studied quantitatively by the rat growth method in the author's laboratory.[1] Filtered canned tomato juice was taken as the source of the vitamin to supplement a basal diet deficient in vitamin B but otherwise adequate. Young rats were divided into groups, some of which received the basal diet and others the basal diet supplemented by the solutions of vitamin B to be tested. These were (1) the filtered tomato juice unheated and at its natural acidity, (2) the juice at its natural acidity but which had been heated for one or four hours at 100° C., (3) portions of the juice which

[1] Burton. Dissertation, Columbia University, 1925.

had been adjusted to various hydrogen ion concentrations and then heated at 100° C. for one or four hours.

A comparison of the weight curves of the animals thus differently fed, gave a quantitative measure of the influence of changes in the hydrogen ion concentrations of its solutions upon the destruction of the vitamin B. At a given temperature (100° C.) this was found to increase with decreasing acidity or increasing alkalinity of the solutions and with the length of time of heating.

The total destruction of the vitamin B due to one hour of heating at 100° C., at the different concentrations of hydrogen ion, was as follows: at pH 5.2, about 10 per cent; at pH 7.9, 30–40 per cent; at pH 9.2, 60–70 per cent; and at pH 10.9, 90–100 per cent. When the heating was continued for four hours at 100° C., the total destruction was 25–35 per cent at pH 5.2, and 65–75 per cent at pH 7.9.

Oxidation was found not to be an appreciable factor in the destruction of vitamin B under the conditions of these experiments.

Summary of Physical and Chemical Properties

Vitamin B is readily soluble in water, glacial acetic acid, and 70 per cent alcohol; it can be extracted by stronger alcohol if used hot. It dialyzes readily through a collodion membrane. It is adsorbed from solution by Lloyd's reagent, a special form of fuller's earth (hydrous aluminium silicate).

The study of the chemical nature of vitamin B has been undertaken by two different methods of investigation: (1) testing substances of known chemical constitution for antineuritic action; (2) fractioning natural materials known to contain the vitamin with the object of obtaining a maximum concentration of either the antineuritic or the growth-promoting activity, and then determining the chemical nature of the substance when isolated.

Neither of these methods has yet given an entirely conclusive result; but both tend to indicate that vitamin B is probably a nitrogenous base (which may perhaps also contain an acid group in the molecule).

The probability that this vitamin is a basic organic compound is consistent with the observation that it is more stable in acid and less stable in alkaline medium.

In acid or neutral solution this vitamin is only slowly destroyed by heating even at temperatures up to 130° C.

In the dry state it is still more stable.

It is not appreciably susceptible to destruction by atmospheric oxidation. As above noted, Sherman and Spohn found no appreciable lowering of the vitamin B content when dried milk was heated in air for 48 hours at 100° C.; and Jansen has reported that rice which had been kept in an ordinary dry granary for 100 years showed an apparently undiminished vitamin B content, compared with rice recently harvested.

Distribution of Vitamin B in Plant and Animal Tissues; Its Nutritive Functions and Relation to Health

Vitamin B is widely distributed in both plant and animal tissues and doubtless has many functions in the nutritional processes of both plant and animal organisms.

Cowgill and other workers in Mendel's laboratory have repeatedly demonstrated the marked effect of vitamin B upon the appetite. Moreover, the suggested relationship of vitamin B to purine or pyrimidine compounds (characteristic of nucleins and "extractives") and the fact that yeast extract, similar to meat extract in general composition, flavor, and property of stimulating the appetite, is rich in B vitamin have favored the view that vitamin B may function as a "physiological stimulant." It has been pointed out that the effect of the vitamin may be a stimulation of the metabolic processes which promote growth rather than of the appetite alone.

There is considerable evidence of a relation of vitamin B to the proper functioning of the digestive tract. Prolonged feeding of diets deficient in vitamin B has been shown to lead to serious impairment of the digestive functions; polyneuritis is accompanied by practical cessation of digestion. An inadequate supply of vitamin B in the diet of test animals leads to intestinal stasis, attributed by McCarrison to diminished neuromuscular control of the intestine.

Although the ways in which this vitamin was discovered have tended to emphasize its conspicuous functions in promoting growth and in protecting the body from nerve disease, it is well to keep clearly in mind also that it is needed at all ages, and that when the food furnishes too little of the vitamin for health, the body may suffer in several other ways before any signs of nerve disease appear. As Plimmer has recently expressed it: "Before the symptoms of beriberi appear, there is a period of ill-health in which occur common symptoms met with every day in medical practice. The first signs are loss of appetite. . . . Weakness, loss of weight, lack of vigor follow. . . . Later gastrointestinal derangements appear — indigestion, constipation, colitis; finally, there are symptoms due to the malnutrition of the nervous system. The onset of these symptoms varies according to the degree of shortage of vitamin B. The greater the shortage, the sooner they appear. If the shortage is slight, the nervous symptoms may never appear, and the organism suffers only from dyspepsia, constipation, and other intestinal troubles. The body is thus weakened and offers no resistance to invading organisms. . . ." Findlay observed a lowering of body temperature and a heightened susceptibility to bacterial infection in pigeons on diets lacking in vitamin B.

Vitamin B and the Reproductive Functions. — McCarrison, working chiefly with pigeons, found a characteristic change in weight of certain organs as a result of vitamin B starvation. The adrenals were enlarged but other organs lost weight — the

thymus most, and then, in order, testes, spleen, ovary, pancreas, heart, liver, kidneys, stomach, thyroid, and brain. These observations of marked atrophy of testes and ovaries are in line with the finding of Drummond that sterility followed quickly in a male rat deprived of vitamin B, and with the reported tendency to amenorrhea among beriberi women (Blunt and Wang).

Evans and Bishop found that ovulation is affected more readily by lack of vitamin B than by the general condition of the body. In connection with the evidence obtained by direct observation upon testes and ovaries of an influence exerted by the vitamin upon the reproductive organs, it is of interest to note that a number of somewhat scattered observations in feeding experiments tend to indicate that normal mammalian reproduction with successful suckling of the young probably requires higher proportions of both A and B vitamins in the food than are required for even rapid growth. Since abundant food intake favors early maturity, fecundity, and lactation, it seems possible that a liberal intake of the B vitamin may result in more successful reproduction and lactation, partly through stimulating the appetite and thus inducing a larger consumption of all the constituents of the diet, and partly through stimulating or directly nourishing the glands and organs directly concerned in the reproductive processes.

Distribution of Vitamin B in Nature. In plants, as Osborne and Mendel have shown, vitamin B appears in relative abundance in all the chief organs — leaves, stems, roots, tubers, seeds, and fruits. A table showing the comparative vitamin content of common foods will be found in Appendix C.

Vitamin B appears to be more widely and more regularly distributed in plant and animal tissues than any of the other recognized vitamins. All the organs of plants appear to contain it, unless it has been removed by artificial refining processes as in the milling of grains. The following table is an estimate

of the distribution of vitamin B in the milling products of one lot of wheat tested by Bell and Mendel.

DISTRIBUTION OF VITAMIN B IN MILLING PRODUCTS OF WHEAT[1]

PRODUCT	MINIMUM PERCENTAGE ADEQUATE IN DIET	CONCENTRATION OF VITAMIN B REFERRED TO WHOLE WHEAT	PERCENTAGE WHOLE WHEAT MILLED INTO EACH PART	PERCENTAGE VITAMIN B CONTAINED IN EACH PART
Whole wheat . .	40	1	100	100
Patent flour . .	(?)	0–0.10(?)	50	0–5
First clear . . .	40(?)	1–0.67(?)	15	15–10
Second clear . .	40	1	5	5
Low grade . . .	20	2	8	16
Middlings . . .	10	4	10	40
Bran	20	2	12	24
Total			100	100

More recent investigations in Mendel's laboratory indicate that the distribution of vitamin B in the wheat kernel should not be considered representative of cereal grains as a whole but that each grain must be studied separately.

The concentration of vitamin B in the embryo of the seed suggests its importance to the early life of the seedling. Later the plant doubtless synthesizes vitamin B; but how or in what organs we do not know.

Animals probably derive their vitamin B either directly or indirectly from plants and the animal organism does not seem capable of storing vitamin B to a sufficient extent to render it independent of the intake for any considerable length of time; yet in milk and in eggs we find a considerable concentration of vitamin B provided by the parent organism for the nutrition of the young. Perhaps it is because under natural conditions animals are receiving this vitamin so regularly in their food that they do not seem to have developed much capacity for storing it. The concentration may be increased in the liver if

[1] Bell and Mendel. *American Journal of Physiology*, Vol. 62, page 145 (1922).

the food has been rich in this vitamin and may be depleted on a deficient diet; but apparently neither the liver nor the body as a whole ever stores enough vitamin B to meet the requirements of nutrition for any considerable time without receiving new supplies in the food.

Undoubtedly the best condition of nutrition and health requires that the food furnish much more vitamin B than is needed to prevent beriberi or even to sustain normal growth. Recent experiments demonstrate fully that, prominent as is the growth requirement, the amount of vitamin B needed by the body continues to increase as the body increases in size, and indicate further that still more vitamin B is needed during reproduction and lactation than at any other time.

There should be, however, no difficulty in so selecting the food as to furnish an abundance of vitamin B under all normal conditions. Milk and the whole grain cereals contain vitamin B in concentrations about three to five times as high as is needed for the support of normal growth, so that even the diet of an infant or invalid restricted to these foods should contain ample amounts of vitamin B unless the foods mentioned are diluted by vitamin-deficient foods such as sugar or highly milled cereal products. The fruit juices now commonly used with the cereal and milk diet of an infant or invalid will add to the vitamin B content; and in the more varied diet of the healthy adult many fruits and vegetables should add to the richness of the intake of this vitamin. Hence, there is little if any danger of a deficiency of vitamin B except in those cases in which the diet consists too largely of artificially refined food products. Yet the use of much of our food in highly refined forms has become customary in this country, and the custom is strongly fostered by the manufacturers because the refined foods are relatively immune to spoilage. So long as this custom persists, vitamin B will constitute a factor of very real practical importance in food values. A larger use of fruit and vegetables, whole wheat

bread and other whole-grain cereals, milk, and eggs may often be advantageous in increasing the intake of vitamin B as well as for other reasons.

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CHAPTER XV

VITAMIN C AND THE ANTISCORBUTIC VALUES OF FOODS

Introductory

For centuries scurvy was one of the most common diseases in Europe and at times among people of European races in North America. It was most frequent and most severe in the more northern regions, where the people were often largely confined to a limited and monotonous diet of bread or other grain products and meat or fish through a large part of the year, fruits and vegetables being abundant only during their short natural season.

On the long voyages which followed the discovery of America, sailors were often obliged to subsist for many months at a time on food even more restricted in variety than that of the winter diet of Europe because they were cut off not only from supplies of fresh fruits and vegetables but also from fresh meat. Their food supplies thus often consisted essentially of breadstuffs and salted meats. On such voyages there were many exceedingly severe outbreaks of scurvy and it gradually came to be recognized that scurvy might be expected when men were kept for a long time on diets which lack fresh food.

The European sailors whose experiences on their long voyages to America did so much to establish the relationship between diet and scurvy and the fact that fresh foods, particularly fresh fruits and vegetables, have antiscorbutic properties, were also instrumental in bringing about a great diminution of the disease. They introduced into Europe from America the cultivation of

the potato, and since that time, as potato culture and the use of potatoes as food throughout the year have become more common in Europe, scurvy has become less common.

Three hundred years ago, however, scurvy was as common in Northern Europe as beriberi was in the Orient. In the case of scurvy, as of beriberi, opinion was divided, down to within recent years, as to whether the disease was nutritional or infectious. Here, however, the general trend of opinion probably favored the view that the disease was nutritional. That scurvy was not only due to faulty diet, but was attributable to the lack of some specific thing, was clearly stated even earlier than the time of Takaki's work on beriberi; for, in 1847, Budd[1] wrote that scurvy was due to the lack of what he called " an essential element, which it is hardly too sanguine to state, will be discovered by organic chemistry or the experiments of physiologists in a not far distant future." Just about seventy years later, when the World War had again made scurvy a disease of great practical importance, the combined experimental work of chemists and physiologists brought evidence which established beyond the possibility of doubt that the essential cause of scurvy is the lack of a specific substance which then came to be called the *antiscorbuic substance* or *vitamin C*.

As early as 1895, Theobald Smith noted that guinea pigs kept upon a diet of oats developed a hemorrhagic disease, but this observation did not attract the attention of students of nutrition at the time.

Later when it had been found by Eijkmann and others that beriberi could be reproduced experimentally in fowls by feeding them upon polished rice, Holst and Frölich undertook to study ship-beriberi in a similar way, using guinea pigs instead of fowls with the idea that experiments upon a mammal would be more directly applicable to human experience. The guinea pigs on the rice diet developed scurvy instead of beriberi. And scurvy

[1] Quoted by Hess: *Scurvy, Past and Present.*

developed in a similar way when either polished or unpolished rice or any other grain was the sole food. This we now attribute to the lack of a necessary antiscorbutic substance (vitamin C) in these grains.

A lack of this substance brings about the same results in man, monkey, or guinea pig (and doubtless also in many other species, which, however, have been less studied in this respect).

Among the more readily noticeable symptoms are soreness and stiffness of joints with a tendency to hemorrhage in them, soreness and hyperemia of the gums leading to looseness of teeth, and bone changes (observable, for instance, in fragility of the jaw). For full description of the pathology of scurvy the reader is referred to the work of Hess (*Scurvy, Past and Present*).

Because of its relative immunity to scurvy, the rat is not used in studies of vitamin C.

Most of our knowledge of vitamin C has been obtained by observation in connection with clinical scurvy in man, or experimental scurvy in guinea pigs or monkeys. For reasons of economy and convenience the guinea pig is chiefly used in experiments upon vitamin C.

When the food is *devoid* of vitamin C, well marked scurvy appears in about two weeks in the guinea pig, or in about four months in a man.

Experimental diets have been developed which, while either wholly or practically devoid of vitamin C, are adequate to meet all other nutritive requirements. By observing with quantitative accuracy and in sufficiently numerous experiments the effects of such diets with and without the addition of measured amounts of any food which we wish to test for vitamin C, it becomes possible not only to ascertain the presence or absence of this vitamin but also to determine the relative quantities present in different foods, or in the same food before and after any desired treatment. Having learned to measure it by its effects, we are now able to study the distribution of this vitamin

and its behavior under various chemical and physical conditions and are thus gaining considerable information regarding its nature and properties.

Quantitative Determination

The method used in the writer's laboratory is, in outline, as follows:

Young guinea pigs, 6 to 8 weeks of age and weighing 250 to 350 grams, are placed individually in cages or in groups in a small experimental pen, and fed *ad libitum* a basal diet consisting of: Oats, sound whole grain ground in the laboratory as needed, *or*, a mixture of equal parts commercial rolled oats and wheat bran, 59 per cent; skimmed milk powder heated on open trays at 110° C. until all antiscorbutic vitamin is destroyed,[1] 30 per cent; butter fat, freshly prepared, 10 per cent; table salt, 1 per cent. Water is also given *ad libitum*. During a preliminary period the animals receive in addition to this basal diet a liberal allowance of some fresh succulent vegetable such as cabbage, lettuce, or raw spinach. This is continued for a week or more in order to make sure that each animal is healthy and capable of making a normal rate of growth.

Then the experimental period is begun by withholding the fresh food. Some of the animals are left as " negative controls " upon the basal diet only; others are fed graduated weighed amounts of the food to be tested in order to find whether the food supplies vitamin C (the only thing lacking in the basal diet), and if so how much of the food must be fed to meet the vitamin C requirement of the standard test animal.

The daily allowance of the food in question which just meets the need of the guinea pig for complete protection from scurvy is sometimes called the " minimum protective dose," and this is one basis for the expression of the relative antiscorbutic values

[1] A frequent and serious source of error has been the use of basal diets not entirely freed from vitamin C.

of foods. Another way is to express the antiscorbutic value of a food in comparison with that of some well-known antiscorbutic such as orange juice, which for convenience of comparison may be given an arbitrary value of 100 as in the table on page 429.

Or we may consider that *the unit of vitamin C* is that amount which when fed as a daily allowance just suffices to afford complete protection from scurvy to a standard guinea pig [1] as above described. Vitamin C values of some typical foods expressed in such units will be found in the table on page 432.

As has been more fully explained elsewhere,[2] it is possible by careful grading of the effects observed both in the living animal and at autopsy, to form a judgment as to what fraction of complete protection the animal had received. This should be attempted only by skilled and experienced observers; but in such hands it has the advantage of bringing into the quantitative interpretation a larger proportion of the test animals than simply those which received exactly the minimum protective dose.

The following summary of results upon guinea pigs, obtained with different amounts of tomato juice, while necessarily far from a complete account of the observations, may serve to illustrate this point:

With no antiscorbutic there is usually good initial growth followed after about 2 weeks by onset of scurvy symptoms, cessation of growth, and great loss of weight before death from scurvy which occurs at 26 to 34 days. Autopsy reveals in severe form all the typical signs of scurvy, notably hemorrhages, fragility of bones, and looseness of teeth.

With 1.0 cc. of tomato juice per day the duration of life is prolonged and becomes less uniform than on the completely

[1] Whatever the mode of expression of values, there is probably some gain in accuracy if the amounts fed are first calculated to the basis of 300 grams body weight of guinea pig protected. The difference should not be great, for animals much larger or smaller than this should not be used.

[2] Sherman, LaMer, and Campbell, *Journal of the American Chemical Society*, Vol. 44, page 165 (January, 1922). Sherman and Smith. The Vitamins, 1922.

scorbutic basal diet. The animals become lame and stiff before death and at death show severe hemorrhages, fragile bones, and loose teeth.

With 1.5 cc. of tomato juice per day the animals usually live out the experimental period of 70 to 90 days, after which it is unlikely that death from scurvy will occur. Such animals develop scurvy symptoms, conspicuously sensitiveness and stiffness in the joints, and usually loss in body weight. Hemorrhages and enlargements of rib junctions may become quite as pronounced as in the previous cases. (Since the animals live longer there is more time for these abnormalities to develop.) Fragility of bones and looseness of teeth are less marked than when less antiscorbutic is given.

With 2.0 cc. of tomato juice per day growth after 15 days is subnormal and animals show soreness of joints without noticeable stiffness. When the animals are killed and examined after 70 to 90 days on this diet they show hemorrhages, but not to a pronounced degree. Jaws and teeth appear normal and bones usually so.

With 3.0 cc. or more of tomato juice per day there is complete protection from scurvy, as judged by examinations both during life and at autopsy. Growth is fully normal in all animals that eat the basal diet well. Hence 3 cc. appears to furnish a fully adequate allowance of the antiscorbutic vitamin.

Quantitative methods such as have just been described are now being used in studying the distribution and properties of vitamin C.

At present, however, any attempt at comprehensive summary of existing knowledge on these points must make use both of data which are really quantitative and of other observations which are not strictly so. This is true of the general statements on occurrence and properties which follow; but wherever possible the results of the more strictly quantitative work have been used as the basis for statements to be made in this book.

Occurrence in Vegetable Materials

Mature, resting seeds evidently contain very little if any of this vitamin. In a diet otherwise adequate the amount of grain fed has no appreciable effect upon the appearance or severity of scurvy. But if properly sprouted grain be fed it is found to have pronounced antiscorbutic properties. Evidently in the sprouting of the grain (and this applies to legumes as well as cereals) there occurs a marked development of vitamin C. As nothing but water and air need enter the seed, it is plain that something preëxisting in the seed must become transformed into vitamin C as the seed sprouts.

This formation of vitamin C in the seed as it passes from the resting stage into a condition of active metabolism is undoubtedly of fundamental significance. From which of the preëxisting substances in the seed the vitamin C is formed, we do not know. It has been suggested that it may be formed from vitamin B, but there does not seem to be adequate evidence either to establish or to disprove this suggestion.

Where and when the plant forms its vitamin C, beyond the amount produced in the sprouting of the seed, is not yet determined; but there seems to be no room for doubt that this vitamin is in some way connected with the active life processes of plants as well as animals.

Being readily soluble in water, the vitamin C formed in the sprouting seed passes as a constituent of the juice or sap into the growing parts of the plant, and in general the studies of the distribution of this vitamin in plant materials show it to occur most abundantly in the actively functioning and the succulent parts, — in the fresh green leaves, the growing shoots, and the juicy stems, roots, tubers, bulbs, and fruits. It is to be mentioned, however, that in any such simple grouping there may be considerable variation within any one group, and this is not to be avoided by a more elaborate subdivision of plant products

according to biological classification or general chemical composition, since in some cases foods very similar from either of these points of view appear to have quite different antiscorbutic values. Thus the juice of the lemon has shown much higher antiscorbutic value than that of the lime and the Swedish turnip is reported to be much richer in vitamin C than the common white turnip. In how far such differences are to be regarded as fixed for the species and varieties concerned and in how far they may have been due to difference in soil, climate, season, handling, or storage of the particular specimens used in the feeding experiments reported, or even to the technique of the experiments and the individual variability of the experimental animals employed, it would at present be exceedingly difficult and probably impossible to decide.

Among the richest sources of vitamin C are oranges, lemons, tomatoes, and raw cabbage; apples, bananas, carrots, and potatoes do not contain such a high concentration of the vitamin but are important as antiscorbutics because of the quantities eaten. Thus potatoes become a very important source of vitamin C because of the large extent to which they enter into the daily dietaries of many people, and in practice it is often largely upon potatoes that the adequacy of the supply of vitamin C in low-cost winter dietaries depends, so that, as stated by Hess, "A failure of the potato crop is followed by scurvy in the spring."

The accompanying table shows how some familiar fruits and vegetables compare, weight for weight, as sources of vitamin C.

Occurrence in Animal Materials

Broadly speaking, animals doubtless depend upon plants for their supplies of vitamin C. Whether some species of animals (such as the rat, which seems so independent of antiscorbutic food and so immune to scurvy) are able to utilize the inactive form or related substance such as we have referred to as existing in seeds, is still an open question. The fact, however, that rats

RELATIVE RICHNESS IN VITAMIN C OF CERTAIN FRUITS AND VEGETABLES COMPARED WITH THAT OF ORANGE JUICE AS 100

Apple, raw	10 to 20
Banana, raw	20 to 40
Cabbage, raw	100
Cabbage, slightly cooked	30
Cabbage, fully cooked	5 to 10
Carrots, raw	5 to 50 (?)
Carrots, cooked	very variable
Grapes or grape juice	4 to 5
Lemon juice	100
Lime juice, fresh	25
Onions, raw	100
Oranges or orange juice	100
Pineapple juice, fresh, raw	70
Potatoes, raw	50 (?)
Potatoes, cooked	10 to 30
Spinach, raw	100 (?)
Spinach, cooked	20–25 (?)
Sprouted beans or peas, raw	70
String beans, raw	70
Tomatoes, fresh or canned	100
Turnip juice, raw	30–70

For other comparisons the tables on page 432 and in Appendix C may be consulted.

thrive so well on diets made up almost entirely of highly purified foodstuffs would seem to suggest that their needs for this vitamin are small, rather than merely that they are able to utilize it in a different form from that required by the guinea pig, the monkey, and man. These species, and doubtless most other animals as well, must have vitamin C as such in their food and cannot store it to any large extent in their bodies. Muscle tissue contains very little vitamin C.

Repeated attempts to show the presence of vitamin C in ordinary meats by the usual tests have given negative results. On the other hand, observations upon human scurvy have sometimes indicated that meat, if eaten sufficiently fresh, raw, or "rare," and in large quantities, has an appreciable though small

antiscorbutic value. However, it would not appear safe to depend upon cooked meat, as ordinarily eaten, to furnish any significant amount of the antiscorbutic vitamin. There is a somewhat higher concentration in the glandular organs and perhaps also in the blood. Hess suggests that blood may be comparable in this respect with milk, and milk normally contains a much higher concentration of vitamin C than does muscle.

Since the animal body probably does not synthesize vitamin C nor carry any large store of it, the concentration of this vitamin in milk must be more or less dependent upon the food of the nursing mother or of the lactating animal as the case may be. In order that the milk of the mother may furnish the child an abundance of vitamin C and that the child may actually receive the benefit from maternal nursing that is usually and properly expected, the mother's diet must contain adequate amounts of antiscorbutic food.

That cows' milk may be made to vary widely in its vitamin C content according to the nature of the food which the cow receives, has been strikingly shown by Hess. It is not to be expected, however, that market milk will show such wide variations, for farmers engaged in producing milk for market do not use rations so extremely poor in vitamin as was used for the purpose of the experiment; if they did the cows so fed would produce so little milk that it would but slightly influence the average of the milk coming to market.

Dutcher, Eckles, *et al* compared the antiscorbutic values of samples of milk obtained from the same cows, first on winter and then on summer feed. They concluded that the summer milk was three times as rich in vitamin C as the winter milk. They report also that when the diet is low in vitamin C, the vitamin value of the milk decreases slowly; while when the diet is made rich in vitamin, the vitamin value of the milk rises rapidly. This is in accordance with the view that the body can-

not store much vitamin C but does carry a limited store which during lactation gradually passes to the milk in case the amount furnished by the food is subnormal. In other words, a vitamin deficiency during lactation may be expected to be divided between the lactating mother and the suckling young, and both must be expected to suffer as the result of such deficiency even though distinct symptoms of scurvy may not appear. On the other hand, when food rich in vitamin C is fed, the body is able to store only a relatively small amount and the surplus entering the body from the food is immediately utilized in the enrichment of the vitamin content of the milk.

These principles doubtless apply as well to the milk of the nursing mother as to cow's milk.

According to Hess, a pint per day of average milk supplies the antiscorbutic requirement of a child. If the antiscorbutic value is materially lessened by heating or aging of the milk or by faulty feeding of the cow, more milk may be required; and, conversely, fresh milk from a cow properly fed will usually be somewhat richer and may be considerably richer in vitamin C than previously estimated.

It is probably best to recommend that all artificially fed infants be given some such antiscorbutic food as orange juice or the juice of canned tomato, and if this is done the possible variations in vitamin C content among the different forms of milk (raw, pasteurized, canned, or dried) need not be further considered.

Roughly quantitative comparison of the vitamin C contents of some typical foods, animal as well as vegetable, is attempted in the table on page 432.

Physical and Chemical Properties of Vitamin C

Vitamin C is freely soluble in water or in alcohol (Hess and Unger), dialyzes through parchment (Holst and Frölich), passes through a porcelain filter, and is not adsorbed as is vitamin B by

TENTATIVE APPROXIMATIONS OF VITAMIN C CONTENTS OF CERTAIN FOODS

FOOD	UNITS OF VITAMIN C * (APPROXIMATE)	
	Per pound	*Per ounce*
Apple, raw	25 to 50	1.5 to 3.0
Banana, raw	50 to 100	3.0 to 6.0
Beef, Eggs	Somewhat conflicting views indicate amounts too small to measure.	
Lemon juice	150 to 300	9. to 19.
Milk	5 to 25	0.3 to 1.5
Orange juice	150 to 300	9. to 19.
Potatoes, cooked	25 to 75	1.5 to 4.5
String beans, raw	100 to 200	6. to 13.
Tomatoes, raw or canned	150 to 300	9. to 19.
Wheat and other grains, dry, mature	Too little to measure, if any.	
Wheat and other grains, dry, sprouted	Fairly rich; variable with stage of sprouting.	

fuller's earth or Lloyd's reagent (Harden and Zilva). Because of the ready solubility of vitamin C, the juices of antiscorbutic foods, and the liquor or " water " of such foods when canned, may be expected to contain as high a concentration of the vitamin as does the solid part of the food. In general the proportion lost from food by being dissolved away in cooking or canning is likely to be fully as great for vitamin C as for vitamin B.

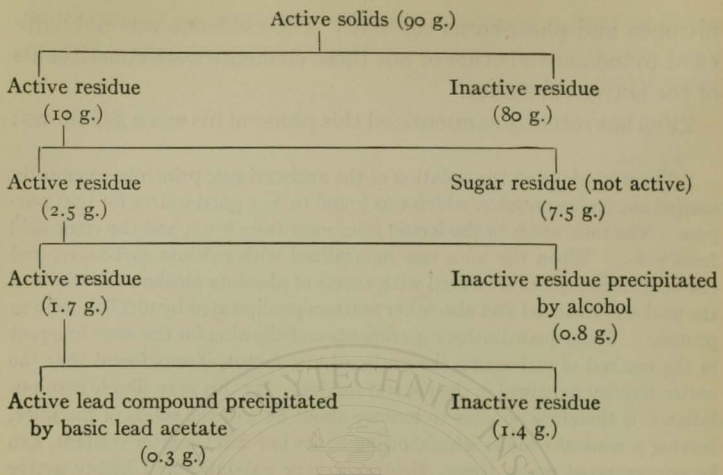
Several investigators have succeeded in preparing highly potent concentrates of the antiscorbutic vitamin. On removal of the invert sugar from decitrated lemon juice by fermentation in an atmosphere of carbon dioxide, Zilva found that the active substance remained in the residue which comprised only about one thirtieth of the total solids of the original lemon juice. By submitting this active residue to further fractionation, utilizing various precipitation methods, he was able to concentrate practically all of the antiscorbutic principle in 0.03% to 0.07% of the total solids. The purest fractions showed an extremely low

* For definition of this unit see page 425.

nitrogen and phosphorus content. The evidence was not sufficient to indicate whether or not these elements were constituents of the active substance.

Zilva has recently summarized this phase of his work as follows:

"The work done on the isolation of the antiscorbutic principle was mostly carried out on lemon juice, which was found to be a good source for that purpose. The total solids of the lemon juice vary from 8–9% and the citric acid from 7–8%. When the juice was neutralized with calcium carbonate, and the neutralized solution treated with excess of absolute alcohol, the whole of the acid was removed and also other matters precipitated by alcohol, such as proteins. From quantitative experiments and allowing for the error inherent in the method of estimating the antiscorbutic factor, it was found that the entire activity remained in the neutral residue. By this very simple manipulation it is therefore possible to remove about 85% of the solids of the juice, leaving a neutral residue which, owing to its low solid matter content, can be concentrated many times, yielding a very palatable and highly active preparation. Such preparations have been tried therapeutically by Dr. Still on several occasions during the last few years with good results, especially on children in whose case the scorbutic condition is complicated by digestive disturbances. As already mentioned, utilizing our knowledge of the influence of oxidation and reduction on the stability of the antiscorbutic factor, it is now possible to prepare such concentrated antiscorbutic solutions which will retain their activity for some time. Although by this first step in the isolation of the vitamins it was possible to remove the major part of the non-active solids of lemon, the real difficulties, from a chemical point of view, were found only to begin at this stage. The neutral active residue of the lemon juice was ascertained to consist mainly of invert sugar. To remove sugar by ordinary chemical means is associated with drastic treatment not suitable for the fractionation of such an unstable principle as the antiscorbutic factor. Recourse was therefore had to a biochemical method. I have shown that by fermenting this neutral fraction with yeast under certain conditions the sugar is almost entirely removed, leaving behind the total activity in the residual fraction. This definitely disposed of the possibility that the activity was in any way associated with the bulk of the sugar of the juice. Moreover, this new fraction was most suitable for further manipulation. Numerous precipitating agents were next tried, and one of these reagents, namely, basic lead acetate, was found to precipitate the entire vitamin content. After removing the lead by suitable means a very active fraction was obtained. The table illustrates my scheme of fractionation."



. . . "The chief chemical characteristic of the most potent fraction is that it contains very little nitrogen, traces of phosphorus, and that it decolorizes potassium permanganate and reduces ammoniacal silver nitrate in the cold. The last reaction was found so far by every active fraction. The reducing properties of active solutions cannot, however, be taken as criteria for their activity." (*Journal of the Society of Chemical Industry*, Vol. 44, pages 445–450 *T*, 1925.)

Oxidation destroys vitamin C, but the conditions determining such oxidative destruction have not yet been worked out satisfactorily. Oxygen itself may be involved; or other oxidizing substances, some of which, apparently, occur naturally in food materials. Such drastic oxidizing agents as potassium permanganate and hydrogen peroxide show marked destructive action even at room temperature. In the case of oxidation by contact with air the destruction becomes rapid only on heating. Thus heating is much more destructive of vitamin C in the presence of air than in its absence.

Kohman and Eddy consider that the amount of respiratory oxygen in the cells of apples is a factor in the destruction of

vitamin C during canning. They report that by covering the peeled and quartered apples with a solution of salt in water, thereby shutting off the supply of atmospheric oxygen and causing the consumption of the stored oxygen, the subsequent processing could be carried on without appreciable loss of vitamin C.

The destruction of vitamin C by heating in water solution (as in the juices of the typical antiscorbutic foods) is a process which proceeds at a rate which can be measured experimentally and which has been studied quantitatively with reference to the influence of temperature, time of heating, and the hydrogen ion concentration (acidity or alkalinity) of the solution.

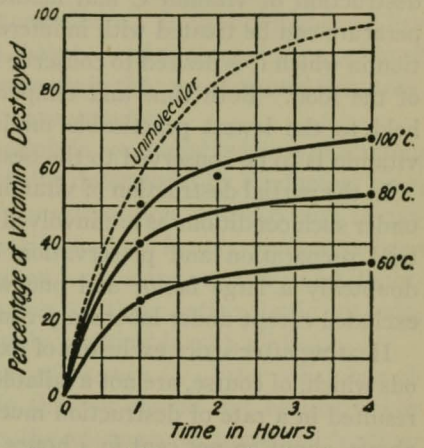


FIG. 17. — Time curves showing the progress of the destruction of vitamin C in tomato juice of natural acidity (pH 4.3) when heated at 60°, 80°, and 100° C. The broken line shows how the destruction would proceed if it were a simple unimolecular reaction of the same initial rate at 100° C. (Courtesy of the *Journal of the American Chemical Society.*)

In the case of tomato juice of natural acidity (pH = 4.3), it was found by LaMer, Campbell, and Sherman that boiling for one hour destroyed practically 50 per cent, and boiling for 4 hours destroyed practically 68 per cent of the antiscorbutic vitamin. At lower temperatures there was less destruction in a given time. Figure 17 shows the time curves of the destruction of the vitamin at 60°, 80°, and 100° C. It will be noted that throughout the entire range of times and temperatures covered by these experiments, the destruction was always greater the higher the temperature, whatever the time of

heating selected for the comparison, and also that at whatever temperature the material was heated the destruction of the vitamin also became greater the longer the heating was continued.

Thus both time and temperature are important factors in the destruction of vitamin C and neither the time nor the temperature can be treated with indifference in any heating operation in which it is desired to conserve the antiscorbutic property of the food. Both time and temperature of heating must be held to the lowest practicable minimum if the antiscorbutic vitamin is to be conserved to the best advantage.

In the partial destruction of vitamin C which thus takes place under such conditions as are involved in the household or industrial preparation and preservation of food, oxidation is undoubtedly a large factor and one which can not be entirely excluded except under laboratory conditions.

Heating after strict exclusion of oxygen, by laboratory methods which, of course, are not available in the kitchen or factory, resulted in a rate of destruction much lower than that charted above, about 20 per cent in 4 hours at 98° C. (experiments by LaMer, Kenny, and Sherman, not yet published).

The best conservation of the antiscorbutic value of the food also demands avoidance of the use of soda or other alkali. Not only is vitamin C more readily destroyed in an alkaline than in an acid solution; but any decrease of acidity, even though the material may still remain acid, means that a greater percentage of the vitamin will be destroyed under the same conditions of time and temperature. Thus tomato juice, which lost 50 per cent of its vitamin C in one hour at 100° C. at natural acidity, lost 58 per cent under the same heat treatment when it had been about half neutralized, and about 65 per cent when it had been made very faintly alkaline (pH = about 9, *i.e.* at a reaction which would appear "neutral" to the indicator phenolphthalein).

The fact that Hess and also Hume have found the antiscor-

butic vitamin to be well preserved in sweetened condensed milk is evidence that vitamin C in a nearly neutral medium can be preserved through a moderate heat treatment and subsequent storage when both heating and exposure to air are reduced to a minimum by evaporating under vacuum and immediately placing the product in airtight containers. In food of higher acidity the antiscorbutic vitamin may be well preserved even without the use of vacuum, as for example in the case of canned tomatoes, which are among our best antiscorbutics.

Drying and aging often result in considerable losses of vitamin C, but these losses are probably determined by the factors time, temperature, hydrogen ion concentration, and exposure to oxidation. By sufficient attention to these factors, foods have been dried or canned and preserved for months certainly, and doubtless for years, with little loss of their antiscorbutic vitamin. Apparently this is not equally practicable with all foods.

Preserved foods as sources of vitamin C. From what has already been said it is plain that we should not be justified in generalizing broadly as to the adequacy of preserved foods as sources of vitamin C, for this will depend upon several factors which vary greatly in different foods. The amount of vitamin C which the food contains at the beginning; its hydrogen ion concentration; the time and temperature of heating to which it is subjected; the extent to which, and temperature at which, it is exposed to air; the presence and concentration of oxidizing substances or of substances which may catalyze the oxidative or other destruction of the vitamin; — all these are factors which may influence the antiscorbutic value of a preserved food, and there may be other factors not yet recognized.

Nutritive Functions and Relations to Health

Cases of well marked, or so-called " manifest " scurvy resulting from practically complete deprivation of the vitamin, are not often seen in this country. More important to us are the

results of diets *poor in* vitamin C rather than wholly devoid of it. Plimmer has recently summarized these as follows:

" Before definite symptoms of scurvy appear there is a period of ill-health, characterized by certain symptoms which may also be looked for in those who habitually take too little vitamin C, though they get enough to prevent acute scurvy. These symptoms are a sallow, muddy complexion, loss of energy, fleeting pains in the joints and limbs, especially in the legs, usually mistaken for rheumatism. So-called rheumatism in infants and young children has often been proved to be due to insufficient vitamin C, and is really scurvy. . . . During the war the slow healing of wounds was found to be associated with shortage of vitamin C."

Study of the literature, not only of experimental investigation but also of food and health conditions in many times and places, together with observations in the scurvy ward of the military hospital at Petrograd during the World War, and the writer's recollection of general conditions and specific cases in the rural regions in which he formerly lived, all combine to suggest that much of the so-called rheumatism which afflicts such a large proportion of our people in winter and spring, is due at least in large part to the use of diet too poor in vitamin C.

Careful experimental work both by Zilva and Wells in England and by Howe at Harvard, has shown to a surprising degree how deficiencies not only in the fat-soluble vitamins usually associated with bone development, but also in vitamin C, are closely connected with tooth defects. They find that the tooth is one of the first, if not the first, part of the body to be affected by a deficiency of vitamin C and that the teeth may be seriously affected, even when scorbutic symptoms are so slight as to be almost unrecognizable. Applying their observations to problems of human nutrition, these authors suggest that the use of food poor in vitamin C may be a very important factor in the great prevalence of tooth decay.

The absence of scurvy does not prove that the food is supplying all the vitamin C that the body needs for its best health and vigor. Thus the rat does not develop scurvy when kept on a " scorbutic " diet such as would induce the disease in man, monkey or guinea pig; yet we have evidence that the rat is not indifferent to the vitamin C furnished by his food. Harden and Zilva and almost simultaneously Drummond tried the addition of vitamin C in the form of orange juice to the diet of rats and found this to result in better growth and vigor. Further light has been thrown upon this subject by the finding of Parsons in McCollum's laboratory and of Carrick and Hauge that livers of rats and of chickens contain vitamin C even after long periods on " scorbutic " diets. It is highly improbable that this can be accidental. All these observations indicate strongly that vitamin C plays a part in the physiology even of those animals which are not subject to scurvy. Similarly in the human body the absence of distinct symptoms of scurvy is not proof that as much vitamin C is being furnished by the food as is needed for the best fulfillment of all the physiological functions.

Hess has repeatedly pointed out how frequently children without showing any distinct scurvy symptoms are irritable, lacking in stamina and more or less retarded in growth, and can be restored to better growth, higher stamina, and better general health and disposition by the feeding of additional vitamin C in the form of orange or tomato juice or other suitable antiscorbutic food, showing that the food supply had been too poor in vitamin C for the maintenance of really good health although no distinct symptoms of scurvy had appeared. It now appears probable that a similar condition of " latent scurvy " is rather common among grown people also, and recently Findlay has definitely shown that long-continued low intake of vitamin C increases the susceptibility to infectious disease, and conversely that the resisting power of the body is increased by a diet containing ample supplies of vitamin C.

Werkman, Nelson, and Fulmer found that resistance of guinea pigs to infection was diminished on diets deficient in vitamin C. They consider the accompanying reduction in body temperature of primary significance in accounting for the reduced resistance.

Thus it is gradually becoming clear that vitamin C not only protects from scurvy but has important functions also in normal nutrition and in the maintenance of the body's defences against the attacks of infectious disease.

It has been estimated that the daily amount of vitamin C that a man must have to protect him from scurvy is about the amount contained in one ounce of orange or lemon juice or canned tomato or raw cabbage or onion, or in about one pound of cooked cabbage or potato, or in a pint of milk. But it is now becoming plain that the amount of vitamin C which we must have to protect us from scurvy is only a fraction of the amount which is really needed for full health and vigor; and instead of reliance upon any one of the items just mentioned as possible minimum sources of vitamin C, the equivalent of several of them should be supplied in each day's food.

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CHAPTER XVI

THE FAT-SOLUBLE VITAMINS WITH SPECIAL REFERENCE TO VITAMIN A

Evidence of the Existence of a Fat-Soluble Substance or Substances Essential to Growth and Health

It is of more than historical interest that the existence of a fat-soluble substance or substances essential to normal nutrition was discovered through experiments, made simultaneously in 1913 by McCollum and Davis and by Osborne and Mendel, in which it was found that young animals on food mixtures alike in all other respects would continue to grow and thrive or would soon cease growing and shortly thereafter sicken and die, according as the fat in these food mixtures was butter fat or lard. Plainly this was evidence of the presence in milk and butter fat of a fat-soluble, growth-promoting substance which in lard is either absent or present only in much smaller amounts.

Almost immediately it was further found by McCollum and Davis that egg-yolk fat, and by Osborne and Mendel that cod-liver oil, resemble butter fat in containing notable amounts of this substance while most vegetable fats are, like lard, nearly if not quite devoid of it. The relatively high concentration of this vitamin in the fat of milk and eggs at once suggests its importance in reproduction and in the development of the young.

In the same year that the existence of this substance was demonstrated, Osborne and Mendel pointed out that animals suffering for lack of it become subject to an eye disease which has

since been widely discussed in connection with this dietary deficiency under the names of ophthalmia, xerophthalmia, keratomalacia, and conjunctivitis. Because of its characteristic relation to the eye disease, the substance has sometimes been called the antiophthalmic or antixerophthalmic vitamin; but this, while accurately descriptive so far as it goes, seems too narrow a name because, as will appear more fully further on in this chapter, the same substance which prevents the ophthalmia has other very important functions in nutrition as well.

In the case of each of the recognized vitamins, the characterization of the substance, and in fact the belief in its actual existence, depends chiefly upon the physiological disturbances observed to follow the use of a diet good in all other respects but deficient with respect to the vitamin in question. When a diet lacking vitamin A but adequate in all other respects is given to a young growing rat, growth continues for a shorter or longer time, probably because of stores of this vitamin which the body already possesses, after which growth ceases and, if the diet is entirely devoid of the vitamin, there usually sets in a loss of body weight and a condition of general decline leading to death. Sometimes, perhaps because the experimental diet contains unrecognized small amounts of vitamin A, the body weight remains nearly constant and the animal apparently in health for some time after growth has ceased. By the time the body's reserves of vitamin A have been exhausted and growth ceases the animal shows increased susceptibility to bacterial infection, and this lowered resistance results in a large proportion of the experimental animals developing the characteristic disease of the eye. This usually begins with a swelling of the lids of one or both eyes or with indications that the eye is becoming unduly sensitive to light. Following this there commonly develops an inflamed and catarrhal condition of the conjunctivae, with a bloody or purulent discharge, the lids becoming scabby or sticky. This, with the swelling of the lids and the sensitiveness to light,

sometimes results in the eye being found completely closed. If the eye condition is not treated, and the animal continues to live, the cornea may become affected and blindness result. The typical condition undoubtedly involves infection and, according to Bulley, may be prevented by careful treatment with antiseptics, though Osborne and Mendel found that their cases were not cured by such treatment. Since eye trouble resulting from lack of the A vitamin involves infection, it is not purely a deficiency disease, yet it is essentially such inasmuch as the dietary deficiency enormously increases the susceptibility of the animal to the infection, and thus practically determines whether or not the disease will occur. It is also very significant that without any local treatment whatever the eye disease, if not too far advanced, usually disappears quickly upon feeding the animal with any food containing an adequate amount of vitamin A. Thus Osborne and Mendel stated as early as 1913 that " Another type of nutritive deficiency exemplified in a form of infectious eye disease prevalent in animals inappropriately fed is speedily alleviated by the introduction of butter fat into the experimental rations," and in 1914 they reported " uniform success by substituting codliver oil for a portion of the lard in our standard diets. . . . Not only was growth resumed in most cases at a very rapid rate, but all evidence of malnutrition, especially the affection of the eyes, promptly disappeared."

That lack of vitamin A may lead to weakness or abnormality of other tissues as well as those of the eye has been shown by the work of several investigators. Osborne and Mendel refer to diarrhea and diminished appetite as frequently resulting from this lack; and they have definitely correlated it with the occurrence of phosphatic renal calculi among their experimental animals. McCollum and Davis and also Drummond report increased susceptibility to infections including lung disease. Steenbock confirms this and suggests that the development of the characteristic eye disease or of lung trouble be regarded as

the distinctive sign of this dietary deficiency. Again, however, it may be said that a wider definition would be required if all danger of ambiguity were to be avoided, for Evans and Bishop find equally distinctive the peculiar type of reproductive failure (in this case a failure of ovulation) which results from lack of vitamin A; and it is certain that such a lack affects nutrition in other ways as well, as will be discussed in the latter part of this chapter.

Significance of the Term Vitamin A

The term vitamin A was for some years used interchangeably with fat-soluble vitamin; but in recent years it has been shown that there are (at least) three fat-soluble substances belonging to the general category to which the term vitamin is commonly and conveniently applied, and so as the evidence has become adequate there have been differentiated from vitamin A first an antirachitic and then an antisterility vitamin as explained in the next section.

In its present significance, therefore, the term Vitamin A stands for a substance (or possibly a closely related group of substances) which, independently of the two other fat-soluble substances just mentioned, is essential to growth and health.

The most common criteria of its absence are cessation of growth on a diet adequate in all other respects and a more or less characteristic susceptibility to eye disease. But this latter is only one phase of a widespread weakening of the body tissues and increased susceptibility to infections of several kinds including, as mentioned above, lung, skin, and bladder infections, and, as more recently demonstrated, inflammation and formation of pus in the ears, the sinuses, and particularly in the glands near the base of the tongue. Hence, even after the complete differentiation of the two other fat-soluble substances mentioned, vitamin A remains much more than merely the antiophthalmic substance.

Differentiation of the Antirachitic Substance ("Vitamin D") and of the Antisterility Vitamin "E" or "X" from Vitamin A

For several years it has been known that codliver oil contains an antirachitic substance, that is, something which tends to prevent or cure rickets. At first it appeared that this might be one of the properties of vitamin A, but further work showed that the oil still retained its antirachitic property after its vitamin A had been destroyed by oxidation. The antirachitic property was thus evidently due to some other substance than vitamin A. Our study of the antirachitic substance will be deferred until the next chapter.

Evans and Bishop have also differentiated from vitamin A another fat-soluble substance which *in addition to vitamin A* and other nutritional essentials is apparently required for the support of reproduction. This substance, which they first designated as "X" but which is now generally called "Vitamin E," differs from vitamin A not only in its distribution but also in that a lack of it causes a *different kind of failure in reproduction.* Lack of vitamin A causes failure in ovulation; lack of vitamin E causes failure in placental function; and these two types of failure Evans and Bishop find that they can distinguish with entire certainty by means of the anatomical and histological methods which they have developed. Vitamin E will be considered further in Chapter XVIII.

Physical and Chemical Properties of Vitamin A

Vitamin A was first described as a fat-soluble substance and Osborne and Mendel early showed that when a natural fat is fractioned by removing the glycerides of higher melting point, the vitamin is not removed to a proportional extent with them but remains dissolved in the more fluid part of the fat.

A little later it was found that the vitamin belongs to the "unsaponifiable matter" of the natural fat and not to the

triglyceride or the fatty acid itself. This "unsaponifiable matter" consists chiefly of unsaturated monatomic alcohols of high molecular weight which are solid at ordinary temperatures and hence (as explained in Chapter II) have been given the group name of *sterols*. The well-known members of the group are cholesterol and phytosterol occurring in animal and vegetable fats respectively. As animal fats have served as the starting point in most of the studies of vitamin A, the findings regarding its properties tend to be given largely in such terms as to permit of comparison with cholesterol.

Drummond in 1924 reported the first results of the studies which he was conducting with a view to the isolation of the fat-soluble vitamin and determination of its physical properties and chemical nature. In this he did not claim to have entirely excluded the admixture of the antirachitic substance but it was to vitamin A that his attention was chiefly directed. In his experiments the unsaponifiable matter prepared from codliver oil represented about one per cent of the weight of the oil but contained the vitamin activity. "Oxidation of this material rapidly destroys the growth-promoting substance A and rather more slowly the antirachitic factor D." Further he found that by crystallization from methyl alcohol at low temperature and by subsequent precipitation with digitonin (which combines with cholesterol to form an insoluble digitonide) "all the cholesterol present may be removed without affecting the vitamin activity of the residue, which is a red-brown oil corresponding to about 0.4 per cent of the original codliver oil." Attempts to fraction this material directly were rendered unsatisfactory by the presence of resinous matters formed during the saponification, but these substances were removed by subjecting the material to distillation in superheated steam in an atmosphere of nitrogen. The purified material appeared to consist very largely of unsaturated alcohols. At a pressure reduced to only 2 mm. it distilled (without loss of its vitamin activity) at approximately

255° C. This distilled material consisted almost entirely of an unsaturated alcohol (sterol) containing two double bonds and one hydroxyl group and having a molecular weight of about 350. This fraction represented about 0.1 per cent (1 part in 1000) of the original codliver oil. Drummond considered the evidence inconclusive as to whether the sterol of which this fraction was chiefly composed was or was not the actual fat-soluble vitamin. Further experiments showed that the vitamin value was not lost when the material was submitted to reactions of acetylation and benzoylation. Drummond adds: " It might be mentioned in passing that the old sulphuric acid test for codliver oil is invariably given by fractions containing the active substance, which lends support to the view, tentatively put forward by Drummond and Watson (*Analyst*, 1922, *47*, 341) that the vitamin may be responsible. If this should prove true we will at last have one chemical test for the presence of a vitamin."

Takahashi, Nakamiya, Kawakami, and Kitasato, in No. 32 (Vol. 3, pages 81–146) of Scientific Papers of the Institute of Physical and Chemical Research (Tokyo) issued June, 1925, report a very extended and interesting study of the chemical, physical, and physiological properties of vitamin A, for which they suggest the name " biosterin " or " biosterol."

In a paper published since the foregoing text was written (1925), Drummond, Channon, and Coward have carefully examined the data of the Japanese investigators in the light of new work of their own. They conclude that the degree in which the vitamin has been concentrated is of the same order in the two investigations, and they point out a number of observations which in their judgment indicate that " biosterin " cannot be regarded as representing an isolation of vitamin A.

The harmful effect reported by the Japanese workers as resulting from the administration of excessive doses of " biosterin " was not confirmed by the English investigators who consider that this was probably due to a toxic impurity mixed with

vitamin A in the biosterin preparation. Drummond, Channon, and Coward also point out that some of the chemical and physical properties described as belonging to biosterin are not consistent with the evidence of their own work upon the chemical nature of vitamin A, and conclude that: "It has not been possible to decide whether vitamin A is identifiable with one of the unsaturated alcohols."

Question of the resistance of vitamin A toward heating and oxidation. Early statements regarding the thermostability or thermolability of vitamin A were somewhat conflicting, and doubtless for a number of reasons. (1) Until methods for the quantitative determination of the vitamin by the feeding of carefully limited and regulated amounts of the material under investigation to a properly prepared experimental animal had been worked out, it was not unlikely that the destruction of a considerable percentage of the vitamin might escape detection. (2) Attempts to compare different experiments upon the effect of heating might be vitiated by differences in the experimental conditions which gave more opportunity for oxidation in one case than the other; *e.g.* heating in contact with air as compared with heating by means of a current of steam. (3) The vitamin may have existed in different forms in the different materials studied.

In 1920 it was shown by Hopkins, and independently by Drummond and Coward, that the rate of destruction of vitamin A in butterfat, and presumably in other fats, was very greatly dependent upon exposure to oxygen during the heating. Since that time the tendency has been to attribute the destruction, which occurs upon heating, to an oxidation accelerated by high temperature.

Apparently, however, account must also be taken of the further fact that the form of the vitamin may differ and that this may influence its susceptibility to destruction by either heat or oxidation.

Recent quantitative experiments have indicated a destruction of only about 18 per cent of the vitamin A of tomato juice when heated at approximately 100° C. for 4 hours,[1] and careful comparative experiments with and without rigorous exclusion of oxygen showed that oxidation is less destructive of vitamin A in the form in which it is found in this primary (vegetable) source than had been supposed in view of the experiments upon heating and aërating animal fats.

Hence the results of these experiments upon heat destruction afford added evidence for the view that vitamin A may exist in nature in more than one chemical form. This view will be considered further in the section which follows.

Formation and Distribution in Nature

Vitamin A is formed in the green leaves of plants and these leaves are richer in this vitamin than any other part of the plant. The thinner and greener the leaf the richer it is apt to be in vitamin A. Next in order of richness in vitamin A among the organs of the plant usually come the growing shoots, including some roots, the embryos of the seeds, and in general the more actively functioning parts of the plant, while the storage organs such as the endosperms of seeds and the starchy or fleshy roots and tubers usually contain relatively little of this vitamin. It is chiefly to McCollum that we are indebted for this general view of the relation between the functional activity of the parts of plants and their richness in vitamin A. Steenbock on the other hand has pointed out that the storage organs of some plants contain more vitamin A than others. Thus yellow maize is richer in vitamin A than white maize, sweet potatoes are richer than white potatoes, and carrots than parsnips or beets. In all these cases the richness in vitamin A is associated with yellow color, and while it is not yet known whether this is more than a coincidence,

[1] Quinn. Dissertation, Columbia University, 1925, and subsequent experiments not yet published.

it serves at least to assist us in remembering these cases of apparent storage of vitamin A in the storage organs of the plant.

Animals obtain their vitamin A from plants, either directly or through other animals. Vitamin A can be stored in the animal body to a much greater extent than can vitamin B or C. The storage occurs most strikingly in the liver, but significant amounts may also be stored in adipose tissue. The amount thus stored in body fat depends upon the food of the animal and the rapidity of fattening. The adipose tissue of the pig usually contains very little vitamin A because the animal has fattened rapidly on grain and other food relatively poor in this vitamin; whereas the adipose tissue of the beef animal which has fattened slowly on a diet of grass and alfalfa rich in vitamin A may be expected to show appreciable amounts of it. Muscle tissue contains little vitamin A even when the food has furnished it in abundance. Glandular organs usually contain more, the concentration depending largely upon the feeding. Eggs and milk are much more important sources of vitamin A than are any of the body tissues.

In view of the great importance of liberal supplies of vitamin A for tissue formation and healthy development, it is natural to find that the maternal organism has evolved the capacity to transfer rich stores of this material to the milk or the egg, the functions of which in nature are to furnish tissue material and growth-promoting substances to the young. And as cattle are furnished with a digestive apparatus permitting them to consume leaf foods in very large amounts, and have been bred to high efficiency and great capacity in milk production, the milch-cow performs a most important service in bringing into form for human consumption large stores of vitamin A which have been formed in the green leaves of plants.

Milk is the most important of all foods as a source of vitamin A, with egg yolks and green vegetables as a meritorious second group. McCollum classes these three types (milk, eggs, and

green vegetables) as " protective foods " because of their richness in fat-soluble vitamin and in calcium — the two factors in which American dietaries are so often liable to be deficient because they consist too largely of the products of seeds, tubers, muscle meats, and sweets.

Such foods make up much the largest part of most American dietaries, and from them it is all too easy to make meals which are entirely acceptable to the eye, to the appetite, and to the traditional menu requirements, but are too poor in vitamin A for entirely satisfactory nutrition. McCollum has especially emphasized the view that such dietaries are common throughout America and Europe, that they give rise to a relative impoverishment of the body in vitamin A as the result of which it becomes more susceptible to any one of many infections to which it may be exposed — perhaps tuberculosis, perhaps pellagra. Whatever the degree of emphasis that one may lay upon this view, it is certain that the foods found by long experience to be especially helpful in combating tuberculosis are milk and eggs, both of which are now found to be rich in vitamin A.

Codliver oil is believed to owe its high content of vitamin A to the synthesis of this substance in very minute algae and other sea plants, under the influence of sunlight. These become food for the small sea organisms (plankton) and these in turn for larger, up to the crustaceans and small fish upon which the cod feeds. The cod thus obtains food which is relatively rich in vitamin A and this vitamin when stored is carried almost entirely in the fat of the liver; the cod has very little fat elsewhere in its body.

Vitamin A may occur in different forms in its animal and plant sources. In the former it is freely soluble in fat and readily dissolved with the fat by ether, while in the latter this is not always true. McCollum suggested that the vitamin may exist in plants in combination with some substance which is separated by digestion when consumed by the animal, so that in the animal

the vitamin occurs in its free form, which is more soluble in fats and fat solvents than is the combined form in which the vitamin occurs in its primary sources, the plants.

The Quantitative Measurement of Vitamin A Values of Foods

Quantitative measurements of the relative amounts of vitamin A in food can be made by means of sufficiently numerous and carefully controlled feeding experiments with rats *amply provided with antirachitic vitamin.*

The general plan is to start with normal young rats 25 to 29 days old, by placing them upon a basal diet good in all other respects but free from vitamin A. When any surplus store of vitamin A which the body contained at the beginning has been exhausted (or sufficiently diluted by the growth of the animal) the body weight ceases to increase and usually remains nearly stationary for a few days. At this time, if not before, each of the animals is placed in a separate cage with a raised wire screen bottom to prevent access to excreta. One rat or more of each litter is left upon the basal ration only, until it dies from lack of vitamin A, thus serving as a "negative control." Other animals are fed in addition to the basal diet various fixed daily amounts of the food to be tested in order to find what amount of this food furnishes just that quantity of vitamin A which is required to give a standard result in a standard test animal in or for a standard time.

The accompanying chart (Fig. 18) shows the average weight curves of animals fed different amounts of tomato as sole source of vitamin A.

Any advantage in survival period or maintenance or gain in weight shown by a test animal over its "negative control" must be attributed to the vitamin A received from the weighed portions of the food or other material which is being tested, provided the experiments are conducted properly and in sufficient numbers to avoid vitiation of results through individual varia-

bility of the animals. Hence, animals making equal gains in weight during the experimental period may be regarded as receiving equal amounts of vitamin A, and the richness of different foods in vitamin A may be taken as inversely proportional to the amounts of the foods required to furnish that fixed amount of the vitamin which will produce a given result in a standard experimental animal, for example, a given rate of gain in weight during the experimental period in a rat that has been prepared for the purpose in the manner described above. As was pointed out by Drummond, Coward, and Zilva, a definite small gain in weight funishes the best basis for quantitative comparison. A suitable standard for this purpose is an average gain of 3 grams per week during an experimental period of eight weeks' duration.

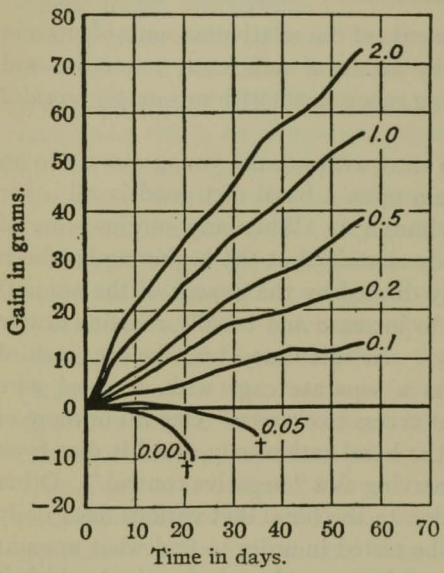

FIG. 18. — Curves showing gains in weight during test period, made by groups of rats fed graded portions of tomato as sole source of vitamin A. The amount in grams of tomato fed to each rat six times weekly is shown at the termination of each curve. All received the vitamin-A-free basal diet *ad libitum*. Each curve expresses the average result obtained from several tests. (Courtesy of the *Journal of the American Chemical Society*.)

A unit of vitamin A may then be taken as that amount which when fed daily just suffices to support this limited rate of gain (3 grams per week) in a standard test animal prepared as indicated above. If such a unit be adopted, then the relative

vitamin-A contents of foods may be expressed as units of vitamin A per gram, per ounce, per pound, or per 100-Calorie portion of the food.

The table[1] on page 458 shows the relative vitamin A values of different foods expressed in terms of such units.

Vitamin A in Nutrition and Health

While vitamin A may not hold the direct controlling relation to any one disease that vitamin B does to beriberi or vitamin C to scurvy, yet it is probable that of the three, vitamin A is the factor of greatest practical importance to nutrition and health, because so many of our staple foods are poor in vitamin A and because a dietary poor in this vitamin causes such widespread weakening of the body and increases its susceptibility to so many infectious diseases. Repeatedly, by many different investigators and in many different species including man, it has been found that a diet poor in fat-soluble vitamin leads to weakness in many respects. What was first observed, aside from cessation of growth in young animals, was an increased susceptibility to eye disease; but further study has shown that not only the eyes but also the appetite, the digestion, the air-passages, the lungs, the bladder, the skin, the sinuses, and the ear are liable to suffer when the food is poor in respect to fat-soluble vitamin. These observations have been made chiefly upon rats, not as is sometimes supposed because the rat is particularly susceptible to a shortage of this vitamin, for as a matter of fact both rabbits and chickens have been found to need relatively more of it than the rat does, but because at the time of the discovery of this vitamin the rat had already become established as an eminently

[1] Based chiefly on data determined in the writer's laboratory by Dr. H. E. Munsell and Miss M. P. Burtis working under grants from the Milbank Memorial Fund through the New York Association for Improving the Condition of the Poor. For permission to use representative results of this work in preliminary form the writer is indebted to the courtesy of Mr. Bailey B. Burritt, General Director of that Association.

APPROXIMATIONS TO VITAMIN A CONTENTS OF FOODS

FOOD	UNITS OF VITAMIN A * (APPROXIMATE)	
	Per Pound	*Per Ounce*
Apple, raw (one test)	250	16
Bacon	125 (?)	8 (?)
Banana	1600 (?)	100 (?)
Beans, canned baked	250	16
Beans, green string, fresh	1600	100
Beef, raw, clear lean	250	16
Beef fat	2500 (?)	150 (?)
Beets	80	5
Butter	8000 to 22,000	500 to 1400
Cabbage, new, raw or cooked	270	18
Cabbage, old (one test)	90	6
Carrots	10,000 to 30,000	600 to 2000
Cauliflower	270	18
Celery, bleached stems	Too little to be measured by present methods.	
Celery, bleached leaves	about 1000	about 65
Celery, green leaves	about 3000	about 200
Cheese, hard (one test)	about 10,000	about 600
Codfish (muscle)	Too little to be measured by present methods.	
Codliver oil	80,000 or more	5000 or more
Eggs	about 9000	about 550
Egg yolk	about 27,000	about 1700
Egg white	Too little to be measured by present methods.	
Flour, white	Too little to be measured by present methods.	
Grapes	250 to 350	16 to 22
Lemon juice	about 50	about 3
Lettuce	750 to 3000	50 to 200
Milk (whole)	about 1000	about 65
Milk, whole, dried	about 7500	about 500
Oatmeal or rolled oats	Too little to be measured by present methods.	
Olive oil	about 200	about 13
Orange juice	about 350	about 20
Peas, green	2500 to 3500	150 to 200
Pork (roast cut, raw)	about 96	about 6
Potatoes, baked	about 130	about 8
Sweet potatoes, yellow	about 3000	about 200
Spinach, fresh	about 25,000	about 1500
Tomato, raw or canned	2700	170
Turnip	25 to 150	1.5 to 10
Wheat	Too little to be measured by present methods.	

* For definition and discussion of this unit see page 456.

practicable laboratory animal for use in nutrition research. There is no reason to doubt and ample reason to believe that the omnivorous rat is a good representative of mammals generally, including man, in his need for fat-soluble vitamin and in his physiological response to the abundance or scarcity of this factor in his nutrition.

Because the animal body can store vitamin A in relatively much greater proportion than vitamin B or C, and because the effects of the shortage of vitamin A are less specific and more widespread, the results of experiments with vitamin A have in many cases given confusing results or failed of correct interpretation so that the great importance of this vitamin has not been fully appreciated even by many of the investigators themselves.

But while the storage of vitamin A in the body is an inconvenience in experimentation it is of very great nutritional importance in enabling the carrying over of this nutritional asset from a time of abundance to one of scarcity; and we now have good reason to believe that a surplus of vitamin A in the body is not simply a reserve asset to be used at some future time but also actively increases the vigor and the ability of the body to resist disease. The conditions and extent of such storage of vitamin A in the body have therefore seemed worthy of careful study.

Storage of Vitamin A in the Body. Even at weaning time young animals may already have a considerable store of vitamin A in the body and thus be able to continue to grow for some time upon a diet carefully freed from vitamin A but adequate in all other respects. Young rats separated from their mothers at a uniform " weaning " age of four weeks show very different growth curves and survival periods on the same experimental diet free from vitamin A, according to the vitamin A content of the mother's diet. The differing stores of vitamin A in the bodies of experimental animals, even at early ages, has undoubtedly been a very large factor, not fully appreciated, in

previous experiments dealing with this vitamin and in attempts to determine the vitamin A content of different foods.

The body can also store vitamin A at later ages. Thus two male rats of the same litter and of the same weight at four weeks of age were placed on two diets one of which was richer in vitamin A because of a higher proportion of milk than the other. After completion of growth, both were placed at the same time upon the same experimental diet deficient in vitamin A, and the survival period was found to be nearly twice as long in the case of the one which had received the diet containing the larger proportion of milk.

In a recent investigation, laboratory animals of known nutritional history were killed and dissected and carefully graded weighed amounts of their tissues were fed as sole sources of vitamin A to young experimental animals. In this way it was shown directly by feeding experiments that there is an actual storage of vitamin A in the tissues, and it became possible to study quantitatively the distribution of the stored vitamin in the body and also to determine directly the extent to which the vitamin A content of the body tissues is influenced by the intake of this vitamin in the food. If adipose tissue and skin be ignored, about nine tenths of the total vitamin A in the body of a well nourished adult rat was found in the liver and the remaining tenth about equally divided between the muscles as a whole, the blood, the kidneys, and the lungs. Weight for weight, kidney was about 40 times, lung more than 40 times, and liver between 200 and 400 times as rich in this vitamin as is muscle. Moderate differences in the vitamin A content of the food, such as are well within the range of variation likely to be encountered in human experience, resulted in large differences in concentration of this vitamin in the liver, and distinct differences in the amount of it found in lung tissue. That the vitamin content of the lung tissue is thus dependent upon the abundance of this vitamin in the food is of special interest because it has also been found that

the higher intake of the vitamin makes the lung more resistant to disease.

It has been suggested that vitamin A may play both a *structural* and a *humoral* rôle in the body, or in other words may serve both as a tissue-building and as a regulating substance.

As there is unquestionably an actual storage of vitamin A which is of great importance to the body, and which depends upon the body's opportunity to store the vitamin from surplus received in the food, a study has been made of the bodily store of vitamin A as influenced by age and conditions of feeding.

Among animals coming from the same previous diet, age was found to have an important influence upon the length of the survival period upon food devoid of vitamin A. The maximum survival period and, therefore, presumably the maximum body store of vitamin A (or at least the maximum store in relation to daily need) was found at six months of age in the rat, or at about the beginning of full adult life.

A moderate difference in the vitamin-A content of the food was found to cause a marked difference in the amount of this vitamin stored in the body as indicated by the survival period on vitamin-A-free food.

The incidence of the characteristic symptoms was influenced by the age at which the experimental animal is subjected to the vitamin deficiency, the younger rats being more likely to develop eye trouble while those which were older when subjected to the vitamin-A deficiency more often developed lung disease.

In a typical series of observations, three fourths of the rats placed upon vitamin-A-free food at the age of about one month developed the characteristic ophthalmia before death; but it developed in only about one fourth of the rats that were from two to nine months old when subjected to the same dietary deficiency. Thus it is plain that, with all other conditions uniform, the older animal is distinctly less likely to develop the ophthalmia even though he dies from the vitamin-A deficiency.

The older rats, however, proved more susceptible than the younger ones to lung infection, the frequency of which, in our experience as in that of Steenbock and other investigators, is increased by diet deficient in vitamin A. In this series, the lungs of the rats that had been transferred to the vitamin-A-free diet at from four weeks to four months of age appeared normal at autopsy in nearly all cases, while nearly half the rats that were six or nine months old when subjected to the same dietary deficiency developed lung trouble.

Two-thirds of all the rats included in this series, and dying from vitamin A deficiency, showed pus in one or more of the glands near the base of the tongue. The younger rats showed this sign rather oftener, and the older animals distinctly oftener, than the ophthalmia (Sherman and Storms).

In another series of experiments the placing of the animals upon the test diet devoid of vitamin A was preceded by the feeding for different lengths of time of a diet bountifully supplied with the vitamin. This resulted in very great prolongation of life upon the vitamin-A-free food, showing that large surpluses of vitamin A can be stored by the body. The maximum storage of vitamin A in rats of different ages was closely approached by feeding a ration containing 4 per cent of codliver oil, which supplied about 80 times as much vitamin A as is usually considered necessary for adequate nutrition. However, the store does not increase in arithmetical proportion to the richness of the diet in vitamin A, for apparently the animal uses the vitamin provided in the food more freely or less economically as the maximum store is approached. Feeding of this ration which promoted greater storage of vitamin A did not alter the age of maximum storage (six months in the rat). This was shown by the fact that the maximum survival period on a ration deficient in the vitamin was not observed in the animals younger than 6 months, regardless of the nature of the previous diet. Apparently, then, given the same opportunity for storage,

or the same concentration of the vitamin in the ration, it is impossible to make young animals attain as large a reserve of vitamin A as may be present in older animals. However, it was possible to build up as large a reserve in a younger animal on this diet as in a mature animal of less fortunate dietary history. (Cammack. Dissertation, Columbia University, 1925.)

Vitamin A in Adult Nutrition. Since the storage of vitamin A in the body has thus been shown to be such an important factor, it becomes probable that the supposed lesser need for this vitamin by the adult than by the young is apparent rather than real, and is largely attributable to the fact that the adult has had more opportunity to provide himself with a bodily store of this vitamin which then carries him over periods of deficient intake. That vitamin A plays an extremely important part in adult nutrition is now well established.

Sherman and MacLeod have described experiments in which parallel groups of rats of identical previous history were fed upon two types of diet, one rather low and the other fairly high in vitamin A, from soon after weaning-time until natural death. The smaller amount of vitamin A proved sufficient for normal growth up to nearly normal adult size, but not for successful reproduction, and rarely did it support satisfactory longevity. The parallel animals receiving the more liberal allowance of vitamin A grew to fully average adult size, were successful in reproduction and the rearing of young, and lived on the average a little over twice as long as those on the diet equally good in all other respects but lower in vitamin A.

These experiments show strikingly that a proportion of vitamin A in the food sufficient to support normal growth and maintain every appearance of good health, for a long time at least, may still be insufficient to meet the added nutritive demands of successful reproduction and lactation.

Along with the failure to reproduce successfully there usually also appeared in early adult life an increased susceptibility to

infection and particularly a tendency to break down with lung disease at an age corresponding to that at which pulmonary tuberculosis so often develops in young men and women. The bacillus involved is different; but the close parallelism of increased susceptibility of the lung to infection at this stage of the life history appears very significant, especially in view of the fact already mentioned that the vitamin-A content of lung tissue varies with that of the food.

Especially noteworthy was the repeated observation of young females growing normally and presenting every appearance of good health throughout youth on a diet low in vitamin A, but failing utterly to succeed in the rearing of a second generation, and showing a strong tendency to break down in health at an age at which they should have been in the prime of life.

Thus it is clearly shown that vitamin A is an even more important factor in the chemistry of food and nutrition than has previously been appreciated, for it must be supplied in liberal proportion not only during growth but in the food of the adult as well, if a good condition of nutrition and a high degree of health and vigor are to be maintained.

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CHAPTER XVII

THE ANTIRACHITIC VITAMIN AND THE PREVENTION OF RICKETS

The Nature of Rickets

According to Park's definition which is now being generally accepted, rickets is a condition in which the mineral metabolism is disturbed in such a way that calcification of the bones does not take place normally.

To guard against confusion, one must keep in mind in reading the literature of rickets that the term has often been given a narrower definition than Park gives it, and one turning more upon the histology of the bone tissue, while Park's is expressed practically in terms of the precipitation of calcium phosphate in the developing bone — an essentially chemical phenomenon.

Starting then with this chemical conception of rickets (the theoretical aspects of which are fully treated from the standpoint of physical chemistry in the papers by Holt, LaMer, and Chown cited at the end of this chapter), one can readily outline in an elementary descriptive way the main features of the condition as seen from the point of view of the chemistry of nutrition.

The failure of normal deposition of calcium phosphate in the young bone is not to be attributed to fault of the bone tissue itself but rather of the fluid which bathes and feeds it; for Shipley found that a rachitic bone placed in normal blood serum begins at once to calcify normally; and Kramer and Howland showed that in rickets there is always a subnormal concentration of either calcium or phosphorus (or both) in the blood serum.

Since the normal concentration of calcium or phosphorus (inorganic phosphate) in the blood serum is essentially the maximum also, it follows that whenever the concentration of calcium or phosphorus or both falls seriously below the normal, the product of the concentrations of these two elements will fall below the normal product. The conception of an essential connection between the condition of rickets in the bone and the occurrence of a subnormal concentration-product of calcium and phosphorus in the blood serum, suggested by Kramer and Howland, has been both amplified and rendered more precise by the physico-chemical investigations of Holt, LaMer, and Chown as noted above.

There are different types of rickets which may be classified according to the nature of the chemical deficiency in the blood.

When the calcium content of the serum is normal but its phosphorus content is reduced there results the so-called *low-phosphorus rickets* which corresponds most closely and completely to the classical histological descriptions of the disease such as those of Schmorl. There is in such cases not only a characteristic pathological histology but also a tendency to overgrowth of the cartilaginous or osteoid tissue at the ends of the long bones and at the rib-junctions. Because the retarded calcification prevents the bones from acquiring normal rigidity, the pressure of the body weight tends further to enlarge the ends of the bones. Together these abnormalities make a typical (and all too familiar) picture recognized in part by the obvious clinical signs of enlarged joints and the " rachitic rosary " and in part by the more recently developed laboratory methods of diagnosis by means of the Roentgen ray photograph and the determination of inorganic phosphorus in the blood serum; and confirmable at autopsy by histological examination. There has sometimes been a tendency to confine the term rickets or " true rickets " to this low-phosphorus type.

When the phosphorus content of the serum is normal but its cal-

cium content is reduced there results a similar gross abnormality but a somewhat different histology of the bones. This has been called a " rickets-like condition " or " a second type of rickets," but is now commonly designated as *low-calcium rickets*. Low-calcium rickets is often accompanied by tetany; and it seems not improbable that in such cases the trouble with the nerves as well as with the bones is attributable (at least in part) to the deficiency (subnormal concentration) of calcium in the blood.

When both calcium and phosphorus are reduced below normal concentration in the blood serum calcification is retarded, but the structural abnormality of the bone differs from that of the two types described above. The condition constitutes a third type of rickets under Park's definition, but because of the differences in clinical and histological appearance it is sometimes called an osteoporosis instead.

The low-phosphorus type of rickets which is believed to be most frequent clinically is also the type most readily produced experimentally in rats under controlled conditions, consequently it is this type which has generally been employed in the experimental investigation of rickets which has been pursued so actively in this country during the past few years. The term " anti-rachitic " as commonly used is apt to refer, therefore, primarily to the prevention or cure of low-phosphorus rickets, whether occurring clinically in children or produced experimentally in rats, though in many cases the observations have been found applicable to the other types of rickets also. For instance, in Steenbock's work upon experimental rickets in puppies, it was shown that their blood serum was low in both calcium and phosphorus, but that their rickets was preventable by the same means as is the low-phosphorus rickets of rats and the clinical rickets of children, which is believed to be chiefly of the low-phosphorus type.

Rickets had been produced and studied experimentally in puppies, notably by Mellanby in England, before the American

work here referred to. For the sake of brevity the present discussion is based on the experimental evidence which now seems the clearest and most conclusive, without so much regard to chronological development and recognition of the work of individual investigators as would be appropriate in a more technical treatment of the subject.

The Causes of Rickets. Codliver Oil and Ultraviolet Light as Antirachitic Agents

It has been fully demonstrated, especially in well-controlled laboratory experiments upon rats, that rickets may be induced or prevented at will in any of three ways, the experimental conditions being suitably chosen in each case: (*a*) by changes in the amounts and proportions of calcium and phosphorus supplied through the blood, (*b*) by the deficiency or the abundance of the antirachitic vitamin, (*c*) by the deficiency or the adequacy of the ultraviolet irradiation received (usually) either as direct sunlight or from a quartz mercury vapor lamp.

Since any one of three factors may of itself be sufficient to turn the scale between rickets and a normal development of the bone, it is plain that, in any given case of rickets, any one or any combination of these three factors may have been at fault.

In one sense it might be said that the fault is always with the mineral content of the blood. Such a statement would be true so far as it goes, but would be misleading in its implication unless the importance of light and of the antirachitic vitamin were also recognized. Furthermore, it is important to recognize that a subnormal phosphorus or calcium content in the blood is not always due to an insufficient amount of the element in the food. The amount in the food may be normal but the utilization of the phosphorus or calcium in the body may be perverted. In some cases the deficiency in the blood has been traced to excessive losses in the digestive tract; and in one investigation these losses were reduced and the rickets was improved by simply

giving hydrochloric acid. Low-phosphorus rickets has also been " cured " by starvation, which doubtless acted by throwing an increased amount of phosphate into the blood through the destruction of muscle tissue as fuel. It may be helpful also to keep in mind that the muscles and soft tissues generally are fairly rich in phosphorus and so in the growing body these tissues are competing with the bones for the available phosphorus brought by the blood; a fact which may readily have a bearing upon the observation that uncomplicated clinical rickets is most frequently of the low-phosphorus type and the impression of many physicians that the worst rickets often accompanies the most rapid growth. In so far as rapid growth means that the muscles and other soft tissues are outstripping the bone tissue in the competition for phosphorus this would tend to aggravate the rickets; also the greater the body weight which has to be borne by the still-soft bones the more likely that the bones will be bent and their ends (the joints) enlarged so that if other things were equal the more rapidly growing rickety child would be apt to develop worse bowlegs and knock-knees. But it must also be remembered that malnourished children are apt to suffer from more than one nutritional defect so that rickets and retarded growth often occur together.

Thus it appears that a number of intervening factors may prevent the bone from receiving phosphorus and calcium in the same relative abundance in which they may be contained in the food; and both clinical experience and laboratory experimentation have shown that without special direct attention to the mineral content of the food, rickets may often be prevented or cured by the antirachitic vitamin of codliver oil or by the direct[1] rays of the sun or other form of ultraviolet irradiation. Treatment with either the vitamin or the ultraviolet irradiation has

[1] Here and in all such cases, "direct" sunlight means that which has not passed through glass, because ordinary glass does not transmit (or only slightly) the ultraviolet rays which are the ones chiefly effective against rickets.

been found to maintain or restore the normal concentration of phosphorus (or calcium, or both) in the blood. Hence the antirachitic vitamin of the codliver oil and the ultraviolet rays of direct sunlight or of the mercury-vapor quartz lamp are said to exert their antirachitic effects through enabling the body to effect a better mobilization of its calcium and phosphorus supplies.

Concentration of the Antirachitic Substance from Codliver Oil and its Production in the Laboratory from Cholesterol and Phytosterol

That codliver oil and sunlight should each " act as a specific against rickets " seemed at first a remarkable coincidence; but the connection now seems plain, for it has been found on the one hand that the antirachitic substance is apparently closely related to but not identical with cholesterol or phytosterol, and on the other hand, that ultraviolet light acts upon these substances in such a way that they undergo some, as yet (1925) not fully defined, chemical change and acquire antirachitic properties. As the wave-lengths of ultraviolet light which cure rickets when allowed to impinge directly upon the skin are the same [1] which convert cholesterol into an antirachitic substance, and as the skin always contains cholesterol, it becomes altogether probable that the ultraviolet rays exert their antirachitic effect by acting upon cholesterol in the skin, changing it to antirachitic vitamin. This is then carried into the circulation and acts in the same way as if it had been acquired through the taking of codliver oil by mouth.

The production in the laboratory of an antirachitic substance presumably identical with the antirachitic vitamin of codliver oil, by irradiation first of certain foods and then of individual

[1] The investigations of Hess and Weinstock indicate that the effective rays are those of a wave length around 300 $\mu\mu$ (milli-microns or millionths of a millimeter) or shorter.

chemical substances (cholesterol and phytosterol) was accomplished independently and simultaneously by Hess and by Steenbock with their respective co-workers. This line of research is still in course of active development at the time this is written and the reader desiring a full account must be referred to the papers cited at the end of the chapter and to subsequent papers which are probably being published as this is being printed.

Similarly the work upon the isolation or concentration of the antirachitic substance of codliver oil is at present in such a formative state, and a recital of methods thus far developed would so nearly repeat what has been given in the corresponding section on vitamin A in the last chapter, that it seems best to refer the reader who wishes to make a study of this point to the original articles listed at the end of the chapter and to the subsequent ones which may confidently be expected.

Inter-relations of Antirachitic Factors

The mineral metabolism, as affecting the concentrations of phosphorus (inorganic phosphate) and of calcium in the blood, the antirachitic vitamin now known to exist in codliver oil, egg-yolk, whole milk, and doubtless other foods, and the ultraviolet rays of direct sunshine or of the mercury vapor quartz lamp are all important factors in the causation and prevention of rickets; and a correct interpretation of the influence of any one of them must take account of the others also, and of the interrelations existing among these different antirachitic factors.

Rickets can be produced experimentally by diets of faulty mineral content; but usually only when there is also a relative deficiency of the regulating factors — antirachitic vitamin and ultraviolet light.

The light and the vitamin appear to be interchangeable as antirachitic agents, and we have seen that there is now reason to believe that the light acts by formation of antirachitic vitamin

from cholesterol in the skin. It may therefore be that the antirachitic action which we attribute to the light is more directly due to the vitamin formed under its influence. If such should prove to be the case these two factors would reduce to one in the consideration of the immediate chemical problem of the calcification of the bone; but even then the antirachitic vitamin would not hold the same all-controlling relation to rickets that vitamin B does to beriberi or vitamin C to scurvy, for so long as the mineral metabolism remains undisturbed there is no absolutely conclusive evidence that the antirachitic vitamin is playing an essential part in the normal calcification, though it is doubtless helpful at all times, and in other ways than merely in the prevention of rickets.

Many investigators have noticed the prevalence of rickets among children living in dark, crowded quarters; and the greater incidence of the disease in winter than in summer. Especially are we indebted to Huldschinsky and to Hess for the demonstration of the efficiency of the ultraviolet rays of direct sunshine or of the mercury-vapor quartz lamp in preventing and curing the disease. But such rays cannot themselves be strictly essential, because rats, which are well known to be subject to rickets, have thrived through many successive generations without the benefit either of direct sunlight or the ultraviolet lamp. These rats, of course, received antirachitic vitamin in their food (a mixture of whole wheat and whole milk).

Also it may be noted that new-born babies are rarely if ever rachitic although they have made a considerable bone development where there could have been no possible penetration of ultraviolet rays. The unborn baby probably profits by the influence of sunshine upon its mother; but as the rays cannot penetrate very deeply the benefit is presumably derived indirectly. Probably the light produces antirachitic vitamin, some of which passes to the unborn baby; then both in the body of the mother and of the child the vitamin aids in the mobiliza-

tion of calcium and phosphorus. Doubtless also antirachitic vitamin can be stored in the body of the baby before birth and thus augment the nutritional endowment with which it is born and upon which its early development may very largely depend.

Storage of Antirachitic Vitamin in the Body and the Influence of Parental Nutrition upon the Occurrence of Rickets

Recent work leaves no room for doubt that the antirachitic vitamin can be stored in the animal body, and to a very important extent. Certain other observations which have been interpreted as throwing doubt upon the ability of the body to store this vitamin are probably to be attributed to the use of so drastic a rickets-producing diet that the body store of the experimental animal did not suffice for appreciable protection.

Recognizing the important part which storage in the body may play, we can readily understand how the nutritive condition of the mother may influence the amount of this vitamin which she can provide for storage in the body of the baby before its birth; and also the effect which her bodily store of this vitamin (or lack of it) may have upon the antirachitic potency of her milk. Hence the important influence of parental nutrition upon the susceptibility to rickets in the young which has been especially emphasized by Daniels and Byfield. It does not necessarily require two generations to produce rickets, but undoubtedly the parental nutrition is an important factor in determining whether or not rickets will appear in the young.

The Practical Problem of the Prevention of Rickets

Park has written, " Personally, I believe that if pregnant women received ample well-balanced diets, in which green vegetables were abundantly supplied and cows' milk was regularly taken, and kept a sufficient part of their time in the open air and sun, and if their infants were placed in the direct rays of the sun for a part of each day and were fed codliver oil for the first

two or three years of life, more could be accomplished in regard to the eradication of caries of the teeth than in all other ways put together, and that rickets would be abolished from the earth."

This declaration of the practicability of complete abolition of rickets is all the more impressive in that it comes from one of the most careful, critical, and conservative students of the disease, who deals with it not only under experimental conditions subject to laboratory control but also clinically under the complexities of actual human experience, and whose definition of rickets is notably broad and inclusive. It would be well if this statement by Park could be given wider circulation and greater emphasis than it has yet received; for if the means of certainly preventing rickets is thoroughly understood and acted upon the question of whether or in what sense cereals are rickets-producing will be seen to become of little real practical importance.

That too exclusive a dependence upon cereals in the feeding of infants and young children may increase the danger of rickets is undoubtedly true. Whether the cereal in any case has any directly injurious action such as to justify the belief that it is " rickets-producing " in any other sense than that it tends to make the diet one-sided and induce a greater gain in size than in bone development is not so clear.

The dominant and practically important aspect of rickets is certainly not a matter of a direct food toxicity; it is a nutritional deficiency or perversion which affects particularly the skeletal tissues, a disturbance of the mineral factors in nutrition which results in a retarded deposition of calcium phosphate in the developing bone.

The antirachitic vitamin of codliver oil, egg-yolk, whole milk, and fresh vegetables probably acts by aiding, in some way not yet fully understood, the mobilization of these mineral elements in the body.

Sunlight (or its equivalent in ultraviolet rays from other sources) probably acts by forming antirachitic vitamin from the

cholesterol always present in the skin. That the outer layers of the human skin are rich in cholesterol has been shown by Eckstein and Wile.

Our present view is that rickets is essentially a matter of nutritional deficiency or defect which shows itself in a diminution of calcium or phosphorus or both in the blood serum, and can be prevented by maintenance of the normal calcium and phosphorus content of the serum whether this be accomplished by direct attention to the metabolism of calcium and phosphorus as such, or their more advantageous mobilization through the aid of antirachitic vitamin or ultraviolet rays. It is best ensured through attention to all three of these factors as recommended by Park in the statement above quoted.

This view is now so well established and of such well-proven adequacy that attention should not be diverted from it by overemphasis upon subsidiary phases of the rickets problem.

Does Rickets Predispose to Other Diseases, and is the So-called Antirachitic Vitamin a Factor in Adult Nutrition?

On the ground of clinical statistics the question has been raised whether children who have suffered from rickets are not more subject than others to respiratory diseases. The distortion of the chest which often results from rickets naturally gives weight to this suggestion; and there is also another possible connection. It is certain that diets deficient in fat-soluble vitamin lead to increased susceptibility to respiratory infections, and, while this has usually been attributed simply to shortage of vitamin A, it is possible that shortage of the antirachitic vitamin may tend to accentuate the same weakness. Or it may be that in practice, children suffering from a shortage of antirachitic vitamin so often encounter a shortage of vitamin A also, as to establish a correlation between rickets and respiratory disease without the first having of necessity any direct causative relation to the second.

Another danger is that malformation of the pelvis resulting from rickets in girls may be permanent and, in adult life, cause difficulties in childbearing.

Aside from the question of direct causative relation between rickets and other diseases, there now seems to be ample evidence that the so-called antirachitic vitamin is an important factor in adult nutrition as well.

It is apparently the same antirachitic vitamin which prevents rickets and improves calcium balance in babies (Steenbock and Daniels) and which also improves the calcium balance of healthy young women on low calcium diet (Bogert *et al*) and in lactating animals whose large output of calcium in milk makes calcium equilibrium difficult even with a liberal amount offered in the food (Hart, Steenbock, *et al*).

A substance which conserves the body calcium and regulates mineral metabolism can hardly fail to be an important nutritional asset at all stages of the life history.

It is probably in part through the formation of this vitamin from cholesterol in the skin, but not necessarily in this way only, that direct sunshine exercises its widespread favorable influence upon metabolism which Park has recently summarized by a reference to experiments in which it was " found that the sunlight also brought about a greater consumption of food, stimulated activity, improved the appearance, and increased the reproductive capacity."

Antirachitic Values of Foods

The antirachitic potency of codliver oil and of ultraviolet irradiation are so great that they have absorbed attention to perhaps a greater extent than is desirable; for the antirachitic values of some common foods should not be ignored.

The presence of important amounts of the antirachitic vitamin in egg yolk, whole milk, butterfat, and green vegetables has now been demonstrated in sufficient numbers of cases so that we may

regard it as fully established. That in some cases the evidence of its presence has failed to appear is probably due in part to the variability of the foods, depending upon the conditions of light and perhaps other conditions surrounding their production and handling, and in part to the use in many experiments of so drastic a rickets-producing diet that symptoms of rickets developed in the experimental animals notwithstanding the presence of some antirachitic vitamin in the food fed for test.

It is a serious mistake, met in some otherwise good writings on rickets, to deny antirachitic value to a food merely because rickets has sometimes been observed in cases in which it was fed. Such faulty reasoning has led to the placing of much too low an estimate upon the antirachitic value of whole milk.

Whole milk has important antirachitic value, as has been shown by large numbers of feeding experiments in the writer's laboratory. Its value for the prevention of rickets is doubtless due in part to the considerable amount of antirachitic vitamin which it contains when produced and handled under favorable conditions, and in part to the fact that it contains liberal and well balanced proportions of calcium and phosphorus so that an abundance of milk in the diet makes it very unlikely that such other foods as may be eaten will seriously distort the ratio of these two elements in the diet as a whole.

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CHAPTER XVIII

CHEMICAL FACTORS IN GROWTH, REPRODUCTION, AND LACTATION

Nutritive Requirements of the Growing Organism

"THE upper limit of the size of an animal is determined by heredity. The stature to which an animal may actually attain, within this definitely fixed limit, is directly related to the way in which it is nourished during its growing period." (Waters.)

While feeding experiments upon growing animals and the influence of growth upon food requirements have been discussed to some extent in previous chapters, the great importance of adequate nutrition during the growing period demands special consideration. Recent investigations upon nutrition in growth and in maintenance tend on the whole to the view that the same chemical substances are involved in both; but that in growth the needs for specific substances are accentuated. The amounts required in growth may be greater not only as compared with the size of the body but also as compared with its total food requirement, and also the needs of the growing organism are more urgent so that a shortage of any essential factor becomes more quickly and strikingly apparent and is likely to be more harmful than in the case of the maintenance metabolism of the adult.

It is well to keep in mind that the effect of a shortage of one nutritional factor may be quite different from that of another. It may, therefore, be well at this point to pass the different factors in brief review with no attempt to repeat the quantitative

consideration of requirements which has been taken up in previous chapters and will be referred to again in the chapter next following, but rather with the object here of comparing the nature of the effects produced by shortages of the different nutritional essentials.

(1) ENERGY

When a diet of such character as would ordinarily meet all requirements is fed to a growing animal but in amounts too small to meet the growth requirement, it is plain that such restriction may result in a deficiency of one, several, or all of the essential factors. If the diet is so selected as to be relatively rich in proteins, ash constituents, and vitamins, then restriction of the amount of food will result primarily in an energy deficit. Waters has described experiments which appear to have been of this character. He reports numerous cases of young cattle kept on restricted amounts of food of suitable kinds, the restriction being such as to materially retard the increase in weight as compared with that of a full-fed animal of the same age, or even to hold the young animal at stationary weight at an age when it should have been growing rapidly. In such cases of insufficiency of the total food (energy) intake, the skeleton continues to grow, in height at least, while adipose tissue steadily disappears, and the muscles become more or less depleted. In a young animal subjected to this type of under-nourishment the skeleton grows in height to a much greater extent than in width. Thus in a full-fed steer the increase in length of foreleg and in width of chest were about equal, while in one whose rate of growth was retarded by sparse rations, the width of chest increased only one third as much as the length of foreleg, and in another animal of the same age whose food was so restricted as to permit no increase in weight the increase of chest-width was only one eighth as much as the increase in length of foreleg. The ratios actually measured in typical cases were as follows:

Condition of Animal	Width of Chest :	Length of Foreleg
I — full fed	1 :	0.97
II — retarded	1 :	3.13
III — maintenance *	1 :	8.00

* Just enough food to maintain constant weight in an animal which should have been growing rapidly had he been more liberally fed.

Along with the narrower skeleton the underfeeding resulted in muscles of smaller diameter, absence of subcutaneous fat, and a general appearance of emaciation. Young animals thus held at constant weight when they should be growing are in reality undergoing starvation. To quote from Waters' paper:

" Apparently the animal organism is capable of drawing upon its reserve for the purpose of sustaining the growth process for a considerable time and to a considerable extent. Our experiments indicate that after the reserve is drawn upon to a certain extent to support growth, the process ceases and there is no further increase in height or in length of bone. From this point on, the animal's chief business seems to be to sustain life. This law applies to animals on a stationary live weight as well as those being fed so that the live weight is steadily declining, and indeed to those whose ration, while above maintenance, and causing a gain in live weight, is less than the normal growth rate of the individual. Such an animal will, while gaining in weight, get thinner, because it is drawing upon its reserve to supplement the ration in its effort to grow at a normal rate.

" On all the animals under observation the retardation in height growth did not manifest itself at all until after the sparse nourishment had been continued for several months. On the other hand, the influence upon the width development was observable much earlier, and width development ceased altogether, in the case of animals on a maintenance or sub-

maintenance ration, long before the height development had ceased.

" Our experiments have shown that within certain limits which are not yet at all well defined, retarded growth means retarded development of the organism. Thus an animal at twelve months of age and weighing on account of sparse nourishment only 400 pounds, when it should under natural nourishment have weighed 800 pounds, has not its tissues as fully developed and matured as they would have been had the nourishment been normal. For example, we find that the flesh of steers 14–16 months old that had been sparsely fed throughout their lives presented the general characteristics, such as color, flavor, etc., of veal or the flesh of calf. At this age the flesh of a highly nourished animal possessed the characteristic color, texture, and flavor of beef. Professor Eckles has shown that dairy heifer calves heavily fed reach sexual maturity at from eight to ten months of age, whereas similarly bred individuals that were sparsely fed did not reach the stage of puberty under from 16 to 19 months of age.

" An animal which has been retarded and which in its earlier life has shown an asymmetric development may tend later to correct this asymmetry, and it is not inconceivable that this may be fully corrected before the animal has reached a state of complete maturity, or a point where growth ceases altogether."

Somewhat similar experiments have been performed upon dogs by Aron. Here also when the food was suitable in character but too limited in amount to support normal growth the young animals grew in length and height but became thinner. Because of the " growth impulse " such an underfed young animal burns his reserve of body material to cover the deficit in the energy intake " in his endeavor to grow at a normal rate." Such a condition continued indefinitely results after a time in cessation of all growth and finally in death from starvation. A dog, which by underfeeding had been kept for a year at the

weight which he had when 5 weeks old, had become long, tall, and very thin. When he was then fed liberally he immediately gained in weight and circumference but appeared to have lost the capacity for further growth in length and height. If, however, the period of underfeeding be not too prolonged, the animal on subsequently receiving ample food may regain normal proportions and grow to full normal size.

Since stationary weight in the young animal which is attempting to grow with an insufficient energy supply does not mean cessation of all growth, but growth of bone and brain at the expense of adipose tissue and to some extent also of muscle, it follows that the body of such an animal gradually changes in composition, the percentage of fat and perhaps protein becoming less while the percentages of water and ash increase. If, however, the diet is rich in fat, as in experiments upon mice reported by Mendel and Judson, a simple diminution of the amount of food to a point where gain in weight ceases may not result in any such general replacement of fat by water, perhaps because in such a case the stunting may be due to insufficiency of some of the other factors rather than to an energy deficit.

(2) PROTEIN

As explained in earlier chapters, it was shown by Osborne and Mendel that, with a diet adequate in all other respects, any one of a number of purified proteins such as casein, lactalbumin, or edestin may, if used in sufficient amounts, serve as the sole protein both for maintenance and for growth, while gliadin as sole protein food sufficed for maintenance but not for growth, and zein as sole protein did not suffice even for maintenance. Gliadin contains adequate tryptophane but only about 1 per cent of lysine; addition of lysine to the gliadin ration made it adequate for growth. Hence it is certain that the failure to grow when gliadin is the sole protein in an otherwise adequate diet is a specific stunting due to shortage of lysine.

When growth is retarded by inadequate intake of this particular amino acid, the emaciated appearance characteristic of animals attempting to grow on an insufficient energy intake is not to be expected. Osborne and Mendel have recorded numerous cases of suspension of growth of young rats, especially when kept on rations containing gliadin as a sole protein food. Here the inadequacy of the lysine intake results in retardation or even complete suspension of growth, but the animal may remain quite healthy and symmetrical. Moreover, rats may be subjected to this type of stunting for a remarkably long time (even as long as would normally cover the entire growth period) and still retain their capacity to grow when given an adequate diet.

In some cases,[1] " after periods of suppression of growth, even without loss of body weight, growth may proceed at an exaggerated rate for a considerable period. This is regarded as something apart from the rapid gains of weight in the repair or recuperation of tissue actually lost. Despite failure to grow for some time the average normal size may thus be regained before the usual period of growth is ended." Statistical studies on children also indicate that retardation in early growth can often be made up by extra rapid growth later.

In contrast to the striking extent to which lysine deficiency may result in simple suspension of growth without apparent injury, it has repeatedly been shown that a tryptophane deficiency is much more speedily disastrous; and the recent experiments of Rose and Cox indicate that this must also be anticipated in case of a deficiency of histidine; while the cystine stunting described by Woods, like the lysine stunting of Osborne and Mendel, resulted in retardation or suspension of growth with no evidence of a pathological condition in the body.

Hence it is plain that the results of protein stunting may be

[1] Osborne, Mendel, Ferry, and Wakeman. *American Journal of Physiology*, Vol. 40, pages 16–20 (1916).

different according to the particular amino acid shortage which is involved.

In general, however, the evidence indicates that stunting from shortage of protein is apt to involve less injury than any other form of stunting.

(3) ASH CONSTITUENTS

Ash constituents have long been recognized as playing an important part in the growth of young animals, and of these, as we have already seen, the elements most likely to be deficient are calcium, phosphorus, and iron. Infants (and young mammals generally) are born with a reserve store of iron usually sufficient to supply the growth requirement up to about the end of the normal suckling period. At any time after this initial reserve supply has been used, the iron in the body will be found very largely localized in the blood. The blood constitutes less than 7 per cent of the weight of the body but contains more than 70 per cent of its iron content. Hence a deficit of iron becomes more noticeable in the blood than in the other tissues — growth may not cease but the child (or young animal) may grow anemic. It must also be pointed out that with a diet good in all other respects, and especially if it contains an abundance of calcium, the healthy body is able to cope with a low intake of iron for a relatively long time without any appreciable development of anemia. Apparently this is because with a liberal supply of calcium, in a diet good in other respects also, the iron in the body is used over and over again with only very small losses through excretion.

To an even greater extent than the iron is localized in the blood, the calcium of the body is localized in the bones; it is estimated that the bones contain over 99 per cent of the body calcium. An inadequate supply of calcium in the food during growth retards the development and calcification of the bones. Bone development may also be interfered with by inadequacy

of the phosphorus supply. Several investigators, in studying the effect of diet upon growth of bone, have found that the bones formed in a young animal kept on diet poor in phosphorus and calcium are apt to be soft, spongy, and weak (of low breaking strength), and that this may be prevented by the simple addition of calcium phosphate to the food.

Moreover, in the experiments of Sherman and MacLeod in which the development of the bones was retarded by a low-calcium diet of average phosphorus content, it was definitely shown that normal results could be obtained by the addition of calcium salt to the food but not by the feeding of codliver oil. Confidence in the value of the antirachitic vitamin must not lead to any lack of care in providing a liberal supply of calcium and phosphorus during growth.

A shortage of either calcium or phosphorus during growth is apt to result in a permanent loss of the long lithe form, the skeleton becoming unduly stocky if not actually distorted.

If, however, the shortage of mineral elements has not been too severe or too prolonged, there may be complete recovery when the mineral content of the diet is corrected, as in the case recorded by McCollum and illustrated in Fig. 19.

(4) WATER-SOLUBLE VITAMINS

The water-soluble vitamins required in growth are the same as for healthy maintenance in kind, and probably not very different in relative amount, *i.e.* relative to total calories of food required.

But while the adult may endure a shortage for a considerable time before showing distinct injury, the younger organism, under the added strain of growth and with the resisting powers of its tissues not yet fully developed, is apt to show detrimental effects more quickly (See Chapters XIV and XV).

An important fact to be kept in mind is that vitamin B certainly, and probably vitamin C also, has a pronounced effect in stimulating the appetite, so that a liberal supply results in the

eating of more food and thus increasing the intake of all other nutrients that the food contains. Thus the intake of other factors may be raised from an inadequate to an adequate level as the result of the mere addition of more vitamin B to the food.

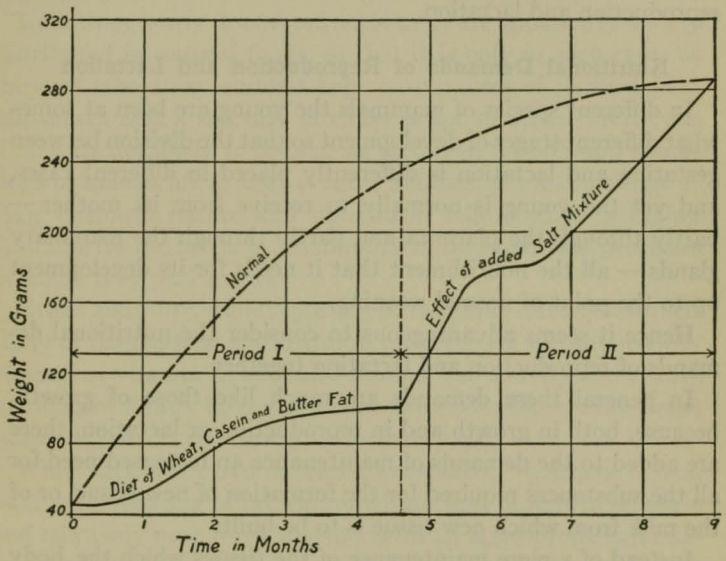

FIG. 19. — Growth at much less than half the normal rate through the greater part of the normal growth period, followed by accelerated growth upon adding a suitable salt mixture to the diet. Courtesy of Dr. E. V. McCollum.

(5) FAT-SOLUBLE VITAMINS

The importance of the fat-soluble vitamins — vitamin A and the antirachitic substance — to the growing organism has been so fully discussed in the last two chapters that it seems unnecessary to do more than allude to it here.

Particularly in the case of vitamin A is the abundance of the supply throughout the whole period of growth of the highest importance, both because of the actual using up of this vitamin in the construction of body tissue (Chapter XVI) and because

during growth, when appetite is usually good and the body is increasing in size, the intake should supply a surplus of vitamin A to be stored in the body against the possibility of future shortages or other contingencies, such as the increased demands of reproduction and lactation.

Nutritional Demands of Reproduction and Lactation

In different species of mammals the young are born at somewhat different stages of development so that the division between gestation and lactation is differently placed in different cases, and yet the young is normally to receive from its mother — partly through the placenta and partly through the mammary glands — all the nourishment that it needs for its development up to the point of normal weaning.

Hence it seems advantageous to consider the nutritional demands of reproduction and lactation together.

In general these demands are much like those of growth, because, both in growth and in reproduction or lactation, there are added to the demands of maintenance an increased need for all the substances required for the formation of new tissue, or of the milk from which new tissue is to be built.

Instead of a mere maintenance of the tissues which the body already has, whole new protein molecules must be made. Hence along with the increased need for energy and for protein in general, there is special need for liberal supplies of the nutritionally essential amino acids for the construction of the proteins of milk or of new tissue.

Also the mineral and vitamin requirements are increased in much greater ratio than is the total food or energy requirement.

And Evans and his coworkers have shown that besides all the substances previously known as essential to maintenance and growth, reproduction requires an additional fat-soluble substance now generally known as the antisterility vitamin or vitamin E.

Furthermore, the work of Evans also indicates that still another chemically unidentified substance is, perhaps not strictly essential, but at least very important, in the nutritional process of lactation.

Both these newly discovered substances are apparently widely distributed in natural foods, so that it is only in such cases as those of laboratory animals kept on mixtures of purified food substances that a shortage of either is apt to occur. But even if they are not likely to be limiting factors in the ordinary conditions of human life or that of farm animals, yet a knowledge of their chemical nature and nutritional relationships is highly desirable for the better understanding of the important processes in which they play a part.

Evans and Burr, while reporting in the paper cited below their study of the physical and chemical properties of the antisterility vitamin or vitamin E, have also summarized so fully and clearly the work of Evans' laboratory upon the entire subject that what follows is based very closely upon their paper.

When rats are reared on various so-called " synthetic " food mixtures consisting of fat, carbohydrate, and protein in separate and relatively pure form, together with an appropriate salt mixture and the vitamins A and B, they grow normally and appear healthy but sooner or later exhibit sterility, although in many instances a period of fertility, variable in its length, follows the attainment of sexual maturity. The sterility has been definitely shown to be due to dietary deficiency. The addition of certain single natural foods high in vitamin E,[1] or the addition of very much smaller amounts of suitable extracts of those foods, prevents or cures this sterility. The sterility disease affects males and females differently.

In the male it eventually leads to destruction of the germ cells; but this is not the case with the female. The ovary and ovula-

[1] The substance was first designated tentatively by the letter X; but is now usually known as vitamin E.

tion are found by Evans to be unimpaired throughout life, but a highly characteristic disturbance occurs in gestation. This is a failure of placental function with death and resorption of the developing young. Evans emphasizes the fact that it is only by ascertaining the existence of typical " resorption gestations " that one may be assured that he is dealing with a deficiency of the specific substance vitamin E. " Many other dietary delinquencies cause sterility in the female, but they all do so by interference with other steps in the reproductive mechanism than those involved in lack of E, usually by preventing oestrus, ovulation, fertilization, or implantation, but not by resorption after implanation has occurred."

In Evans' laboratory large numbers of females have been reared on various mixtures of purified food substances and bred shortly after attaining sexual maturity. Only those exhibiting a typical resorption were then employed to trace the distribution and abundance of vitamin E in natural foods. Shortly after the resorption gestation had shown a deficiency of vitamin E, a small amount of a single natural food was added to the ration or fed separately from it and the fate of the next gestation was carefully studied. " In many instances a normal sized litter of vigorous young resulted. In others, no alteration of the sterility was secured." In this way they showed that some foods are good sources of vitamin E while others either lack it or contain relatively little. It was found to be present but never highly concentrated in a great variety of animal tissues, including muscle, fat (adipose tissue), viscera, and milk. Nine per cent of butter fat, together with 15 per cent lard, failed to prevent sterility; but 24 per cent of butter fat made the diet adequate. Whole milk powder as the sole food was also found to furnish adequate amounts of vitamin E. Evans and Burr also state that there is definite evidence of a higher vitamin E content of milk from cows on fresh alfalfa pasturage. They did not find any concentration of vitamin E in the viscera, liver showing less

than muscle or adipose tissue, and codliver oil failing to prevent the sterility when fed to the extent of 9 per cent of the weight of the food mixture.

Seeds and green leaves were found to furnish vitamin E in abundance. Study of the wheat seed showed that the vitamin E is concentrated in the embryo, and that the oil of the wheat embryo is rich in it. Evans believes that vitamin E is probably present in most vegetable oils.

As proof of the existence of vitamin E in the tissues of animals reared upon natural foods and of its depletion in those reared upon diets of purified food substances, Evans and Burr report experiments upon rats in which sterile females reared upon diets of purified food constituents were killed and their tissues (liver, muscle, and fat) fed to other females reared in an identical fashion and likewise of proven sterility. At the same time, normal females of proven fertility were similarly slaughtered and fed to other females which had been made sterile by lack of vitamin E. In all instances, the tissues of rats reared on natural food were able to invoke fertility in their sterile sisters; and in no instance could a cure be obtained by the administration of the same tissues from the sterile females.

The survival of fertility in animals shifted from a diet possessing vitamin E to one deprived of it was shown to be sufficient to permit of successful reproduction for a limited time. This is doubtless due to the functioning in reproduction of the vitamin E previously stored in the body. Moreover, vitamin E is evidently transferred from mother to offspring during intrauterine life, for the tissue of new-born rats acts as a cure of female dietary sterility induced by feeding rations artificially freed from vitamin E.

That there is some normal use or wastage of vitamin E in the usual metabolic processes of the body was inferred by Evans and Burr from observations which indicated that shielding females from the nutritional demands of reproduction did not

result in any marked prolongation of the period of retention of fertility on vitamin-E-free food.

The administration of foods or extracts of foods known to be twice to twenty times as rich in vitamin E as is required for normal reproduction did not result in any increase in the number or size of the young or in any other way improve the performance of the reproductive mechanism beyond normal limits.

Since the work above mentioned showed a definite if transitory storage of vitamin E by the body, Evans experimented to find whether a sufficiently high feeding of vitamin E on a single occasion — early in gestation — might suffice for that particular gestation. He reports that success resulted from a single administration of the same total amount which he had found to suffice when fed in separate daily allowances throughout the period of gestation. He also found that vitamin E need not be given by the mouth but may be supplied by subcutaneous injection.

Physical and Chemical Properties of Vitamin E

Vitamin E may be called fat soluble, though its range of solubility is not restricted to fats. According to Evans and Burr:

"While this solubility range may really be due to the solubilities of impurities as yet associated with the vitamin, it is a fact that the most concentrated fractions yet obtained have been almost completely miscible with solvent representing such a range as methyl alcohol, ethyl alcohol, ether, pentane, benzene, acetone, ethylacetate, carbon disulphide, etc. The vitamin is almost insoluble in water, yet we have repeatedly encountered its presence in watery solutions. There is enough left in the water after precipitation of calcium soaps, for instance, to be extracted with ether and effect cures. The distribution ratio between water and ether is very large, for a few extractions with an equal volume of ether effects quantitative removal. This has been established by a large number of feedings of the nonsaponifiable fraction, the residual soap always failing to produce fertility. The solubility of E in such substances as alcohol and pentane shows a large temperature coefficient and is so much greater than some of the contaminating substances — the sterols for example — as to permit separation of the vitamin from them.

"Vitamin E is remarkably stable to heat, light, air, and many of the ordinary chemical reactions. As regards temperature, while the ashing of wheat germ completely destroyed the vitamin, yet heating of the germ to 170° C. so that it was greatly charred left the E unimpaired. Distillation of wheat germ oil, or a fraction out of it, in superheated steam at 180° C. for several hours has not destroyed it. Distillation in vacuo up to 233° C. has not in fact caused any lowering of the potency of the fractions so treated nor have any physical changes like changes in solubility been detected. We have not encountered evidence that daylight affects E in wheat germ oil, but there would appear to be partial destruction by one hour exposure in thin layers to a powerful quartz mercury lamp. As regards oxidation, exposure of wheat germ oil for as many as twelve hours to a stream of air washed with acid and alkali and at 97° C. has not destroyed E. At normal temperatures the vitamin is remarkably stable to both acid and alkali and many chemical treatments. It dissolves unchanged, for instance, in saturated alcoholic hydrogen chloride. We have hydrogenated wheat germ oil in the presence of palladium at 75° C. and no injury to the vitamin resulted. . . . It resists the action of boiling 20 per cent alcoholic potassium hydroxide though partial destruction would appear to occur on very prolonged hot saponification. The saponification with 20 per cent alcoholic potassium hydroxide can be carried out at 30° C. without great loss of the vitamin which goes into the nonsaponifiable quota, five per cent of the oil, so that by this step alone a notable concentration of E is always attained. The nonsaponifiable quota is in turn chiefly (73 per cent) sitosterol, which is largely insoluble in pentane in the cold, an excellent solvent for vitamin E, which together with pigments and other materials can thus be washed out of the sterols, leaving them white. The sterols are inactive. The orange-red viscous oil obtained from the pentane can be treated with methyl alcohol, removing more extraneous material, the vitamin going in the alcohol portions which now can be mixed with petroleum ether and diluted to 90 per cent methyl alcohol, allowing an immediate separation into two layers, the petroleum ether invariably securing more of the vitamin, in fact all of it, if the distribution be done with successive fresh portions of the petroleum ether. Further purifications can now be carried out both with digitonin, boiling methyl alcohol, and finally distillations in vacuo. (See accompanying diagram.)

"The final yellow viscous oil does not develop crystals on long standing. It contains only a trace of ash and no nitrogen, sulphur, phosphorus, or halogen. It is remarkably potent. When five milligrams are fed or injected under the skin of a female of proven sterility at the inception of a new gestation, normal litters of vigorous young are born and have been reared to adulthood. Sister control rats invariably continue sterile. Furthermore,

Outline of Fractionation of 6 Kilos of Wheat Germ

6 kilos Wheat Germ.

Extracted with U. S. P. Ether in Soxhlet.

Ether extract. Active. Yield: 600 grams — 10%.	Ether insoluble residue. Inactive.

Saponified in the cold with 20% alcoholic KOH.

Nonsaponifiable matter (N. S. M.). Yield: 5%. Contains all the active material.	Soaps and Glycerol. Inactive.

Crystallized from cold pentane.

Pentane-soluble red oil. Yield: 33% of the N. S. M. Contains all the active material.	Pentane insoluble solids. Sitosterol. Yield: 66% of N. S. M. Inactive.

Extracted with hot MeOH.

Hot methyl alcohol solution. Active.	Methyl alcohol insoluble residue. Inactive. Yield: 3% of N. S. M.

Crystallized from cold MeOH.

Cold MeOH solution. Active.	Precipitate from cold MeOH. Almost entirely inactive. Yield: 10% of N. S. M.

Distributed between dilute MeOH and petroleum ether.

the daily administration of only three tenths of a milligram of this substance throughout the life of the male (rat) results in the retention of complete normality when animals are reared and held on pure foods — a normality

Petroleum ether soluble. Active. Yield: 13% of N. S. M.

Dilute MeOH soluble. Inactive. Yield: 4% of N. S. M.

Sterols precipitated by digitonin.

Sterol-free orange oil. Yield: 2–3 grams. Active.

Sterols from digitonide. Inactive.

Refluxed in hot 20% alcoholic KOH.

N. S. M. Contains all of the active material.

Fatty acids. Inactive.

Sterols again precipitated by digitonin.

Sterol-free oil. Active.

Sterols. Inactive.

Treated with boiling MeOH.

Orange solution in MeOH. Active. Yield: 700–1000 mgms. (Proven active in single doses of 5–10 mgs.)

Residue insoluble in hot MeOH. Inactive. Yield: 50–100 mgs.

Distilled in vacuo.

Fraction I.	Fraction II.	Fraction III.
Up to 200° at 0.8 mm. Low activity. 38% of the total.	200–233° at 0.5 mm. Highly active. 27% of the total.	Residue above 233°. Highly active. 35% of the total.

proven from the weight and histological condition of the testis and by weekly functional tests throughout a year, and controlled by the invariable development of sterility at the end of three months in littermate brothers held on the identical ration save for omission of the vitamin trace."

The work of Evans and Burr, in thus so greatly concentrating vitamin E together with the excellent physiological work also recorded by Evans and his associates, would seem to leave neither reason nor room for further skepticism as to the reality of the existence of this substance as an essential to reproduction. Evidently also it can be stored for a relatively long time in the animal body. This, together with the fact (at first not clearly understood) that it occurs as a fat-soluble substance in milk and so is contained in the butter fat which enters into so many so-called "synthetic" diets, has caused considerable confusion during the past few years (1922 to 1925). It is largely in the hope of clearing up this confusion that the work of Evans has here been reported somewhat fully. The reader is not to suppose, however, that vitamin E is of great importance from a purely practical point of view. Its wide distribution in foods and the extreme smallness of the amounts needed make it unlikely that vitamin E will prove an actual limiting factor. Failures in reproduction encountered in actual experience are more apt to be due to shortage of some other factor, such as vitamin A.

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CHAPTER XIX

DIETARY STANDARDS

The General Problem of a Dietary Standard

It is sometimes asked whether a normal appetite does not indicate, as well as can any dietary standard, the amount of food which is desirable for an individual in any given circumstances.

In considering such a question we shall hardly expect the phrase " amount of food " to indicate equally the energy value, the protein content, the content of each of the necessary mineral elements, and of each of the vitamins. Since different articles of food vary greatly in the relative amounts of the various nutrients which they contain, some one aspect of food value must be chosen as a basis in order to give definite meaning to the phrase " amount of food." Inasmuch as the most prominent of the nutritive requirements is the need for energy and the yielding of energy is the one function in which practically all articles of food take part, it is logical to expect that " amount of food " will correspond more nearly to the energy requirement than to any other one factor of food value or nutritive requirement. Observation confirms this impression and shows that men or other animals when eating varied food under the unrestricted guidance of hunger and appetite tend to take such quantities as are proportioned to the energy requirement whether or not this amount meets also the requirements as to each of the substances known to be necessary in nutrition.

If then hunger and appetite be regarded as guides, primarily to the eating of the right amount of food to meet the energy requirement, we may determine their adequacy in any given case by the fatness of the person concerned, since excess of fuel food of whatever kind can contribute to the storage of body fat.

If from year to year the body keeps in good condition for its work and maintains a fairly constant weight which bears such a proportion to the height as to show that a proper amount of fat is being carried, it is reasonably certain that the amount (fuel value) of food eaten in the course of the year is substantially that which is suited to the degree of activity maintained. If, however, by following the appetite, one becomes unduly stout or unduly thin, or does not get sufficient fuel for the energy required for the day's work, or is annoyed by digestive disturbances indicative of improper feeding, it is certain that the appetite is in this case not a perfect standard. Still more often will the individual appetite prove an inadequate guide to such a quantitative combination of the different types of food as shall lead to a well-balanced intake of each of the many essential food constituents. Here the customs and traditions which govern the food economics of the household and which undoubtedly to some extent reflect the accumulated experience of the race serve an extremely important purpose in checking the caprices of the palate and guiding the individual into food habits which are more likely to conform to actual needs than are the dictates of the individual appetite. But the fullest appreciation of the value of household and social traditions in the formation of good dietary habits does not justify the conclusion that such traditions will always lead to the best results, either physiologically or economically. Even if these traditions represented the experience of past generations to the fullest imaginable extent, they could not be expected to guide us in the use of foods which were not available to our predecessors but have now within a genera-

tion become a common part of the dietary. Nor is it reasonable to suppose that dietary habits adapted to people engaged chiefly in outdoor occupations under frontier conditions will be equally suited to the sedentary city worker of to-day. Under modern conditions scientific dietary standards, based on a knowledge of food chemistry and nutritive requirements such as the preceding chapters have attempted to give, constitute the most rational guide to the formation of hygienic and economic habits in the use of food.

The earliest attempts to set dietary standards in terms of nutrients were those of the German physiologists, among whom the most influential was Voit. He suggested as a proper daily allowance of foodstuffs for a man at moderate muscular work:

Protein,	118 grams.
Fat,	56 grams.
Carbohydrates,	500 grams.

This dietary would have a fuel value of approximately 3000 Calories. The allowance of 118 grams of protein, which has since provoked considerable discussion, is said to have been based upon the average protein metabolism of many laboring men who were living apparently upon unrestricted diet, so that it was practically the result of dietary study. In the division of the remaining calories between fat and carbohydrate, Voit made the allowance of fat low and of carbohydrates high in order to cheapen the dietary.

In America, dietary standards in this sense have been discussed chiefly by Atwater, Chittenden, and Langworthy. Atwater, in his later writings,[1] ceasing to make distinction between fats and carbohydrates as sources of energy in ordinary dietaries, but making allowances for different degrees of muscular activity, recommended the following standards:

[1] Farmer's Bulletin No. 142, U. S. Department of Agriculture. Also Fifteenth Annual Report Agricultural Experiment Station, Storrs, Conn., 1903.

Standards For	Protein, Grams	Fuel Value, Calories
Man at hard muscular work	150	4150
Man at moderately active muscular work . .	125	3400
Man at sedentary or woman with moderately active work	100	2700
Man without muscular exercise or woman at light to moderate work	90	2450

That these standards were not intended simply as expressions of the actual needs of the body is plainly shown by the allowance of 150 grams of protein for a man at hard work, as against 100 grams for a sedentary man. By his own experiments with men at rest and at work in the respiration calorimeter Atwater had demonstrated that muscular work need not increase protein metabolism, if a sufficient amount of fuel be provided in the form of carbohydrates and fats. Hence, when, in providing for muscular work, he proposes to increase the protein in practically the same ratio as the calories, the idea evidently is not that such an increase is necessary, but simply that it was considered advisable on general grounds not to alter very greatly the nature of the diet in increasing its amount.

In explanation of the liberality of his standards Atwater suggested that " the standard must vary not only with the conditions of activity and environment, but also with the nutritive plane at which the body is to be maintained. A man may live and work and maintain bodily equilibrium on either a higher or a lower nitrogen level, or energy level. One essential question is, What level is most advantageous? The answer to this must be sought, not simply in metabolism experiments and dietary studies, but also in broader observations regarding bodily and mental efficiency and general health, strength, and welfare."

Langworthy, maintaining a similar point of view, has col-

lected the data of large numbers of dietaries believed to be fairly representative of the food habits of people of different occupations in the United States and other countries, and stated them in terms of protein and calories per man per day with the following results:

LANGWORTHY'S COMPILATION OF RESULTS OF DIETARY STUDIES

OCCUPATION OF HEAD OF FAMILY	FOOD PER MAN* PER DAY	
	Protein, Grams	Fuel value, Calories
United States:		
Man at very hard work (average 19 studies)	177	6000
Farmers, mechanics, etc. (average 162 studies)	100	3425
Business men, students, etc. (average 51 studies)	106	3285
Inmates of institutions, little or no muscular work (average of 49 studies)	86	2600
Very poor people, usually out of work (average of 15 studies)	69	2100
Canada: Factory hands (average 13 studies)	108	3480
England: Workingmen	89	2685
Scotland: Workingmen	108	3228
Ireland: Workingmen	98	3107
Germany: Workingmen	134	3061
Professional men	111	2511
France: Men at light work	110	2750
Japan: Laborers	118	4415
Professional and business men	87	2190
China: Laborers	91	3400
Egypt: Native laborers	112	2825
Congo: Native laborers	108	2812

* In calculating these results it is assumed that women consume 0.8 as much food as men, and children of different ages from 0.3 to 0.8 as much as the man of the family.

Langworthy concludes that the results obtained, the world over, for persons of moderate activity, "do not differ very markedly from a general average of 100 grams of protein and

3000 Calories of energy, and that it is fair to say that, although foods may differ very decidedly, the nutritive value of the diet in different regions and under different circumstances is very much the same for a like amount of muscular work." He also points out that in some cases this may not be apparent until allowance is made for differences in body weight. Thus he estimates the average weight of the Japanese professional and business men at 105 pounds, so that their food consumption of 87 grams protein and 2190 Calories corresponds to 105 grams protein and 3120 Calories for a man of 150 pounds, which agrees well with the American average for similar employment.

As a standard for men with more muscular activity, such as mechanics at moderately active work, Langworthy suggests 3500 Calories including 105 grams of protein.

Chittenden differs from those whose standards have been quoted in giving almost no weight to the results of dietary studies, holding that these serve chiefly as a measure of self-indulgence, and that the true measure of what the body will most profitably use is to be found in the results of experiments upon the protein metabolism, such as have been described in Chapter VIII. On the basis of these experiments he proposes as a standard allowance for the man of 70 kilograms body weight, 60 grams of protein and 2800 Calories per day. For business and professional men such as Chittenden evidently has in mind, the allowance of 2800 Calories is in substantial agreement with earlier estimates. Sixty grams of protein for a man of 70 kilograms is, however, decidedly lower than any standard previously current.

Energy Allowances for Adults

It has been shown in a previous chapter that different normal individuals of similar age and physique are substantially alike in their energy requirement when performing equivalent amounts of muscular work, and that it is primarily the muscular activity,

and not personal idiosyncrasy or the amount of food eaten, which determines the amount of energy transformed in the body. A dietary standard of high fuel value, and designed to maintain metabolism on a high energy level, provides, therefore, primarily for a large amount of muscular work. If this work is not performed and the food continues to be eaten and digested, we may expect to find a storage of fuel in the body chiefly in the form of fat, and this is true whether the surplus food eaten is carbohydrate, fat, or protein. Thus the store of body fat which a person carries is the most reliable indication as to whether the amount of food habitually eaten is or is not properly adjusted to the work performed. The storage of fat does, however, in itself modify the food requirement. While it is true, as has been shown, that, as between a lean and a fat man having the same weight, the lean man will have the greater food requirement, yet it is also true that when any given man becomes fat, his increased size of body calls for increased metabolism of energy. The work involved in walking, for example, will increase in proportion to the weight moved (*i.e.* to the weight of the body as a whole); and the work of respiration will increase about in proportion to the weight of that part of the body which must be moved with the expansion and contraction of the lungs; while, if fat is deposited in such a way as to interfere directly with the free play of the muscles, there may be an actual lowering of muscular efficiency, so that a larger expenditure of energy may be required in order to produce a given amount of work. If the liberal diet is continued and the digestion remains normal, the storage of fat will continue until it raises the energy expenditure of the body to a point where the food is no longer in excess for the maintenance of weight at the higher level. If the store of fat carried when this point is reached is excessive, the fuel value has been too high; if the store of fat is not excessive, the fuel value of the diet, although greater than would have been necessary to maintain the body at

its former weight, has not been too high, and the body has acquired an asset whose utility may not always be recognized in health, but which may be of great value in case of accident, illness, exposure, or any unusual strain.

Opinions differ somewhat as to the desirable degree of fatness as indicated by the relation of height to body weight.

Hill estimates the average height at 25 years of age as 5 feet 3 inches for women and 5 feet 8 inches for men, and the corresponding average weights as 119 and 150 pounds respectively. He considers that variations of 10 to 15 per cent above or below the average should be considered normal. According to this estimate the woman of 5 feet 3 inches should weigh not less than 102–107, nor more than 131–136 pounds, and the man of 5 feet 8 inches not less than 128–135, nor more than 165–173 pounds. These figures are exclusive of clothing. Hill considers as " fat " those persons whose weight exceeds the average by 15 to 30 per cent, and as " over fat " those who exceed by more than 30 per cent, *i.e.* over 155 pounds for a woman 5 feet 3 inches or over 195 pounds for a man 5 feet 8 inches.

From a study of the records of body weight in relation to the mortality records as collected by life insurance companies in the United States and Canada, Symonds concludes that among young people the greatest vitality coincides with a weight somewhat above the generally accepted averages, while with middle-aged and elderly people a condition of less than average fatness is most favorable to vitality and longevity. Another way of stating the same facts is: That the average of healthy men and women keep themselves slightly too thin while young, and allow themselves to grow slightly too stout as they grow older.

Since Symonds' publication of his results in 1908[1] several similar studies have been made, all of which lead to the same general conclusion.

The tables here reproduced are those of Symonds and of the

[1] *Medical Record*, September 5, 1908.

Symonds's Table of Height and Weight for Men at Different Ages

based on 74,162 accepted applicants for life insurance

(*Medical Record*)

Ages	15–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69
5 ft. 0 in.	120	125	128	131	133	134	134	134	131	
1 in.	122	126	129	131	134	136	136	136	134	
2 in.	124	128	131	133	136	138	138	138	137	
3 in.	127	131	134	136	139	141	141	141	140	140
4 in.	131	135	138	140	143	144	145	145	144	143
5 in.	134	138	141	143	146	147	149	149	148	147
6 in.	138	142	145	147	150	151	153	153	153	151
7 in.	142	147	150	152	155	156	158	158	158	156
8 in.	146	151	154	157	160	161	163	163	163	162
9 in.	150	155	159	162	165	166	167	168	168	168
10 in.	154	159	164	167	170	171	172	173	174	174
11 in.	159	164	169	173	175	177	177	178	180	180
6 ft. 0 in.	165	170	175	179	180	183	182	183	185	185
1 in.	170	177	181	185	186	189	188	189	189	189
2 in.	176	184	188	192	194	196	194	194	192	192
3 in.	181	190	195	200	203	204	201	198		

Symonds's Table of Height and Weight for Women at Different Ages

based on 58,855 accepted applicants for life insurance

(*McClure's Magazine*)

Ages	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64
4 ft. 11 in.	111	113	115	117	119	122	125	128	128	126
5 ft. 0 in.	113	114	117	119	122	125	128	130	131	129
1 in.	115	116	118	121	124	128	131	133	134	132
2 in.	117	118	120	123	127	132	134	137	137	136
3 in.	120	122	124	127	131	135	138	141	141	140
4 in.	123	125	127	130	134	138	142	145	145	144
5 in.	125	128	131	135	139	143	147	149	149	148
6 in.	128	132	135	137	143	146	151	153	153	152
7 in.	132	135	139	143	147	150	154	157	156	155
8 in.	136	140	143	147	151	155	158	161	161	160
9 in.	140	144	147	151	155	159	163	166	166	165
10 in.	144	147	151	155	159	163	167	170	170	169

Life Extension Institute. In both cases the height includes ordinary shoes and the weight includes ordinary indoor clothing.

Table of Heights and Weights based upon the report of the Medico-Actuarial Investigation, 1912, covering an analysis of 221,819 men and 136,504 women.

TABLE OF AVERAGE HEIGHTS AND WEIGHTS—MEN

Age	5 ft. 0 in.	5 ft. 1 in.	5 ft. 2 in.	5 ft. 3 in.	5 ft. 4 in.	5 ft. 5 in.	5 ft. 6 in.	5 ft. 7 in.	5 ft. 8 in.	5 ft. 9 in.	5 ft. 10 in.	5 ft. 11 in.	6 ft. 0 in.	6 ft. 1 in.	6 ft. 2 in.	6 ft. 3 in.	6 ft. 4 in.	6 ft. 5 in.
15	107	109	112	115	118	122	126	130	134	138	142	147	152	157	162	167	172	177
20	117	119	122	125	128	132	136	140	144	148	152	156	161	166	171	176	181	186
25	122	124	126	129	133	137	141	145	149	153	157	162	167	173	179	184	189	194
30	126	128	130	133	136	140	144	148	152	156	161	166	172	178	184	190	196	201
35	128	130	132	135	138	142	146	150	155	160	165	170	176	182	189	195	201	207
40	131	133	135	138	141	145	149	153	158	163	168	174	180	186	193	200	206	212
45	133	135	137	140	143	147	151	155	160	165	170	176	182	188	195	202	209	215
50	134	136	138	141	144	148	152	156	161	166	171	177	183	190	197	204	211	217
55	135	137	139	142	145	149	153	158	163	168	173	178	184	191	198	205	212	219

TABLE OF AVERAGE HEIGHTS AND WEIGHTS—WOMEN

Age	4 ft. 8 in.	4 ft. 9 in.	4 ft. 10 in.	4 ft. 11 in.	5 ft. 0 in.	5 ft. 1 in.	5 ft. 2 in.	5 ft. 3 in.	5 ft. 4 in.	5 ft. 5 in.	5 ft. 6 in.	5 ft. 7 in.	5 ft. 8 in.	5 ft. 9 in.	5 ft. 10 in.	5 ft. 11 in.	6 ft. 0 in.
15	101	103	105	106	107	109	112	115	118	122	126	130	134	138	142	147	152
20	106	108	110	112	114	116	119	122	125	128	132	136	140	143	147	151	156
25	109	111	113	115	117	119	121	124	128	131	135	139	143	147	151	154	158
30	112	114	116	118	120	122	124	127	131	134	138	142	146	150	154	157	161
35	115	117	119	121	123	125	127	130	134	138	142	146	150	154	157	160	163
40	119	121	123	125	127	129	132	135	138	142	146	150	154	158	161	164	167
45	122	124	126	128	130	132	135	138	141	145	149	153	157	161	164	168	171
50	125	127	129	131	133	135	138	141	144	148	152	156	161	165	169	173	176
55	125	127	129	131	133	135	138	141	144	148	153	158	163	167	171	174	177

In the Keep-Well Leaflets of the Life Extension Institute from which the table on this page is quoted, the leading comments on under- and over-weight are as follows:

" Pronounced underweight before the age of 25 is an unfavorable condition, as it is often associated with lack of resistance to pulmonary affections and to other diseases of youth. After the age of 30, underweight, unless extreme, is not an unfavorable condition. Indeed, an investigation of the combined mortality experience of forty life insurance companies shows that the lowest mortality at middle life is found among those a few pounds under weight.

"When underweight is associated with a tendency to frequent colds and there is a condition of debility, anemia, or general malnutrition, it is, at any age, an unfavorable symptom, especially if there has been recent loss in weight. Under such conditions, dieting should be directed to improving nutrition and inducing a gain in weight."

"A moderate degree of overweight is well borne before the age of 35. After the age of 35 overweight is associated with an increasingly high death rate and at or after middle life it becomes a real menace to health, either by reason of its mere presence as a physical handicap or because of the faulty living habits that are often responsible for its development."

Except for the rather general tendency to acquire excessive fatness in middle life, the optimum weight for height and age is very near the average of the accepted applicants as shown in the tables, and Symonds uses these figures as standards in his computations and discussions of the influence of overweight and underweight on longevity and on mortality from specific diseases. The best available data therefore support the opinion that the average degree of fatness of healthy American people is just about the most advantageous fatness for them to maintain. Whatever we accept as the ideal relation of weight to height, it is obvious that the proper standard for fuel value of the diet is that which will preserve the desired degree of fatness while sustaining the desired amount of activity. If good authorities differ in standards for fuel value, it is because, consciously or unconsciously, they contemplate different amounts of muscular activity or the maintenance of a different physique.

That the amount of food required per day to maintain a healthy adult at the desired body weight will vary considerably with age and size and enormously with extremes of muscular activity has already been explained at some length in Chapter VII and need not be discussed further here. Unless it is desired to increase or decrease the body weight, the optimum energy

intake of the healthy adult will be that which coincides with the total energy expenditure; in other words the " standard " and the " requirement " will in this case be the same.

In the above discussion, the energy requirement has been considered as a whole without reference to the question of the most desirable distribution of the total calories among the different foodstuffs. As protein is to be discussed separately, only the problem of the ratio and interchangeability of the fats and carbohydrates presents itself here. All competent students of the question are agreed that fats and carbohydrates are isodynamically interchangeable within wide limits but not to an unlimited extent. In the matter of emphasis, as distinguished from fundamental opinion, we find differences even among the highest authorities, Hopkins having recently emphasized the idea of caution in accepting the " so-called law of isodynamic equivalence," while Osborne and Mendel have described experiments in which they obtained good nutritional results both with diets practically devoid of fats and with those in which carbohydrates were reduced to a vanishingly small proportion. In the actual feeding of people, the problem is probably more largely one of expediency than of actual nutritional need. During the World War the European nations found that a shortage of fat which reduced the available allowance to less than 75 grams per man per day was likely to cause not only discontent but a tendency toward a lowering of efficiency and morale, while their Oriental allies were entirely satisfied and highly efficient on even smaller amounts. This was probably not a racial difference in true physiological need but a matter of a long-standing difference in habits and preferences.

Energy Allowances for Children

Food allowances or dietary standards for children differ from those for adults in that they must provide not only for all expenditures but also for growth. Recently a considerable number of

accurate measurements of energy expenditure of children have been made — especially of infants in the first year of life and of adolescent boys and girls. These data, whether obtained by the method of direct or indirect calorimetry, give precise information as to the energy output at the time of the experiment, but naturally the observations cannot cover the entire 24 hours of the day, nor can experiments of a few hours' duration give any direct information as to how much the intake must exceed the output in order to provide amply for a normal rate of growth. Observations of the unrestricted food consumption (ordinary dietary studies) of healthy children who are making normal growth, and nitrogen balance experiments which show both gain in weight and storage of nitrogen (growth of protein tissue), may be expected to furnish evidence of some value though of a somewhat inferential nature. As a result of compilation and study of data from dietary studies, nitrogen balance experiments, observations of the respiratory exchange, and direct measurements of energy output, the following standards are suggested:

FOOD ALLOWANCES FOR HEALTHY CHILDREN — (Gillett)

AGE	CALORIES PER DAY	
Years	Boys	Girls
Under 2	900–1200	900–1200
2–3	1000–1300	980–1280
3–4	1100–1400	1060–1360
4–5	1200–1500	1140–1440
5–6	1300–1600	1220–1520
6–7	1400–1700	1300–1600
7–8	1500–1800	1380–1680
8–9	1600–1900	1460–1760
9–10	1700–2000	1550–1850
10–11	1900–2200	1650–1950
11–12	2100–2400	1750–2050
12–13	2300–2700	1850–2150
13–14	2500–2900	1950–2250
14–15	2600–3100	2050–2350
15–16	2700–3300	2150–2450
16–17	2800–4000	2250–2600

too low.

. In earlier allowances no distinction was made between boys and girls below ten years of age. The averages of recorded data show, however, a slightly higher energy exchange (or metabolism) in boys than in girls of the same age, though the difference is often less than the range allowed to cover differences of size and activity at a given age. Beyond 10 years of age, the energy exchange in boys evidently increases more rapidly than in girls, probably because of their greater restlessness and muscular activity through this period of development and their greater average rate of growth during and after the fifteenth year.

Many attempts have been made to secure an easily applied and accurate measurement of the nutritional development of the child. Baldwin voices the opinion of many American investigators in his statement, " Normal growth in weight and height is probably the best single index of good health and good nutrition during childhood." The accompanying table showing the rate of growth for children of different builds at all ages is taken from the Baldwin-Wood *Weight-Height-Age Tables for Boys and Girls of School Age,* published by the American Child Health Association, also printed in full by Rose in *Feeding the Family* (Revised Edition).

Although a normal rate of growth is considered more significant than close agreement with the average weight for height and age, yet a comparison of a child's actual weight with the accepted standards gives some indication of his nutritional condition. Certain deviations from the average weight for height and age must undoubtedly be considered normal. More investigation is needed before the border line between normal weight and underweight or overweight can be drawn with finality. The " normal zone " seems to vary for different ages and with different economic and racial groups.

Children, like adults, will vary in muscular activity and this will influence their energy requirements irrespective of other conditions. Among other conditions to be considered are

RATE OF GROWTH FOR BOYS OF SCHOOL AGE *

Age–Year		6	7	8	9	10	11	12	13	14	15	16	17	18	19
Average Height (inches)	Short	43	45	47	49	51	53	54	56	58	60	62	64	65	65
	Medium	46	48	50	52	54	56	58	60	63	65	67	68	69	69
	Tall	49	51	53	55	57	59	61	64	67	70	72	72	73	73
Average Annual Gain in Weight (lbs.)	Short	3	4	5	5	5	4	8	9	11	14	13	7	3	
	Medium	4	5	6	6	6	7	9	11	15	11	8	4	3	
	Tall	5	7	7	7	7	8	12	16	11	9	7	3	4	

RATE OF GROWTH FOR GIRLS OF SCHOOL AGE *

Age–Year		6	7	8	9	10	11	12	13	14	15	16	17	18
Average Height (inches)	Short	43	45	47	49	50	52	54	57	59	60	61	61	61
	Medium	45	47	50	52	54	56	58	60	62	63	64	64	64
	Tall	47	50	53	55	57	59	62	64	66	66	67	67	67
Average Annual Gain in Weight (lbs.)	Short	4	4	4	5	6	6	10	13	10	7	2	1	
	Medium	5	5	6	7	8	10	13	10	6	4	3	1	
	Tall	6	8	8	9	11	13	9	8	4	4	1	1	

* Measurements are taken in indoor clothing, without shoes, sweaters or coats. Age is taken at the nearest birthday, height at the nearest inch; and weight at the nearest pound.

differences in size and physical development among children of the same age and sex. Children of more than average size, if normally active and not over-fat, will require somewhat more food than an average child of the same age. An estimate of energy requirement per unit of weight at different ages has been given in Chapter VII (page 223). A child who has become somewhat emaciated, either through rapid growth[1] or other

[1] Large as are the appetites of growing children, it is not uncommon for the "growth impulse" to outrun the food intake so that the child although always having had access to ample food may as the result of very rapid growth be brought into a condition somewhat resembling that of the young animals described in the preceding chapter (page 495) which become emaciated through "attempting to grow" on rations sufficient only for maintenance, *i.e.* through the growth of some tissues at the expense of others. As Aron points out, a child in this condition has an abnormally low percentage of fat and high percentage of water in his body content. Hence he

causes should have a larger food allowance than would ordinarily be required either for his age or for his weight.

Energy Requirements of Family Groups [1]

In computing the food requirements of family groups, it has been customary to regard the man as the unit and assume that the food of each child may be represented by some appropriate fraction of the food of the father. This practice naturally arose from the fact that the food requirements of men had been longer studied and were better known than those of children, few investigations having been made upon children by accurate laboratory methods until within comparatively recent years.

But, as the writer has previously pointed out, the food requirement of a man varies so greatly, according to his occupation, that it seems hardly logical to make this the basis for estimating the dietary needs of a family. Thus a carpenter may require 3500 Calories per day; a tailor, 2500; a fourteen year old son of either of these men, 2800 Calories. With the carpenter as the unit, the boy's requirement would be represented by 0.8 of that of the father; but with the tailor as the unit, the allowance of 0.8 would obviously provide far too little food for the boy's needs. The Atwater dietary standards for children were stated as decimal fractions of the requirement of a man at moderately active muscular work such as a carpenter requiring 3400 or 3500 Calories.

In practice, the dietitian who makes use of these decimal fractions in computing the food requirements of the family finds it difficult to avoid the tendency to reckon the child's food requirement according to that of the father, which works serious injustice to the child if the father has a low food requirement

needs extra food not only to increase his weight up to that which corresponds to his height, but also to restore the normal percentage of fat in the body weight which he already has.

[1] Quoted from the writer's introduction to Gillett's *Food Needs of Healthy Children.*

because of being engaged in an occupation which does not involve active muscular work. Obviously, it is equally inaccurate to assume that the needs of all men are nearly the same, in order that the allowances for the children may approximate uniformity. In our opinion the food requirement of each member of the family should be determined on his own merits rather than in terms of the man's requirement.

The Problem of a Standard for Protein

In attempting to set a standard for the amount of protein in the dietary we find no such definite and satisfactory basis for judgment as in the case of total food (or fuel) value. There is no indication that work necessarily increases the expenditure of protein as muscular work increases the expenditure of fuel, and the body cannot store up protein to anything like the extent that it stores fuel in the form of fat; the feeding of protein above what is required for maintenance increases only slightly the store of protein which the body carries.

When one writer proposes an amount of protein but little above the minimum required for equilibrium, while another advocates a much larger amount, there is implied a difference of view regarding protein such as no longer exists with respect to the energy metabolism. The difference, it is true, is hardly so great as might appear from a casual examination of the proposed standards. It may perhaps be most fairly expressed in terms of the relation between protein and energy in the different standards. Protein would contribute, according to the standards of Voit, Playfair, and Gautier, about 16 per cent of the fuel value of the food; of Atwater, about 15 per cent; of Langworthy, 12 per cent; of Chittenden, 8½ per cent.

It will be of interest to examine some of the arguments which have been advanced in favor of a high protein or of a low protein diet. The following extracts, given in chronological order, are from writings of those who had given special study to the sub-

ject and chiefly from the literature of the first decade of this century, when Chittenden's investigation of the protein requirement was a subject of active discussion. The time of publication of these opinions must not be overlooked, since some of the phenomena then attributed to differences in protein intake might perhaps now be attributed, in part at least, to the ash constituents and vitamins of the food.

Opinions Regarding the Value of Liberal Protein Diet

Liebig believed that fats and carbohydrates were burned in the body primarily to supply it with warmth, and that protein alone served as the source of muscular work and other forms of tissue activity. He therefore classed the non-nitrogenous as " respiratory " and the nitrogenous as " plastic " foodstuffs, and treated the proteins as playing a " nobler " part in nutrition than can be taken by fat or carbohydrate. Although it was soon demonstrated that carbohydrates and fats as well as protein serve the body in the production of muscular energy, yet the influence of Liebig's teaching, and of the great attention given to protein in Voit's classical researches on nutrition, together with the fact that protein is the most prominent constituent of protoplasm, has resulted in a strong tendency to associate high protein feeding with increased stamina and muscular power.

The reasoning of those who appreciated the results of more recent experimental work, and yet believed the general attitude of Liebig and Voit to have been largely sustained by experience, is well expressed by Von Noorden, who wrote in 1893 : [1]

" When one considers that the dietary habits of peoples are the results of biological laws, it would seem that the action of these laws, extending through the thousands of years of existence of the species, would have resulted in the establishment of suitable habits regarding the amounts of protein consumed. The

[1] Freely translated from the first edition of Von Noorden's Pathologie der Stoffwechsel.

data gathered by Voit may be taken as showing that this normal habit involves the consumption of about 105 grams of digestible protein [1] per day, a smaller protein consumption being usually associated with weak individuals or inactive peoples. While men can maintain equilibrium on less, still it can rightly be said that a liberal protein consumption makes for a full development of the man. A single individual may for years, or even decades, offend against this biological law unpunished. When, however, the small consumption of protein continues for generations, there results a weak race.

" On the other hand, the importance of protein must not be overestimated. A diet is not necessarily good because the amount of protein is right; it must have the proper proportions of the non-nitrogenous nutrients as well, since the protein is not to be depended upon for the necessary fuel value. Better somewhat less protein with a liberal amount of total food than more protein with insufficient fuel value; the latter brings a rapid loss of strength, the former can be endured very well, at least for a long time, and very likely throughout the life of the individual."

Chittenden, in 1905, had reached exactly the opposite conclusion, — that the products of protein metabolism are a constant menace to the well-being of the body, and that any excess of protein over that which the body actually needs is likely to be directly injurious, and at best puts an unnecessary and useless strain upon the liver and kidneys. Chittenden had satisfied himself by his numerous and long-continued experiments that both physical and mental stamina are promoted by decreasing the amount of protein in the food: " Greater freedom from fatigue, greater aptitude for work, greater freedom from minor ailments, have gradually become associated in the writer's mind

[1] Corresponding to Voit's allowance of 118 grams of total protein when the food for the sake of economy, as contemplated by Voit, is taken somewhat largely from vegetable sources.

with this lowered protein metabolism and general condition of physiological economy." . . . (*Physiological Economy in Nutrition*, pages 51, 127.)

Hutchison, in 1906, concluded that the normal amount of protein in a diet furnishing 3000 Calories should be placed at about 75 grams. This allows some margin above the results of Chittenden's experiments and agrees with the relation of protein to calories in mother's milk, which Hutchison regards as nature's hint as to the proper balance of nitrogenous and non-nitrogenous food for the human species (*Chemical News*, Vol. 94, page 104).

The loss of body nitrogen which occurs in the early periods of restricted protein feeding, and which was not determined nor specifically discussed by Chittenden, is treated by Folin as follows: "All the living protoplasm in the animal organism is suspended in a fluid very rich in protein, and on account of the habitual use of more nitrogenous food than the tissues can use as protein, the organism is ordinarily in possession of approximately the maximum amount of reserve protein in solution that it can advantageously retain. When the supply of food protein is stopped, the excess of reserved protein inside the organism is still sufficient to cause a rather large destruction of protein during the first day or two of protein starvation, and after that the protein catabolism is very small, provided sufficient non-nitrogenous food is available. But even then, and for many days thereafter, the protoplasm of the tissues has still an abundant supply of dissolved protein, and the normal activity of such tissues as the muscles is not at all impaired or diminished. When 30 grams or 40 grams of nitrogen have been lost by an average-sized man during a week or more of abstinence from nitrogenous food (but with an abundance of carbohydrate and fat) the living muscle tissues are still well supplied with all the protein that they can use. . . . The continuous excessive use of protein may lead, however, to an accumulation of a larger

amount of reserve protein than the organism can with advantage retain in its fluid media. It is entirely possible that the continuous maintenance of such an unnecessarily large supply of unorganized reserve material may sooner or later weaken one, or another, or all, of the living tissues. At any rate, it seems scarcely conceivable that the human organism, having all the time access to food, can gain in efficiency on account of such an excess of stored protein. The carrying of excessive quantities of fat is considered as an impediment, the carrying of excessive quantities of unorganized protein may be none the less so because more common and less strikingly apparent" (*American Journal of Physiology*, Vol. 13, pages 131–132, 136–137).

Benedict argued that general experience in animal feeding favors the use of liberal quantities of protein, and that " while men may for some months reduce the proportion of protein in their diet very markedly and apparently suffer no deleterious consequences, yet, nevertheless, a permanent reduction of the protein beyond that found to be the normal amount for man is not without possible danger. The fact that a subject can so adjust an artificial diet as to obtain nitrogenous equilibrium with an excretion of nitrogen amounting to about 2 or 3 grams per day is no logical argument for the permanent reduction of the nitrogen in food for the period of a lifetime. . . . Dietary studies all over the world show that in those communities where productive power, enterprise, and civilization are at their highest, man has instinctively and independently selected liberal rather than small quantities of protein" (*American Journal of Physiology*, Vol. 16, page 409).

A similar position was taken by Meltzer, who compared the appetite for a liberal surplus of protein with the liberal way in which the body is provided with organs and tissues for nearly all of its functions, and concludes that " valuable as the facts which Chittenden and his colaborer found may be, they do not make obvious their theory that the minimum supply is the optimum —

the ideal. The bodily health and vigor which people with one kidney still enjoy does not make the possession of only one kidney an ideal condition. The finding that the accepted standard of protein diet can be reduced to one half can be compared with the finding that the inspired oxygen can be reduced to one half without affecting the health and comfort of the individual, but no one deduces from the latter fact that the breathing of air so rarefied would be the ideal. . . . The storing away of protein, like the storing away of glycogen and fat, for use in expected and unexpected exceptional conditions is exactly like the superabundance of tissues in an organ of an animal, or like an extra beam in the support of a building or a bridge — a factor of safety " (*Science*, Vol. 25, page 481).

In view of the arguments of Benedict and of Meltzer, it is of especial interest that in his later book Chittenden says: " It is certainly just as plausible to assume that increase in the consumption of protein food follows in the footsteps of commercial and other forms of prosperity, as to argue that prosperity or mental and physical development are the result of an increased intake of protein food. Protein foods are usually costly and the ability of a community to indulge freely in this form of dietetic luxury depends in large measure upon its commercial prosperity." Moreover, Chittenden contends that his allowance of 60 grams of protein per day for a man of average size is a perfectly trustworthy figure, with a reasonable margin of safety; that " dietetic requirements, and standard dietaries, are not to be founded upon the so-called cravings of appetite, but upon reason and intelligence reënforced by definite knowledge of the real necessities of the bodily machinery "; that " we must be ever mindful of the fact, so many times expressed, that protein does not undergo complete oxidation in the body to simple gaseous products like the non-nitrogenous foods, but that there is left behind a residue not so easily disposed of "; and that " there are many suggestions of improvement in bodily health,

of greater efficiency in working power, and of greater freedom from disease, in a system of dietetics which aims to meet the physiological needs of the body without undue waste of energy and unnecessary drain upon the functions of digestion, absorption, excretion, and metabolism in general . . ." (*The Nutrition of Man*, pages 160, 164, 227, 269).

McCollum and Simmonds commenting upon Chittenden's opinions in the light of their investigations with laboratory animals say: " These (Chittenden's) experiments on men covered approximately 1 per cent of the normal span of life for individuals of their age. It has been abundantly demonstrated on animals that diets which fall below the standard of quality necessary to maintain life and vigor over a very great fraction of the span of life of which the species is capable of living, may cause no noticeable deterioration in the physical condition, during even 5 per cent of the normal expectation of life. In order to produce noticeable effects by faulty diet during 4 or 5 per cent of the average life, it is necessary that the faults shall be relatively severe. Yet diets which are just good enough to permit a young animal to develop in what appears to be an approximately normal manner, may cause early aging and instability of the nervous system.

" The experiments of Chittenden . . . can scarcely be expected to be used as a basis of deductions of a far reaching nature concerning the dietary practices which man may safely adhere to throughout long periods " (*The Newer Knowledge of Nutrition*, Third Edition, page 57).

Plainly the dietary habit of well-to-do people and the dietary standards which have been generally accepted in the past tend to be decidedly liberal with respect to protein, and to prescribe it in quantities which may be believed to be beneficial but certainly are not known to be necessary. It does not seem advisable, however, to adopt as a standard the lowest amount of protein to which the body can adjust itself, but rather to regard

as the normal requirement an amount which will enable the body to maintain not only its equilibrium, but also some such reserve store of protein as we are accustomed to carry. An allowance of one gram of protein daily per kilogram of body weight, about 70 grams per average man, which is 50 per cent above the present estimate of the actual requirement (page 247) seems a reasonable one to use as a general guide.

The feeding of experimental animals on diets containing large quantities of protein has not thrown much light upon the possible desirability of keeping the protein intake within a maximum allowance. Osborne and Mendel[1] secured normal growth of rats for a limited period on diets containing more than 90 per cent protein, but the rats did not reach full size.

Squier and Newburgh[2] found signs of renal irritation after feeding 150 grams of protein to 4 normal young men at both the noon and evening meal. This amount, however, is greatly in excess of the protein content even of a diet very liberal in that respect. " At present one is probably not justified in making too sweeping deductions as to the effects of proteins *per se* fed with great liberality."

A reasonable surplus of protein, from suitable food materials, can hardly be injurious and may be advantageous. Whether such a surplus should be especially recommended or not is largely an economic question. Where little can be spent for food and there is danger that too little food may be eaten, it would be a mistake to use a surplus of protein which could economically be replaced by other food of greater fuel value. In such cases one must not be misled by the popular statement that " protein builds tissue " into supposing that a liberal amount of protein can keep the body strong in spite of a deficiency in the

[1] Osborne and Mendel. Feeding Experiments with Mixtures of Foodstuffs in Unusual Proportions. *Proceedings of the National Academy of Sciences*, Vol. 7, page 157 (1921).

[2] Squier and Newburgh. Renal Irritation in Man from High Protein Diet. *Archives of Internal Medicine*, Vol. 28, page 1, (1921).

total food. This impression is still somewhat prevalent, but is certainly incorrect.

The body is weakened through getting too little food, because body material must then be burned for fuel. So long as the total food be deficient, the loss of body substance will continue, because not only the food protein, but body tissues as well, must be burned to meet the energy requirement. To strengthen the body through the diet we must increase, not the protein alone, but primarily the total calories.

Strengthening or weakening of the body by feeding, ordinarily depends much more upon the sufficiency or insufficiency of the energy value of the total food than upon the amount of protein which it contains.

Careful study of the food habits of large numbers of American families believed to be representative of the population as a whole shows much more reason to fear deficiencies of energy or of calcium than of protein. Amounts of protein actually consumed usually run well above actual requirements.

If the average daily food requirement of a man at rest be taken as 2000 Calories including 50 grams of protein, the same man at work may require 3000 or 4000 Calories while his actual requirement for protein will not be appreciably increased. If the protein be held at 50 grams while the food is increased from 2000 to 3000 or 4000 Calories, the protein in percentage of total calories would be in the three cases 10 per cent, 7 per cent, and 5 per cent respectively. Thus it is plain that when the energy requirement is subjected to considerable variations by differences in muscular activity, the protein requirement cannot be taken as constituting a fixed proportion of the total calories, since muscular work increases the energy requirement very greatly and the protein requirement very little if at all. In practice, however, a diet of 2000 Calories would usually contain somewhat over 50 grams of protein; and when the man increased his activity and his total food consumption, he would probably in-

crease his protein intake in almost the same proportion, for he would in most cases simply eat a larger quantity of his usual kind of food. Thus there is rational basis for the custom of allowing enough protein to furnish from 10 to 15 per cent of the total energy value of the diet.

Protein Standards for Children and for Family Dietaries

Little can be said with confidence regarding the best amount of protein for children after the nursing period. In practice well-planned dietaries for children usually contain between 10 and 15 per cent of the total energy in the form of protein. During the years of rapid growth a considerable fraction of the protein of the food is utilized in the synthesis of body protein; and since the amount of food protein required to form a gram of body protein is variable, depending upon the amino acid make-up of the former, it is evident that the kind of protein supplied becomes a matter of great importance. Here, chemical and physiological laboratory evidence, clinical experience, and its evident place in nature, all indicate plainly the superiority of milk as source of supply of protein for growth, whether the case be that of the growing child after weaning or of the nursling fed through the mother. In cases where the nutritive requirements of growth, pregnancy, or lactation are to be met, the kind of protein is perhaps as important as the amount. The recommendation that family dietaries should whenever possible include " a quart of milk a day for every child " was aimed primarily to ensure an appropriate protein supply. Needless to say, the milk also supplies important amounts of many other substances essential to growth.

Since the energy requirement is greatly increased by muscular activity and the protein requirement is not, it is evident that in the metabolism of normal adults the energy and protein requirements will not run parallel. The protein requirement of the healthy adult depends chiefly upon his size, while his energy requirement depends chiefly upon his activity.

In childhood both the energy requirement and the protein requirement are high — often two to three times as high per unit of weight as for adults without muscular work. Moreover, the high protein and energy requirements of the child as compared with the man are found to run approximately parallel and, as shown in a previous chapter, the same proportion of protein in terms of the total energy which seems rational for the adult dietary suffices also for the food requirements of the child, provided in the latter case the food is of appropriate kind.

In most family groups the differences in age and size will constitute a more prominent factor than the differences in activity, and since the former affect energy and protein requirements in about the same proportion, it becomes feasible and convenient to set the protein allowance for ordinary family groups in terms of a proportion of the total energy. To allow for varying conditions and for individual preferences as well as to provide a liberal margin for safety it is customary to consider that from 10 to 15 per cent of the total calories may be in the form of protein.

Standards for the Calcium, Phosphorus, and Iron Content of the Dietary

Formerly dietary standards took no account of the ash constituents because it was assumed that dietaries furnishing sufficient energy and protein would always be adequate as regards the " inorganic " elements. As explained in previous chapters, this assumption is not safe in the case of calcium, phosphorus, or iron. In the light of present knowledge, adequate dietary standards must provide for these elements.

The evidence thus far available indicates an average minimum requirement for equilibrium, per man per day, of 0.45 gram calcium (0.63 gram CaO), 0.88 gram phosphorus (2.02 grams P_2O_5), and about 0.010 gram (10 milligrams) of iron.

To allow only these quantities in the daily food would cor-

respond to an allowance of only 44 grams per man per day of protein.

If the standard allowance be set 50 per cent above the indicated average minimum, corresponding to an allowance of 67 grams of protein, we obtain:

Calcium,	0.68 gram	(equivalent to 0.95 gram of CaO)
Phosphorus,	1.32 grams	(equivalent to 3.03 grams of P_2O_5)
Iron,	0.015 gram	(15 milligrams)

If it be desired to provide as liberal a margin of safety here as in the case of a protein allowance of 100 grams per man per day, then the above figures must obviously be increased by one half.

Because of the increased demands for calcium during growth, pregnancy, and lactation, the allowance suggested above can not be considered adequate for children or for women during the reproductive period. A study of calcium and phosphorus metabolism in 12 children from 3 to 13 years of age indicated a requirement of at least 1 gram of calcium and phosphorus per day for the growing child. These findings furnish an additional argument for considering the child separately from the adult in planning the family dietary. That the source of the calcium affects its utilization was shown by the failure to secure the most favorable balances when half the calcium was supplied by vegetables. Optimum storage of calcium and phosphorus took place only when each child received 1 quart of milk a day.

The Vitamins

Obviously the chemical identification of the vitamins must precede any statement of the quantities by actual weight in which they should enter into the dietary. But statements in terms of the vitamin units suggested in Chapters XIV, XV, and XVI would serve the same purpose in practice. In fact, these very units are in a sense dietary standards for the experimental

animals by means of which the vitamin values of foods are determined. The expression of dietary standards for vitamins in terms of such units instead of actual weights need be no more distressing than the use of the units now employed to express dosages of antitoxins, or of insulin; or for that matter than the use of the now familiar electrical units.

Quantitative studies of the vitamin values of foods are developing rapidly and it probably will not be long until the number of units of vitamin A in a dietary can be computed with much the same definiteness with which one can now compute the number of milligrams of iron.

It will then be possible to suggest dietary standards for vitamins, not in terms of actual weights, but nevertheless in numerical and quantitative terms. But the basis for such standards will probably of necessity be less direct than the basis of our present standards for energy, protein, calcium, phosphorus, and iron; for these latter rest upon the direct experimental determination of actual physiological need in the human subject and such vitamin experiments upon humans will not be feasible so long as our only methods of determining the vitamin need are through experiments which must show stunting of growth or some departure from health. Hence inference and judgment will probably have to play an even larger part in the matter of standard allowances of vitamin units than in the dietary standards which we have been discussing in the earlier sections of this chapter. Nevertheless, to be able to discuss vitamin intakes in quantitative terms will be another important step in the evolution of dietetics to the status of an exact science.

Meanwhile the presence of sufficient amounts of vitamins in the dietary is best assured by giving ample prominence to those foods which are known to be good sources, notably milk and its products, eggs, vegetables, and fruit. And this is all the more to be emphasized because these foods are such excellent sources of other important nutrients as well.

In the preceding discussion of dietary standards, we have followed the order of the scientific development of our knowledge and of the best sequence for the study of dietary needs in placing the energy requirement (calories) first and the mineral and vitamin requirements last. In the practical planning of a diet or a family food supply, however, it may often seem best to provide first of all those foods upon which we chiefly depend for the necessary mineral elements and vitamins, after which the remainder of the protein and energy requirement may be covered by almost any type of food so far as strictly nutritive needs are concerned. When this plan is followed, the one responsible for the dietary should first of all provide an adequate supply of milk, vegetables, and fruit, after which breadstuffs, meats, fats, and sweets may be added according to taste, purse, energy requirement, and individual digestive powers.

Limitations of Dietary Standards

At the risk of repetition let it be clear that too much weight must not be attached to any of the so-called dietary standards, *i.e.* to any attempt to state the requisites of an adequate diet in terms of quantities of certain nutrients. As Atwater sought strongly to emphasize, a dietary standard at best is "only an indication, not a rule." Some of those who have been most active in recent investigations are most emphatic in warning against the expectation that dietary standards can be made to embrace all the qualities which a diet must have in order to be permanently adequate. Thus Hart, McCollum, Steenbock, and Humphrey say:[1]

"With this recognition of all the normal factors for adequate nutrition there must not simultaneously arise a desire for a mathematical expression of these factors in feeding standards. It is doubtful if this can ever be done, at least for certain of them. . . . We need more effort placed on the accumulation

[1] *Proceedings of the National Academy of Sciences*, Vol. 3, page 374 (May, 1917).

of information on the physiological behavior of feeding stuffs than on the attempts to bring out new mathematical expressions of feeding standards."

More recently McCollum and Simmonds comment:

"We have reached the conclusion that it is not possible to state the amount of any food factor which constitutes the minimum, or the optimum, or an excess, without taking into consideration the biological values of each of the other factors in the diet. The minimum amount of any dietary factor of constant quality which will just serve to maintain apparently normal well-being will fail to suffice when another factor is modified so as to be unsatisfactory, and after such a modification of the diet increasing either of the two factors under consideration will suffice to protect the body from harm in some measure. The diet must be considered as made up of a number of factors, and an effort made to adjust each factor at the optimum."

Hopkins has written:

"What we have actually to recognize is that each of several factors may become that which limits efficiency and that no one of these is in any strict sense more important than any other. Normal nutrition calls for a certain minimum of each one and every one. If a diet is harmoniously balanced in a chemical sense, then indeed energy does become the sole limiting factor. Nutrition then fails, of course, only when too little of the diet is eaten to yield the essential minimum of energy. Or again, when the supply of energy consumed is ample, with fat and carbohydrate duly adjusted, the circumstance that a single essential amino acid in one case, or a vitamin in another, is present in amount below the necessary minimum, converts each of these in turn into the factor which limits utilization. Small as the necessary minimum in either case may be, unless it is reached the proper use of the rest of the diet is reduced to a degree which is proportional to the deficiency. If the deficiency be complete normal utilization is altogether impossible."

What seems most important to the writer in this connection is that we realize the complexity of the subject, the inter-relations of the many factors, and the need of a many-sided view, and yet be not discouraged by our difficulties and limitations (nor even deterred by the skepticism of some of the prominent workers in the field) from the effort to put our knowledge of nutrition and its professional application upon as quantitatively accurate a basis as is possible.

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CHAPTER XX

THE PROBLEM OF THE BEST USE OF FOOD

The problem of the best use of food plainly presents both individual and community aspects.

It is an economic problem in the broad sense in which true economy in the use of food contemplates physiological as well as pecuniary economy. And by physiological economy we should mean the adaptation of the food supply to the best possible nutrition and not merely the meeting of conventional standards of adequacy.

Food, then, should be so chosen and used that the health and efficiency of the individual shall be served in the highest degree, and at the same time used with such regard to the economics of the food supply as a whole that this ideal of optimal nutrition shall be brought within the reach of all.

To a certain extent public opinion already demands that the food supply shall be such as to serve the interests of health. But in the minds of many this does not yet extend beyond the requirement that food shall not in itself be directly detrimental to health.

What may be called the negative relations of food to health have been more widely recognized than the positive. That, either as the result of adulteration or of deterioration, food may conceivably contain deleterious substances; or that toxic or unsuitable material may conceivably be offered as food; or that food materials may sometimes be the carriers of disease germs — all these possibilities the public has recognized, and has insured itself against them by means of the "pure food"

laws. While these laws obviously cannot enforce themselves and may not always be perfectly enforced, especially if appropriations for their enforcement are not adequate, yet in general it may be said that the food legislation and inspection of the present day have relieved the individual consumer of the problem of avoiding food which for any of the reasons mentioned is likely to be directly injurious. Cases of food allergy, the hypersensitiveness which may make an ordinary staple food a source of distress or even of danger to the individual consumer, are highly individual in their nature and of infrequent occurrence when the population as a whole is considered. However important such phenomena may be to the few individuals who suffer from them, they cannot be regarded as of major importance in the general problem of the best use of food.

Of much broader and deeper significance in the relation of food to health is the problem as to how perfectly the food, even if freely chosen, meets all the best nutritional interests of the body; and whether this is accomplished with such conservation of the food supply as best to serve the community or the people as a whole.

In the preceding chapters an effort has been made to show how food supplies, which superficially might seem adequate, may yet fail to supply a sufficient amount of some nutritionally essential factor such as a mineral element or a vitamin. And it has been seen that such a nutritional defect in the diet involves a double danger to health; for it introduces both the danger of a deficiency disease and an increased susceptibility to bacterial (and perhaps other) infections. The recognition of the more elusive factors of nutritional requirement, and of the fact that many superficially acceptable combinations of staple foods (such as the freely chosen dietaries of many people) may need supplementing in order to make them adequate in these respects, is the central feature of what has been frequently referred to as "the newer knowledge of nutrition." This is of fundamental

importance and has marked a great advance in our understanding of the right use of foods. But already we are beginning to see that adequacy alone is not a sufficient aim. It is true that we should seek to make all dietaries adequate; but having found or attained the adequate we should not rest content with that as if there were nothing more to be learned or accomplished. Not merely adequate but rather optimal nutrition should be the aim.

Nutritional Characteristics of Common Foods

How best to draw upon the different types of food in meeting the nutritive requirement must always remain to some extent an individual problem, but the solution of the problem can be greatly facilitated by a consideration of the nutritional characteristics of our different staple articles of food. In recent years, our knowledge of the contribution of individual foods to the diet has been greatly increased, partly by analytical studies more complete than the routine food analyses of the past, and partly by systematic animal feeding experiments upon the dietary deficiencies of single articles or types of foods, and the extent to which these deficiencies may be overcome by the supplementary relations of two or more foods fed in combination. It has been found, for instance, that seeds in general do not contain adequate proportions of calcium and vitamin A for normal growth and reproduction, but that seeds may be supplemented by the leaves of plants, so that it is feasible, if desired, to construct a diet adequate for all the requirements of an omnivorous animal, entirely from plants. Milk has been found to be especially effective in supplementing diets consisting largely of the products of cereal grains or other seeds.

Most of the common articles and types of food may be helpfully grouped according to their most significant nutritive characteristics from the standpoint of our present knowledge of food values, as follows:

(1) Breadstuffs and other grain products. Economical

sources of energy and protein, but not satisfactory in their mineral and vitamin content.

(2) Sugars and fats. Chiefly important from the nutritional standpoint as supplementary sources of energy, although some fats are also important as sources of fat-soluble vitamin.

(3) Meats (including fish and poultry). Rich in protein or fat or both, but showing, in general, the same mineral and vitamin deficiencies as do the grains.

(4) Fruits and vegetables. Greatly variable in their protein and energy values, but highly important as sources of mineral elements and vitamins.

(5) Milk. Important as source of energy, protein, mineral elements, and vitamins. The most efficient of all foods in making good the deficiencies of grains and in insuring the all-round adequacy of the diet.

It becomes apparent that a dietary made up, as so many American dietaries are, too largely of breadstuffs, meats, sweets, and fats, may be satisfying to the palate and to the traditional demand for variety; may furnish ample quota of protein and calories, with fats and carbohydrates in any desired proportions, and yet may be inadequate because of faults in its mineral and vitamin content. We now understand how it is that fruits, vegetables, and especially milk in its various forms, serve (in ways which until recently could not be fully appreciated) to make good the deficiencies of breadstuffs, meats, sweets, and most fats.

Eggs are not included in the above grouping of articles of food because their nutritional character seems too distinctive to warrant their being merged with either meat or milk, while to put them in a group by themselves would tend to give an exaggerated impression of their practical and quantitative importance. In most respects, eggs may be regarded as intermediate between meat and milk, but the recent discovery that the egg yolk has special value for the prevention of rickets lends new

significance to its inclusion in the dietaries of young children and of nursing mothers.

Food Values of American Dietaries

The character of the average American dietary may be studied from the data of detailed and intensive dietary studies. Calculations based on the data from 224 families, taken as typical and studied carefully by trained field workers from the United States Department of Agriculture and the New York Association for Improving the Condition of the Poor, show the average food value per man per day to be as follows:

Energy	3256. Calories
Protein	106. Grams
Phosphorus	1.63 Grams
Calcium	0.74 Grams
Iron	0.0179 Grams

Comparing these averages with the amounts actually required for normal nutrition (as summarized in previous chapters), it will be seen that the freely chosen dietaries contained a liberal surplus of protein and a fair supply of phosphorus and iron but scarcely more than is actually necessary of calories or of calcium. Correspondingly we find that the number of individual family dietaries actually deficient in calcium and in total food value (calories) is high enough to cause serious concern, while the cases of deficiency of phosphorus or iron were considerably less frequent and there were few if any cases showing an actual deficiency of protein.

Many careful students of the subject believe that over one hundred grams of protein per man per day may perhaps be in excess of the best amount for people so largely devoted to sedentary and indoor occupations as we now are; but if we disregard this possibility and think only of the margin by which the average intake exceeds the actual requirement as an insur-

ance, even then we are faced with the inevitable conclusion that the insurance carried by average American food habits is quite disproportionate as between the different factors of nutritional need. Our protein need is less than 100 times our calcium need while our protein intake is over 140 times our calcium intake; or conversely, our calcium need is over 1.0 per cent of our protein need but our calcium intake is only 0.7 per cent of our protein intake. Either we are over-insured as regards protein or we are under-insured as regards calcium.

And, while not susceptible of the same mathematical demonstration, there is reason to believe that we are also under-insured as regards vitamin A and perhaps also as regards vitamin C.

Since it is well within the power of the consumer to alter the distribution of his expenditure among the different articles or types of food, and indeed we often find considerable divergencies in the relative prominence of different foods in the dietaries of neighboring families, it becomes important to consider what influence such variations in the food budget may be expected to have upon the food value of the resulting dietary.

Nutritional Significance of the Distribution of Expenditure for Food

From dietary studies made by the United States Bureau of Labor Statistics, by the United States Department of Agriculture, and by the New York Association for Improving the Condition of the Poor, it can be estimated that of the money devoted to the purchase of food the average American family spends from 30 to 40 per cent for meats and fish (including poultry and shellfish when used), about 5 or 6 per cent for eggs, about 7 to 10 per cent for milk, from 7 to 12 per cent for butter and other fats, from 10 to 20 per cent for bread and other cereal and bakery products, 3 to 7 per cent for sugar and other sweets, 7 to 10 per cent for vegetables, 2 to 8 per cent for fruit, and less than 2 per cent for cheese and nuts. At the same time it is plain that such a food

budget, however prevalent, need not be regarded as fixed. Many people occasionally, and some frequently, put the last and smallest of the items just mentioned in the place of the first and largest by using cheese or nuts as so-called "meat substitute," more properly as an alternative to meat, — a custom which on the whole appears to be growing. The place of each type of food in the diet has been discussed in a general way elsewhere [1] and space does not permit us to go over the same ground here.

Just what prominence should be given to each type of food in the provisioning of a given family or community is a problem calling for consideration of many factors. One important feature of the problem is to ascertain how the normal variations in the distribution of expenditure among the various types of food materials affects the relative proportions of nutrients in the resulting mixed diet. The accompanying table permits a comparison between the expenditures for the different types of food and the returns from each in terms of energy, protein, calcium, phosphorus, and iron in the case of the series of 224 family dietaries mentioned above. In individual dietaries the returns will naturally vary according as an economical or an expensive food of its kind is chosen, but in the average of 224 different dietaries, each of a week's duration, the danger of error due to such individual variations is minimized.

These 224 studies represent a wide range of American conditions — urban and rural, well distributed over the country, over a number of years, and all seasons of the year, and including families showing a normal distribution of levels of income and of expenditure for food. The average of this entire group is, therefore, believed to present a true picture of average American food economy. Furthermore, if this entire group of 224 studies be subdivided into a few large sub-groups according to the rela-

[1] Sherman, *Food Products, Revised Edition*, pages 86–93, 245–250, 284–285, 328–341, 396–416, 460–464, 509–513, 552, 555, 572.

AVERAGE PERCENTAGE DISTRIBUTION OF COST AND NUTRIENTS IN 224 AMERICAN DIETARIES

TYPE OF FOOD	RELATIVE COST	CALORIES	PROTEIN	PHOSPHORUS	CALCIUM	IRON
Meat and fish . . .	32.19	18.99	35.34	26.36	3.86	30.37
Eggs	5.47	1.77	4.64	4.02	3.64	6.25
Milk and cheese . . .	10.59	8.08	11.56	20.61	55.76	5.11
Butter and other fats .	9.55	10.32	.31	.32	.73	.33
Grain products . . .	18.29	38.20	37.25	30.27	15.67	25.87
Sugar and molasses . .	4.57	10.06	.14	.20	1.81	1.80
Vegetables	10.55	9.05	9.55	15.58	14.87	26.42
Fruit	5.31	2.99	.78	1.82	3.15	3.29
Nuts	.15	.14	.11	.13	.07	.09
Food adjuncts . . .	3.33	.40	.32	.69	.44	.47

tive prominence of any one article or type of food, the differences appearing in the average data of the large sub-groups thus obtained may be taken as a true indication of the actual nutritional outcome of such differences in choice of food as do actually exist within the range of typical normal conditions.

In order to find what differences of this kind normally exist in American food budgets and how they affect the food economy, certain conventional limits were employed to divide the 224 dietaries into three groups with reference to each type of food according as the place of this type of food was "low," "medium," or "high" in the dietary as compared with average American practice.

By the use of this means of classification, the effect exerted by any one food or type of food in actual American experience can be studied by comparing the effect of the prominence of this food upon the prominence of other foods and upon the relative cost and nutritive value of the dietary as a whole.

The results of such a study of these data have been described in Chapter XIII of the writer's *Food Products, Revised Edition*, and only the outstanding features can be summarized here.

Grain products (including all breadstuffs, cereals, etc.) are found to be most prominent in the dietaries of lowest cost. If the amounts of nutrients obtained *in proportion to cost* be considered, the increasing prominence of grain products in the food budget is found to give an increasing return in calories, protein, and phosphorus, while calcium and iron remain at about the same level. Both calcium and iron could readily be somewhat increased by the use of a larger proportion of whole grain products in place of a part of the highly milled materials which are so prominent in most American dietaries, but even then prominence of grain products in the dietary must be expected to lead to a relatively low calcium content, as would be expected in view of the data both of ash analyses and of the feeding experiments to determine the nutritional characteristics and dietary deficiencies of the different types of food. From the table on page 555 it will be seen that calcium is the only one of the factors of food value there measured of which the return from the grain products was less than proportional to the money cost. If, however, we take account of the vitamins also we may safely conclude from the results of many laboratory feeding experiments that an increasing prominence of grain products in the dietary tends also to a diminishing intake of vitamins A and C. In order, then, that the economy of the grain products as food may be fully availed of without detriment to the nutritional value of the dietary as a whole, it is important that they be balanced by giving prominence to milk, vegetables, and fruits in order to assure ample supplies of calcium and of vitamins A and C.

The relation between the prominence of milk in the diet and the intake of calcium is shown graphically in Fig. 20, from which it is evident that under ordinary conditions of actual American practice the calcium of the dietary depends more largely upon the milk than upon any other factor, or indeed upon all other factors combined. Since American dietaries are probably more

often deficient in calcium than in any other chemical element, the value of milk as a source of calcium is a large factor in its unique importance in the dietary.

When the 224 dietaries here considered were divided into three groups according as the place of milk in the food budget was low, medium, or high, it was found that an increase in the prominence of milk, from an average of 10.30 per cent of the

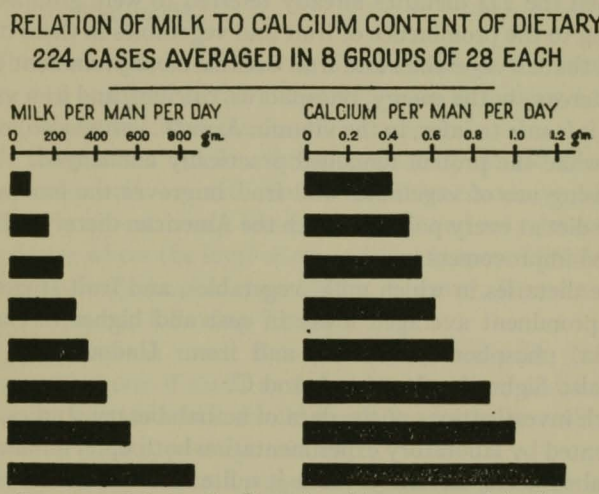

FIG. 20. — Relation of milk to the calcium content of the dietary.

total expenditure for food in the " medium," to an average of 19.38 per cent in the so-called " high milk " dietaries, resulted in an increase in all the factors of food value with no increase in the total cost. Furthermore the improvement was greatest at the point at which it was most needed, *i.e.* in the calcium content of the dietary; and there was doubtless a very important increase in the vitamin A value as well.

The cheapness of breadstuffs and the efficiency with which milk supplements them, give rise to the saying that " the dietary should be built around bread and milk."

Vegetables and fruit taken as a group may be ranked next after grain products and milk in importance as constituents of an economical and well-balanced diet. They tend to correct both the mineral and the vitamin deficiencies of the grain products and in a sense they supplement the milk also in that many of the vegetables and fruits are rich in iron or vitamin C, or both.

When the 224 dietaries already referred to were grouped according to the prominence of fruit and vegetables in them it was found that as vegetables and fruit became more prominent there was increase in the energy, phosphorus, calcium, and iron values (and, it is safe to infer, in the vitamin A and C values also) of the diet, while the protein remained practically unchanged. Thus increasing use of vegetables and fruit improves the food value of the diet at every point at which the American dietary is likely to need improvement.

The dietaries in which milk, vegetables, and fruit (together) were prominent averaged lower in cost and higher in energy, protein, phosphorus, calcium, and iron. Undoubtedly they were also higher in vitamins A and C.

Such investigations of the data of actual dietary studies, supplemented by laboratory experimentation both upon human and animal subjects, have now made it quite clear that the average or typical American food budget can be easily improved by giving greater prominence to milk, vegetables, and fruit, the money needed for this being obtained if necessary by reducing the expenditure for meats and sweets.

A record kept in the writer's household showed that one fourth or more of the money spent for food was spent for milk, about one fifth for vegetables and fruit, and less than one sixth for meats, poultry, and fish.

Based apparently upon the experience of Miss Gillett in her work upon family nutrition problems for the New York Association for Improving the Condition of the Poor, the Food Adminis-

tration issued as a part of its educational program, a simple suggested family food budget essentially as follows:

Divide your food money into fifths:
One fifth, more or less, for vegetables and fruit;
One fifth, or more, for milk and cheese;
One fifth, or less, for meats, fish, and eggs;
One fifth, or more, for bread and cereals;
One fifth, or less, for fats, sugar, and other groceries and food adjuncts.

The recommendation that a fifth or more of the total food-money be spent for bread and cereals aims at making a dietary more economical than the American average.

In the writer's opinion the proportion spent for bread and cereals may well vary with the need for strict economy. It must be high in an extremely low-cost dietary and may be considerably lower where the level of expenditure is more liberal.

Whatever the level of expenditure, however, it seems wise to observe the two following "rules":

(1) At least as much should be spent for milk (including cream and cheese if used) as for meats, poultry, and fish, and

(2) At least as much should be spent for fruits and vegetables as for meats, poultry, and fish.

These simple "rules," suggested by the writer several years ago, are said to have been found useful as a guide in both low-cost and liberal-cost food budgets and can obviously be used in all cases in which even the simplest of records of expenditure are kept. They tend to make milk, vegetables, and fruits more prominent and meat somewhat less prominent than in the average American dietary for reasons which have been discussed in this and in previous chapters.

From among the dietary records above discussed, 25 were taken at random and a calculation was made to see how their nutritive values would have been affected if, with no change in the amount of money spent or in the nature of the foods selected,

the quantities had been simply readjusted in accordance with the two " rules " just given. It was found that such readjustment without change in cost would leave the protein practically unchanged in amount while the Calories and iron would be slightly increased and the calcium and phosphorus materially increased and brought into better quantitative relations with each other. There is also ample evidence that the dietaries thus adjusted would be improved in their content of vitamins A and C.

Dietary Adjustments and the National Food Supply

General adoption of a dietary such as we now believe to be best would call for more milk and perhaps more vegetables and fruit than now come to our city markets; but more of these foods will be produced and marketed as the demand for them increases. Moreover, an increased demand for these foods and a correspondingly decreased (per capita) demand for meat, so far from causing any serious " dislocation of industry," will help to facilitate natural evolution of American agriculture. With increasing population on stationary area farming necessarily becomes more intensive. Beef is produced less by the grazing of cattle on free ranges of unbroken prairie and more by the feeding of grain and other cultivated crops. For a given amount of food consumed, a dairy herd yields a product of greater food value than does a herd of beef animals. Armsby estimates that of the energy value of grain about 18 per cent is recovered for human consumption in milk and only about 3.5 per cent in beef. An increasing ratio of milch cows to beef cattle is naturally to be expected with the development of a more intensive agriculture and will be to the advantage of producer and consumer alike. In regions adapted to dairy farming but too remote from large markets to ship milk in the fresh state we may anticipate an increasing production of condensed and dried milk and of butter and cheese. An increased production of fruit and vegetables

should also be a natural result of a more stable and intensive agriculture. At the same time the concentration of population in large cities increases the expense of transportation and makes the cost of retail distribution a serious item, especially in the case of bulky products with a relatively low value per pound. Cabbage, potatoes, and root crops can be produced at a low cost per ton, but the percentage of the cost of production which must be added when they are distributed through modern retail agencies tends constantly to increase.

The more highly perishable fruits and vegetables having a higher cost per pound or ton are now successfully transported in transcontinental carload shipments. Precooling and lowered temperatures in refrigerator cars promise to reduce still further the losses incident to their transportation.

Cold storage tends to equalize prices throughout the year on such perishable foods as butter, cheese, and eggs, and secures a supply of other fresh foods, such as apples of good quality, throughout almost the entire year. With the perfection of facilities for more rapid distribution in cities after removal from freezing temperatures, the number and quantity of vegetables and fruits so preserved should increase greatly. The canning industry has already developed to enormous proportions.

The physical and economic wastes in marketing are being reduced by various agencies in the United States Department of Agriculture, now largely consolidated in the Bureau of Agricultural Economics, and, in general, the supply may be trusted to keep pace with the demand in the gradual shifting of emphasis from meat toward dairy products, vegetables, and fruit, which seems to be clearly desirable both in view of our present knowledge of nutrition, and in the light of our agricultural situation.

Adequate versus Optimal Nutrition

The above suggestions for adjustment of food habits, in the light of present knowledge of nutrition and food values, gain

greatly in cogency when it is realized that we now have ample justification for setting our nutritional aim higher than mere adequacy, and for anticipating that further improvement in food habits will bring about still higher degrees of health with increased efficiency and power to resist infectious disease.

This higher significance of the relation of food to health becomes fully apparent only as feeding experiments upon laboratory animals are followed throughout the entire lifetime of the individuals under observation and even through successive generations of families kept upon different diets but identical living conditions in all other respects.

By means of such experiments it is possible to demonstrate and measure differences of degree in the positive attributes of health such as are included, for example, in the following definition from the Century dictionary: "Health: Soundness of body; that condition of a living organism and of its various parts and functions which conduces to efficient and prolonged life; a normal bodily condition. Health implies also, physiologically, the ability to produce offspring fitted to live long and to perform efficiently the ordinary functions of their species."

This is both a positive and a comprehensive conception of health, and it is of much interest to note that all its phases have now been covered in studies of the influence of foods upon the health of experimental animals. In human experience so many factors may enter to influence health in the course of a lifetime that it is hard to separate and measure the effects of food alone upon the whole duration and efficiency of life. But this can be and has been done with laboratory animals of rapid growth and early maturity, such as the rat, and it has been possible to determine, under conditions uniform in all other respects, the influence of modifications of the diet upon the various factors of health comprised in the broad definition above quoted. And among the recent findings of nutrition experiments, carried

through successive generations of such laboratory animals, is the fact that starting with a dietary already adequate according to current standards, we may, by improvement of the diet, induce a higher degree of health and vigor. This has been repeatedly and conclusively shown to result from an improvement consisting of increasing the proportion of milk in an already adequate diet; and the diet thus improved was rendered still more effective by the addition of a fresh green vegetable.

It may be of interest here to cite in brief the actual results of that comparison for which the data are most abundant and the findings clearest and most conclusive.

In the writer's laboratory certain families of experimental rats are still thriving after 16 generations on a diet of which dry whole milk constitutes one sixth and ground whole wheat five sixths of the food mixture. Although this diet is unquestionably adequate, an increase in the proportion of milk, from one sixth to one third of the solids of the food mixture, results in a marked improvement in nutrition. The animals on the diet containing the larger proportion of milk have, by actual measurements upon large numbers of cases, fully demonstrated the following evidences of advancement of health through improved nutrition as the result of increasing the proportion of milk in a dietary which was already adequate.

1. An increased rate of gain during the period of active growth.

2. More efficient growth as shown by greater gain per 1000 Calories of food consumed.

3. Somewhat larger average size at all ages.

4. Earlier maturity.

5. Longer duration of the prime of life, *i.e.* of active adult life, old age being deferred in the same individuals in which earlier maturity was induced by the improvement in the already adequate diet.

6. Greater success in the rearing of young as shown by increase

both in the numbers and in the percentage of young reared, and by decrease in the percentage of families dying out.

7. Better growth of the young during the nursing period.

Each of these seven advances in health through improvement of nutrition habit has been measured on large numbers of cases in which inheritance and all the factors of the environment except the food were exactly the same. And these large numbers of measurements have been subjected to statistical analysis with results which establish with entire certainty the fact that all of these differences are real and are due to the improvement in diet — none of them accidental or due to chance or physiological variability.

Further observations and statistical studies are showing in like manner that the improvement in diet has lowered the death rate in all of the various age periods studied and improved the longevity of those who attain to a really long life, as well as the average length of life for the community as a whole.

And all of these improvements are even more noticeable in the second generation than in the first.

The better of these two diets is probably capable of further improvement. There is no reason to doubt that all these findings, as thus stated in qualitative terms, will apply equally in human experience and that a higher degree of vigor will follow an improvement in the dietary of the individual or the food supply of the community, even where the original dietary was already adequate according to all current standards.

Evidently there is not only a line to be drawn but a wide zone to be explored between adequate and optimal nutrition.

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APPENDIX A

NOMENCLATURE AND CLASSIFICATION OF PROTEINS

Joint Recommendations of the Committees on Protein Nomenclature of the American Physiological Society and American Society of Biological Chemists

SINCE a chemical basis for the nomenclature of the proteins is at present not possible, it seemed important to recommend few changes in the names and definitions of generally accepted groups, even though, in many cases, these are not wholly satisfactory. The recommendations are as follows:

First. — The word " proteid " should be abandoned.

Second. — The word " protein " should designate that group of substances which consist, so far as at present is known, essentially of combinations of α-amino acids and their derivatives, *e.g.* α-amino acetic acid or glycocoll; α-amino propionic acid or alanine; phenyl-α-amino propionic acid or phenylalanine; guanidin-α-amino valerianic acid or arginine, etc., and are therefore essentially polypeptids.

Third. — That the following terms to be used to designate the various groups of proteins:

I. SIMPLE PROTEINS. — Protein substances which yield only α-amino acids or their derivatives on hydrolysis.

Although no means are at present available whereby the chemical individuality of any protein can be established, a number of simple proteins have been isolated from animal and vegetable tissues which have been so well characterized by constancy of ultimate composition and uniformity of physical properties

that they may be treated as chemical individuals until further knowledge makes it possible to characterize them more definitely.

The various groups of simple proteins may be designated as follows:

(*a*) *Albumins.* — Simple proteins soluble in pure water and coagulable by heat.

(*b*) *Globulins.* — Simple proteins insoluble in pure water, but soluble in neutral solutions of salts of strong bases with strong acids.[1]

(*c*) *Glutelins.* — Simple proteins insoluble in all neutral solvents but readily soluble in very dilute acids and alkalies.[2]

(*d*) *Alcohol-soluble Proteins.* — Simple proteins soluble in relatively strong alcohol (70–80 per cent), but insoluble in water, absolute alcohol, and other neutral solvents.[3]

(*e*) *Albuminoids.* — Simple proteins which possess essentially the same chemical structure as the other proteins, but are characterized by great insolubility in all neutral solvents.[4]

(*f*) *Histones.* — Soluble in water and insoluble in very dilute ammonia, and, in the absence of ammonium salts, insoluble even in an excess of ammonia; yield precipitates with solutions of other proteins and a coagulum on heating which is easily soluble in very dilute acids. On hydrolysis they yield a large number of amino acids, among which the basic ones predominate.

(*g*) *Protamins.* — Simpler polypeptids than the proteins included in the preceding groups. They are soluble in water, uncoagulable by heat, have the property of precipitating aqueous solutions of other proteins, possess strong basic properties, and

[1] The precipitation limits with ammonium sulphate should not be made a basis for distinguishing the albumins from the globulins.

[2] Such substances occur in abundance in the seeds of cereals and doubtless represent a well-defined group of simple proteins.

[3] The subclasses defined (*a*, *b*, *c*, *d*) are exemplified by proteins obtained from both plants and animals. The use of appropriate prefixes will suffice to indicate the origin of the compounds, *e.g.* ovoglobulin, myoalbumin, etc.

[4] These form the principal organic constituents of the skeletal structure of animals and also their external covering and its appendages. This definition does not provide for gelatin, which is, however, an artificial derivative of collagen.

form stable salts with strong mineral acids. They yield comparatively few amino acids, among which the basic amino acids greatly predominate.

II. Conjugated Proteins. — Substances which contain the protein molecule united to some other molecule or molecules otherwise than as a salt.

(*a*) *Nucleoproteins.* — Compounds of one or more protein molecules with nucleic acid.

(*b*) *Glycoproteins.* — Compounds of the protein molecule with a substance or substances containing a carbohydrate group other than a nucleic acid.

(*c*) *Phosphoproteins.* — Compounds of the protein molecule with some, as yet undefined, phosphorus-containing substance other than a nucleic acid or lecithin.[1]

(*d*) *Hemoglobins.* — Compounds of the protein molecule with hematin or some similar substance.

(*e*) *Lecithoproteins.* — Compounds of the protein molecule with lecithins (lecithans, phosphatids).

III. Derived Proteins.

1. *Primary Protein Derivatives.* — Derivatives of the protein molecule apparently formed through hydrolytic changes which involve only slight alterations of the protein molecule.

(*a*) *Proteans.* — Insoluble products which apparently result from the incipient action of water, very dilute acids, or enzymes.

(*b*) *Metaproteins.* — Products of the further action of acids and alkalies whereby the molecule is so far altered as to form products soluble in very weak acids and alkalies, but insoluble in neutral fluids.

This group will thus include the familiar " acid proteins " and " alkali proteins," not the salts of proteins with acids.

(*c*) *Coagulated Proteins.* — Insoluble products which result

[1] The accumulated chemical evidence distinctly points to the propriety of classifying the phosphoproteins as conjugated compounds, *i.e.* they are possibly esters of some phosphoric acid or acids and protein.

from (1) the action of heat on their solutions, or (2) the action of alcohols on the protein.

2. *Secondary Protein Derivatives.*[1] — Products of the further hydrolytic cleavage of the protein molecule.

(*a*) *Proteoses.* — Soluble in water, uncoagulated by heat, and precipitated by saturating their solutions with ammonium sulphate or zinc sulphate.[2]

(*b*) *Peptones.* — Soluble in water, uncoagulated by heat, but not precipitated by saturating their solutions with ammonium sulphate.[3]

(*c*) *Peptids.* — Definitely characterized combinations of two or more amino acids, the carboxyl group of one being united with the amino group of the other, with the elimination of a molecule of water.[4]

[1] The term "secondary hydrolytic derivatives" is used because the formation of the primary derivatives usually precedes the formation of these secondary derivatives.

[2] As thus defined, this term does not strictly cover all the protein derivatives commonly called proteoses, *e.g.* heterproteose and dysproteose.

[3] In this group the kyrins may be included. For the present we believe that it will be helpful to retain this term as defined, reserving the expression "peptid" for the simpler compounds of definite structure, such as dipeptids, etc.

[4] The peptones are undoubtedly peptids or mixtures of peptids, the latter term being at present used to designate those of definite structure.

APPENDIX B

COMPOSITION OF FOODS

Explanation of Tables

Food as purchased may or may not consist entirely of edible material. When an article of food contains inedible matter or refuse, this may be stated separately and the composition of the edible portion then given, or the percentages of refuse and of edible nutrients in the original matter may be given so as to show directly the percentage of each edible nutrient obtained in the material as purchased. For example; 100 pounds of beef contains 16 pounds of bone and 84 pounds of moist flesh, of which 15.4 pounds are protein, 15 pounds fat, 53 pounds water, and 0.6 pound ash. The composition may be stated in either of the following forms:

Composition of Beef

Refuse Per Cent	Water Per Cent	Protein Per Cent	Fat Per Cent	Ash Per Cent
16.0	53.0	15.4	15.0	0.6

Composition of Beef

Refuse	Edible Portion			
Per Cent	Water Per Cent	Protein Per Cent	Fat Per Cent	Ash Per Cent
16.0	63.1	18.3	17.9	0.7

For most purposes it is convenient to include in one table the nutrients calculated both on the basis of edible material and of material as purchased. In such a case the percentage of refuse in the material as purchased may be given or may be omitted as in the following form:

COMPOSITION OF BEEF

	WATER	PROTEIN	FAT	ASH
Edible portion (E. P.)	63.1	18.3	17.9	0.7
As purchased (A. P.) .	53.0	15.4	15.0	0.6

In order to avoid confusion and possible errors in taking data from tables of composition it is important to note in which form the percentages are stated. Data given in either form are of course readily convertible into the other. In Table I which follows, the percentages of nutrients and the corresponding energy values are stated in the form last illustrated above. Table II shows percentages of ash constituents in the edible portion only. Table III shows grams of protein and of calcium, phosphorus, and iron in 100-Calorie portions, which estimates may obviously be used equally well whether the food be originally recorded in terms of edible material or of material as purchased.

A word of explanation regarding the sources and reliability of the data may also be offered. The percentages of proteins, fats, and carbohydrates given in Table I are in the great majority of cases taken from the tables of composition of American food materials compiled by Atwater and Bryant and published in Bulletin 28 of the Office of Experiment Stations, U. S. Department of Agriculture. By reference to this bulletin the reader may find the number of analyses on which the average is based and the maximum and minimum of the recorded percentages

of each constituent, as well as the percentages of moisture, ash, and in some cases crude fiber. The energy values given in Table I are computed from the average percentage of protein, fat, and carbohydrate by the use of the latest and most accurate factors (see page 168). The data for ash constituents given in Tables II and III are based on a critical compilation of all available ash analyses, both American and European. In some cases only a single ash analysis could be found; in other cases the data given are averages of many fairly concordant analyses. Between these extremes are data of all degrees of probable reliability. It does not seem feasible to indicate the relative accuracy of the estimates for different articles of food. In general it may be said that only in the cases of the more important foods are the ash analyses as yet sufficiently numerous and concordant to justify one in laying great emphasis upon comparisons of one article of food with another. More emphasis can properly be laid upon estimates of the ash constituents of rations or dietaries made up of several food materials, since in such cases accidental errors will tend to offset each other. It is chiefly to facilitate such calculations that the tables have been made as complete as seemed practicable even though this necessitated including estimates of differing reliability on apparently equal terms.

Data which are based in part at least upon assumptions are inclosed in parenthesis. They are not necessarily less accurate as estimates of average composition than are some of the directly determined data of individual analyses.

Since many unpublished ash analyses have been included in the present averages, Tables II and III will be found to present many differences in detail from those published elsewhere, or in the first edition of this book. The general trend of the averages has, however, not been materially altered by the results of recent work.

Attention may also be called to the fact that in Table II the

data are uniformly given as percentages of the elements and not of their oxides. For the convenience of those who may prefer to continue to calculate calcium and phosphorus in terms of the oxides as has been customary in the past, Table III shows the weights of CaO and P_2O_5 as well as of protein, calcium, phosphorus, and iron in 100-Calorie portions of foods.

TABLE I

Edible Organic Nutrients and Fuel Values of Foods*

Food		Protein (N×6.25) per cent	Fat per cent	Carbohydrate per cent	Fuel value per pound Calories	100 Calorie portion grams
Almonds	E. P.†	21.0	54.9	17.3	2940	15
	A. P.†	11.5	30.2	9.5	1615	28
Apples	E. P.	.4	.5	14.2	285	159
	A. P.	.3	.3	10.8	214	212
Apricots	E. P.	1.1	—	13.4	263	174
	A. P.	1.0	—	12.6	247	184
Artichoke, French	E. P.	3.4	.5	12.0	300	151
	A. P.	1.7	.3	6.0	150	302
Asparagus, fresh	A. P.	1.8	.2	3.3	100	450
cooked	A. P.	2.1	3.3	2.2	213	213
Avocado	E. P.	2.1	20.1	7.4	993	46
	A. P.	1.4	13.2	4.8	652	70
Bacon, smoked	E. P.	10.5	64.8	—	2840	16
	A. P.	9.5	59.4	—	2372	19
Bananas	E. P.	1.3	.6	22.0	447	101
	A. P.	.8	.4	14.3	290	156
Barley, pearled		8.5	1.1	77.8	1615	28
Beans, dried		22.5	1.8	59.6	1565	29
Lima, dried		18.1	1.5	65.9	1586	29
Lima, fresh	E. P.	7.1	.7	22.0	557	82
	A. P.	3.2	.3	9.9	250	182

†E. P. signifies edible portion; A. P. signifies as purchased.

* The percentages of nutrients are taken from Bull. 28, Office of Experiment Stations, U. S. Department of Agriculture. The fuel values are calculated from these percentages by the use of the factors explained in Chapter V, viz. — protein, 4 calories; fat, 9 calories; carbohydrate, 4 calories per gram.

TABLE I — EDIBLE ORGANIC NUTRIENTS (*Continued*)

FOOD		PROTEIN (N×6.25) PER CENT	FAT PER CENT	CARBO-HY-DRATE PER CENT	FUEL VALUE PER POUND CALO-RIES	100 CALORIE PORTION GRAMS
Beans — *Continued*						
string, fresh	E. P.	2.3	.3	7.4	184	241
	A. P.	2.1	.3	6.9	176	259
baked, canned	A. P.	6.9	2.5	19.6	583	78
red kidney, canned		7.0	.2	18.5	471	96
Beef, brisket, medium fat	E. P.	15.8	28.5	—	1449	31
	A. P.	12.0	22.3	—	1130	40
chuck, average	E. P.	19.2	15.4	—	978	46
	A. P.	15.8	12.5	—	797	58
corned, average	E. P.	15.6	26.2	—	1353	34
	A. P.	14.3	23.8	—	1230	37
cross ribs, average	E. P.	15.9	28.2	—	1440	32
	A. P.	13.8	24.8	—	1262	36
dried, salted, and smoked,	E. P.	30.0	6.5	.4	817	56
	A. P.	26.4	6.9	—	760	60
flank, lean	E. P.	20.8	11.3	—	838	54
	A. P.	20.5	11.0	—	821	55
fore quarter, lean	E. P.	18.9	12.2	—	842	54
	A. P.	14.7	9.5	—	655	69
fore shank, lean	E. P.	22.0	6.1	—	647	70
	A. P.	14.0	3.9	—	414	110
heart	E. P.	16.0	20.4	1.0	1140	40
	A. P.	14.8	24.7	.9	1292	35
hind quarter, lean	E. P.	20.0	13.4	—	907	50
	A. P.	16.7	11.2	—	757	60
hind shank, lean	E. P.	21.9	5.4	—	617	75
	A. P.	9.1	2.2	—	255	179
hind shank, fat	E. P.	20.4	18.8	—	1171	40
	A. P.	9.9	9.1	—	552	83
liver	E. P.	20.4	4.5	1.7	584	78
	A. P.	20.2	3.1	2.5	537	85
loin	E. P.	19.7	12.7	—	877	52
	A. P.	17.1	11.1	—	764	60
neck, lean	E. P.	21.4	8.4	—	732	62
	A. P.	15.1	5.9	—	493	93
neck, medium fat	E. P.	20.1	16.5	—	1040	44
	A. P.	14.5	11.9	—	749	61

TABLE I — EDIBLE ORGANIC NUTRIENTS (*Continued*)

FOOD	PROTEIN (N×6.25) PER CENT	FAT PER CENT	CARBO-HY-DRATE PER CENT	FUEL VALUE PER POUND CALO-RIES	100 CALORIE PORTION GRAMS
Beef — *Continued*					
plate, lean . . . . . E. P.	15.6	18.8	—	1051	43
A. P.	13.0	15.5	—	867	52
Porterhouse steak . . . E. P.	21.9	20.4	—	1230	37
A. P.	19.1	17.9	—	1077	42
rib rolls, lean . . . . . A. P.	20.2	10.5	—	795	57
ribs, lean . . . . . E. P.	19.6	12.0	—	845	54
A. P.	15.2	9.3	—	654	69
ribs, fat . . . . . E. P.	15.0	35.6	—	1721	26
A. P.	12.7	30.6	—	1480	31
round, lean . . . . . E. P.	21.3	7.9	—	709	64
A. P.	19.5	7.3	—	649	70
round, free from visible fat	23.2	2.5	—	512	87
rump, lean . . . . . E. P.	20.9	13.7	—	940	49
A. P.	19.1	11.0	—	796	57
rump, fat . . . . . E. P.	16.8	35.7	—	1763	26
A. P.	12.9	27.6	—	1361	33
sides, lean . . . . . E. P.	19.3	13.2	—	890	51
A. P.	15.5	10.6	—	715	64
sirloin steak . . . . . E. P.	18.9	18.5	—	1099	41
A. P.	16.5	16.1	—	960	48
sweetbreads . . . . . A. P.	16.8	12.1	—	799	57
tenderloin . . . . . A. P.	16.2	24.4	—	1290	35
tongue . . . . . E. P.	18.9	9.2	—	717	63
A. P.	14.1	6.7	—	529	86
Beets, cooked . . . . . E. P.	2.3	.1	7.4	180	252
fresh . . . . . E. P.	1.6	.1	9.7	209	217
A. P.	1.3	.1	7.7	167	271
Blackberries . . . . . A. P.	1.3	1.0	10.9	262	173
Blackfish . . . . . E. P.	18.7	1.3	—	393	116
A. P.	7.4	.7	—	163	279
Bluefish . . . . . E. P.	19.4	1.2	—	402	113
A. P.	10.0	.6	—	206	220
Boston crackers	11.0	8.5	71.1	1835	25
Brazil nuts . . . . . E. P.	17.0	66.8	7.0	3162	14
A. P.	8.6	33.7	3.5	1591	28

TABLE I — EDIBLE ORGANIC NUTRIENTS (*Continued*)

FOOD		PROTEIN (N×6.25) PER CENT	FAT PER CENT	CARBO-HY-DRATE PER CENT	FUEL VALUE PER POUND CALO-RIES	100 CALORIE PORTION GRAMS
Bread, Boston brown		6.0	6.3	54.0	1345	34
graham		8.9	1.8	52.1	1189	38
rolls, water		9.0	3.0	54.2	1268	36
toasted		11.5	1.6	61.2	1385	33
white, homemade		9.1	1.6	53.3	1199	38
milk		9.6	1.4	51.1	1158	39
Vienna		9.4	1.2	54.1	1199	38
average white		9.2	1.3	53.1	1182	38
whole wheat		9.7	.9	49.7	1113	41
Buckwheat flour		6.4	1.2	77.9	1580	29
Butter		1.0	85.0	—	3491	13
Buttermilk		3.0	.5	4.8	162	280
Butternuts	E. P.	27.9	61.2	3.5	3065	15
	A. P.	3.8	8.3	.5	417	109
Cabbage	E. P.	1.6	.3	5.6	143	317
	A. P.	1.4	.2	4.8	121	376
Calf's-foot jelly		4.3	—	17.4	394	115
Carrots, fresh	E. P.	1.1	.4	9.3	204	221
	A. P.	.9	.2	7.4	158	286
Cauliflower	A. P.	1.8	.5	4.7	139	328
Celery	E. P.	1.1	.1	3.3	84	542
	A. P.	.9	.1	2.6	68	672
Celery soup, canned		2.1	2.8	5.0	243	187
Cerealine		9.6	1.1	78.3	1640	28
Chard	E. P.	3.2	.6	5.0	173	262
Cheese, American pale		28.8	35.9	.3	1990	23
American red		29.6	38.3	—	2102	22
Cheddar		27.7	36.8	4.1	2080	22
cottage		20.9	1.0	4.3	499	91
full cream		25.9	33.7	2.4	1890	24
Fromage de Brie		15.9	21.0	1.4	1170	39
Neufchâtel		18.7	27.4	1.5	1484	31
pineapple		29.9	38.9	2.6	2180	21
roquefort		22.6	29.5	1.8	1645	28
Swiss		27.6	34.9	1.3	1945	23
Cherries, fresh	E. P.	1.0	.8	16.7	354	128
	A. P.	.9	.8	15.9	337	134

TABLE I — EDIBLE ORGANIC NUTRIENTS (*Continued*)

FOOD		PROTEIN (N×6.25) PER CENT	FAT PER CENT	CARBO-HY-DRATE PER CENT	FUEL VALUE PER POUND CALO-RIES	100 CALORIE PORTION GRAMS
Cherries — *Continued*						
canned	A. P.	1.1	.1	21.1	407	112
Chestnuts, fresh	E. P.	6.2	5.4	42.1	1098	41
	A. P.	5.2	4.5	35.4	920	49
Chicken, broilers	E. P.	21.5	2.5	—	493	92
	A. P.	12.8	1.4	—	289	157
Chocolate		12.9	48.7	30.3	2768	16
Cocoa		21.6	28.9	37.7	2258	20
Cod, dressed	A. P.	11.1	.2	—	209	217
salt	E. P.	25.4	.3	—	473	96
	A. P.	19.0	.4	—	361	126
Consommé, canned	A. P.	2.5	—	.4	53	862
Corn, green, canned		2.8	1.2	19.0	455	102
sweet, fresh	E. P.	3.1	1.1	19.7	459	99
	A. P.	1.2	.4	7.7	178	255
Corn meal		9.2	1.9	75.4	1620	28
Cowpeas, dried		21.4	1.4	60.8	1550	29
green	E. P.	9.4	.6	22.7	603	76
Crackers, butter	A. P.	9.6	10.1	71.6	1887	23
cream	A. P.	9.7	12.1	69.7	1938	23
graham	A. P.	10.0	9.4	73.8	1905	24
soda	A. P.	9.8	9.1	73.1	1875	24
water	A. P.	10.7	8.8	71.9	1855	24
Cranberries	A. P.	.4	.6	9.9	212	212
Cream		2.5	18.5	4.5	883	50
Cucumbers	E. P.	.8	.2	3.1	79	575
	A. P.	.7	.2	2.6	68	666
Currants, fresh		1.5	—	12.8	259	175
dried Zante		2.4	1.7	74.2	1455	31
Dandelion greens		2.4	1.0	10.6	277	164
Dates, dried	E. P.	2.1	2.8	78.4	1575	29
	A. P.	1.9	2.5	70.6	1416	32
Doughnuts		6.7	21.0	53.1	1941	23
Eggplant	E. P.	1.2	.3	5.1	126	349
Eggs, uncooked	E. P.	13.4	10.5	—	672	68
	A. P.	11.9	9.3	—	594	76

TABLE I — EDIBLE ORGANIC NUTRIENTS (*Continued*)

FOOD		PROTEIN (N×6.25) PER CENT	FAT PER CENT	CARBO-HY-DRATE PER CENT	FUEL VALUE PER POUND CALO-RIES	100 CALORIE PORTION GRAMS
Farina		11.0	1.4	76.3	1640	28
Figs, dried		4.3	.3	74.2	1437	32
Flounder	A. P.	5.4	.3	—	110	412
	E. P.	14.2	.6	—	282	161
Flour, rye		6.8	.9	78.7	1590	29
wheat, California fine		7.9	1.4	76.4	1585	29
wheat, entire		13.8	1.9	71.9	1630	28
wheat, graham		13.3	2.2	71.4	1628	28
wheat, patent baker's grade		13.3	1.5	72.7	1623	28
wheat, straight grade		10.8	1.1	74.8	1608	28
wheat, average high and medium		11.4	1.0	75.1	1610	28
wheat, average low grade		14.0	1.9	71.2	1625	28
Fowls	E. P.	19.3	16.3	—	1017	45
	A. P.	13.7	12.3	—	752	60
Gelatin		91.4	.1	—	1660	27
Grape butter		1.2	.1	58.5	1088	42
Grapes	E. P.	1.3	1.6	19.2	437	104
	A. P.	1.0	1.2	14.4	328	138
Grapefruit	E. P.	.6	.1	12.2	235	193
	A. P.	.4	.1	8.9	172	264
Haddock	E. P.	17.2	.3	—	324	140
	A. P.	8.4	.2	—	160	283
Halibut steaks	E. P.	18.6	5.2	—	550	83
	A. P.	15.3	4.4	—	457	100
Ham, fresh, lean	E. P.	25.0	14.4	—	1042	44
	A. P.	24.8	14.2	—	1030	44
fresh, medium	E. P.	15.3	28.9	—	1458	31
	A. P.	13.5	25.9	—	1303	35
smoked, lean	E. P.	19.8	20.8	—	1209	38
	A. P.	17.5	18.5	—	1073	42
Herring, whole	E. P.	19.5	7.1	—	644	70
	A. P.	11.2	3.9	—	362	125
smoked	E. P.	36.9	15.8	—	1315	35
	A. P.	20.5	8.8	—	731	62
Hominy		8.3	.6	79.0	1609	28

TABLE I — EDIBLE ORGANIC NUTRIENTS (*Continued*)

FOOD		PROTEIN (N×6.25) PER CENT	FAT PER CENT	CARBO-HY-DRATE PER CENT	FUEL VALUE PER POUND CALO-RIES	100 CALORIE PORTION GRAMS
Honey		.4	—	81.2	1481	31
Huckleberries		.6	.6	16.6	336	135
Kohl-rabi	E. P.	2.0	.1	5.5	140	324
Koumiss		2.8	2.1	5.4	234	194
Lamb, breast	E. P.	19.1	23.6	—	1311	35
	A. P.	15.4	19.1	—	1058	43
chops, broiled	E. P.	21.7	29.9	—	1614	28
fore quarter	E. P.	18.3	25.8	—	1385	33
	A. P.	14.9	21.0	—	1127	40
hind quarter	E. P.	19.6	19.1	—	1149	40
	A. P.	16.5	16.1	—	953	48
leg, roast		19.7	12.7	—	876	52
side	E. P.	17.6	23.1	—	1263	36
	A. P.	14.1	18.7	—	1015	45
Lard, refined		—	100.0	—	4080	11
Lemon juice		—	—	9.8	178	255
Lemons	E. P.	1.0	.7	8.5	201	226
	A. P.	.7	.5	5.9	140	323
Lettuce	E. P.	1.2	.3	2.9	87	525
	A. P.	1.0	.2	2.5	72	633
Liver, beef	E. P.	20.4	4.5	1.7	583	78
	A. P.	20.2	3.1	2.5	538	84
veal	E. P.	19.0	5.3	—	562	81
Lobster, whole	E. P.	16.4	1.8	.4	379	120
	A. P.	5.9	.7	.2	139	326
canned	A. P.	18.1	1.1	.5	382	119
Macaroni		13.4	.9	74.1	1625	28
Macaroons		6.5	15.2	65.2	1922	24
Mackerel	E. P.	18.7	7.1	—	629	72
	A. P.	10.2	4.2	—	356	127
salt	E. P.	21.1	22.6	—	1305	35
	A. P.	16.3	17.4	—	1005	45
Marmalade, orange		.6	.1	84.5	1548	29
Milk, condensed, sweetened		8.8	8.3	54.1	1480	31
skimmed		3.4	.3	5.1	167	273
whole		3.3	4.0	5.0	314	145

TABLE I — EDIBLE ORGANIC NUTRIENTS (*Continued*)

FOOD		PROTEIN (N×6.25) PER CENT	FAT PER CENT	CARBO-HYDRATE PER CENT	FUEL VALUE PER POUND CALORIES	100 CALORIE PORTION GRAMS
Mince meat, commercial		6.7	1.4	60.2	1280	36
homemade		4.8	6.7	32.1	942	48
Molasses, cane		2.4	—	69.3	1302	35
Mushrooms	A. P.	3.5	.4	6.8	204	223
Muskmelons	E. P.	.6	—	9.3	180	252
	A. P.	.3	—	4.6	89	510
Mutton, fore quarter	E. P.	15.6	30.9	—	1543	29
	A. P.	12.3	24.5	—	1223	37
hind quarter	E. P.	16.7	28.1	—	1450	31
	A. P.	13.8	23.2	—	1197	38
leg	E. P.	19.8	12.4	—	863	52
	A. P.	16.5	10.3	—	718	63
side	A. P.	13.0	24.0	—	1215	37
	E. P.	16.2	29.8	—	1512	30
Nectarines	E. P.	.6	—	15.9	299	152
	A. P.	.6	—	14.8	280	162
Oatmeal		16.1	7.2	67.5	1811	25
Okra	E. P.	1.6	.2	7.4	172	264
	A. P.	1.4	.2	6.5	152	300
Olives, green	E. P.	1.1	27.6	11.6	1357	33
	A. P.	.8	20.2	8.5	995	46
ripe	E. P.	1.7	25.0	4.3	1130	40
	A. P.	1.4	21.0	3.5	947	48
Onions, fresh	E. P.	1.6	.3	9.9	220	206
	A. P.	1.4	.3	8.9	199	228
Oranges	E. P.	.8	.2	11.6	233	195
	A. P.	.6	.1	8.5	169	268
Oxtail soup, canned	A. P.	3.8	.5	4.2	166	274
Oysters	E. P.	6.2	1.2	3.7	228	199
in shell	A. P.	1.2	.2	.7	43	1065
canned	A. P.	8.8	2.4	3.9	328	138
Parsnips	E. P.	1.6	.5	13.5	294	154
	A. P.	1.3	.4	10.8	236	192
Pea soup, canned	A. P.	3.6	.7	7.6	232	196
Peaches, canned	A. P.	.7	.1	10.8	213	213
fresh	E. P.	.7	.1	9.4	188	242
	A. P.	.5	.1	7.7	153	297

TABLE I — EDIBLE ORGANIC NUTRIENTS (*Continued*)

FOOD		PROTEIN (N×6.25) PER CENT	FAT PER CENT	CARBOHYDRATE PER CENT	FUEL VALUE PER POUND CALORIES	100 CALORIE PORTION GRAMS
Peanuts	E. P.	25.8	38.6	24.4	2490	18
	A. P.	19.5	29.1	18.5	1877	24
Pears, fresh	E. P.	.6	.5	14.1	288	158
	A. P.	.5	.4	12.7	256	177
Peas, canned	A. P.	3.6	.2	9.8	252	180
dried		24.6	1.0	62.0	1611	28
green	E. P.	7.0	.5	16.9	454	100
	A. P.	3.6	.2	9.8	252	180
Peppers, green	E. P.	1.1	.1	4.6	109	417
Persimmons	E. P.	.8	.7	31.5	615	74
Pies, apple		3.1	9.8	42.8	1233	37
custard		4.2	6.3	26.1	806	56
lemon		3.6	10.1	37.4	1156	39
mince		5.8	12.3	38.1	1300	35
squash		4.4	8.4	21.7	817	56
Pineapples, fresh	E. P.	.4	.3	9.7	196	232
canned	A. P.	.4	.7	36.4	695	65
Pine nuts (pignolias)		33.9	49.4	6.9	2757	16
Pistachios, shelled		22.3	54.0	16.3	2900	16
Plums	E. P.	1.0	—	20.1	383	118
	A. P.	.9	—	19.1	363	125
Pomegranates	E. P.	1.5	1.6	19.5	447	102
Pork, chops, medium	E. P.	16.6	30.1	—	1530	30
	A. P.	13.4	24.2	—	1230	37
chuck ribs and shoulder	E. P.	17.3	31.1	—	1585	29
	A. P.	14.1	25.5	—	1298	35
fat, salt	A. P.	1.9	86.2	—	3555	13
sausage	A. P.	13.0	44.2	1.1	2030	22
side	E. P.	9.1	55.3	—	2423	19
	A. P.	8.0	49.0	—	2145	21
tenderloin	A. P.	18.9	13.0	—	875	52
Potato chips	A. P.	6.8	39.8	46.7	2598	17
Potatoes, white, raw	E. P.	2.2	.1	18.4	378	120
	A. P.	1.8	.1	14.7	302	149
sweet, raw	E. P.	1.8	.7	27.4	558	81
	A. P.	1.4	.6	21.9	447	102

TABLE I — EDIBLE ORGANIC NUTRIENTS (*Continued*)

FOOD		PROTEIN (N×6.25) PER CENT	FAT PER CENT	CARBO-HY-DRATE PER CENT	FUEL VALUE PER POUND CALO-RIES	100 CALORIE PORTION GRAMS
Prunes, dried	E. P.	2.1	—	73.3	1368	33
	A. P.	1.8	—	62.2	1160	39
Pumpkins	E. P.	1.0	.1	5.2	117	389
	A. P.	.5	.1	2.6	60	753
Radishes	E. P.	1.3	.1	5.8	133	341
	A. P.	.9	.1	4.0	91	488
Raisins	E. P.	2.6	3.3	76.1	1562	29
	A. P.	2.3	3.0	68.5	1407	32
Raspberries, red		1.0	—	12.6	247	184
black		1.7	1.0	12.6	300	151
Rhubarb	E. P.	.6	.7	3.6	105	433
	A. P.	.4	.4	2.2	63	714
Rice		8.0	.3	79.0	1591	29
Salmon, dressed	A. P.	13.8	8.1	—	582	78
whole	E. P.	22.0	12.8	—	923	49
	A. P.	15.3	8.9	—	642	71
Sausage, Bologna	E. P.	18.7	17.6	.3	1061	43
	A. P.	18.2	19.7	—	1135	40
farmer	E. P.	29.0	42.0	—	2240	20
	A. P.	27.9	40.4	—	2156	21
Shad, whole	E. P.	18.8	9.5	—	727	61
	A. P.	9.4	4.8	—	367	124
roe		20.9	3.8	2.6	582	78
Shredded wheat		10.5	1.4	77.9	1660	27
Spinach, fresh	A. P.	2.1	.3	3.2	109	417
Squash	E. P.	1.4	.5	9.0	209	217
	A. P.	.7	.2	4.5	103	443
Strawberries		1.0	.6	7.4	169	269
Succotash, canned		3.6	1.0	18.6	444	102
Sugar		—	—	100.0	1815	25
Tomatoes, fresh	A. P.	.9	.4	3.9	104	438
canned	A. P.	1.2	.2	4.0	103	443
Tuna (tunny fish)	E. P.	26.6	11.4	—	946	48
Turkey	E. P.	21.1	22.9	—	1320	34
	A. P.	16.1	18.4	—	1042	43
sandwich, canned		20.7	29.2	—	1568	29

TABLE I — EDIBLE ORGANIC NUTRIENTS (*Continued*)

FOOD		PROTEIN (N×6.25) PER CENT	FAT PER CENT	CARBO-HYDRATE PER CENT	FUEL VALUE PER POUND CALORIES	100 CALORIE PORTION GRAMS
Turnips	E. P.	1.3	.2	8.1	178	256
	A. P.	.9	.1	5.7	124	367
Veal, breast	E. P.	20.3	11.0	—	817	56
	A. P.	15.3	8.6	—	629	72
cutlet	E. P.	20.3	7.7	—	683	66
	A. P.	20.1	7.5	—	670	68
fore quarter	E. P.	20.0	8.0	—	690	66
	A. P.	15.1	6.0	—	517	88
hind quarter	E. P.	20.7	8.3	—	715	64
	A. P.	16.2	6.6	—	534	85
side	E. P.	20.2	8.1	—	697	65
	A. P.	15.6	6.3	—	539	84
Vegetable soup, canned		2.9	—	.5	62	735
Walnuts, California or English	E. P.	18.4	64.4	13.0	3199	14
	A. P.	4.9	17.3	3.5	859	53
black	E. P.	27.6	56.3	11.7	3011	15
	A. P.	7.2	14.6	3.0	780	59
Watermelons	E. P.	.4	.2	6.7	136	332
	A. P.	.2	.1	2.7	57	800
Wheat, cracked		11.1	1.7	75.5	1635	28
Whitefish	E. P.	22.9	6.5	—	680	67
	A. P.	10.6	3.0	—	315	144
Zwieback		9.8	9.9	73.5	1915	24

TABLE II

ASH CONSTITUENTS OF FOODS IN PERCENTAGE OF THE EDIBLE PORTION
(Compiled from Various Sources)

FOOD	CALCIUM (Ca)	MAGNESIUM (Mg)	POTASSIUM (K)	SODIUM (Na)	PHOSPHORUS (P)	CHLORINE (Cl)	SULPHUR (S)	IRON (Fe)
Almonds	.239	.251	.741	.019	.465	.037	.160	.0039
Apples	.007	.008	.127	.011	.012	.005	.006	.0003
dried	.032	.037	(.623)	(.050)	.048	(.025)	?	(.0015)
Apricots	.014	.010	.248	.038	.025	.002	.010	(.0003)
dried	(.066)	(.047)	(1.157)	(.177)	(.117)	(.009)	?	(.0014)
Asparagus	.025	.011	.196	.007	.039	.039	.041	.0010
Bacon (10% protein)	.006	.012	.169	.042	.108	.038	.115	.0015
Bananas	.009	.028	.401	.034	.031	.125	.010	.0006
Barley, entire	.043	.141	.477	.076	.400	.016	.153	.0041
pearled	.020	(.070)	(.241)	(.037)	.181	(.016)	(.120)	(.0020)
Beans, dried	.160	.156	1.229	.097	.471	.032	.215	.0070
kidney, dry	.132	.139	1.144	.041	.475	.041	.227	.0072
Lima, dry	.071	.188	1.741	.249	.338	.026	.161	.0070
Lima, fresh	.028	(.070)	(.613)	(.088)	.133	(.009)	(.057)	.0020
string, fresh	.046	.025	.247	.019	.052	.024	.030	.0011
Beef lean (20% protein)	.012	.024	.338	.084	.216	.076	.230	.0030
Beets	.029	.021	.353	.093	.039	.058	.016	.0006
Blackberries	.017	.021	.169	(.007)	.034	(.010)	.020	.0006
Blood (avg.)	.008	.004	.075	.261	.031	.280	.137	.0526
Blueberries	.020	.007	.051	.016	.008	.008	.011	.0009
Bluefish (See Fish)								
Bread,								
Boston brown	.129	.078	(.232)	(.394)	.185	(.607)	.201	(.0030)
"entire wheat"	(.05)	(.05)	(.208)	(.394)	(.175)	(.607)	(.120)	(.0016)
graham	(.05)	(.05)	(.291)	(.394)	(.218)	(.607)	.150	(.0025)
rye	.024	.039	.151	.701	.148	1.025	.104	(.0016)
white	.027	.023	.108	(.394)	.093	(.607)	.105	.0009
Breadfruit	.084	.007	.235	.027	.068	.100	.049	
Brussels sprouts	.027	.040	.375	.004	.120	.040	.194	(.0011)
Buckwheat flour	.039	.048	.130	.027	.226	.012	.071	.0012
Butter	.015	.001	.014	(.788)	.017	(1.212)	(.010)	.0002
Buttermilk	.105	.016	.151	.064	.097	.099	.026	.00025

TABLE II — ASH CONSTITUENTS IN PERCENTAGE (*Continued*)

FOOD	CALCIUM (Ca)	MAGNESIUM (Mg)	POTASSIUM (K)	SODIUM (Na)	PHOSPHORUS (P)	CHLORINE (Cl)	SULPHUR (S)	IRON (Fe)
Cabbage	.045	.015	.247	.027	.029	.024	.066	.0011
Cabbage greens	.106	.030	.512	.025	.099	.068	.173	.0018
Cantaloupe	.017	.012	.235	.061	.015	.041	.014	.0003
Capers	.122	.022	.209	.051	.062	—	—	—
Carp (See Fish)								
Carrots	.056	.021	.287	.101	.046	.036	.022	.0006
Cauliflower	.123	.014	.222	.068	.061	.050	.086	.0006
Caviar	.137	.022	.422	.874	.176	1.819	—	—
Celery	.078	.014	.316	.084	.037	.156	.022	.0005
Chard	.150	.071	.318	.086	.040	.039	.124	(.0025)
Cheese	.931	.037	.089	.606	.683	.880	.263	.0013
Cherries	.019	.016	.213	.023	.031	.014	.011	.0004
Cherry juice	.017	.011	.200	.013	.018	.003	.006	(.0003)
Chestnuts	.034	.051	.560	.065	.093	.006	.068	.0007
Chicken (See Meat)								
Chocolate	.092	(.293)	(.563)	.012	.455	(.051)	.085	(.0027)
Cider	.008	.011	.095	.020	.009	.006	.006	(.0002)
Citron	.121	.018	.210	.011	.033	.003	.020	—
Clams, round	.106	.098	.131	.705	.046	1.220	.224	—
soft, long	.124	.079	.212	.500	.122	.910	.213	—
Cocoa	.112	.420	.900	.059	.709	.051	.203	.0027
Coconut, dried	.059	.059	.597	.073	.155	.239	(.056)	—
fresh	.024	.020	.300	.036	.074	.120	.028	—
Coconut milk	.020	.009	.144	—	.010	—	.008	—
Cod (See Fish)								
Corn(maize),mature	.020	.121	.339	.036	.283	.045	.151	.0029
meal	.018	.084	.213	.039	.190	.146	.111	.0009
sweet	.006	.033	.113	.040	.103	.014	.046	.0008
sweet, dried	.021	.121	.414	.146	.376	.050	.167	.0029
Cotton-seed meal	.265	.462	1.390	.234	1.193	.037	.485	—
Cowpeas	.100	.208	1.402	.161	.456	.040	.240	—
Crackers	.022	.011	.100	(.594)	.102	(.910)	.125	.0015
Cranberries	.018	.007	.077	.010	.013	.009	.007	.0006
Cream	.086	.010	.126	.035	.067	.080	.030	.00022
Cucumbers	.016	.009	.140	.010	.033	.030	.020	.0002
Currants, dried	.082	.044	.873	.081	.195	.060	.044	(.0025)
fresh	.026	.017	.211	.007	.038	.006	.014	.0005

TABLE II — ASH CONSTITUENTS IN PERCENTAGE (*Continued*)

FOOD	CALCIUM (Ca)	MAGNESIUM (Mg)	POTASSIUM (K)	SODIUM (Na)	PHOSPHORUS (P)	CHLORINE (Cl)	SULPHUR (S)	IRON (Fe)
Currant juice . .	.021	.010	.185	(.006)	.018	.004	.005	—
Dandelion . . .	.105	.036	.461	.168	.072	.099	.017	.0027
Dates	.065	.069	.611	.055	.056	.228	.070	.0030
Duck (See Meat)								
Eggplant	.011	.015	(.140)	(.010)	.034	.024	.016	.0005
Eggs	.067	.011	.140	.143	.180	.106	.195	.0030
Egg white . . .	.015	.010	.160	.156	.014	.155	.216	.0001
Egg yolk	.137	.016	.115	.075	.524	.094	.166	.0086
Endive	.104	.013	.380	.109	.038	.167	.035	—
Farina	.021	.025	.120	.065	.125	.076	.155	.0008
Figs, dried . . .	.162	.071	.964	.046	.116	.043	.056	.0030
fresh	.053	.022	.303	.012	.036	.014	.010	—
Fish *								
Flaxseed	.204	.252	.901	.050	.627	.022	.170	—
Flour, buckwheat .	.010	.048	.130	.027	.176	.012	.071	.0012
"entire wheat" .	.031	(.090)	(.274)	(.037)	.238	(.070)	(.180)	.0025
graham . . .	.039	(.133)	(.457)	(.037)	.364	(.070)	.183	.0037
white	.020	.018	.115	.060	.092	.074	.177	.0010
rye	.018	.081	.463	.019	.289	.055	.123	.0013
Fowl (See Meat)								
Gluten feed . . .	.247	.221	.250	.420	.542	.090	.558	—
Goose (See Meat)								
Gooseberries . .	.035	.014	.197	.038	.031	—	.011	.0005
Grapefruit . . .	.021	.009	.161	.004	.020	.005	.010	.0003
Grapejuice . . .	.011	.009	.106	.005	.011	.002	.009	.0003
Grapes	.019	.010	.197	.015	.031	.005	.024	.0003
Guava	.014	.008	.384	—	.030	.045	—	—
Haddock (See Fish)								
Halibut (See Fish)								
Ham (See Meat)								
Hazelnuts . . .	.287	.140	.618	.019	.354	.067	.198	.0041
Herring (See Fish)								
Hominy	.011	.058	.174	.020	.144	.046	(.136)	(.0009)

* Average fish is estimated to contain **per 100 grams of protein** as follows: 0.109 gram Ca; 0.133 gram Mg; 1.671 grams K; 0.373 gram Na; 1.148 grams P; 0.528 gram Cl; 1.119 grams S; 0.0055 gram Fe.

TABLE II — ASH CONSTITUENTS IN PERCENTAGE (*Continued*)

FOOD	CALCIUM (Ca)	MAGNESIUM (Mg)	POTASSIUM (K)	SODIUM (Na)	PHOSPHORUS (P)	CHLORINE (Cl)	SULPHUR (S)	IRON (Fe)
Honey	.004	.018	.386	.001	.019	.029	.001	.0007
Horseradish	.096	.039	.468	.062	.076	.016	.190	—
Huckleberries	.020	.007	.051	.016	.008	.008	.011	.0009
Huckleberry wine	.009	.004	.042	.006	.004	.001	.006	—
Jam *								
Jelly	.014	(.010)	(.100)	(.013)	.008	(.004)	(.007)	(.0003)
Kohl-rabi	.077	.030	.370	.050	.071	.053	.057	.0006
Lamb (See Meat)								
Leeks	.058	.014	.199	.081	.006	.024	.072	—
Lemons	.036	.007	.175	.004	.022	.002	.011	.0006
Lemon juice	.024	.010	.127	.009	.010	.003	.006	—
Lemon, sweet	.030	.006	.442	—	.042	.013	.016	—
Lentils, dry	.107	.101	.877	.062	.438	.050	.277	.0086
Lettuce	.043	.017	.339	.027	.042	.074	.014	.0007
Limes	.055	.014	.350	.062	.036	.039	.010	—
Lime juice	—	—	—	—	—	—	.003	—
Linseed meal	.413	.432	1.083	.251	.741	.085	.396	—
Lupins, dry	.191	.191	.840	.073	.520	.034	—	—
Macaroni	.022	.037	.130	.008	.144	.073	.172	.0012
Mackerel (See Fish)								
Mamey	.009	.012	.345	—	.028	.140	—	—
Mango	.021	.007	.235	—	.017	.019	.013	—
Mangolds	.026	.030	.334	.071	.038	.082	.026	—
Maple syrup	.107	.034	.208	.010	.013	(.010)	(.005)	(.003)
Meat †								
Meat extract, solid	.085	.363	7.347	2.394	2.800	3.117	—	—
Meat peptone	.025	.124	2.440	.641	1.130	.561	.222	—
Milk (cow's), whole	.120	.012	.143	.051	.093	.106	.034	.00024
(cow's), skimmed	(.122)	(.012)	(.149)	(.052)	(.096)	(.110)	(.035)	.00025
(cow's), condensed	(.300)	(.032)	(.374)	(.134)	.235	(.280)	(.090)	.0006

* The percentages of the ash constituents in jams are believed to average about two thirds those of the corresponding fruits.

† Average meat is estimated to contain **per 100 grams protein** as follows: 0.058 gram Ca; 0.118 gram Mg; 1.694 grams K; 0.421 gram Na; 1.078 grams P; 0.378 gram Cl; 1.146 grams S; 0.0150 gram Fe.

TABLE II — ASH CONSTITUENTS IN PERCENTAGE (*Continued*)

FOOD	CALCIUM (Ca)	MAGNESIUM (Mg)	POTASSIUM (K)	SODIUM (Na)	PHOSPHORUS (P)	CHLORINE (Cl)	SULPHUR (S)	IRON (Fe)
Milk — *Cont.*								
buffalo	.203	.016	.099	.038	.125	.062	—	—
camel's	.143	.021	.114	.019	.098	.105	—	—
goat's	.128	.013	.145	.079	.103	.014	.037	—
human	.034	.005	.047	.010	.015	.035	—	—
mare's	.083	.007	.081	.010	.054	.029	—	—
sheep's	.207	.008	.187	.030	.123	.071	—	—
Millet	.014	.167	.290	.085	.327	.019	—	—
Molasses	.211	.068	1.349	.019	.044	.317	.129	.0073
Mushrooms	.017	.016	.384	.027	.108	.021	.051	—
Muskmelon	.017	.012	.235	.061	.015	.041	.014	.0003
Mustard	.492	.260	.761	.056	.755	.016	1.230	—
Mutton (See Meat)								
Oatmeal	.069	.110	.344	.062	.392	.069	.202	.0038
Okra	.071	.010	.035	.043	.019	—	—	—
Olives	.122	.002	1.526	.128	.014	.004	.027	.0029
Onions	.034	.016	.178	.016	.045	.021	.070	.0006
Oranges	.045	.012	.177	.012	.021	.006	.011	.0002
Orange juice	.029	.011	.182	.008	.016	.003	.009	.0002
Oysters	.052	.037	.091	.459	.155	.590	.187	.0045
Paprika	.229	.164	2.075	.178	.341	.155	—	—
Parsnips	.059	.034	.518	.004	.076	.030	.036	.0006
Peaches	.016	.010	.214	.022	.024	.004	.009	.0003
dried	.034	.056	(.830)	.082	.146	—	.212	(.0012)
Peanuts	.071	.180	.654	.050	.399	.056	.224	.0020
Pears	.015	.011	.132	.016	.026	.011	.010	.0003
Pear juice	.009	.008	.140	—	.011	—	.009	—
Peas, dried	.084	.149	.903	.104	.400	.035	.219	.0057
fresh	.028	.038	.285	.013	.127	.024	.063	.0017
Pecan nuts	.089	.152	(.332)	—	.335	.050	.113	.0026
Pepper, green, fresh	.006	.010	(.139)	—	.026	.013	.014	.0004
Pepper, black, dry	.440	.156	1.140	.131	.188	.312	—	—
Pepper, white, dry	.425	.113	—	—	.233	.029	—	—
Perch (See Fish)								
Persimmons	.022	.009	.292	.011	.021	.002	.005	—
Pineapple	.018	.011	.321	.016	.028	.051	.009	.0005
Plums	.020	.011	.203	.019	.032	.002	.009	.0005

TABLE II — ASH CONSTITUENTS IN PERCENTAGE (*Continued*)

FOOD	CALCIUM (Ca)	MAGNESIUM (Mg)	POTASSIUM (K)	SODIUM (Na)	PHOSPHORUS (P)	CHLORINE (Cl)	SULPHUR (S)	IRON (Fe)
Pomegranate . .	.011	.005	.063	.085	.105	.003	—	.0004
Pork (10% protein)	.006	.012	.169	.042	.108	.038	.115	.0015
Potatoes	.014	.028	.429	.021	.058	.038	.030	.0013
sweet	.019	.028	.397	.039	045	.094	.024	.0005
Prunes, dried . .	.054	.055	1.030	.069	.105	.017	.037	.0030
Pumpkin	.023	.008	(.320)	.065	.059	—	.021	(.0008)
Radishes . . .	.021	.012	.218	.069	.029	.054	.041	.0006
Raisins	.064	.083	.820	.133	.132	.082	.051	.0021
Raspberries . . .	.049	.024	.173	—	.052	—	.017	.0006
Raspberry juice .	.021	.016	.134	.005	.012	—	.009	—
Rhubarb	.044	.017	.325	.025	.031	.036	.013	.0010
Rice, brown . . .	—	—	—	—	.207	—	—	.0020
white	.009	.033	.070	.025	.096	.054	.117	.0009
Romaine (salad) .	.045	.032	.306	.016	.053	.073	.019	—
Rutabagas . . .	.074	.018	.399	.083	.056	.058	.083	—
Rye, entire . . .	.055	.130	.453	.035	.385	.025	.170	.0039
(See also Bread and Flour)								
Salmon (See Fish)								
Sapato	.026	.008	.179	—	.006	.087	—	—
Shredded wheat .	.041	.144	—	—	.324	—	—	.0045
Shrimp	.096	—	—	—	—	—	—	—
Soup, canned . .	.036	—	.033	—	.030	—	—	—
canned vegetable	.025	.013	.101	—	.038	—	.025	—
Spinach	.067	.037	.774	.125	.068	.074	.038	.0036
Squash, summer, seeds removed	.018	.008	.150	.002	—	—	—	(.0006)
with seeds . .	.024	.012	.180	.004	—	—	—	(.0006)
Squash, winter . .	.019	.011	.320	.004	—	—	—	(.0006)
Strawberries . .	.041	.019	.147	.050	.028	.006	.014	.0008
Tamarind . . .	.007	.021	—	—	.072	.007	.009	—
Tapioca	.023	—	—	—	.090	.018	.029	.0016
Tomatoes . . .	.011	.010	.275	.010	.026	.034	.014	.0004
Tomato juice . .	.006	.010	.310	.015	.015	.055	—	—
Truffles	.024	.018	.404	.077	.062	.039	—	—
Turnips	.064	.017	.338	.056	.046	.041	.065	.0005
Turnip tops . .	.347	.028	.307	.082	.049	.168	.069	—

TABLE II — ASH CONSTITUENTS IN PERCENTAGE (*Continued*)

FOOD	CALCIUM (Ca)	MAGNESIUM (Mg)	POTASSIUM (K)	SODIUM (Na)	PHOSPHORUS (P)	CHLORINE (Cl)	SULPHUR (S)	IRON (Fe)
Veal (See Meat)								
Vinegar (cider) .	.016	.008	.165	—	.013	—	.017	(.0003)
Walnuts	.089	.134	(.332)	—	.358	.040	.172	.0021
Water cress . . .	.187?	.034	.287	.099	.005	.061	.167	.0019
Watermelon . .	.011	.003	.073	.008	.003	.008	.007	
Wheat, entire . . (See also Bread and Flour)	.045	.133	.473	.039	.423	.068	.181	.0050
Wheat bran . . .	.120	.511	1.217	.154	1.215	.090	.247	.0078
Wheat germ . .	.071	.342	.296	.722	1.050	.070	.325	—
Wheat gluten . .	.078	.045	.007	.028	.200	.050	.920	—
Whey	.044	.008	.157	.038	.035	.119	.009	?
Whortleberries, entire	.031	.021	.261	.021	.042	—	—	—
flesh only . . .	.020	.011	.087	—	.018	—	—	—
Wine (avg.) . . .	.009	.010	.104	.008	.015	.011	.015	(.0003)

TABLE III

PROTEIN, CALCIUM, PHOSPHORUS, AND IRON IN GRAMS PER 100 CALORIES OF FOOD MATERIAL

(Estimated from data compiled from various sources)

FOOD	PROTEIN	CALCIUM (Ca)	PHOSPHORUS (P)	IRON (Fe)	CaO	P_2O_5
	Grams	Grams	Grams	Grams	Grams	Grams
Almonds	3.22	.037	.072	.00060	.052	.165
Apples	0.64	.012	.020	.00048	.016	.045
Apricots	1.90	.023	.044	.00052	.033	(.100)
Asparagus	8.10	.122	.177	.00451	.171	.405
Bacon (See Meat)						

TABLE III — AMOUNTS PER 100-CALORIES (*Continued*)

FOOD	PROTEIN	CALCIUM (Ca)	PHOSPHORUS (P)	IRON (Fe)	CaO	P_2O_5
	Grams	Grams	Grams	Grams	Grams	Grams
Bananas	1.32	.009	.031	.00061	.012	.072
Beans, dried	6.52	.047	.137	.00203	.065	.314
kidney	5.83	(.040)	(.143)	(.00216)	(.056)	(.326)
Lima	5.80	.020	.096	.00200	.028	.221
string	5.55	.110	.126	.00265	.154	.289
Beef (See Meat)						
Beer	—	.008	.061	.00217	.011	.140
Beets	3.47	.064	.084	.00130	.089	.193
Blackberries	2.25	.029	.058	.00104	.042	.133
Blueberries	(0.8)	(.027)	(.011)	(.0012)	(.038)	(.025)
Bluefish (See Fish)						
Bread, Boston brown	2.64	.056	.082	(.0013)	.079	.187
"entire" wheat	3.95	(.020)	.071	(.00065)	(.028)	(.163)
graham	3.42	(.020)	.084	(.00096)	(.028)	(.192)
rye	3.54	.009	.058	.00063	.013	.133
white	3.50	.011	.035	.00035	.015	.081
Brussels sprouts	(7.30)	(.086)	(.380)	(.00349)	(.121)	(.870)
Buckwheat flour	1.85	.011	.065	.00034	.015	.148
Butter	0.13	.002	.002	.00003	.003	.005
Buttermilk	8.40	.294	.271	.00070	.411	.621
Cabbage	5.07	.143	.092	.00349	.200	.210
Cantaloupe	1.51	.044	.038	.00071	.061	.088
Carp (See Fish)						
Carrots	2.42	.124	.101	.00133	.173	.232
Cauliflower	5.90	.403	.200	.00197	.564	.459
Celery	1.28	.421	.201	.00270	.589	.460
Chard	8.37	.393	.105	(.00655)	.550	.240
Cheese	6.05	.212	.156	.00030	.297	.357
Cherries	1.20?	.025	.039	.00051	.035	.090
Chestnuts	2.55	.014	.044	.00029	.019	.088
Chicken (See Meat)						
Chocolate	2.11	.015	.075	(.00044)	.021	.171
Citron	0.15	.037	.010	.00099	.052	.023
Clams, long	19.82	.285	.282	(.00970)	.399	.645
round	14.01	.229	.100	(.00970)	.321	.228
Cocoa	4.35	.023	.143	.00054	.032	.327
Coconut	0.95	.006	.018	(.00030)	.009	.041
Cod (See Fish)						

TABLE III — AMOUNTS PER 100-CALORIES (*Continued*)

FOOD	PROTEIN	CALCIUM (Ca)	PHOSPHORUS (P)	IRON (Fe)	CaO	P_2O_5
	Grams	Grams	Grams	Grams	Grams	Grams
Corn	3.06	.006	.102	.00079	(.008)	(.233)
Corn meal	2.59	.005	.053	.0003	.007	.121
Cotton-seed meal	12.80	.066	.298	—	.092	.682
Cowpeas	6.20	.029	.132	—	.041	.303
Crackers, "soda"	2.37	.006	.025	.00036	.008	.057
Cranberries	0.85	.039	.027	.00129	.054	.062
Cream, 18.5 per cent fat	1.27	.050	.044	.0001	.072	.100
40 per cent fat	0.58	.020	.020	.00005	.032	.045
Cucumbers	4.60	.090	.191	.00115	.126	.437
Currants, dried (Zante)	0.75	.026	.061	.00087	.036	.139
fresh	2.62	.045	.066	.00087	.063	.150
Dandelion greens	3.93	.172	.117	.0044	.241	.269
Dates	0.60	.019	.016	.00086	.026	.037
Duck (See Meat)						
Eggplant	4.30	.041	.122	.00184	.057	.280
Eggs	9.05	.045	.122	.00205	.063	.279
Egg white	24.12	.020	.022	.00020	.028	.050
Egg yolk	4.32	.036	.118	.00230	.050	.270
Farina	3.05	.006	.035	.00022	.008	.079
Figs	1.35	.051	.037	.00095	.072	.084
Fish (See footnote on page 589)						
Flour, buckwheat	1.84	.011	.065	.00034	.015	.148
"entire" wheat	3.85	.009	.066	.0007	.012	.152
graham	3.71	.011	.101	.00100	.015	.232
white (wheat)	3.20	.006	.026	.00023	.008	.060
rye	1.95	.005	.082	.00037	.007	.188
Fowl (See Meat)						
Goose (See Meat)						
Grapefruit	1.15	.040	.036	.00058	.056	.083
Grapes	1.35	.019	.032	.00031	.027	.074
Grapejuice	0.35	(.011)	.011	.0003	.015	.025
Haddock (See Fish)						
Halibut (See Fish)						
Ham (See Meat)						
Hazelnuts	—	.041	.050	.00057	.057	.115
Herring (See Fish)						
Hominy	2.35	.002	.027	.00025	.002	.063

TABLE III — AMOUNTS PER 100-CALORIES (*Continued*)

FOOD	PROTEIN	CALCIUM (Ca)	PHOSPHORUS (P)	IRON (Fe)	CaO	P_2O_5
	Grams	Grams	Grams	Grams	Grams	Grams
Honey	0.12	.002	.006	.0003	.002	.013
Huckleberries	0.82	.027	.011	.0012	.038	.025
Kohl-rabi	6.48	.249	.186	.00194	.349	.426
Lamb (See Meat)						
Lemons	2.25	.081	.049	.00135	.113	.112
Lemon juice	—	.060	—	—	.084	.059
Lentils	7.37	.031	.126	.00247	.043	.288
Lettuce	6.27	.224	.224	.00367	.314	.513
Linseed meal	—	—	—	—	—	—
Lupins	—	—	—	—	—	—
Macaroni	3.70	.006	.040	.00033	.008	.092
Mackerel (See Fish)						
Maple syrup	—	.037	(.003)	(.001)	.053	(.007)
Meat (See footnote on page 590)						
Milk, whole	4.75	.174	.134	.00035	.243	.308
skimmed	9.25	(.331)	.262	(.00068)	(.463)	(.600)
condensed, sweetened	2.70	(.096)	.072	(.0002)	(.135)	.165
condensed, unsweetened	5.75	.189	.146	(.0004)	(.264)	.335
Molasses	0.83	.074	.015	.00255	.102	.035
Muskmelon	1.51	.043	.038	.0008	.060	.088
Mutton (See Meat)						
Oatmeal	4.20	.017	.099	.00096	.024	.226
Olives	0.37	.041	.004	.00097	.057	.010
Onions	3.30	.069	.093	.0010	.097	.212
Oranges	1.55	.088	.040	.00039	.123	.091
Orange juice	1.44	.067	.037	.00046	.093	.082
Oysters	12.30	.106	.306	.00893	.149	.702
Parsnips	2.47	.091	.117	.0009	.128	.268
Peaches	1.70	.038	.057	.00073	.053	.130
Peanuts	4.70	.013	.073	.00036	.018	.166
Pears	0.95	.024	.041	.00047	.033	.093
Peas	6.92	.026	.127	.00165	.036	.274
Pecans	1.30	.012	.045	.00035	.017	.104
Pepper, green	4.59	.034	.145	.00222	.047	.333
Perch (See Fish)						
Persimmons	—	—	—	—	—	—
Pineapple, fresh	0.92	.041	.064	.00116	.058	.146

TABLE III — AMOUNTS PER 100-CALORIES (*Continued*)

FOOD	PROTEIN	CALCIUM (Ca)	PHOSPHORUS (P)	IRON (Fe)	CaO	P_2O_5
	Grams	Grams	Grams	Grams	Grams	Grams
Plums	1.20	.024	.038	.00059	.033	.087
Pork (See Meat)						
Potatoes	2.65	.016	.069	.00156	.023	.158
sweet	1.45	.016	.037	.00041	.023	.084
Prunes	0.70	.018	.035	.00100	.025	.080
Pumpkin	3.90	.089	.229	(.00130)	.125	.525
Radishes	4.42	.073	.098	.00205	.102	.225
Raisins	0.75	.019	.038	.00061	.026	.088
Raspberries	2.57	.074	.078	.00091	.104	.178
Rhubarb	2.60	.189	.134	.00433	.264	.307
Rice, brown	2.52	(.003)	.060	.00058	(.004)	.138
white	2.27	.001+	.027	.00026	.003	.063
Rutabagas	3.15	.185	.140	—	.259	.322
Rye, entire	—	—	—	—	—	—
Salmon (See Fish)						
Shredded wheat	3.50	.011	.089	.00123	.016	.203
Spinach	8.79	.281	.285	.01506	.393	.653
Squash, summer	3.05	.039	.035	(.0013)	.054	.080
winter	3.10	.040	.061	(.0013)	.056	.139
Strawberries	2.56	.104	.072	.00205	.146	.164
Tapioca	0.11	.004	.025	.00045	.006	.058
Tomatoes	3.95	.050	.113	.00175	.070	.259
Turnips	3.30	.161	.117	.00127	.226	.269
Turnip tops	—	—	—	—	—	—
Veal (See Meat)						
Vinegar (cider)	—	.111	.090	.00213	.156	.206
Walnuts, California or English	2.60	.013	.015	.00030	.018	.116
Water cress	—	—	—	—	—	—
Watermelon	1.32	.038	.010	(.00099)	.053	.023
Wheat, entire	3.63?	.013	.118	.00140	.018	.270
Wheat germ	—	—	—	—	—	—
Wheat gluten	—	—	—	—	—	—
Whey	3.74	.165	.131	?	.231	.300
Whortleberries	—	—	—	—	—	—
Wine (average, 10 per cent alcohol)	—	.011	.021	.00167	.016	.047

APPENDIX C

FOODS AS SOURCES OF VITAMINS A, B, AND C

+ indicates that the food contains the vitamin.

++ indicates that the food is a good source of the vitamin.

+++ indicates that the food is an excellent source of the vitamin.

– indicates that the food contains no appreciable amount of the vitamin.

? indicates doubt as to presence or relative amount.

* indicates that evidence is lacking or appears insufficient.

Food	Vitamin A	Vitamin B	Vitamin C
Alfalfa	+++	++	*
Almonds	+	++	*
Apples, raw	+	+ to ++	++
Apples, canned	*	*	– to ++
Apples, dried, cooked	*	+	+
Artichoke, French	++	+	*
Asparagus	*	+++?	*
Avocado (alligator pear)	+	++	*
Bacon	– to +	+ to ++	?
Bananas	+ to ++	+ to ++	++
Barley, whole	+	++	–
Beans, kidney	+	+++	*
Beans, navy, dry or canned	+	+++	*
Beans, soy	+	+++	*
Beans, sprouted	+	++(?)	++
Beans, string, fresh, raw	++	++	++
Beans, string, cooked	++	++	*
Beechnuts	*	++	*
Beef	+	++	– to +

FOODS AS SOURCES OF VITAMINS A, B, AND C (*Continued*)

FOOD	VITAMIN A	VITAMIN B	VITAMIN C
Beef juice	*	+	− to +
Beef fat	++	−	−
Beets (roots)	+	+	+
Beet leaves	++	++	*
Beet stems	*	+	*
Brains	+	++	*
Brazil nuts	+	++	*
Bread, white, water	?	+	−
Bread, white, milk	+	+	− to +
Bread, whole wheat, water	+	++	?
Bread, whole wheat, milk	++	++	− to +
Butter	+++	−	−
Buttermilk	+	++	+variable
Butternuts	+	++	*
Cabbage, green, raw	++	++	+++
Cabbage, head, raw	+	++	+++
Cabbage, head, cooked	+	++	+
Cabbage, head, dried	+ to ++	++	+
Cabbage, head, canned	+	++	+
Cantaloupe	++	++	*
Carrots, fresh, young	+++	++	++
Carrots, old, raw	+++	++	+
Cauliflower	+	++	+
Celery, bleached stems	− to +	++	*
Celery, bleached leaves	+	++	*
Celery, green leaves	++	++	*
Chard	++	+ to ++	*
Cheese, whole milk	++ to +++	−?	*
Cheese, cottage (skim)	+	*	*
Clover, young	+++	++	*
Chestnuts	*	+	*
Cloudberries	*	*	+++
Cloudberries, canned	*	*	+++
Coconut	+	++	*
Coconut oil	− to +	−	−
Codliver oil	+++	−	−
Corn (maize) white	+	++	−
Corn (maize) yellow	++	++	−
Corn meal	− to +	−	−

FOODS AS SOURCES OF VITAMINS A, B, AND C (*Continued*)

FOOD	VITAMIN A	VITAMIN B	VITAMIN C
Corn oil	+	−	−
Cottonseed (flour or meal)	+	++	*
Cottonseed oil	?	−	−
Cranberry (or juice)	*	*	+
Cream	+++	++	+variable
Cress	*	*	+
Cucumber	− to +	+	++?
Dandelion greens	++	++	+
Dasheens	*	+	+
Eggs	+++	+ to ++	−?
Egg white	−	*	−?
Egg yolk	+++	++	−?
Egg plant	+	+	*
Endive	+	*	+
Escarole	+++	*	*
Filberts	*	++	*
Fish, fat	+	+	*
Fish, lean	− to +	+	*
Flour, white	−	− to +	−
Flour, whole wheat	+	++	−
Glucose	−	−	−
Grains, whole, dry	+	++	−
Grains, sprouted	+	++	++
Grapefruit (or juice)	+	++	+++
Grapes	+	+ to ++	+
Grape juice	+	+ to ++	+?
Ham	− to +	++	−
Heart	+	++	+?
Hickory nuts	*	++	*
Honey	−	+?	−
Horse fat	+	−	−
Ice cream (genuine)	++	++	+?
Kale	++	*	*
Kidney	++	++	+?
Kohlrabi	*	*	+
Lard	− to +	−	−
Legumes, sprouted	*	++?	++
Lemon juice	+	++	+++
Lemon juice, dried	?	++	+++

FOODS AS SOURCES OF VITAMINS A, B, AND C (*Continued*)

FOOD	VITAMIN A	VITAMIN B	VITAMIN C
Lentils (dry)	+	++ to +++	−?
Lettuce	+ to ++	++	+++
Limes (or juice)	*	*	++
Liver	++ to +++	++	+
Malt, green	+	++?	++
Mangoes	*	*	+
Margarine	− to ++	−	−
Meat, canned	− to +	+	−
Meat extract	−	−?	−
Milk (whole)	+++	++	+variable
Milk, "scalded"	+++	++	+variable
Milk, condensed	+++	++	+variable
Milk, evaporated	+++	++	−?
Milk, dried, whole	+++	++	+variable
Milk, dried, skim	+	++	+variable
Milk, fresh, skim	+	++	+variable
Millet	+	++	*
Molasses	−	+	*
Mulberries	*	*	+
Mushrooms	−	++	−
Mutton	− to +	++	*
Mutton fat	+	−	−
Oatmeal	− to +	++	−
Okra	*	+++?	*
Oleo oil	+	−	−
Olive oil	− to +	−	−
Onions, raw	− to +	+	++
Onions, cooked	− to +	+	+
Orange juice	+	++	+++
Orange peel	+	+	++
Oysters	*	*	+
Palm oil	+	−	−
Parsley	*	++	*
Parsnips	− to +	++	*
Peaches, raw	+	+ to ++	++
Peaches, dried	*	*	+
Peanuts	+	++	*
Peanut butter	+	++	*
Peanut oil	?	−	−

FOODS AS SOURCES OF VITAMINS A, B, AND C (*Continued*)

FOOD	VITAMIN A	VITAMIN B	VITAMIN C
Pears	*	+	*
Peas, young, green, raw	++	++	+++
Peas, canned	++	++	++
Peas, dry	+	++	?
Peas, sprouted	+	++?	++
Pecans	+	+	*
Peppers, green	++	*	*
Pig kidney fat	++	–	–
Pine nuts	+	+	*
Pineapple, fresh, raw	++	++	++
Pineapple, canned	++	++	++?
Pork	– to +	+++?	–
Potatoes, sweet	++	++	++
Potatoes, white, raw	+	++	++
Potatoes, white, boiled 15 minutes	+	++	++
Potatoes, white, boiled 1 hour	+	++	+
Potatoes, white, baked	+	++	+ to ++?
Prunes	++	++	–
Pumpkin	+	+	+
Radish	– to +	+	*
Raisins	–	+	–
Raspberries	*	*	++
Rhubarb	*	*	+
Rice ("polished") white	–	–	–
Rice, whole grain	+	++	–
Roe, fish	++	++	?
Romaine	++	++	*
Rutabaga	– to +	++	+++?
Rye, whole	+	++	–
Salmon, canned	+	*	*
Sauerkraut	+	+	–?
Shrimp	+	*	*
Spinach, raw	+++	+++	+++
Spinach, cooked	+++	++	+
Squash, Hubbard	++	*	*
Starch	–	–	–
Strawberries	*	+	+ to ++
Sugar	–	–	–
Swede	*	++	+++?

FOODS AS SOURCES OF VITAMINS A, B, AND C (*Continued*)

FOOD	VITAMIN A	VITAMIN B	VITAMIN C
Sweetbreads	+	+	*
Tangerines	*	*	+++
Tomato, raw or canned	++	++	+++
Tomato, dried	++	++	++
Turnip, white	− to +	++	+
Veal	− to +	+?	*
Walnuts	+	++	*
Whale oil	++	−	−
Wheat bran	+	++?	−
Wheat embryo	++	+++	−
Wheat endosperm	−	+?	−
Wheat flour, white	−	+?	−
Wheat middlings	*	++?	−
Wheat, whole	+	++	−
Yeast	−	+++	−
Yeast extract	−	+++	−

APPENDIX D

SIMPLE STATISTICAL TREATMENT OF THE DATA OF NUTRITION INVESTIGATIONS

In quantitive scientific work, whether experimental or observational, it is important not only to bring the data of experiments or observations into comparable numerical form but also to measure, as definitely as may be, the validity of the mean results obtained.

In most good modern laboratory courses in physics or in quantitative analysis, the student is taught to compute for a series of measurements or determinations some measure of the precision of the mean result. The *precision measure* thus taught in connection with physics and chemistry may or may not be exactly the same as the classical *probable error* which is so much used in the statistical analysis of observational data (as in economics, sociology, and public health work) and which is equally applicable to the results of laboratory experimentation.

Investigations in the chemistry of food and nutrition, especially since the use of laboratory animals in the study of food values and nutrition problems has become common, often deal with data which involve both the errors of measurement which apply to all laboratory work and also the physiological or individual variability of the experimental animals used — both precision of measurement in the ordinary laboratory sense and the elements of biometrics or of vital statistics as well.

As a matter of fact these two kinds of errors are not so distinct as the ordinary statement of them might seem to imply, the " errors " (variations) in a series of " purely physical "

measurements being largely due to physiological variations such as those of eyesight and steadiness of hand in the measurer or measurers. Without elaboration of this consideration, however, it suffices for our present purpose to emphasize the fact that the particular form of precision measure known as probable error (P.E., E., or p. e.) is applicable to the ascertainment of the degree of certainty or trustworthiness of the result of an investigation whose data are subject to either or both of these kinds of errors or variations. And the computation of the probable error yields incidentally a measure of the variability of the data which (expressed usually in the form of *standard deviation* or *coefficient of variation*) is often of added value in the interpretation of the results.

$$\frac{\text{Standard Deviation}}{\text{Mean}} \times 100 = \textit{Coefficient of Variation}$$

Jevons in his *Principles of Science* has given the following concise rules for the computation of probable error,[1] and definition of the value thus found:

Probable Error[2]

"The following are the rules for treating the mean result, so as thoroughly to ascertain its trustworthiness.

1. Draw the mean of all the observed results.
2. Find the deviation of each result from the mean.
3. Square each of these deviations.
4. Add together all these squares, which are of course all positive.

[1] The term probable error (like many other technical terms) may be regarded as more or less of a misnomer, and partly on this account some writers are inclined to criticize and minimize it. But the term is so well established that even ridicule seems hardly likely to displace it, and whatever the ambiguity of the *term* "probable error" the *conception* is one of very great importance which can and should be clearly understood by all students of science.

[2] Jevons' *Principles of Science*, page 387.

5. Divide by one less than the number of observations.[1] This gives the *square of the mean error.*
6. Take the square root of the last result; it is the *mean error of a single observation.* (Also called *Standard Deviation.*)
7. Divide now by the square root of the number of observations, and we get the *mean error of the mean result.* (Standard deviation *of the means*).
8. Lastly multiply by the natural constant 0.6745 (or even by $\frac{2}{3}$) and we arrive at the *probable error of the mean result.*

"The probable error is taken by mathematicians to mean the limits within which it is as likely as not that the truth will fall."

The advantage of computing the probable error rather than some other precision measure has been mentioned above. So far as concerns applicability to different types of data, one might equally well use the mean error omitting the final step in the calculation, which consists simply in multiplying this by a constant. The probable error has, however, the added convenience over the mean error (1) of being easily and simply defined in words as the limits within which it is as likely as not that the truth will fall; (2) of easy statement of the numerical likelihood of finding the truth within such wider limits as may be selected,

[1] In the fifth of the numbered steps above quoted from Jevons, there is a difference of usage as to whether the division indicated therein shall be by the number of observations or one less than this number. (That is by a divisor which represents, (1) the number of objects observed, or (2) the number of intervals which one would have if these objects were set up in a row, "in array," for the purpose of observing the extent to which they differ.) This difference should never appreciably affect the conclusions drawn, for if there were one hundred observations the probable error would be affected to the extent of only one hundredth of its numerical value whereas the differences which one would be concerned to interpret would usually be relatively larger. The larger the number of observations the smaller the influence of this deviation of usage, and as the validity of this or any other precision measure is dependent upon the number of observations being large enough to ensure a "fair sample" of the data concerned, it is only when the number of observations is fairly large that it is worth while to compute the probable error. For full and recent discussions of the gathering of data, the judgment of their adequacy for statistical treatment, and related problems see Chaddock's *Principles and Methods of Statistics* (1925).

and of the likelihood as to whether or not the difference between the means of two series of observations or measurements is a real or an accidental difference.

The probable error of the difference between two means is the square root of the sum of the squares of their respective probable errors.[1] If the difference between two means were exactly the same as the probable error of this difference, then the chances would be exactly even as to whether the difference were a real or an accidental one; but if the difference were three times its probable error, then (assuming that the sampling was adequate and the data of such a nature as to make the rule applicable) the chances would be better than 20 to 1 that the observed difference was a real one and not attributable to accident or to individual variations.

A probable error is significant only in connection with the mean or the difference to which it applies.

Reitz and Mitchell[2] summarize as follows the rapidly increasing probability of the validity or reality of a finding or a difference according as the difference of two means is found to be equal to or several times greater than its probable error. They say:

"The exponential equation affords us a means of determining not only certain limits within which the probability is one half that a deviation will fall, *i.e.* those set by the probable error, but also with what probability we may expect a deviation not to exceed *any* assigned limit. Thus, taking limits that are multiples of the probable error, Gauss's Law of Error enables us to assert that, for variates that follow this law, the chances that

[1] For a more precise form of statement, see Chaddock, page 239.

[2] Reitz and Mitchell, *Journal of Biological Chemistry*, Vol. 8, page 305. This paper as a whole argues strongly for the general applicability of Gauss's Law of Error, and the rules derived from it, to the interpretation of the data of nutrition experiments. A relatively skeptical view is presented by Wilson, *Science*, Vol. 58, page 93 (August 10, 1923). Chaddock in his *Principles and Methods of Statistics* gives full and judicial discussions both of the uses and of the possible misuses and limitations of the ordinary statistical methods.

another random observation or the mean of any equal random sample will fall within the range ± E, ± 2E, etc., are as follows:

± E	the	chances	are	even
± 2E	"	"	"	4.5 to 1
± 3E	"	"	"	21 to 1
± 4E	"	"	"	142 to 1
± 5E	"	"	"	1,310 to 1
± 6E	"	"	"	19,200 to 1
± 7E	"	"	"	420,000 to 1
± 8E	"	"	"	17,000,000 to 1
± 9E	"	"	"	about 1,000,000,000 to 1

" It is improbable, therefore, that the deviation of another random observation will exceed the probable error many times."

Applicability. — It is presumably not necessary to remind the reader that all these considerations apply only to " chance " or " compensating " errors and to normal individual variations; not to " constant " or " cumulative " errors or errors due to the use of incorrect methods or to biased or inadequate sampling. A more picturesque way of making the distinction is the statement that the Law of Error " applies only to errors and not to mistakes."

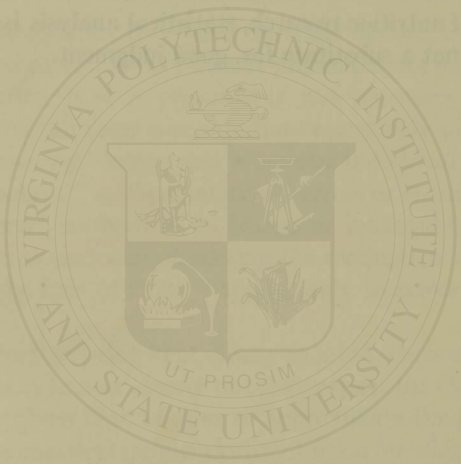
This distinction being kept clearly in mind, there still remains the question as to how closely one may expect the chance variations of any given kind of data to approximate the ideal of the perfectly symmetrical frequency distribution for which alone the above rules and ratios hold strictly and precisely true.

For critical discussions of this problem the reader may refer to the papers of Reitz and Mitchell and of Wilson and the text book of Chaddock already cited as well as to many other books on statistical method written from various points of view.

Of special significance to the student of nutrition is the fact that variations which result from a multiplicity of causes, which plainly include the individual variations encountered in nutrition experiments, have quite regularly been found to

approximate closely to the ideal distribution when studied on sufficient numbers of cases; from which it follows that the computation of probable error by the method above cited and its use as here indicated in the interpretation of findings and differences is in full accord with our best scientific knowledge. But unless or until it be actually demonstrated that the particular data at hand do show a symmetrical frequency distribution, the computed probable error should be taken as an indication or approximation rather than as a precisely determined value to be stated dogmatically to several significant places of figures.

And always it is to be remembered that, in the interpretation of the data of nutrition research, statistical analysis is an important aid to, not a substitute for, good judgment.



SUBJECT INDEX