BIOCHEMISTRY OF THE FATS

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By the Biochemistry of Fats is meant the chemistry of the fats in their relation to living organisms.[1] It involves, therefore, not only the fatty acid tri-esters of glycerol which are the fats proper, but other compounds of the fatty acids which are associated with them in the life processes of living things. On this basis consideration must be given to such substances as the lecithins and cephalins, and a number of others in which the central figure is the fatty acid. In recognition of their relationship a classification of these substances was presented some years ago by Gies and Rosenbloom (1), and adapted with slight modifications by Mathews in his text book (2). The classification included a number of substances, such as the essential oils and fat colors, which are not concerned in any way with the biochemical relations of the fats, and besides seemed unnecessarily complicated. A somewhat simpler classification based more strictly on the relationships of the various substances to the fats was later proposed (3), and is given in a modified form below. Three terms have been suggested for the group, namely, "Lipins"

[1] Reviewer's note: In writing this review attention has been confined largely to those phases of the subject which are of definite interest to the student of life processes of living things. Many substances and reactions that are of mainly chemical or technical interest have, for this reason, been omitted or dealt with briefly. For reasons of space, other topics which have been reviewed adequately elsewhere within a brief period have been passed over lightly.

The literature on this subject has grown to be enormous and it was felt that little would be gained by reference to all the articles on a given topic. Consequently only those which in the reviewer's opinion are of outstanding importance in the discussion are quoted but care has been taken to include those which contain full references so that the reader who wishes more detailed information may know where to find it.

by Gies and Rosenbloom, "Lipides" by the International Congress of Applied Chemistry, and the old term "Lipoids" by the author. The term lipins has been used in a different sense by Leathes, and was later adopted by McLean in his monograph as a name for a subgroup containing the cerebrosides and the phosphatides. The term lipoids is understood by many to exclude the fats, although used in the wider sense by many workers on the continent. For these reasons, and for the sake of uniformity, the author recommends the use of the term Lipides as the general group name. The modified classification is as follows:

Lipides

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.

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, sulfolipides, 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.

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 dispersons 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 difficulty 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.

THE SIMPLE LIPIDES

Fats

Esters of the fatty acids with glycerol. These are commonly called oils when they remain liquid at ordinary temperatures, and fats when solid. They are the most important of the

lipoids from the point of view of quantity, wideness of distribution, food value, and commercial interest. They constitute the main form of food storage in animals, and share with carbohydrates and to a less extent with proteins this function in plants. As they occur naturally they are always mixtures of triglycerides of various fatty acids, and their properties vary with the nature of the fatty acids in the separate glycerides, and with the nature of the glycerides composing the mixture.

The glycerides of the higher fatty acids are insoluble in water, those of the lower fatty acids, e.g., butyric, are slightly soluble. In the organic solvents such as ether, chloroform, benzene, all are readily soluble even in the cold and much more soluble hot. In ethyl and methyl alcohol and acetone they are slightly soluble in the cold but readily soluble when hot. In fact boiling ethyl alcohol is one of the best solvents for use in extracting tissues, giving a cleaner extraction than ether, chloroform or benzene, probably because, owing to its affinity for water, it penetrates the tissue better. The solubility in alcohol, like the melting point, varies with the nature of the combined fatty acid, the glycerides of the unsaturated and the lower fatty acids being more soluble than those of the higher and saturated acids. The glycerides of the hydroxy fatty acids like the acids themselves are insoluble in petroleum ether.

Melting point and solidifying point. In general the melting points of the glycerides are higher than those of the contained fatty acids and vary with the fatty acids, the glycerides of the higher saturated acids having the highest melting points, those of the lower fatty acids lower, and those of the unsaturated acids still lower. The melting point of a natural fat, which is always a mixture of glycerides, depends on the nature of the component glycerides. Its melting point may be low because it contains either glycerides of the lower acids or glycerides of the unsaturated acids. The melting points of mixtures of pure glycerides cannot be foretold from the melting points of the constituents. Eutectic mixtures are formed of which the melting points pass through a characteristic minimum value below that of either of the constituents. On the other hand, having

determined the curve of melting points of various known mixtures of pure triglycerides it is possible to determine the composition of an unknown mixture with a fair degree of accuracy, a fact which has been made use of by Twitchell (4).

The solidifying point of a glyceride or mixture of glycerides is always lower than the melting point, the difference being generally considerable and often wide. Thus the melting point of tristearin is given as 71.5°, its solidifying point 52.5° (Lewkowitsch). A sample of beef fat (from the heart) melted at 49.5° and solidified at 36° (5). Butter fat melted at 34.5° and solidified at 22.7°. (Stearic acid melts and solidifies at practically the same temperature (69.3°).) Analogous to these findings is the fact that pure triglycerides under certain conditions will exhibit a double melting point. Tristearin, for example, which had just been melted will melt at 55° then on raising the temperature it will solidify and melt again at 71.5° (6). The above peculiarities of the glycerides conflict with the physical law that phenomena of this nature should take place at the same temperature, and a considerable amount of work has been done to clear up the inconsistency. Thus it was found by Guth (6) and Le Chatelier and Cavaigac (7) that well crystallized tristearin has but one melting point (71.5°). If however, it was examined shortly after having been melted it showed two melting points due to a delayed cooling. A similar conclusion was reached by Le Chatelier and Cavaigac (7) who showed that in glyceride mixtures the change from liquid to solid is extremely slow, and that if the observations be carried out with sufficient slowness the melting and solidifying process reverses within 0.1 to 0.2 degrees. Brigl and Fuchs (8) claimed that fatty acids of identical structure may differ in their melting point depending on the different orientation of the carbon atoms in the crystal—the existence of two forms of the same acid which may change into each other. Bömer (9) found that tristearin obtained by crystallization from ether melted at 71.6° to 72.2° and solidified at 70°. If, however, the crystals were heated above this melting point a few degrees they solidify at 52°, and exhibit the double melting point at 55° and 71.6°.

Grün and Schacht (10) prepared synthetically three mixed glycerides which could be prepared in either the lower (labile) or higher (stable) melting forms. The labile form could be gradually converted into the stable by seeding with a crystal of the stable form, but the reverse change was not possible. The results of these workers seem to indicate that some unknown factor comes into play, possibly the existence as suggested, of two forms of the glycerides, although from our present knowledge of the structure of the glycerides it is difficult to see how such forms could be explained. From a practical point of view the rule has originated that to get a true melting point it is necessary for the fat to stand for at least twenty-four hours in the melting point tube before the determination is made.

The delayed solidification appears to be of considerable importance in the living animal since many stored fats have a melting point considerably above body temperature, while the solidifying point is some degrees below [note beef fat above].[2]

Color, odor, etc. The pure triglycerides are colorless, odorless and tasteless, the properties of color, odor and taste when present being due entirely to foreign substances mixed with or dissolved in them. Thus the desirable yellow color of butter is due to plant pigments carried over from the food. Plant pigment is also responsible for most of the color of the stored fat of animals (12) (13). The flavor of food fats is also due to foreign materials absorbed by the fat, either from its natural environment, or formed during the processes of preparation. In modern butter making the bacterial flora is carefully controlled with this point in mind.

Glycerides. The glycerides composing the natural fats may be either simple, containing only one fatty acid, or mixed, containing more than one acid, and, far from being rare constituents of the natural fats as was first believed, more recent investigations tend to show that mixed glycerides form the bulk of many of the natural fats. They have been prepared syn-

[2] A condition has been noted in infants (Sclerema neonatorum) in which the subcutaneous fat has hardened resulting in the death of the infant (11). In this case the abnormality found was a high content of free fatty acid.

thetically in large numbers. The methods of synthesis are not markedly different from those for the simple glycerides, but the procedure is somewhat complicated by the fact that there is more or less shifting of the radicles in the molecule. For example Kreis and Hafner (14) found that when oleic acid was allowed to act on di-palmitin and di-stearin considerable quantities of tri-palmitin and tri-stearin were found, while the yields of oleodi-palmitin and -stearin were correspondingly reduced. It should be noted that the phospholipides, lecithin and cephalin, are naturally occurring mixed triglycerides, containing two different fatty acid radicles and one phosphoric acid radicle.

Optical properties. Certain of the mixed triglycerides should be optically active, since they contain an asymetric carbon atom. Thus:

$$C_3H_5\begin{cases}O.R_1\\O.R_2\\O.R_3\end{cases}, \quad C_3H_5\begin{cases}O.R_1\\O.R_1\\O.R_2\end{cases}, \text{ should be optically active}$$

as a matter of fact the only optically active fats which are found in nature are those which contain optically active fatty acids as, for example, castor oil and chaulmoogra oil, and those which contain optically active non-fat substances such as resins, sterols (cholesterol, phytosterol, etc.). Unsuccessful attempts have been made from time to time to prepare optically active glycerides (15) (16).

Hydrolysis (saponification). Fats are hydrolyzed in the laboratory in the same way and with the same agents as are simple esters. Water at high temperature (under pressure) as in the autoclave may be used either alone, or more advantageously with catalysts such as acids or alkalis. At ordinary pressures the same catalysts do the work, but more slowly. The speed of reaction may be increased by the use of a solvent, such as alcohol, which dissolves the fats. Amyl alcohol is more effective than ethyl alcohol, probably because of the higher temperature which can be obtained.

Enzyme hydrolysis is effective when the fats are emulsified. Fat-splitting enzymes or lipases are present in the gastro-in-

testinal secretions of animals, and in many plants especially in fatty seeds as the castor bean, seeds of chelidonin majus, the rubber plant, etc.

Synthesis. Synthesis of the glycerides is carried out along the same lines as synthesis of the simple esters, but is complicated by the fact that glycerol is a triatomic alcohol with two different positions, and by the fact that there may be shifting of the groups from one position to the other. The special problems involved are reviewed by Amberger and Bromig (17), and the question of optical activity of the glycerides by Bergmann and Sabetay (16) and Abderhalden (15).

Synthesis of fats by enzymes has been successfully demonstrated with castor bean lipase by Welter (18), and Armstrong and Gosney (19), who gave a beautiful demonstration of the reversibility of the synthetic-hydrolytic powers of the castor bean lipase. Synthesis by lipase of the pancreatic juice has been demonstrated by Hamsik (20), Taylor (21) and Foá (22).

Rancidity. Dry air, excluding moisture and light, has apparently no effect on oils and fats. Air in presence of moisture and particularly of light and heat rapidly brings about those changes known collectively as rancidity. Unsaturated fats become rancid more quickly than saturated, and free fatty acid formation appears to be the first essential for rancidity. The stages in the process have been outlined by Kerr and Sorber as follows (23). First the development of free acid, then a drop in free acid coincident with the rancidity, followed again by increased acidity. The iodine value falls, unsaponifiable matter increases, and oxygen is fixed in peroxide form of the following nature

$$CH_3\cdot(CH_2)_7\cdot\underset{\displaystyle O}{\overset{\displaystyle |}{CH}}\!\!-\!\!\underset{\displaystyle O}{\overset{\displaystyle |}{CH}}\cdot(CH_2)_7\,COOH$$

This substance then acts as a carrier of oxygen for the production of various oxidation compounds, the nature of which is unknown. Greenbank and Holm (24) found that metals catalyze powerfully the production of rancidity.

Waxes—esters of the fatty acids with alcohols other than glycerol

Waxes are very widely distributed in the plant and animal kingdom, their main usefulness being apparently as protective agents due to their chemical inertness. Thus, the high lipide content of the tubercle bacillus is due mainly to the waxes and wax alcohols in contains. Insect waxes and leaf waxes are esters of higher alcohols and mainly higher saturated acids. Thus the main constituent of beeswax is myricyl palmitate.

A group of waxes which is of particular importance to the biochemist is that of the esters of cholesterol and related alcohols with various fatty acids. In animals these occur in largest amount in blood plasma. Similar esters may occur in plant tissues, but so far, no mention has been made of them. The cholesterol esters of blood plasma have been found to consist mainly of esters of palmitic, oleic and linolic acids with smaller amounts of stearic and other acids. In addition to the blood plasma the esters are to be found in large amounts only in the suprarenal glands. Small amounts occur in the liver, kidney, heart, and probably in other organs and tissues, but the quantities are generally so small that there is always a question whether they are real constituents of the tissues or are due to the blood plasma present. In some abnormal conditions, such as amyloid kidney, undoubted deposits of esters occur. The waxes are considerably more difficult to hydrolyze than the fats, and this difference has been made use of in separating them, in particular cholesterol esters, from the fats (25). For complete saponification of the cholesterol esters special means must be employed, for instance, the use of sodium ethylate on the esters in ethereal solution (26).

COMPOUND LIPIDES

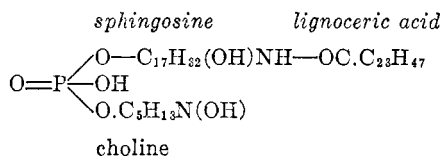
Phospholipides

We are indebted largely to MacLean in England and to Levene in this country for clearing up this previously very complicated subject, and reducing the large number of poorly determined and doubtful compounds to a few which they have been mainly

instrumental in defining. The best characterized members of the group are the lecithins, cephalins, and sphingomyelin.

Sphingomyelin. Composition. The substance as prepared contains two fatty acid radicles, one probably lignoceric acid, and about an equal amount of a low melting acid, probably a hydroxy acid, two bases—sphingosine and neurine or choline, and phosphoric acid. Sphingomyelin apparently contains no unsaturated acid.

A formula suggested by Levene (27) is as follows:

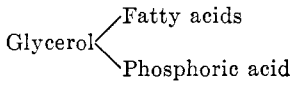


Properties. It is a relatively stable substance, undergoing no change in air or light, is soluble in hot alcohol from which it separates on cooling in crystalline form, relatively insoluble in cold or hot ether, easily soluble in cold or hot chloroform, benzene, pyridine and glacial acetic acid. It is insoluble in cold but somewhat soluble in hot acetone. It mixes with water to form an opalescent suspension from which it is precipitated by acetone. It is dextrorotatory having a specific rotation of about eight.

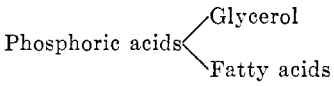
Sphingosine, the chief base, is an unsaturated amino alcohol, containing two hydroxyl groups, one primary amino group and one double bond, with the empirical formula of $C_{17}H_{35}NO_2$ and with the probable composition of $CH_3(CH_2)_{11}\cdot CH = CH\cdot CHOH\cdot CHOH\cdot CH_2\cdot NH_2$. Sphingomyelin occurs in brain, kidney, liver, egg yolk, and in small amounts in blood and muscle.

Lecithin and cephalin. These substances contain, with one exception the same elementary constitutents—two molecules of fatty acid, one of phosphoric acid, one of glycerol and one of base. In lecithin the base is choline, in cephalin it is amino ethyl alcohol. They are found associated with each other in all tissues. Whether their chemical structure is the same apparently remains to be proven, but MacLean is of the opinion first expressed by Thudicum, that the arrangement of the con-

stituents in the molecule is different. Thus the arrangement in the lecithins would be



while in the cephalins it would be



At any rate all workers are agreed that it is much more difficult to get the fatty acids from cephalin free from phosphoric acid than in the case of lecithin. The situation is further complicated by the fact noted by MacLean (28) that lecithin may be changed by the treatment of extraction and purification until it becomes insoluble in alcohol and thus passes for cephalin. True, cephalin, he states, is soluble in alcohol.

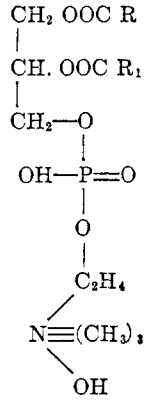
The number of lecithins or cephalins is limited theoretically only by the number of fatty acids, since each new arrangement of fatty acids would mean a new compound. Practically, however the number of different substances of this nature found in the animal body is probably limited to a very few, although very little information is available on this point. The phospholipide of cellular material is probably to be regarded as essential to the life of the cell since it is preserved in constant proportion even in extreme emaciation (29) (30). Its exact function is still unknown although, as indicated below, it probably has mainly to do with the metabolism of the fatty acids.

Properties. The lecithins and cephalins are soluble in the fat solvents with the single and characteristic exception of acetone. They are miscible with water forming cloudy solutions from which they may be precipitated by acetone. Ordinary lecithins and cephalins oxidize readily in air, turning brown and taking on a disagreeable odor. They have no definite melting point but decompose on heating. They combine with both acids and bases and form combinations with the most diverse substances —salts, proteins, carbohydrates,—most of which, are not to be

considered as chemical compounds but rather adsorption mixtures, and it is probable that lecithin exists in tissues in this type of combination. They undergo hydrolysis more readily than the fats.

In preparing them advantage is taken of these properties. Thus lecithin is separated along with cephalin from the other lipides by virtue of its insolubility in acetone. It is separated from cephalin by taking advantage of the insolubility of the latter in alcohol. It is separated from water-soluble impurities by solution in water and precipitation with acetone; from organic impurities by combination with cadmium chloride, etc.

Lecithin. The structure of lecithin generally accepted (MacLean) is as follows:



This is the asymmetrical form. A symmetrical form is possible, with the phosphoric acid radicle attached to the middle carbon of glycerol, but since all known lecithins are optically active the asymmetrical formula is probably the correct one. The mode of attachment of the choline to the phosphoric acid is still a matter of dispute, although the ester form of combination as above is generally accepted.

Lecithin has been synthesized by different workers, Grün and Kade (31), Grün and Limpacher (32) and most recently by Levene and Rolf (33). Grün and Limpacher indicate another possibility in the lecithin formula—the possibility of an anhydride form by loss of water from the phosphoric acid and chlorine

residues. Levene, Rolf and Simms (37) removed one of the fatty acids (the unsaturated acid) by the action of cobra venom on lecithin, and from the lysolecithin so formed built up other lecithins.

The differences between various lecithins is largely if not altogether due to the fatty acids which they contain, and these have as yet been insufficiently studied. As far as present information goes each lecithin contains one saturated and one unsaturated fatty acid. Levene and Simms (34) found that the liver lecithins contained the saturated acids palmitic and stearic, and oleic and arachidonic as the unsaturated acids although linolic acid was not excluded. Levene and Rolf (35) found that egg yolk contained oleic and small amounts of linolic and arachidonic acids. In a lecithin prepared from the soy bean Levene and Rolf (36) found stearic and palmitic acids, and oleic, linolic and linoleic acids. The proportion of unsaturated acids was relatively low as compared with animal lecithins. As noted below the fatty acid composition of the animal lecithins may be altered to some extent by the fat of the food.

Cephalin. The cephalins have been much less studied than the lecithins. They are distinguished from them by their insolubility in alcohol, by containing aminoethyl alcohol in place of choline, and possibly by a difference in molecular structure. Otherwise their properties and behavior are much the same.

Like the lecithins they contain one saturated and one unsaturated acid in each simple molecule, and of these the saturated acid is apparently stearic. The unsaturated acids of brain cephalin were found by Levene and Rolf (38) to be oleic and arachidonic. MacArthur (39) found that cephalin from sheep and beef brain contained fatty acid in the following approximate proportions—stearic acid 30 per cent, oleic acid 55 per cent, cephalinic acid 10 per cent, and clupanodonic acid 5 per cent. However, as MacLean has pointed out, no one else has found as high a percentage of oleic acid in cephalin. Parnas (40) came to the conclusion that the only saturated acid of cephalin was stearic.

Galactolipides

Cerebrosides. These substances, of which only two (phrenosin and cerasin) are at all well characterized, are substances containing galactose, a base spingosine, and a fatty acid, but no phosphoric acid. They differ from each other only in the nature of the fatty acid which they contain, phrenosin containing phrenosinic acid ($C_{25}H_{50}O_3$), and cerasin lignoceric acid ($C_{24}H_{48}O_2$). In solubility they closely resemble sphinogomyelin, and the separation from this substance is difficult. They dissolve readily in hot alcohol, acetone, or benzene, but are almost insoluble in ether, hot or cold. They dissolve readily in pyridine. They occur in largest amounts in the brain, and are of interest to the biochemist because they contain galactose which is not known to occur in any other combination in the tissues, and also because they contain a sugar and a fatty acid in the same molecule, which is of interest from the fact noted below that the fatty acids, in the later stages of their metabolism, seem to require the assistance of the carbohydrates.

The significance of these compounds in the living body is unknown and their mention in the biochemical literature has had almost entirely to do with their separation and characterization.

DERIVED LIPIDES

Fatty acids

The important series of fatty acids are:

1. The saturated, straight chain series $C_nH_{2n}O_2$, including practically all the even numbered carbon atom acids up to and including C_{30}. The more commonly occurring ones in the natural fats are palmitic (C_{16}) and stearic (C_{18}) acids which occur in practically all. Palmitic acid is the most widely distributed, and quantitatively is the most important. Of the others which are of notable importance to the biochemist may be mentioned the lignoceric $C_{24}H_{48}O_2$ found in sphingomyelin and cerasin.

2. The series with one double bond $C_nH_{2n-2}O_2$—oleic or acrylic

series. The only practically important member of this series is oleic acid, although several other members of the series are known, for example, hypogeic (C_{16}), gadoleic (C_{20}), erucic (C_{22}) together with isomers of each, having the double bond in different positions.

Oleic acid is the most widely distributed of all the acids of the fats, and is also quantitatively the most important. It is found in several isomeric forms but the commonly occurring one has the constitution $CH_3(CH_2)_7CH=CH\cdot(CH_2)_7COOH$. Another oleic acid was found by Hartley (41, 42) among the fatty acids of liver, and has the double bond between the 6th and 7th carbons. It is interesting to note that he also found in liver a C_{18} acid with two double bonds, one between the 6th and 7th, and one between the 9th and 10th positions, the occurrence of which would be most readily understood by supposing that the ordinary oleic acid of the food or stores had acquired a new linkage between the 6th and 7th carbons.

The cis-trans type of isomerism is exhibited in ordinary oleic acid by treatment with nitrous acid, the liquid acid changing to the solid elaidic acid. This transformation takes place readily in the case of most other members of the series but strangely enough does not in the case of the isomers of oleic acid with the double bond in a different position. Oleic acid is quite stable in air as ordinarily kept, but becomes rancid when exposed to the combination of light, air and moisture.

The acids more unsaturated than oleic acid are found in vegetable oils and in animal tissues. In the former they are present as tri-glycerides (fats) while in animal tissues very little is present, as fat the larger part being as phospholipides. The presence of these more highly unsaturated acids in the vegetable oils gives them the important commercial property of "drying," i.e., of forming by oxidation a waterproof skin or varnish over surfaces on which they are spread as in painting. The unsaturated acids of animal tissues, although they oxidize in air in a similar way, become sticky instead of forming a smooth skin. These differences may probably be referred to differences either in the length of chain or position and number of the double bonds.

While oleic acid is quite stable in the presence of oxygen at ordinary or body temperatures, the more unsaturated acids take up oxygen and undergo other changes with a readiness dependent on the degree of unsaturation. Leathes has observed that it is rare to find fatty acids in the fat stores of the animal body more unsaturated than oleic acid, which be believes is due to the fact that oleic acid may be stored without oxidation at body temperature, while a more unsaturated fat cannot. When needed for combustion it is further desaturated, and probably combined with phosphorus.

3. *The Linolic Series.* $C_nH_{2n-4}O_2$. The known acids of this series are all C_{18} acids. They were studied first in linseed oil and have since been found to occur rather widely in animal tissues, for example in pigs' liver as shown by Hartley (42), and by recent work in other tissues such as blood (43), egg-yolk (35), muscle, etc. Except in the liver, and then only in relatively small amount, they do not occur in ordinary stored fat of mammals, but are found mainly as phospholipides or as esters with cholesterol.

The structure of these acids is not known. They react with alkaline permanganate in the cold in a similar way to oleic acid, forming hydroxy acids, and on further oxidation yield short chain acids.

Linolenic Series $C_nH_{2n-6}O_2$. A C_{18} acid of this series, or rather two isomeric acids have been prepared from linseed oil (44). Some light on its constitution was obtained by decomposition of the ozonide which yielded sufficient azelaic acid to account for half the molecule. In addition, malonic acid and propionic aldehyde were obtained. Traces of an acid of which the bromine derivative is insoluble in ether but soluble in benzene have been found in blood plasma.

Linolenic acid has been reported among the unsaturated acids of brain by Grey (45).

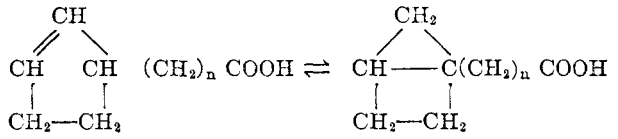
Other series. Of the acids of other series very little of biochemical importance is known.

Ricinoleic acid $C_{18}H_{34}O_3$ is the main constituent of the glycerides of castor oil. Its constitution is probably $CH_3(CH_2)_5$-

$CHOH \cdot CH \cdot CH = (CH_2)_7COOH$. It is optically active, having a specific rotation $\alpha_D = 6.67°$ (46). On oxidation with nitric acid it yields azelaic and sebacic acids.

Hydroxy acids of unknown composition have been found in brain by Grey (45) making up about 25 per cent of the solid acids. Two of the acids found were monohydroxy acids, and therefore cannot have been formed in the separation.

Certain cyclic fatty acids have come into prominence recently because of their therapeutic use in leprosy. These are the acids of chaulmoogra oil, mainly hydnocarpic acid $C_{16}H_{28}O_2$, and chaulmoogric acid $C_{18}H_{32}O_2$. The composition of these acids as indicated by the work of Power and Barrowcliff (47)[3] is



They take up only two atoms of bromine and are strongly optically active.

Odd carbon fatty acids. These do not occur in nature, but since the lower acids have been found to form sugar and not to form derivatives of acetone, their possible usefulness in diabetes has been suggested. Reports on the use of glycerides of margaric acid ($C_{17}H_{34}O_2$) indicate that they are apparently well utilized, but whether they will be found more useful than ordinary fats for diabetics is open to question (48) (49).

Clupanodonic acid, the 18 carbon member of the series $C_nH_{2n-8}O_2$ has been obtained from Japanese sardine oil, herring, and whale oil, and appears to be contained in all fish oils. Arachidonic acid, $C_{20}H_{32}O_2$, was found in pig's liver by Hartley (42), and later in the lecithin from the same source by Levene and Simms (34). Amounts generally less than 5 per cent of the total fatty acid, of acids giving bromine derivatives insoluble in ether and benzene have been found in the cholesterol

[3] Recent work of R. L. Shriner with Roger Adams indicates that these compounds exist in only the first form. The paper will soon be published in J. Am. Chem. Soc.—Editor.

ester fractions of blood plasma, and in the lecithin (acetone insoluble) fractions (50).

Levene has found archidonic acid in brain cephalin and lecithin (38).

Solubility. The lowest members of the various fatty acid series up to C_6 are miscible with water in all proportions. Caproic acid is soluble in water at 15° to the extent of about 0.9 per cent, and the solubility decreases rapidly with increasing length of chain. All the saturated acids above lauric acid are practically insoluble in water. In hot absolute or 95 per cent alcohol all fatty acids are soluble, but from palmitic acid upwards all are sparingly soluble in cold alcohol.

All fatty acids are soluble in ether, chloroform, benzene, etc. All except the hydroxy fatty acids are soluble in petroleum ether (distilling below 60°).

The hydroxy acids are more or less soluble in water depending on the number of hydroxyl groups, thus octohydroxy arachidic acid is easily soluble in water (Lewkowitsch), hexahydroxystearic more difficultly soluble, while tetrahydroxystearic acid requires 2000 parts of boiling water for solution. The highly hydrolylated acids are insoluble in ether and difficultly soluble in alcohol. The dicarboxy acids are more soluble in water than the corresponding monocarboxy acids, and in general less soluble in the fat solvents. The solubilities of the hydroxy and dicarboxylated acids are of particular importance in biochemistry, since all the reactions of living beings take place in a watery medium and probably in water solution. The fact that the fats are insoluble in water imposes a difficulty on their utilization, and makes important any forms which are soluble in water. The hydroxy acids have been found in living things, and dicarboxy acids are among the products of oxidation in vitro of the unsaturated acids, although their presence in the tissues of animals has not been demonstrated (see below).

Salts of glycocholic and taurocholic acids (bile salts) dissolve fatty acids, and greatly increase the solubility of the soaps formed during digestion of the fats (51).

Oxidation. Oxidation of oleic acid yields a variety of prod-

ucts depending on the oxidizing agent and the conditions under which oxidation takes place. With potassium permanganate in alkaline solution at low temperatures it yields a dihydroxystearic acid, and at a higher temperature breaks at the double bond giving two 9 carbon acids, azelaic, a dicarboxy acid and pelargonic, a monocarboxy acid, the stages in oxidation being probably as follows (Leathes):

$$\begin{array}{c} \text{H H} \\ \text{CH}_3\cdot(\text{CH}_2)_7\cdot\text{C}\cdot\text{C}\cdot(\text{CH}_2)_7\cdot\text{COOH} \\ \text{OH OH} \end{array}$$

$$\begin{array}{c} \text{H} \\ \text{CH}_3\cdot(\text{CH}_2)_7\cdot\text{CO}\cdot\text{C}(\text{CH}_2)_7\cdot\text{COOH} \\ \text{OH} \end{array}$$

$$\text{CH}_3\cdot(\text{CH}_2)_7\cdot\text{CO}\cdot\text{CO}\cdot(\text{CH}_2)_7\cdot\text{COOH}$$

$$\text{CH}_3\cdot(\text{CH}_2)_7\cdot\text{COOH} \quad \text{HOOC}\cdot(\text{CH}_2)_7\cdot\text{COOH}$$

It is assumed that the position of the double bond does not shift during the oxidation, and the above is accepted as evidence that the double bond is in the middle of the molecule. That the double bond in oleic acid may be mobile and that therefore the above assumption may be incorrect is believed by Armstrong and Hilditch (52). Ozone acting on oleic acid forms first an addition product, an ozonide, which on heating with potassium alcoholate yields azelaic and pelargonic acids, giving further evidence that the double bond is in the middle of the molecule.

The fact that the double bond is thus shown to be a point of weakness in the chain, at least during oxidation, in vitro is taken by Leathes to indicate that during oxidation in the animal body a similar breaking of the chain takes place. Exception may be taken to this assumption as Leathes himself has pointed out in the fact that there is no evidence for the formation of either azelaic or pelargonic acids in the body, and for other reasons to be discussed below.

Hydrogenation. The unsaturated acids take up hydrogen with the aid of catalysts at the double bonds and become saturated. Sabatier and his co-workers, especially Senderens, were the first to show that finely divided metals and particularly

nickel were the best catalysts. Since that time the process has become of great commercial importance in changing comparatively inedible oils, such as cottonseed oil, into valuable articles of diet. As far as can be determined, the hydrogenated fats are just as well utilized by the animal body as the natural fats (Langworthy). A review of the work on hydrogenation of oils has recently been published by Sabatier (54).

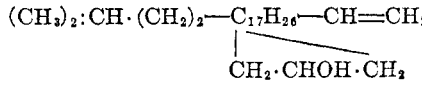
Halogen absorption. The halogens, and particularly iodine, are readily absorbed at the double bonds, and under certain conditions the absorption is quantitative so that the halogen absorption value (iodine number) constitutes one of the most important means of study of the fatty acids. The more unsaturated acids (three and four double bonds) form halogen absorption derivatives which are insoluble in most fat solvents, and this property serves for their identification and approximate determination.

Sterols

The alcohols belonging in this group are those found combined in the waxes, and of these the only ones directly connected with life processes are cholesterol, its isomers and derivatives.

Cholesterols and phytosterols. These are isomeric compounds differing from one another in crystal form, melting point, degree of optical rotations, melting point of esters. Cholesterol is characteristic of animal tissues and phytosterol correspondingly of plant tissues.

Cholesterol. Formula $C_{27}H_{46}O$, constitution (55)—



This formula indicates the presence of only one double bond, but since it absorbs two molecules of ozone there appears to be a second one, perhaps in the ring nucleus. The nature of the nucleus is not known, but the evidence available points to its being related to the terpenes (56). Cholesterol is insoluble in cold water, sparingly soluble in cold alcohol, readily soluble in hot alcohol and in fat solvents generally. Its melting point is

about 148°C. It is levo-rotatory, the rotation varying somewhat with the solvent. For ether $\alpha_D^{15°} = 31.1°$ (Hesse). On exposure to light and air it slowly undergoes changes—its melting point is lowered, its solubility changed, and its color reactions rendered indefinite.

Its iodine absorption value varies with the solution used With Hübls' reagent it gives practically theoretical values (67 to 68 as compared with 65.8, the theoretical value.) Wijs solution gives erratic results.

Methods for detection and determination. The methods which are most useful for small amounts are:

1. *The Liebermann-Burchard method* depending on the color developed in a chloroform solution of cholesterol by acetic anhydride and sulfuric acid. A fine green color is finally produced, which under exactly defined conditions is quantitative. Based on this reaction a considerable number of methods for its determination in blood and other body fluids have been developed, but the fact that there are so many methods indicates that the determination by color production is not particularly satisfactory. Reasons for the difficulty have been indicated, among them the fact that rosin acids and terpenes give similar reactions (57), that the tint and development of the color is influenced by light and temperature (58), and that the esters of cholesterol influence the tint (59). Nevertheless the colorimetric methods take relatively little time as compared with the precipitation method (noted below), and when conditions are carefully controlled give results of sufficient accuracy for most purposes.

2. *The Windaus digitonin precipitation.* Cholesterol unites quantitatively with digitonin to form an addition product which is practically insoluble in alcohol or ether. The esters do not combine with digitonin, so that it is possible by this means to make a quantitation separation of free cholesterol from its esters (60) (61). This useful method is at present almost unavailable owing to the high price of digitonin.

The gravimetric determination of cholesterol in tissues is beset with difficulties owing to the fact than when separated in the ordinary way in the "unsaponifiable" fraction after

hydrolysis of the tissue with alkali, it is contaminated with much other material from which it is difficult to separate it.

Concerning its distribution in the animal kingdom Doree (62) writes as follows: "It is found in the tissues of all animals examined. In warm blooded vertebrates it is the only sterol found but in lower animals and insects somewhat variant forms are present. They all have the same formula and differ only in crystal form and in the melting points of their dibromides and acetates. The unsaturated linkage and the $-OH$ which Hausmann (63) found to be essential for its antitoxic functions are found in all forms. Cholesterol never occurs in plants and in animals appears to be formed almost exclusively from the phytosterol of the plants although recent work indicates that synthesis is possible."

Isocholesterol differs from cholesterol in being dextrorotatory ($\alpha_D = +60°$ in ether), and in having a lower melting point (136°). It is less soluble in cold alcohol than cholesterol and gives a yellow color with the Liebermann-Burchard reagent. It occurs in wool wax.

Phytosterols are closely related to the cholesterols in composition and have many similar properties. They hold a position in plants corresponding to cholesterol in animal tissues. Phytosterol palmitate has been found in corn pollen (64).

The distribution of the sterols in various fats and oils has been reported by Steuart (65).

METHODS OF STUDY OF TISSUE LIPIDES

Two general procedures are available for removal of lipides from tissues:

Extraction—removal of the lipides from the tissues in approximately the form in which they exist there. In the experience of the writer the solvent which gives most nearly complete extraction is boiling alcohol. It has two disadvantages—it extracts other substances than the lipides, and the heat probably decomposes some of the more sensitive ones. Nevertheless it is probably superior to all others for the purpose, since it can

be applied directly to the tissue without previous drying and almost inevitable oxidation, and because it penetrates the tissue readily, breaking up whatever loose combinations there may be between lipide and other cellular constituents. Cold alcohol is preferred by many because there is less danger of decomposition of the lipides. It takes much longer than the hot solvent and gives a less complete extraction. Others of the fat solvents are used for special purposes, e.g., acetone to remove cholesterol and free fat, leaving the phospholipides. Solvents like ether and chloroform extract very little from fresh tissues, and while they give better results with dried material, the extraction is never complete. Preliminary treatment of the tissue with alcohol results in a better extraction with these solvents. The purification and examination of the individual lipides is a matter of great difficulty, and the procedures cannot be described in a brief review. The reader is therefore referred to special articles (66).

Hydrolysis—destroying the tissue by the use of strong alkali, then extracting the separated fatty material. This procedure is to be recommended when a quantitative measure of the lipide content is required, but it has the disadvantage of giving very little information regarding the lipides as they are in the tissue. Two fractions are obtained—the fatty acids and the "unsaponifiable matter," the latter consisting of the sterols and a number of unknown substances, part of which are probably related to them (67). The procedure was first made use of by Liebermann, and developed in detail by Kumagawa and Suto (68), and later by Lemeland (69).

Methods for the determination of the physical and chemical characteristics ["constants"] of the fatty acids have been well worked out, but since they are given in all good treatises on the chemistry of the fats they will not be described here.

PHYSIOLOGICAL ASPECTS

This section of the review will deal with the fats and related substances more particularly in their behavior in the animal organism.

Digestion

Fat differs from the other foodstuffs in that it has very few forms which are water-soluble and probably for this reason an entirely different mechanism is made use of in the animal body for its transport through the intestinal wall and in the blood and tissues (70). In its digestion it follows the general rule of breaking down into its constituent elements, glycerol and fatty acids just as the proteins are reduced to amino acids and the carbohydrates to monosaccharides and while absorption in undigested form cannot at present be excluded, the facilities for digestion are ordinarily so adequate that it is questionable whether any escapes hydrolysis. Enzymes which can accomplish the splitting (lipases) are to be found in the gastric and pancreatic secretions and to a less extent, although still in notable amounts, in the secretions of the glands of the intestine itself. These lipases are all in water solution and can therefore act only on the surface of the fat particles and splitting can take place to a notable extent only when the available surface of the fat is large—as when emulsified. The main emulsifying agent in the intestine is soap formed by the union of the free fatty acid in the fat with the alkali of the bile and pancreatic secretions. The fatty acid present in small amounts in all food fats is increased by the processes of cooking and by the gastric lipase until when the fat reaches the intestine it has enough free fatty acid in it to ensure good emulsification. Mucin in the bile and other secretions and lecithin in the bile act to increase the stability of the soap emulsion.

Ordinarily there is little fat splitting in the stomach, first because a soap emulsion cannot form in an acid medium and second because the gastric lipase is destroyed by acid. Appreciable hydrolysis takes place only when the fat is in emulsified form as in milk and when enough protein or other neutralizing agent is present to reduce the gastric acidity to a point where the lipase destruction is slow. Another condition under which fat splitting may take place in the stomach is when for any reason, such as excess of fat in the food, emptying of the stomach

is delayed, then intestinal fluids containing bile and pancreatic secretions back up into the stomach.

In the intestine the most important factor in digestion and absorption not excepting the pancreatic secretion, appears to be the bile, since when it is missing, fat absorption falls to a lower level than when bile is present and pancreatic secretion is absent. The great importance of the bile rests in its power of dissolving soaps and free fatty acids in which solution they appear to be readily absorbed. The important constituents of the bile which accomplish this end are the bile acids, glycocholic and taurocholic acids (71).

The absence of pancreatic lipase appears to have no great effect on the *hydrolysis* of the fat since owing to the presence of lipases in the intestinal secretion this is practically complete whether pancreatic lipase is present or not (72) and the reason why so much fat escapes absorption under these conditions is probably that hydrolysis is greatly delayed and owing to reabsorption of bile which is known to take place, there is no bile salt left in the intestine to aid in the passage of the fatty acids through the absorbing cells.

During their passage through the intestinal wall the fatty acids and glycerol are resynthesized into fat, in this way differing again from the other foodstuffs which apparently reach the blood stream and are delivered to the tissues as "building stones." It is generally assumed that the agent producing the synthesis is the same agent—the pancreatic lipase—which brought about the hydrolysis. The resynthesized fat does not go directly into the blood stream but passes by the lymph stream through the thoracic lymph duct into the venous circulation. It is still an unsettled question as to whether any part of the fat is absorbed directly from the intestine into the blood stream. Only about 60 per cent can be recovered from the thoracic duct where it empties into the circulation, which leaves 40 per cent to be accounted for. Attempts to demonstrate direct absorption of fat into the blood leaving the intestine have not given satisfactory results (73) (74). Recent work goes to show however, that there are pathways between the lymph and venous cir-

culations other than the main opening of the thoracic duct, a finding which will go far to explain the discrepancy. Thus Lee (75) after ligation of the thoracic duct found that connections were established between the lymphatic duct and the azygos vein or its branches also with the right thoracic duct. He gives also a good review of the literature showing that similar results have been reported previously, concluding that it is well established that the lymph and therefore the absorbed fat does not necessarily all enter the venous system at the base of the neck. These findings are borne out by the recent work of Eckstein (76)

As regards other lipides—lecithin when fed has sometimes been found to give a slight increase in the blood lecithin (77) and in the lecithin of the chyle (78). More recent work has failed to support these findings (76) and goes to show that lecithin, like the fats and simple esters is hydrolysed in the intestine and the resulting fatty acids built up into fats during absorption. Simple esters such as the ethyl esters of the ordinary fatty acids are hydrolyzed in the intestine and the fatty acids are built into glycerides, no evidence of the original ester or its contained alcohol being found in the chyle (79, 80, 81).

Choice of fat during absorption

A statement is being carried in the literature and attributed to Arnschink (82) that the fatty acids of the feces have a higher melting point than those of the fat fed and that therefore the intestine exercises some choice in the absorption of fat. There is no such statement in Arnschink's article and no other support to be found in the literature. The fat of the chyle is however not always the same as the fat of the food. Frank (80) found after feeding ethyl stearate that the melting point of the fatty acids was much below that of either stearic acid or tri-stearin. Bloor (83) after feeding olive oil found that the fat of the chyle melted around 30°. These differences may mean either that the absorbed fat was diluted with body fat from some nearby source or that chemical changes take place—such as desaturation in the case of the stearic acid and saturation in case of oleic acid.

After feeding cocoanut oil Raper found that the fatty acids of chyle showed a higher average molecular weight than that of the oil fed, which he believed indicated that the lower acids of the oil probably reached the blood stream as soluble salts (84).

Digestibility and absorbability of fat

There is a prevailing belief among dietitians that certain fats are more digestible than others, that the vegetable fats and the recently exploited hydrogenated fats are less well utilized by the animal body than the animal fats. In an investigation of this point, Langworthy (53) made use of 23 animal, 34 vegetable and 6 hydrogenated fats in feeding experiments on human beings. The results showed very little difference in the behavior of the fats studied. In general the utilization was found to be inversely proportional to the melting point, the high melting fats being more difficult of utilization. His results show that, aside from melting point, the origin of the fat is not of importance to its utilization.

After long feeding, fat may be less well utilized than at first. Thus Pettenkofer and Voit (85) in a 58 day feeding period of 500 grams meat and 200 grams fat daily to a 32 kilo dog found that the feces contained as high as 38 per cent of fat while at the beginning absorption was complete (98 per cent).

Parenteral absorption

Fat as such or preferably as an emulsion injected under the skin or into the abdominal cavity is absorbed in considerable amounts. It passes into the lymph system and much of it enters the blood by the usual channel—the thoracic duct (86) (87).

Homogenized fat injected intravenously produces a sudden rise of blood fat of 100 per cent or more, then a slow fall. Not over 44 per cent of the injected fat was present in the blood at any time (88). Fat in coarser suspension appears to be treated like other suspended foreign matter—it is removed rapidly, the coarse particles in the lungs, the finer ones in the liver, spleen and bone marrow.

Cholesterol

Free cholesterol alone is absorbed but little if at all from the intestine. When dissolved in fat or fatty acid it is slowly absorbed with partial esterification. Müller (89) found that when cholesterol was fed either free or as esters (together with fat) there was an increase of both in the chyle, the proportion between the two remaining approximately the same as is found normally in blood plasma indicating that esterification or hydrolysis may take place in the intestinal walls. Wacker and Hueck (90) found that both free and bound cholesterol could be increased in rabbits' blood by feeding cholesterol, but they could not get an increase in the blood of cats and dogs. Grigaut and L'Huilliere (91) succeeded in producing these effects in dogs by feeding cholesterol. Knudson (92) found that during the absorption of olive oil there was a marked increase in cholesterol esters but no increase in total cholesterol in the blood. In later work (93) he fed pure cholesterol and cholesterol esters to dogs and found both were absorbed but in every case the increase was in the *free* cholesterol and not in the esters. These results are in disagreement with those of Gardner and co-workers (94) who found that when cholesterol was fed to rabbits there resulted an increase in esterified as well as free cholesterol. Knudson believes that the exclusion of fat from his diets is responsible for the failure of the esters to increase.

Fat—blood

The fat passes from the intestine into the thoracic duct and to the blood in the form of a very fine suspension which persists for a few hours and then disappears. The fine particles of fat are about 1μ in size, are visible with the highest power of the microscope and a count of the number in a given volume gives a measure of the rate at which fat is reaching the blood (95). The measure is only approximate since the number in the blood at any time is the resultant of the number entering and the number leaving the blood during the period of absorption. During the period in which absorbed fat is present in the blood the

lipide phosphorus of the blood increases (96) (97) (98). Cholesterol has been found to increase (generally late in the period of absorption) by some workers (99) but most others have not found any increase unless cholesterol was present in the fat fed.

The manner in which the fat leaves the blood is not known. During fat absorption it accumulates in the blood, in the liver, and to a less extent in other organs. Colored fat in fine emulsions injected into the circulation is found to collect in the liver, bone marrow, spleen and muscles in the order named in which respect it behaves like other foreign material (100).

Since fat is hydrolyzed before passing the intestinal cells, the question has been raised whether a similar hydrolysis was necessary before it could pass into the tissue cells (101). Such a process would require the presence in the blood or in the cells where fat was being deposited, of enzymes capable of splitting the fats. Such has been shown not to be the case (102) and fat passing the tissue cell walls as such to and from the blood, probably does so without the aid of lipases.

In this connection confusion has arisen because of the universal presence of an enzyme which splits simple esters such as ethyl butyrate and glycerides of the lower fatty acids readily, but acts only slowly on the glycerides of ordinary fat. This enzyme is called lipase by many workers. Since it also hydrolyses lecithin and possibly cholesterol esters it is probably of considerable but enough evidence (103) has accumulated to show that it is not a true lipase and should not be so called. The name suggested by Loevenhart for this enzyme is "Esterase" which seems to the reviewer a good one and since there is no longer any question about there being two enzymes, the distinction should be emphasized. While esterases are widely distributed in animal tissues (104) lipases appear to be confined to the pancreas and intestine.

LIPIDES IN THE TISSUES

Stored fat

It has long been known that fat was one of the forms in which energy was stored in plants and animals and the nature of the

fat and the way in which it was formed and laid down have been the object of scientific and commercial interest.

In animals fat may occur in all organs and tissues but the three most important places of permanent deposit are the intermuscular connective tissue, the abdominal cavity, and the subcutaneous connective tissues. In plants the seeds and fruits and in certain cases the roots are rich in fat and in winter, fat is to be found in the trunks of trees.

The nature of the stored fat in warm blooded animals has been extensively studied. It consists ordinarily largely of a mixture in varying proportions of the glycerides of the three fatty acids, palmitic, stearic, and oleic acids. Esters of various other fatty acids such as lauric, myristic, and linolic are sometimes present in small amounts and are probably to be referred to the food.

In cold blooded animals the stored fat contains a larger proportion of the unsaturated acids than does the fat of warm blooded animals.

In plants there occurs the greatest variety of combined fatty acids, practically all the known *even* numbered fatty acids occurring in combination in plant products.

Stored fat in animals originates in the food, representing either material synthesized from carbohydrate or protein or transferred more or less directly from the fat in the intestine.

Each species of animal under normal conditions lays up a fat which is to a considerable extent characteristic of the animal; thus it is not difficult for even the amateur to distinguish between lard and beef tallow and the chemist has been able to work out a set of characteristics for each animal and vegetable fat by means of which he is able to distinguish one from the other with a considerable degree of accuracy. In plants there is only one possible source of the stored fat—carbohydrate and as consequence the "constants" of vegetable fats are much more definite than those of animal fats. In animals the fat of the food may by forced feeding be transferred to the fat stores with little if any change but when the animal has a normal choice of food the fat which is stored is quite different from that

of the food. Modifications may be brought about in the fat absorbed in the following known ways: (a) by changes during absorption, (b) by choice as to which of the constituents of the food fat is burned and which stored, (c) by admixture with fat synthesized from carbohydrate. Very little is known about any of these methods of modification so that their relative importance cannot be estimated. Regarding (a), although changes have been shown to take place in both hard and soft fats, the way in which the change is produced, whether by dilution with fat from bodily sources or by chemical change, is not known. Fat synthesized from carbohydrate is "hard" fat as exemplified in the practice among hog-raisers of "finishing" the animals by a period of grain feeding to harden them, i.e., to produce a firm fat. Scientific foundation for the practice has been furnished by the work of Anderson and Mendel (105) who found that with a preponderance of carbohydrate in the diet a characteristic hard fat was produced, while with a preponderance of various oils, a fat corresponding to these oils could be stored. Anderson (106) showed in addition that in adult animals which had been raised on various oils the oily stored fat may be changed to a hard fat by feeding with starch. Jackson (107) lists the fats of ordinary feeds in the following order of their ability to produce a soft fat—most effective linseed, then soy bean, maize, beechnut, cottonseed, wheat, pea, oat, rice, peanut, barley, rye, and bean. Shioji (108) showed that the phospholipides of the tissues may also be somewhat changed by the fat of the food but to a much less degree than the stored fat.

Fat storage in the liver

The liver of animals ordinarily contains considerable amounts of carbohydrate and not much fat. The fat which it contains is, however, considerably more unsaturated than the fat of the stores. Under certain conditions—fasting, phosphorus poisoning in certain diseases, and to a certain extent after fat feeding the fat content of the liver increases greatly. Fat has been found to accumulate in the liver of young animals before birth.

Imrie and Graham (109) studied the fat content of the livers of embryonic guinea pigs throughout the period of gestation and found that from the time when the embyro is 35 to 40 grams weight to the time of birth the fat content increases from 2 to 3 per cent up to 16 to 18 per cent of the moist tissue and has a comparatively high iodine value. After birth the fat diminishes rapidly in the first 48 to 72 hours. Many reasons have been offered for the accumulation of fat in the liver. Of these one of the earliest, that of fatty degeneration—the transformation of liver protein into fat—has been abandoned since exact methods have provided no evidence to support it. Another was that of an antagonism between glycogen and fat. When the liver is full of glycogen, fat cannot be deposited there in notable amounts, but when the glycogen is exhausted or low, fat flows in to take its place (110). The flow of fat to the liver in diabetes and in general in carbohydrate starvation has been taken as support for the idea that fat is transformed to carbohydrate, which is believed to take place in diabetes by many workers (chiefly European) (111). It should be noted that carefully controlled work in this country gives little support to this view (112). In the writer's opinion the most rational reason for the accumulation of fat in the liver is given by the Leathes hypothesis of fat metabolism, according to which the flow to the liver is a normal process since the first stage of fat catabolism—desaturation—takes place there (see below).

Biochemical synthesis of fatty acids

It has long been known, practically, that animals can synthesize fat from the carbohydrate of the food and the scientific proof has not been difficult to supply. In plants formation from carbohydrate is the only method of fat formation. Nevertheless the details of this all-important synthesis are practically unknown.

Pasteur observed the formation of butyric acid by bacteria from glucose and also from lactic acid. Nencki explained this synthesis in the light of the fact that lactic acid very readily

breaks down to acetaldehyde. Two molecules acetaldehyde condense by the aldol condensation to β hydroxy butyric aldehyde and this by simultaneous oxidation and reduction passes over to butyric acid. Raper (113) showed that small amounts of caproic and caprylic acids are formed at the same time, indicating that the aldol condensation may be repeated with the formation of higher fatty acids.

A. Loeb (114) found, that when a surviving liver containing but little glycogen was perfused with a solution of acetates in blood, diacetic acid was formed, indicating a direct condensation. Since, as noted above, acetic acid is a product of the break-down of lactic acid and therefore probably of glucose, these findings indicate that the aldol condensation or one of a similar nature may be responsible for the synthesis of the fatty acids in the animal body. An objection has been raised to the aldol condensation as applied to the synthesis of the higher fatty acids by Smedley (115) who showed that the higher aldehydes when condensed in vitro tend to form branched chains instead of straight chains. Other facts bear out the assumption that the fatty acids are built up as they are broken down—two carbon atoms at a time. Thus practically all the naturally occurring fatty acids contain an even number of carbon atoms although odd carbon fatty acids are well utilized by the animal organism as shown by the experiments on feeding the lower odd carbon acids mentioned above and the recent work with glycerides of margaric acid ($C_{17}H_{34}O_2$) (116). In milk fat, and cocoanut oil, practically all the even numbered fatty acids from butyric acid up to stearic are present, representing all stages of the progressive synthesis, two carbons at a time.

The suggestion by Emil Fischer that the higher fatty acids are formed by direct condensation of the sugar molecules with reduction and oxidation has no chemical or biological evidence to support it but is nevertheless interesting since the most widely distributed and, from the point of view of quantity the most important fatty acids are those of 18 carbon atoms such as would be formed by the condensation of three glucose molecules.

Synthesis from proteins

It has been shown by feeding experiments with experimentally diabetic animals that about two-thirds (58 per cent) of the protein molecule is convertible into carbohydrate (117). Since carbohydrate is readily convertible into fat it follows that protein should be also. The difficulty of demonstrating the conversion is great, owing to the fact that, because of the bulk of ordinary protein food and also because of its stimulating effect on metabolism, animals cannot readily be made to eat enough to provide a surplus. Then again all protein food contains some fatty material from which it is almost impossible to free it without destroying the protein. Moreover, all animals contain a store of glycogen which is a potential source of fat. One of the most convincing experiments on this point was carried out by Cremer (118). After starving a cat for several days so as to eliminate the glycogen as far as possible, the animal was given all the lean meat it would eat—450 grams per day. For eight days the whole carbon retention was 58 grams, corresponding to a glycogen production of 130 grams. The animal was found, however, to contain only 35 grams of glycogen, leaving the remainder of the carbon therefore as fat.

In recent work Atkinson, Rapport, and Lusk (119) report as follows: When the glycogen reservoirs are low, ingestion of large quantities of meat results in deposition of glycogen. Further meat feeding results in retention of both fat and glycogen while on stuffing with meat, fat alone was formed. After a carbohydrate meal the evening before (glycogen stores full), ingestion of meat produces fat. In an earlier experiment it was found (120) that after a meal of 1000 grams of meat the respiratory quotients of the fifth, six and seventh hour were 0.842, 0.845, 0.845. Computations on the basis of the metabolism of protein which was the equivalent of 1.44 grams of urinary N per hour, appeared to indicate a retention of material which would have shown respiratory quotients in the successive hours of 0.708, 0.688, 0.685, or approximately the same as fat, confirming the earlier work of Atkinson, and Lusk (121). For

the three hours by direct calorimetry 85.32 calories were found. By indirect calorimetry, if the retained carbon be calculated as having been deposited as fat, 83.58 calories may be calculated as the heat which should have been expected to arise. Had the carbon been retained as glycogen the calculated heat production would have been greater (about 4 calories per hour).

In the lower forms of animals and in plants formation of fat from protein can be more easily demonstrated. Weinland (122) showed that the larvae of the blow-fly (calliphora) could split peptone to amino acids, remove the amino and carboxyl groups and form fatty acids from the fragments. Bacteria and Fungi can form fat from protein (123).

On the other hand much of the evidence for fat formation from protein must be rejected. Thus the supposed increase of fat in ripening of cheese was found not to be the case (124) but rather the fat content diminished. The "adipocere" found in animal remains buried in wet, impervious soils was believed to be formed by decomposition of body protein. The work of Ruttan and Marshall (125) has shown that this substance has nothing to do with protein but is formed from the fat present in the animal at death. It consists of a mixture of cholesterol and fatty acids in crystalline and amorphous form and calcium, magnesium and ammonium soaps of palmitic and stearic acid. The oleic acid of the fat seems to disappear except as it is represented by a small amount of hydroxy stearic acid. The material left is light but bulky and gives the impression that most of the body has been transformed into this waxy substance.

As regards the fatty degeneration of tissues during which fat was supposed to be formed at the expense of protein it has been shown that the fat content of the tissue is generally no greater than normal and in those cases where it is greater, as is often the case in the liver, the extra fat can be shown to have originated in the fat stores (126) (127). The apparent increase of fat in degenerated tissues is due to a setting free of fatty material which in normal tissues is "built in" in such a way as to be invisible, unstainable by histologic methods and not extractable with ether.

Tissue lipides

As has just been noted, 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.

In addition to the lipides normally present in tissues others may be deposited in abnormal conditions. Thus the "anisotropic" fat of the pathologists appear to consist characteristically of combinations of cholesterol with the fatty acids and may be produced by excessive feeding of cholesterol (128) (129). Under these circumstances the deposits take place first in the Kupfer cells of the liver and in the adrenals, corpus luteum, spleen and endothelium of the blood vessels. The suprarenal glands are characterized by the large amounts of cholesterol and cholesterol esters which they contain. Cholesterol crystals may be found in any tissue that is undergoing slow destruction, especially where absorption is poor, accumulating as one of the least soluble of the cell constituents.

The lipide percentages of normal blood and tissues are characteristic of the tissue and remain relatively constant over a wide range of bodily conditions including starvation, (130) (131) although with the progress of the latter certain differences appear. In severe diabetes the percentages of blood lipides are generally considerably above normal and in some cases very much above (132) (133). The mechanism of the lipemia of diabetes has been discussed in detail (134).

THE OXIDATION OF THE FATS IN THE LIVING BODY (INTERMEDIARY METABOLISM)

The glycerol

The available evidence indicates that glycerol is utilized in the same way as the carbohydrates. Thus when fed to completely phlorizinized animals it is excreted as sugar (135) (136) and in the consideration of the foodstuffs as to whether they produce or prevent the production of the acetone compounds it is found to behave as an anti-ketogenic substance (137).

The fatty acids

The manner of disposal of the long chains of the fatty acids offers greater difficulties. One of the probable early stages in fat utilization is the formation from it of phospholipides by replacement of one of the fatty acid groups by a phosphoric acid complex—generally either phosphoric acid-cholin as in the lecithins or phosphoric acid-aminoethyl alcohol as in the cephalins. The evidence regarding the phospholipides as intermediary compounds in the utilization of the fats is, first, the universal presence of these compounds in living tissues and organs, second, the increase of lipide phosphorus in the blood during fat absorption when the blood contains much extra fat (alimentary lipemia) (138) (97), third, the increased values for lipide phosphorus in lipemia of other origins (139), and fourth, evidence of formation of milk fat from phospholipide of blood. Meigs, Blatherwick and Cary (140), workers were able to show as a result of the analysis of the blood before and after

passing through the milk gland of the cow, that the fat of milk originated mainly if not entirely in the phospholipide of the blood. In work reported at an earlier date Jordan, Hart and Patten found in cows that a diet low in phosphorus resulted in a lower milk fat production than when the phosphorus supply was adequate. The change affected mainly the glycerides of the volatile and soluble fatty acids—which would require more phosphorus for lecithin formation than the higher acids (141). Lastly it has been possible to show that the phospholipides of the hen's egg may be influenced by the fat of the food (142) and that the fat of the food may have some although slight influence on the phospholipides of the tissues (108). McCollum (142) found with hens that on a fat-free diet the phospholipides of egg yolk had iodine values of 34 to 35 while on normal mixed diet the iodine values were 63 to 64.

The advantages of the phospholipides as intermediate stages in the utilization of the fats are obvious. They mix readily with blood plasma or even with distilled water, forming a dispersion which while not a true solution provides a convenient means of transport of the insoluble fats in a watery medium such as the blood and tissues of the animal body. They readily form loose conbinations with various substances such as salts, glucose, and proteins indicating that they may be a very important stabilizing factor in the complex of living protoplasm. They are chemically more reactive that the fats, undergoing hydrolysis (to at least a considerable extent) more readily and are also more readily oxidizable, which may mean no more than that of the fatty acids they contain are more readily oxidized than those of the fats.

The evidence, while it does not prove that the phospholipides are intermediate stages in the utilization of the fats indicates that such a stage is a strong possibility.

Regarding other early stages in the working up of the long chains of the fatty acids, the theory of Leathes is practically universally accepted. It is stated as follows: "The fat is transported to the liver, unsaturated unions are there introduced into the fatty acids and possibly there, too, the complex compounds

of fatty acids with phosphorus and nitrogen are built up" (143). The data on which Leathes based his theory are as follows:

Hartley found (41) that the fatty acids of the liver, kidney and heart of several animals including man were to a considerable extent acids of a series more unsaturated than oleic acid, having iodine absorption values of 115 to 135 while the fatty acids of the fat stores in the different animals ranged between 35 and 65. In the later part of his work he paid particular attention to the identification of the fatty acids in these organs and found that in addition to the saturated acids, palmitic and stearic, which composed about half the acids, there were present an oleic acid with the double bond between the 6th and 7th carbon atoms, a linolic acid ($C_{18}H_{32}O_2$) with the double bonds between the 6th and 7th and the 9th and 10th carbons and an acid with four double bonds $C_{20}H_{32}O_2$ which has since been called by Levene "arachidonic acid." Arachidonic acid was present to the extent of about 10 per cent of the total fatty acids while there was more linolic that oleic acid. Hartley found also that the unsaturated acids were mainly combined in lecithin and similar complex substances.

Other facts which engaged Leathes' attention were, that in poisoning with phosphorus, chloroform, phlorizin, mineral acids, etc., in starvation and in many pathological conditions (in which an important factor may have been starvation) that the total fatty acid content of the liver which was not ordinarily more than three per cent of the moist weight might reach values up to 20 per cent (144) (145). It was also shown that the greater the fatty acid content of the liver the lower was the iodine value and the more nearly it approached the values for stored fat, indicating that the extra fat in the liver under these conditions was fat brought from the depots. Putting together these observations of mobilization of fat to the liver and the presence there of an unusual proportion of unsaturated acids, Leathes made the generalization that transport of fat to the liver was for the purpose of desaturation and was a normal step in fat utilization. Direct evidence of the desaturating power of the liver was obtained (146) by first determining the iodine value

of the fatty acids of the liver of rats on a normal diet, then feeding a fat of relatively high iodine value (cod-liver oil) and again examining the liver fatty acids. They were found to have an iodine value considerable higher than that of normal liver fatty acids and higher (over 30 per cent) than that of the cod liver oil fed. These experiments showed also that during fat feeding there was an increase of fat in the liver. Similar results were later obtained by Joannovies and Pick (147). Raper (84) obtained further evidence of the desaturating power of the liver. A comparison of the iodine value of the volatile fatty acids of the livers of cats with and without cocoanut oil feeding showed that the iodine absorbing power of the volatile fatty acids of the liver was increased after the feeding, indicating that the volatile fatty acids of the cocoanut oil of 10, 12, or 14 carbon atoms were desaturated in the liver. Hartley had stated that the unsaturated acids of liver were mainly in combination with lecithin and similar complex substances. Kennaway and Leathes were able to show (148) that the acetone soluble fraction of the lipoids of liver—consisting mainly of fat—contained fatty acids with iodine values much higher than those of adipose tissue (often double the values or more), indicating that the desaturating power of the liver was not limited to the phospholipides but extended to the fats also.

Leathes did not try to exclude the possibility of desaturation or phosphorization in other organs and tissues, but believed that both processes and especially desaturation were carried out mainly in the liver. The evidence in support of his theory regarding these early stages of fatty acid metabolism is perhaps not as extensive as might be desired but as far as it goes gives excellent support to his modestly expressed generalization.

Very little is known about the extent to which desaturation is carried. In the blood and most animal tissues, the most unsaturated acid found in quantity is a tetra-unsaturated acid identified by Hartley and Levene (149) as arachidonic acid. Not more than 10 per cent of the acids were found to consist of this one. An acid with three double bonds apparently does not exist in measurable amounts, except in the brain (45), while two

bond acids and of course single bond acids are most prevalent. Acids more unsaturated than the four bond acid have been reported only in the brain and in traces.

Regarding the final stages in the break-down of the fatty acid chains the prevailing conception of the method is that they are first oxidzed at the β carbon atom from the carboxyl group, producing β hydroxy or more probably β ketonic acids and then lose the terminal pair of carbon atoms yielding acetic acid and a fatty acid of two less carbons, the process being repeated until the chain is destroyed. The evidence supporting this conception has been reviewed many times and so need be only briefly referred to at this time. It is based largely on results obtained with phenyl derivatives of the fatty acids, by the use of which it has been shown (150) that the fatty acid side chains are oxidized away in this manner. The conclusion that this is the normal method of oxidation of the fatty acid chains is supported by much other evidence. Thus Embden (151) (152) and his co-workers perfused surviving livers with blood containing even numbered carbon fatty acids containing 6, 8, and 10 carbon atoms obtaining diacetic acid—which could have been produced only by β oxidation. Odd numbered acids did not yield diacetic acid. In various conditions which may be grouped under the general head of lack of available carbohydrate—starvation, diabetes, continued vomiting, etc.—the acetone compounds, acetone, diacetic and β oxybutyric acids are excreted in the urine. These are fatty acid derivatives oxidized in the β position and are regarded as originating mainly in the fatty acids of the fats. The objection that α oxidation and not β oxidation is the common result of oxidation of the fatty acids in vitro was answered by the experiment of Dakin (153) who showed that when neutralized butyric acid was oxidized in vitro under approximately the conditions in the living body, i.e., at about body temperature and with hydrogen peroxide, diacetic acid and acetone were obtained. When the reaction was carried out at higher temperatures the diacetic acid was converted into acetone by loss of carbon dioxide. This reaction was extended by him to higher fatty acids and it was found that every normal higher fatty

acid when neutralized and warmed with hydrogen peroxide gave the corresponding ketone containing one less carbon atom. This reaction demonstrated the occurrence of β oxidation in vitro in the clearest fashion. No good objection has yet been raised to the theory and it remains as the best explanation of the final stages of the oxidation of the fatty acids.

Accepting desaturation as the first stage in the catabolism of the fatty acids and β oxidation as the final stage, what can be said about the steps coming between?

Leathes' opinion was that since the double bonds constituted a point of weakness, oxidation with formation of hydroxy acids and subsequent breaking of the chain would take place in the animal body just as these processes take place in the laboratory with oxidizing agents such as alkaline permanganate. The resulting short chain acids would then be taken care of by β oxidation. Hydroxy acids, although of relatively frequent occurrence in plants, have been reported in the animal body only in the brain (Grey) so that they are probably not of great importance as intermediate steps. Further evidence that the hydroxy acids are not products of oxidation of the unsaturated acids in the animal body has been supplied by Dakin (154) and he is of the opinion that they are not formed, or at any rate not to any important extent. Oxidations in the animal body are limited by the fact that they must be carried on at a constant and relatively low temperature in a neutral medium and with reagents no stronger than the organic peroxides so that the analogy with laboratory reactions cannot be carried too far. Evidence from the behavior of the short chain acids which would be formed by the breaking of the long chains confirms the opinion of Dakin. The short chain fatty acids when fed to normal animals disappear without traces. When fed to animals rendered experimentally diabetic by phlorizin the odd carbon fatty acids yield glucose in proportion to the amount of propionic acid which they would yield by β oxidation (155) and the even carbon acids under the same conditions yield a certain proportion of diacetic and β hydroxybutyric acids and acetone. That odd carbon fatty acids are produced by

breaking the long chains seems unlikely, since feeding fat to experimentally diabetic animals or to diabetics does not produce any sugar (156). Lusk also showed that work sufficient to double the fat catabolism in phlorizinized animals did not increase the sugar output (157). As regards the formation of even-numbered, short chain acids, it is known that these yield acetone compounds in the completely diabetic organism, but since the greatest quantity of acetone compounds ever reported was in the proportion of one molecule of the acetone derivative to one molecule of fatty acid (158) the formation of short-chain even-carbon acids seems equally unlikely since otherwise more acetone compounds would be obtained.

Dicarboxy acids are formed by oxidation of unsaturated acids in vitro. Our information about the behavior of these acids in the animal body is, however, slight. Oxalic acid is resistant to oxidation, malonic and succinic acids are readily oxidized, tartaric acid and glutaric, less readily, while both these acids produce a severe nephritis (159). Baer and Blum (160) reported that adipic, pimelic and suberic acids had the same effect as glutaric in inhibiting sugar secretion in phlorizinized animals and probably, as Rose has pointed out, because they are also nephropathic. Rose believed that the toxic action on the kidney is due to slow oxidation.

A. I. Ringer (161) found that malic and succinic acids yielded large amounts of glucose in phlorizinized animals while glutaric acid yielded neither sugar nor acetone compounds. The available evidence thus goes to show that three and four carbon dicarboxy acids may be used by the animal body but the higher acids only with difficulty if at all. In general they seem to behave in the same way as the monocarboxy acids. Taken altogether, it would seem that the conception of the breaking of the long chains into shorter ones before final oxidation should not be adopted at the present time.

Although the available evidence does not indicate that the long chains are broken yet the presence of double bonds in the chain undoubtedly renders β-oxidation easier, whether the bond is at the point where β oxidation would take place or in the

position next to it, since, as was shown by Knoop's experiments with phenylalanine and tyrosine α oxidation does not hinder and probably aids β oxidation.

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.

Ketogenesis and antiketogenesis

The peculiarity in oxidation of the fatty acids referred to in the last paragraph now calls for attention. In the absence of available carbohydrate, many animals including man are unable to oxidize completely the fatty acids past the four carbon stage and the unburned products, diacetic acid, β hydroxybutyric acid and acetone (the acetone or ketone 'bodies' appear in the excretions. Two of these substances are fairly strong acids and all three are believed by some (162) to be toxic in addition to their acidity. The formation in quantity of these acids which must be neutralized before excretion, puts a severe strain on the ability of the organism to supply alkali for their neutralization and results are often fatal owing to depletion of the fixed alkalies necessary for respiration. The dog and probably carnivorous animals in general are immune to this type of poisoning, a result undoubtedly of long adaptation to the lack of carbohydrate and the presence of large amounts of fat in their diet. Instances of similar adaptation of human beings and other animals to a high fat diet are available in the literature. Thus Wigglesworth in experiments on rats with a straight fat diet found marked ketosis which reached a maximum on the third day, then subsided and adaptation was complete on the fifth

day. Sodium bicarbonate (6 per cent) prevented the adaptation, causing an increased output of lactic and β hydroxybutyric acid, indicating that much alkali may interfere with the normal oxidation of fat (163). Folin (164) found that obese individuals when fasted excreted much less acetone compounds on the second and succeeding fasts than on the first one. The amount of actual or potential carbohydrate necessary in the diet of human beings in order to avoid this "acidosis" or "ketogenesis" has been the subject of many investigations, of which the most extensive have been those of Woodyatt (165) and Shaffer (166).

Without going into the elaborate details of this work, which has been reviewed recently by Shaffer (166), the results may be summed up as follows: One molecule of glucose or its equivalent in other substances which yield sugar in metabolism (antiketogenic substances) is theoretically able to secure the complete combustion of two molecules of fatty acid or other substance yielding ketone compounds (ketogenic substance); but owing probably to uneven distribution and uneven metabolism in different parts of the body it is necessary, in order to be certain of avoiding the production of the acetone derivatives, to allow one molecule of antiketogenic substance, such as glucose, in the diet to one molecule of ketogenic substance such as fatty acid. These results have already been applied satisfactorily in clinical practice (167), (168) (169), which is proof of their essential soundness. Shaffer has also contributed much toward the elucidation of the manner in which the carbohydrates assist in the combustion of these fragments of the fatty acids, finding that the combustion (170) (171) of diacetic acid in the presence of glucose is probably preceded by a condensation of the Knövenagel type between some derivative of glucose and the diacetic acid, the condensation product being much more easily oxidized than the diacetic acid alone.

Acid intoxication

The condition of acid intoxication, due to depletion of fixed alkali noted above, is in man almost always associated with

incomplete combustion of fatty acid residues and for this reason a brief reference should be made to it.

Excess of inorganic acids, HCl, H_3PO_4, which cannot be destroyed or detoxicated, produce in man the following symptoms —stupor, coma accompanied by excessively active respiration (air hunger) with normal oxygen content of blood and low CO_2 values. The urine contains increased quantities of calcium, magnesium, potassium, sodium and ammonia. In dogs the ammonia alone is much increased and this animal is relatively resistant to acid poisoning. Acidosis is therefore an impoverishment of the blood and tissues of bases, thus reducing the capacity to combine with and eliminate CO_2 and other acids formed in metabolism. Practically, acidosis results either from defective oxidation of organic acids formed in metabolism or defective elimination due to impaired kidney function. The first factor is of main interest to us here because the most important source of organic acids is in the failure to oxidize completely the fatty acids.

In man, poisoning with organic acids is not an infrequent occurrence although confined largely to one disease—diabetes mellitus. In this disease the final stage is often coma in which the symptoms are strikingly similar to those noted above—asphyxia, due not to diminished ability of the blood to carry oxygen but a diminished ability to carry carbon dioxide, the reason for which is the production in large amounts of β hydroxybutyric and diacetic acids together with acetone and a resultant depletion of fixed alkali. These compounds are closely related chemically and the acetone and β hydroxybutyric are generally regarded as derived from the diacetic acid, which is thought to be the primary product (172) (173), an opinion which is given substantial support by the work of Wilder (174) who found that while β hydroxybutyric acid was formed in the animal body after injecting diacetic acid, diacetic acid was not formed as the result of corresponding injections of β hydroxybutyric acid.

These acids are not formed in appreciable amounts in the organism as long as a minimum quantity of carbohydrate is being utilized. This carbohydrate may be available as such

or be formed from protein. In the normal individual a lack of available carbohydrate occurs practically only in fasting but in the diabetic it occurs as the result of inability to utilize carbohydrate even though adequate amounts may be supplied.

The excretion of these acids in man often reaches 15 to 20 grams per day and as much as 150 to 200 grams has been claimed. β hydroxybutyric is normally 60 to 80 per cent of the total (175). In normal blood Marriott (176) found about 4 mgm. of β hydroxybutyric and 1.5 mgm. of acetone and diacetic together, while in blood of diabetic coma the figures were 45 and 28 mgm. per 100 cc. of blood respectively.

The tissues and especially the blood are kept at a reaction very near the neutral point (pH 7.3 to 7.4) and the extreme variations consistent with life are only a few tenths either way. In addition to preserving the reaction within these narrow limits, provision must be made of a certain reserve of fixed alkali necessary to take care of the carbon dioxide formation and excretion. To keep the reaction of blood and tissues within these narrow limits of reaction noted and to keep a sufficient "alkali reserve" in the face of acid production various devices are made use of in the animal economy. The more important ones are (1) excretion of a urine as acid as possible, the limit in humans appears to be a pH of about 5 (177); (2) neutralization of the acids by ammonia, making use for the purpose of nitrogen which would otherwise be excreted as urea (178); (3) use of excess alkali in the food and as a last resort the use of the fixed alkali of the blood and tissues down to a point where respiration can no longer be carried on. Animals vary a great deal in their ability to protect their fixed alkali. Herbivorous animals have little power of resistance while carnivorous animals such as the dog are very resistant. Man comes in between these two extremes and, when carbohydrate is not present in the food or cannot be made available from it, as in diabetes, is in danger of death due to depletion of the alkali necessary for respiration.

Intermediary metabolism of other lipides

Very little is known regarding the behavior of the other lipide substances in metabolism.

Cholesterol appears to be necessary for life and is ordinarily supplied in the food in adequate amounts either as such or in the form of other sterols which the body changes into cholesterol. Animals, or at least young animals, appear to be able to synthesize cholesterol. Thus Thannhauser and Schnaber (179) found that the cholesterol of hen's eggs increased on incubation. Stepp (180) reported that pigeons could synthesize cholesterol. Beumer and Lehmann (181) in experiments with new born puppies found that the increase in bodily cholesterol was equal to twenty times the amount in the food. Similar results have been obtained by Knudson (182) with rats. Gamble and Blackfan (183) found that much more cholesterol (up to three times as much) was excreted by infants than was present in the food. There is evidence that when the intake is inadequate it is retained and used over again (184).

Fat excretion

A small amount [generally not over 3 per cent] of fatty material is normally present in the feces of animals and it is ordinarily regarded as material that has escaped digestion. Evidence has, however, accummulated which goes to show that it (185) (186) (187) is not unabsorbed material but represents largely an excretion, whether directly from the blood by way of the intestinal secretions, or indirectly as desquamated cells from the intestinal tract. The possibility of its origin in the bacteria present in the intestine and composing a notable percentage of the fecal mass cannot be excluded but since the bacteria usually present in the intestine contain very little fat they are probably not of great importance as sources of fecal fat. Recent work (188) (189) has shown that the lipide output in the feces, while to some extent related to the amount and kind of fat in the food is in general remarkably independent of it and approaches in composition that of the blood. Under certain conditions such as lack of bile or pancreatic secretion, diarrhorea, and when the food contains a large amount of bulky, indigestible material, fat may escape digestion or absorption. The excretion of fatty material is almost entirely as fatty acids, very little unhydro-

lysed fat appearing except when the secretion of bile is stopped. Of the excretion of other lipides very little is known except of cholesterol.

Excess of cholesterol in the food is apparently largely excreted in the bile. McMaster (190) found that the amount of cholesterol in the bile was greatly increased by feeding it, while in fasting the output is greatly reduced. D'Amato (191) found that cholesterol feeding (as brain and egg) caused a small but constant increase of cholesterol but the increases were so small as to indicate that the bile was not the main path of excretion. The most authoritative statement on this point is that of Gardner (192) as follows: Cholesterol is eliminated by the liver and reabsorbed from the intestine, and whether any is finally excreted depends on the intake. Man reduces it to coprosterol and β cholestenol although some escapes unchanged. In man there is an ordinarily negative balance of 0.3 gram per day so that there is probably some synthesis.

FATS IN THE DIET

Fats are the most concentrated of the food stuffs, about 90 per cent of their weight being available for combustion. Their energy value is consequently the highest of all the food stuffs.

The heats of combustion of the natural food fats vary from 9.0 calories per gram for cocoanut oil to 9.50 calories for goose fat. Castor oil has a value of 8.84 calories per gram, boiled linseed oil 8.81 calories and spermaceti (a wax) 9.95 calories per gram (Lewkowitsch). The average value used in computations of energy in animals in 9.3.

Because of their concentrated nature they are made use of largely in plants and animals as a form in which energy may be stored. In the human diet they are valuable as convenient and concentrated sources of energy, reducing the bulk of the food intake, and adding the factors of flavor and platability as well as for certain valuable vitamins. In view of our present knowledge of tissue constituents, the statement formerly made regarding them that "they cannot serve for the upbuilding or renewal of tissues" must be revised, since such substances as

lecithin are probably as important constituents of tissue as protein.

The fact that fats can be synthesized from other food stuffs such as carbohydrates has raised the question as to whether they can be dispensed with in the diet. Experiments on this point have been carried out by Drummond and Coward (193) who have shown that young rats may grow normally from weaning to maturity on diets as free as possible from neutral fats, indicating that fats are dispensable constituents of a diet, provided the fat soluble vitamin is present in other food stuffs. The same conclusion had been reported by Hindhede (194).

Along with a little carbohydrate [probably to ensure their complete combustion] they act as efficient sparers of protein. Their utilization stimulates metabolism ["specific dynamic action"] less than protein but more than carbohydrate. After ingestion of fat the heat production [over the basal] rises gradually to the sixth hour to a maximum 30 per cent over the basal metabolism then falls slowly to the basal level, which is reached 10 hours after consumption, following the curve of changing fat content of the blood and therefore probably the concentration of metabolites available (195). The increased heat production is entirely at the expense of the ingested fat, the basal respiratory quotient averaging 0.84 and after fat ingestion becoming 0.79. Calculation showed that the amounts of protein and glycogen oxidized during two sets of experiments were identical so that the extra heat production after giving fat was derived from fat itself.

The use of fat in the diet is limited by its difficulty of digestion and combustion and ordinarily it does not compose more than one-fifth of the total energy requirement. In diabetes where carbohydrate [and therefore most of the protein] cannot be utilized it may form a much higher percentage (up to 80 per cent) of the requirement and be well utilized. As has been pointed out many times, however, a distinction must be made in these cases between fat eaten and fat burned since a certain proportion of the large amount of fat fed is probably stored.

In starvation the animal must depend largely on its store of

fat. The carbohydrate stores [glycogen] are exhausted in the first two or three days, then the energy requirement falls chiefly on the fat with a minimum of body protein, and the animal lives as long as his fat stores last. As these near exhaustion, body protein alone is left and since its energy value is less than half that of fat and is moreover, as tissue, mixed with about three times its weight of water, the animal soon dies. The composition of the organs most essential to life such as the heart, kidney, and brain is preserved almost to the end.

VITAMINS ASSOCIATED WITH FATS

Three of the five probable vitamins are associated with fats in natural products and so enhance their importance in diet. These are the fat soluble, or growth vitamin A, the antirachitic vitamin D, and the newly discovered but fairly well established reproductive or fertility vitamin E. The study of these substances has been carried on largely by the use of the domesticated white rat which lends itself readily to this type of experiment, reacting to feeding in most ways like the larger animals and by its short life cycle making it possible to get results quickly. The main disadvantage of this animal so far as is concerned with these vitamins is that it is relatively resistant to rickets. The literature on this subject is very large and since it has been excellently reviewed recently only the outstanding points and the later work will be mentioned (196). (Note: For the more recent developments in this field the reviewer has drawn largely on an unpublished summary made by Dr. H. A. Mattill of this institution.)

The vitamin A is associated with growth and well being and its lack is shown by failure to grow, and by lowered resistance to infection of which the most frequently manifest sign is xeropthalmia—a drying up of tear glands accompanied by purulent infection. The presence of vitamin A in a given food is best tested by determining the amount which will bring about resumption of growth and disappearance of the eye symptoms. Cod liver oil and most other fish liver oils have been found to be the richest source of vitamin A. The fat of egg yolk, alfalfa,

clover, green vegetables, carrots, yellow corn, butter, and milk contain notable amounts. It is low in root vegetables, seeds, and nuts. The amount in milk fat is dependent on the amount in the food of the animal.

Vitamin A is more easily destroyed by oxidation than by heat and the accompanying material appears to influence the rate of destruction. Thus heated animal fats and unheated lard and certain vegetable fats appear to destroy it (197). Young animals require more of the vitamin than old, females more than males, and reproduction and lactation increase the requirement. It may be stored in the organs and tissues of the body, especially in the liver.

The active substance is associated with the unsaponifiable fraction of the fats and is apparently not injured by mild processes of saponification. It is not cholesterol and the active substance may be distilled at low pressure without destruction and may be concentrated in this way (198).

Vitamin D: The disease rickets is characterized by an abnormality in the deposition of calcium and phosphorus in the bones and teeth and the presence of vitamine D is best demonstrated by the beginning of a zone of calcification (line test) in the long bones. The identification of this vitamin has been delayed because of a similarity in distribution with vitamin A and because vitamin D also shows a growth promoting function. The distribution of vitamin D is much the same as vitamin A but the content of the two is generally widely different. The amount of vitamin D varies widely with the history and antecedents of the product which brings us to the most striking characteristic of the vitamin which is its connection with ultraviolet radiation. Light rays of 313$\mu\mu$ or shorter appear to function in place of the vitamin or probably to be the cause of it. Inactive food products of various kinds, but particularly the fats and oils may be activated by exposure to direct sunlight or to ultraviolet radiation and, furthermore, the substance may be produced in the living animals by the same treatment (199) (200) (201).

Cholesterol, itself inactive, becomes an active antirachitic

substance by irradiation. Even the excreta of animals may be activated.

As may be imagined from the above, little can at present be said about the chemical nature and relationship of vitamin D, but like A it appears to be associated with the unsaponifiable fraction of the fats rather than with the fats or fatty acids themselves.

Vitamin E (named by its discoverer, H. M. Evans, Vitamin X) is related to the reproductive function and its absence is shown in female rats by the failure to carry their young to term although impregnation and fertilization may proceed normally. In male rats the germinal cells of the testicles degenerate. The female rat is the best test animal and the presence of the vitamin in a given food product is determined by its ability to bring about normal reproduction.

Wheat germ and dried lettuce leaves are the richest sources of vitamin E yet found. It is found in butter fat but not in cod liver oil.

It is a definitely fat-soluble substance and is again associated with the unsaponifiable fraction but is not cholesterol. It is relatively sensitive to oxidation (202) (203) (204) (205).

Summing up the present knowledge of the vitamins in their relation to the fats it appears that they are not connected with the fats or fatty acids as such but are always found in the unsaponifiable residue and are not cholesterol or any other known substance, although they may be related to some of these. Attention is thus strongly drawn to this hitherto little studied group of substances, indicating that their investigation will well repay the effort of the chemist.

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