THE INTERRELATIONSHIPS OF FOODSTUFFS IN METABOLISM

A Tentative Proposal*

JOHN P. PETERS

Like every panel of physiology or medicine, metabolism has been studied and taught by the categorical method. But, for a full understanding of the subject, synthesis and integration are essential. Energy production and caloric needs are only significant when the purposes to which the energy is directed are taken into account. It may be useful to regard the body as a complex industrial plant or machine. Power to run this plant could be derived from a variety of fuels, such as wood, coal, or oil. The counterparts of these in the physiological factory are carbohydrate, fat, and protein. The mere combustion of these for the production of power may be called the energy metabolism. The efficiency of the power plant will vary—first, with the nature of the fuel; secondly, with the device used for its combustion. This will have to be different and appropriate for each fuel. The mere production of heat, though essential for its operation, is not, however, the object of this plant. The heat must be directed to useful ends. This requires highly specialized machinery for which particular materials must be provided. Some of these can be manufactured on the premises; others must be supplied ready-made. This machinery must be integrated and must be equipped with regulating devices to permit variations in the relative speeds of its multifarious processes. It has been suggested that these phases of metabolism be termed *operative* metabolism, in contradistinction to *energy* metabolism. Until recently operative metabolism has been an almost unpenetrated mystery. This mystery is now being dispelled with such rapidity that it is hard to keep pace with the advances. It is impossible in a single article to detail these discoveries; an attempt will be made through certain examples to convey some impression of the modern dynamic concept of metabolism which deals with chemical compounds not as mere substances, but as instruments.

Since provision of power is the ultimate limiting factor, it may as well receive attention first. Any one of the three foodstuffs—fat, carbohydrate, or protein—may serve as fuel for this plant, but not indifferently. No one of them is altogether indispensible because each has an operative as well as an energy-producing function. A small amount of carbohydrate is essential for purposes that will be outlined later. If adequate and continuous amounts are not supplied in the food, the body is compelled to manufacture it. The only

* From the Department of Internal Medicine, Yale University School of Medicine.
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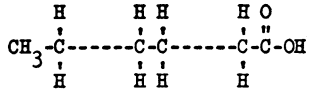
material suitable for this purpose is protein; but to use protein for this purpose is inefficient and wasteful. Species vary in the quantities of carbohydrate required for operative purposes, carnivores being particularly economical in this respect. In the investigation of physiological problems the choice of animals is not a matter of indifference. Inferences with respect to the functions of man cannot be indiscriminately drawn from experiments on rats, rabbits, dogs, hamsters, or even monkeys. Conceptions and misconceptions about diabetes might be very different if von Mehring and Minkowski had chosen another animal than the dog for their classical experiments.

Fat, like carbohydrate, is composed solely of carbon, hydrogen, and oxygen. It is an ideal fuel because its caloric value per unit of mass is so great. It is formed freely in the body from carbohydrate; but, for reasons that will be discussed later, the reverse is not true. The reaction,

1] Carbohydrate ⟶ Fat

is essentially irreversible. Fat would appear to be an indifferent substance which would not have to be provided from the environment. So far as its fuel value is concerned, this is true. Nevertheless, if an animal is given a diet devoid of fat, but containing enough carbohydrate to supply its caloric needs, it will lose weight and ultimately die with characteristic lesions, including a fatty liver and degeneration of the kidneys. The explanation for this lies in the fact that certain types of fatty acids essential for life cannot be formed in the body. A fatty acid consists of a long straight hydrocarbon chain with a carboxyl tail,

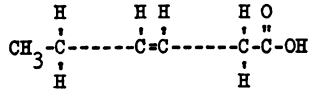
2] Fatty acid, saturated



Stearic acid contains 18 carbons

The length of the carbon chain varies up to about 22 carbons. Of these, fatty acids up to 18 carbons, stearic acid, can be formed in the body. Of the fatty acids in the body, a certain number are unsaturated; that is, they contain carbon-linkages without the full complement of hydrogens.

3] Fatty acid, unsaturated



Oleic acid contains 18 carbons with one unsaturated bond

Oleic acid belongs to this class, being an 18-carbon acid with one unsaturated bond. The body is able to manufacture, from carbohydrate or from fatty acids, oleic acid and other fatty acids with one unsaturated bond. There are, however, in the body certain essential fatty acids containing 18 or more carbons and having more than one unsaturated bond, the principal

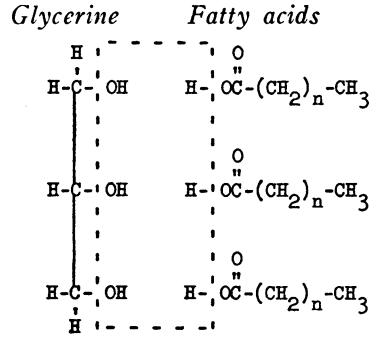
ones being linoleic, linolenic, and arachidonic, which cannot be synthesized in the body, but must be provided in fats in the food.

Essential fatty acids which cannot be synthesized in the body:

4]	Linoleic acid	18 carbons, 2 unsaturated bonds
	Linolenic acid	18 carbons, 3 unsaturated bonds
	Arachidonic acid	20 carbons, 4 unsaturated bonds

A fat is composed of three molecules of fatty acid combined with a molecule of glycerine.

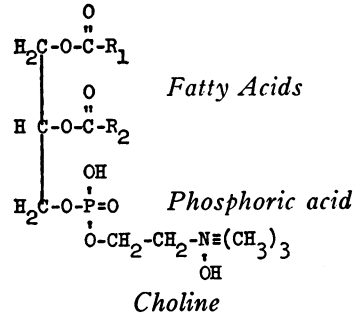
5]



The OH^- and H^+ ions inclosed by the broken lines are lost as water when glycerine and fatty acids combine to form fat.

Fats are found in adipose tissue and are both found in and utilized by the cells of the liver. They do not occur in other tissue cells, however, although fatty acids are burned by these cells. In the tissue cells fatty acids occur in cholesterol esters and phospholipids. The tissue cells apparently burn the fatty acids of the phospholipids, which presumably serve as vehicles for the fatty acids and as instruments that facilitate their oxidation. A characteristic phospholipid, lecithin, has the general composition of a fat, except that one of the fatty acids is replaced by a phosphoric acid and the methylated nitrogen-containing compound, choline.

6]



Of the two fatty acids, R_1 and R_2, one is of the ordinary type, the other belongs to the highly unsaturated types. It is the ordinary saturated fatty acid that is used for fuel, the unsaturated members are conserved with great

economy. They form essential parts of the machinery. It is apparently for the formation of these components of the phospholipids that the essential fatty acids are required.

Carbohydrate and fat are both composed solely of the three elements, carbon, hydrogen, and oxygen, and can, therefore, be completely oxidized to carbon dioxide and water. Protein, on the other hand, contains nitrogen. On this account it cannot be replaced by either carbohydrate or fat. On the other hand, it can, to a certain extent, replace both the other foodstuffs. It is not, however, an efficient fuel because it cannot be completely oxidized. Furthermore, it serves operative functions of such importance that it is not economical to waste it for fuel alone. Proteins are complex combinations of amino acids which have the following general configuration:

7]  The radical R differentiates the amino acids.

Although the amino acids have this common characteristic, they differ widely in the nature of the radical, R. Altogether 23 amino acids are generally recognized. Of these 10 cannot be synthesized in the body, but must be provided in the diet. These are generally termed essential amino acids, not because they are any more essential than the others, but because, if all of them are not provided in sufficient quantities and proper proportions, life cannot be supported. It is, therefore, not only necessary to supply adequate amounts of protein in the diet, but also protein of a satisfactory quality. Furthermore, if protein is consumed as fuel, larger amounts are required. The amino acids generally recognized are listed below with the ten essential ones capitalized:

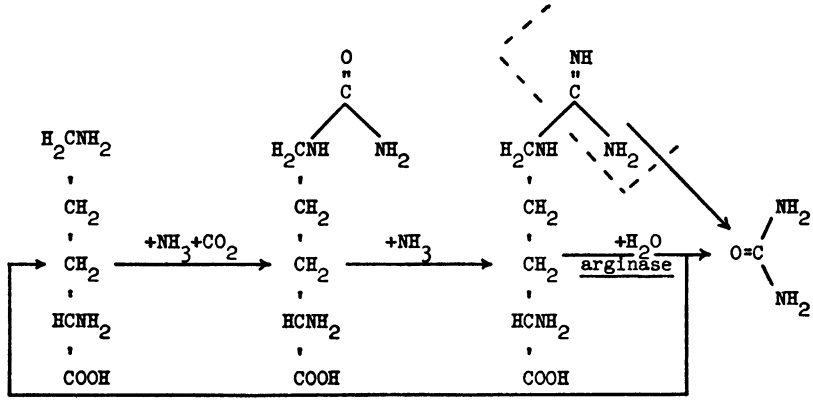
8]

ARGININE	Serine
Citrulline	THREONINE
LYSINE	VALINE
Hydroxylysine	LEUCINE
HISTIDINE	ISOLEUCINE
PHENYLALANINE	Norleucine
Tyrosine	METHIONINE
TRYPTOPHANE	Cystine
Proline	Glutamic acid
Hydroxyproline	Hydroxyglutamic acid
Glycine	Aspartic acid
Alanine	

The precise functions which all of these serve are not known, although most of them occur in varying proportions and in innumerable permutations and combinations in proteins. It may be worth while to point out a few of the processes in which some of them are known to participate. The position of arginine among the ten essential amino acids is open to question. It can apparently be synthesized in the body, but only to a limited extent. It is the

chief vehicle by which ammonia is conveyed to the liver for the formation of urea. The reactions involved in this process are illustrated thus:

9]

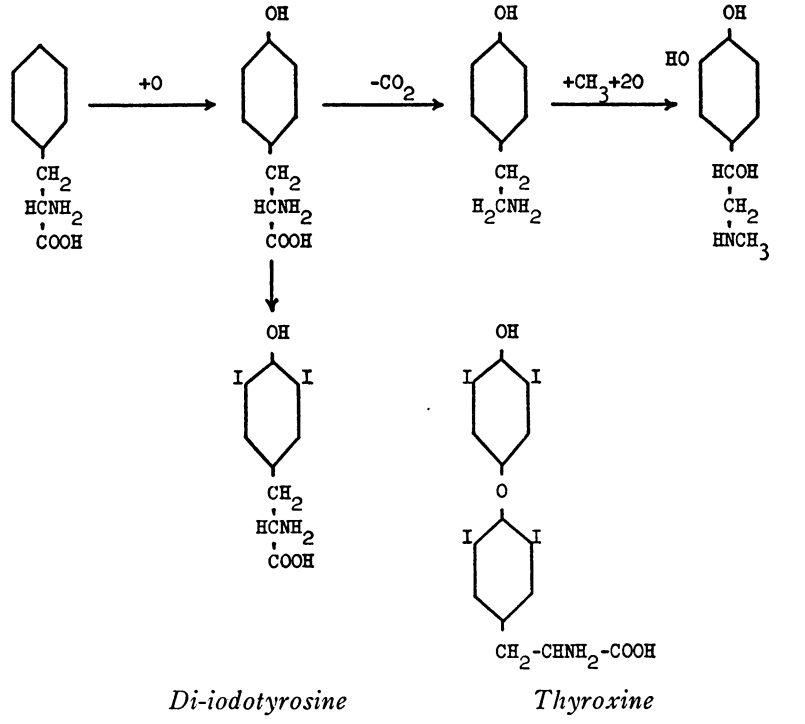


Ornithine *Citrulline* *Arginine* *Urea*

Ornithine, an amino acid, for some reason not included in the regular lists, combines with carbon dioxide and ammonia to form citrulline, which is listed. This, in turn, combines with another molecule of ammonia to form arginine plus a molecule of water. From arginine with the addition of a molecule of water and the action of the enzyme, arginase, the amidine group

Phenylalanine *Tyrosine* *Tyramine* *Epinephrine*

10]



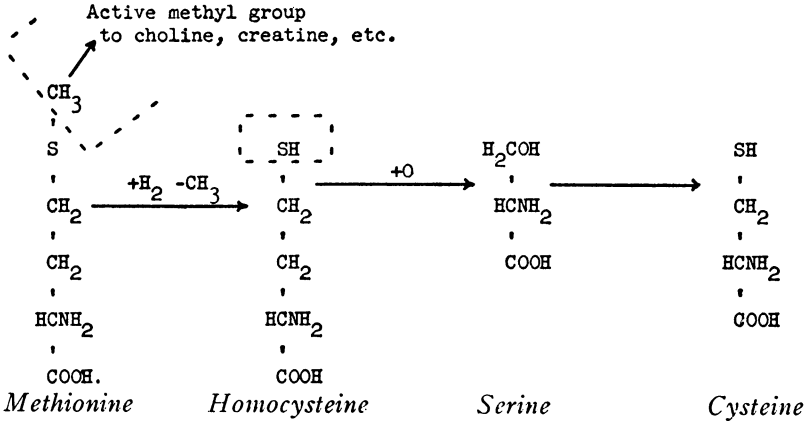
Di-iodotyrosine *Thyroxine*

is broken off to form urea and by this reaction ornithine is again reconstituted to repeat the cycle. Arginine also contributes the amidine group for the formation of creatine (see 11]).

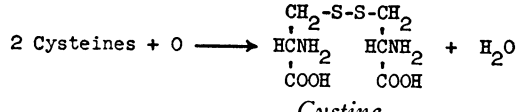
Phenylalanine can be used to form tyrosine, if this is not included in the diet (See 10]).

The former must have also certain independent functions that have not been clearly elucidated, because no amount of tyrosine can be substituted for phenylalanine. The oxidation of phenylalanine to tyrosine is, therefore, an irreversible process. From the latter both epinephrine and thyroxine appear to be elaborated.

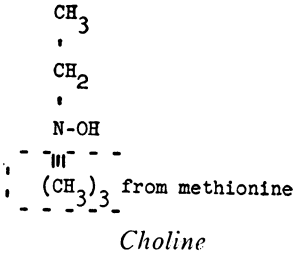
Methionine is peculiar in that it contributes fractions of itself for a variety of purposes.



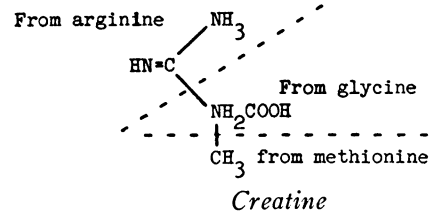
11] *Methionine* *Homocysteine* *Serine* *Cysteine*



Cystine



Choline



Creatine

The methyl group, —CH_3, at the top provides active methyl groups for both of the essential substances, choline and creatine. The sulfur group is used for the formation of cystine, cysteine, and secondarily glutathione and other sulfhydryl compounds, with the aid of another amino acid, serine, which can be synthesized in the body.

In addition to the replacement of protein in the body, therefore, proteins must supply amino acids for all kinds of specific purposes. This entails the continuous degradation of a certain amount of protein. The capacity to store protein is extremely limited in a well-fed individual. In spite of its high value, therefore, any excess of protein is burned. In the process of combustion, the nitrogen is broken off as ammonia which is excreted as such, or as urea; the remainder is converted to materials that can be oxidized through the channels of either carbohydrate or fat.

12]

$$\text{Energy-production} \begin{cases} \longrightarrow \text{Urea or ammonia} \\ \longrightarrow \text{Carbohydrate or fatty acid} \rightarrow CO_2 + H_2O \end{cases}$$

$$\text{Operative} \begin{cases} \longrightarrow \text{Creatine, creatinine, uric acid, etc.} \\ \longrightarrow \text{Carbohydrate} \end{cases}$$

The end-products of the combustion of protein are, therefore, CO_2, H_2O, and urea (for our purposes the small amounts of sulfate can be neglected). In the urine, in addition to urea and ammonia, other less completely catabolized nitrogen-containing compounds, such as creatinine, uric acid, and amino acids are found. When the protein in the diet is varied, the excretion of urea + ammonia varies accordingly; but the excretion of other nitrogenous substances remains almost entirely unchanged. If protein catabolism is reduced to a minimum by administration of a protein-free high calorie diet, urine urea+ammonia diminishes almost to the vanishing point, but creatinine and uric acid are hardly affected at all, even when energy-production is greatly increased by exercise. This is, perhaps, the best illustration of the distinction between energy-producing and operating metabolism. Creatinine and uric acid are products of operational functions (what has been called endogenous metabolism) which must continue uninterrupted to maintain vital activities. There are no substitutes for the compounds used in these operations. Urea and ammonia are products of energy-producing processes. They, therefore, vary with the nature and quantity of food available. In addition, even the energy-producing function of protein is not entirely independent of operative needs. Protein is the only source of carbohydrate when the latter is not provided in the food and must, under these circumstances, take over the operating offices of carbohydrate.

The proteins themselves must not be regarded as mere structural components of the tissues, but as extremely active agents. Perhaps more than any others they are responsible for the differentiation and specialization of vital activities. Schoenheimer showed that their structures are continually changing. Among other things, proteins form the cores of all enzymes.

There is another large and heterogeneous class of substances for the supply of which animals are dependent upon the environment—the vitamins. There is space for only a few remarks about these compounds. It is unfortunate that such a heterogeneous aggregation of substances should continue to be grouped together under a term that was devised as a cloak

for ignorance, and should be distinguished from one another only by letters and numbers. The composition of a large proportion of these substances is now known; some of their functions have been elucidated. They are no longer mysterious vital forces, but identified chemical compounds that participate in the reactions that constitute the operative metabolism. The majority, especially of the members of the B-vitamin group, are components of enzyme systems.

The nature of these enzyme systems requires a few words. Some of the mysteries surrounding these are also being gradually dispelled. They have also proved to be complexes which can be isolated and identified and which participate in the chemical reactions which they facilitate. The term, enzyme systems, is used because such an organism as the yeast cell forms alcohol from sugar by a long series of linked reactions, each one of which is implemented by a separate enzyme. In most instances an enzyme consists of a highly specialized protein, together with one or more organic components (this is the rôle of the vitamins) and frequently inorganic elements, especially phosphorus. These complexes implement chemical reactions. For example, inorganic phosphate mixed with glycogen is altogether inert.

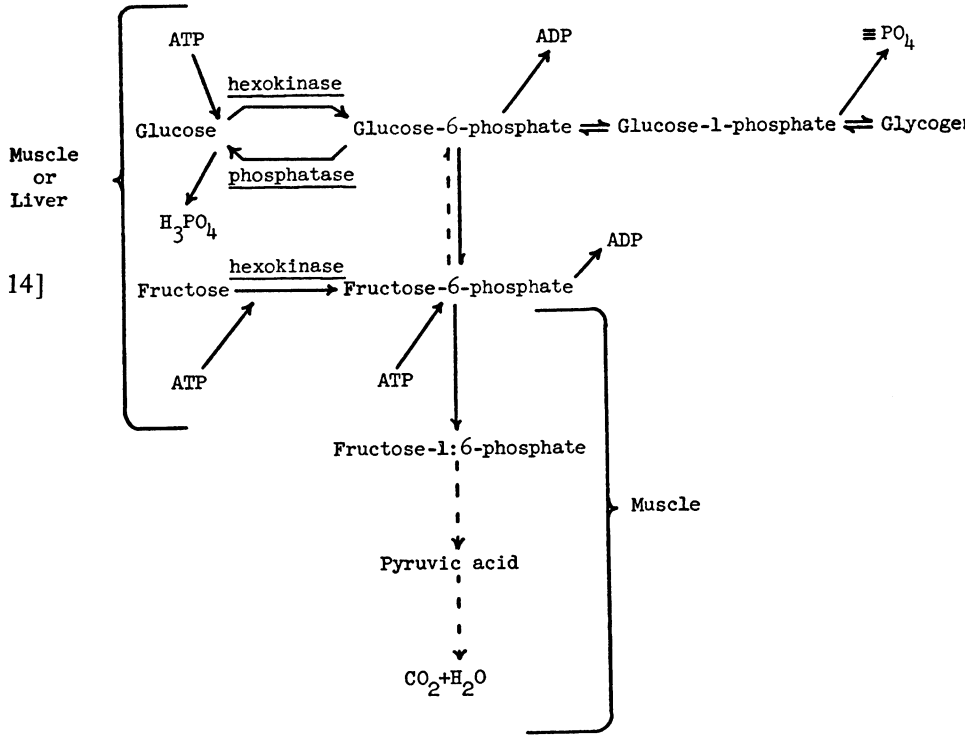
13]

$$\text{Glycogen} + H_3PO_4 = \text{glycogen} + H_3PO_4 \text{ (no enzyme present)}$$

$$\text{Glycogen} + H_3PO_4 \xrightleftharpoons{\text{phosphorylase}} \text{glucose-1-phosphate}$$

But, if the enzyme, phosphorylase, is added to the mixture with a little adenylic acid, glucose-1-phosphate is formed. This reaction is depicted as freely reversible, which it is when this particular set of reagents is allowed to react alone. Under these circumstances, also, the enzyme tends to deteriorate. In its native habitat, however, the reaction is oriented, proceeding predominantly in one direction, because it is linked with other similar reactions. The direction of an equilibrium reaction like the one illustrated in this equation depends upon the relative proportions of reactive materials on the two sides of the equation. Addition of glycogen and phosphoric acid will drive the reaction to the right, whereas addition of glucose-1-phosphate will drive it to the left. If, then, glucose-1-phosphate was withdrawn by another reaction as soon as it was formed, the reaction would tend to run always to the right, forming glucose-1-phosphate. The speed and direction of a process is determined by the relative activity of the various components of the enzyme system as well as the quantities of materials provided. This is the principle by which order is achieved in biological organisms. It is the sum of such a series of reactions that is termed an enzyme system. In such a system provision is also made through these linked reactions to restore to an enzyme the activity which it lost in the course of the single reaction

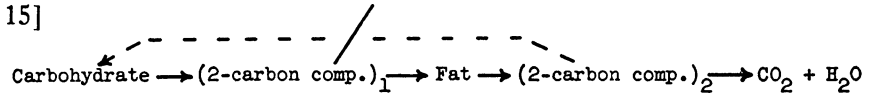
which it implemented. Biological reactions are seldom spontaneously reversible. If, in the course of a series of reactions the original reagents are reconstituted, it is usually by a cyclical or circuitous route. All these points are illustrated in the formation of urea through the agency of arginine (see 9]). It must be recognized that not only the last reaction, but also the first two reactions, ornithine to citrulline and citrulline to arginine, are implemented by enzymes. The reaction begins and ends with ornithine, but not by retracing its steps, which would be futile. In the first two reactions ammonia and CO_2 are accumulated. In the last, through the agency of arginase, they are discharged as urea to be excreted. Furthermore, in this last reaction the molecule of water accumulated in the course of the formation of arginine is used to form the urea, thus leaving the whole system in its original state. In this connection it may be worth while to present a few other examples which will require attention later. Glycogen of the liver is a temporary repository for carbohydrate. Glucose from the blood is built up to glycogen in the liver to be again broken down when required to provide glucose to the blood. The simple equilibrium equation, glucose⇌glycogen, would express the over-all effect of the reaction, but would not be a correct representation of the facts.



Glucose, with the aid of adenosine triphosphate and the enzyme, hexokinase, forms glucose-6-phosphate by an irreversible reaction. From glucose-6-phosphate, glycogen is formed through the intermediate step of glucose-1-phosphate with the aid of the enzymes, phosphoglucomutase, and phosphorylase. These two reactions are apparently reversible. Glucose-6-phosphate can also be changed to fructose-6-phosphate, which may likewise be formed from fructose.* It is evident that through this path fructose can form glycogen. Glucose-6-phosphate can again be converted to glucose by a separate path implemented by the enzyme phosphatase. Glucose-6-phosphate, therefore, occupies a central position through which glucose can pass to glycogen and return to glucose and through which fructose in turn can form either glycogen or glucose. Glycogen of muscle, in contradistinction to glycogen of liver, does not contribute to the glucose of blood. The reason lies in the presence in muscle of another enzyme system for the oxidation of sugar, which begins with the conversion of fructose-6-phosphate to fructose-1 : 6-diphosphate with the aid of adenosinetriphosphate and the enzyme phosphohexokinase. From there the chain of reactions continues through pyruvic acid to carbon dioxide and water. The circuit is, therefore, tapped at the fructose-6-phosphate point by a chain of extremely active reactions. In consequence, the flow through glucose-6-phosphate is entirely diverted from the path, glucose-6-phosphate to glucose, into the channel of oxidation.

Carbohydrate can be converted to fat, but fat does not form important amounts of carbohydrate. Although carbohydrate forms 2-carbon compounds that can be converted to fat,

15]



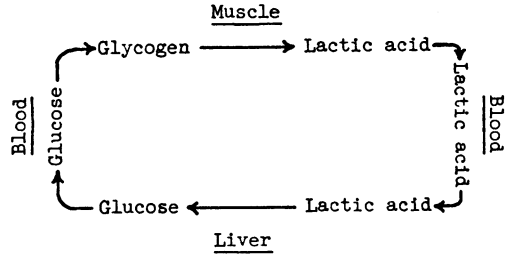
and fat in turn forms 2-carbon compounds that can be burned, the 2-carbon compounds derived from fat cannot be used to form carbohydrate or deriva-

* It has been repeatedly demonstrated that in both normal and diabetic subjects fructose is removed from the blood more rapidly than glucose is, and that in the diabetic subject the blood glucose does not rise so rapidly nor so high after fructose as is does after glucose. Max Miller (personal communication) has recently shown that the liver removes fructose from the blood far more rapidly than it does glucose. At the same time pyruvic acid in the blood increases after fructose, the contribution coming from the liver. This suggests that fructose and glucose are treated differently by the liver; the major part of the former must bypass the common pool of glucose-6-phosphate. This is indicated in equation 14] by the broken line from fructose-6-phosphate to glucose-6-phosphate. This reaction cannot be as freely reversible as has been generally assumed. The chain of reactions proceeding from fructose-6-phosphate to pyruvic acid also must be less strictly confined to muscle than it is here depicted. The tendency for the reaction, glucose-6-phosphate$\rightleftharpoons$fructose-6-phosphate, to proceed predominantly to the right might be referable to inherent characteristics of the enzyme system involved or merely to the fact that fructose-6-phosphate is constantly drawn off for the formation of pyruvate.

tives that can be substituted for carbohydrate. It may be that the 2-carbon compounds formed from carbohydrate are not the same as those formed from fat.

In the absence of oxygen, glycogen of muscle is burned only to lactic acid. The latter is not reconverted to glycogen in the muscle, but is conveyed by the blood stream to the liver where it can be converted to glycogen.

16]



One word about hormones. Although the secretions of the endocrine glands influence chemical reactions, they do not appear to serve as enzymes. They are not the prime movers of metabolism, which does not cease entirely when one or more of the endocrine glands is removed. They appear only to accelerate or retard the actions of the enzyme systems, perhaps by potentiating or inhibiting certain particular enzymes.

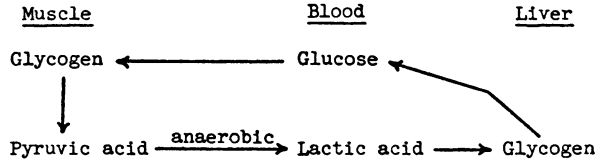
So much for what may be called stage setting. It has been a long and tedious process, but essential to an understanding of the intermediary metabolism and exchange of the various foodstuffs. It is unnecessary to detail all the steps in the estimation of energy metabolism. A few general statements will suffice. Energy is produced by the combustion, that is oxidation, of foodstuffs. The end products of the complete oxidation of carbohydrate and fat, since these substances contain only carbon, hydrogen, and oxygen, are CO_2 and H_2O. The ratio of CO_2 produced to oxygen consumed, the respiratory quotient, as well as the heat produced by consumption of a given amount of oxygen, will vary with the composition of the foodstuff. As far as the two foodstuffs, carbohydrate and fat, are concerned, therefore, the energy produced can be estimated from the oxygen consumed and the CO_2 produced. Protein contains nitrogen and sulfur as well as carbon, hydrogen, and oxygen. In addition to CO_2 and H_2O, therefore, its combustion products will include nitrogen-containing compounds and sulfates which are excreted in the urine, the nitrogen-containing compounds in an incompletely oxidized state. Estimation of the total energy production, then, requires measurement of the oxygen consumed, the carbon dioxide produced and the nitrogen excreted in the urine.

The first steps in the metabolism of carbohydrate have already been described. Not only glucose, but other sugars and some of the products of

the metabolism of protein are converted into glucose or glycogen. The latter is an aggregation of molecules of glucose which is used for the storage of carbohydrate in liver and other tissues. Because of its large molecular weight, it exerts almost no osmotic pressure and, therefore, does not disturb the hydration of cells as glucose itself would. The actual quantity of glycogen in the body is quite small, altogether too small, we now realize, to bear the whole brunt of continuing metabolism. Apart from this, however, there is no other endogenous source of carbohydrate except protein, and the amount ordinarily contributed by protein is quite small. All carbohydrates, as the name implies, are made up of units, each consisting of one atom of carbon and one molecule of water, CH_2O. For example, glucose, $C_6H_{12}O_6$, consists of 6 of these units. It follows that one molecule of oxygen, O_2, is required for the combustion of each unit, forming one molecule of carbon dioxide, CO_2, and one of water, H_2O. Since molecular equivalents of ideal gases occupy equal volumes, the respiratory quotient of carbohydrate is 1.000. The caloric value of 1 gram of carbohydrate equals about 4.1 Calories.

By reference to 14] it will be seen that when glycogen is burned in the muscles it does not pass through glucose as an intermediary step, but through glucose phosphates and fructose phosphates to pyruvic acid. Numerous intermediate steps between fructose-1 : 6-phosphate and pyruvic acid have been omitted. From this point several alternative paths present themselves. If the supply of oxygen is inadequate, complete oxidation of the pyruvic acid is impossible. Instead it is converted to lactic acid.

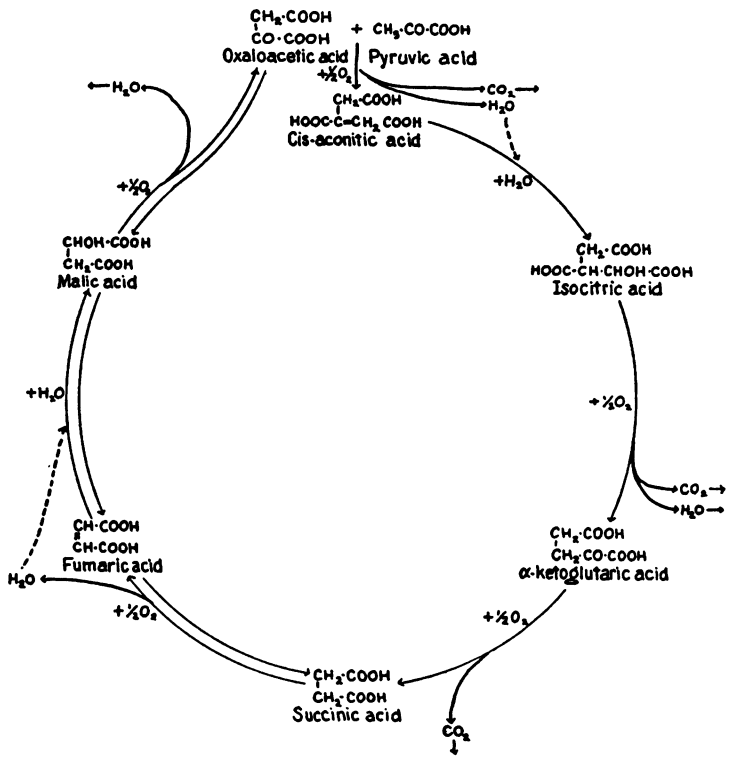
17]



This releases part of the energy of the carbohydrate, but leads to a dead end because the lactic acid cannot be further burned nor can it be reconverted to glycogen in the muscle as Hill and Meyerhoff once conceived. Instead it must be conveyed to the liver for such reconversion. This is apparently the only means of supplying any reasonable amount of energy in the absence of oxygen. In itself it would be a sufficient reason for the provision of a store of carbohydrate in the muscles. Anaerobic exercise is not unusual. It is required for any quick spurt of activity. For instance, a 100-yard sprint is almost entirely anaerobic exercise.

Sustained exercise with carbohydrate as fuel requires oxygen and from pyruvate onwards follows an altogether different channel, the Krebs' cycle, so named from its discoverer.

18]



It has been drawn in circular form to emphasize its cyclical character and to bring out its machine-like nature. It is unnecessary to dwell on all the compounds in this circle, but only some of its characteristics. It is pictured as proceeding clockwise from the top, the 12 o'clock position. At this point oxaloacetic acid, a 4-carbon group, comes up on the wheel to combine with pyruvic acid. Before proceeding further, however, these two acids deserve some attention. In the course of its metabolism, glucose, originally a 6-carbon compound, has been broken in half, leaving pyruvic acid, a 3-carbon compound.

It will be noted first that if a molecule of CO_2 is added to pyruvic acid, it can form oxaloacetic acid.

Pyruvic acid *Oxaloacetic acid*

$$CH_3\text{-}CO\text{-}COOH + CO_2 \longrightarrow \begin{matrix} CH_2\text{-}COOH \\ | \\ CO\text{-}COOH \end{matrix}$$

19] $3C+4H+3O + 1C+2O = 4C+4H+5O$

Pyruvic acid

$$CH_3\text{-}CO\text{-}COOH + 5O \longrightarrow 3CO_2+2H_2O$$

$$3C+4H+3O + 5O = 3C+4H+8O$$

Since oxaloacetic acid is the end and beginning of this cycle, it follows that the cycle can be primed or renewed at any time by means of a combination of pyruvic acid and CO_2. To oxidize pyruvic acid completely will require five atoms of oxygen, five O, producing three molecules of CO_2, and two of water. Let us assume, then, that oxaloacetic acid to start this cycle has been provided by combination of pyruvic acid with CO_2 and that another pyruvic acid has been fed to the oxaloacetic acid on the wheel and that this has started to go around the cycle. At the very beginning, as you will see, it takes on one O and loses a CO_2 and an H_2O as the oxaloacetate and pyruvate combine to form the next component, cis-aconitic acid. Forget for the moment these compounds and consider only the oxygens that enter and the CO_2's that come off. Exactly five oxygen atoms are taken up: one at the very beginning; another at about 3 o'clock, after isocitric acid; a third at 5 o'clock, after α-ketoglutaric; a fourth at 7 o'clock after succinic; and the fifth at 10 o'clock after malic acid. In the same manner three CO_2's are given off: one at the beginning, one at 3 o'clock, and one at 5 o'clock. It can be shown in the same way that two molecules of water are lost. In other words, the pyruvic acid fed to the oxaloacetic acid has been oxidized precisely and completely, leaving the original component, oxaloacetic acid, intact and ready to start another cycle to burn another molecule of pyruvic acid. The cycle can, therefore, be regarded as a machine for the combustion of pyruvic acid, a combustion device. Undoubtedly, the energy required to run this device is derived from the combustion of the pyruvic acid. But all of the energy from this reaction is not dissipated in this futile manner. This is not the actual machinery of muscular activity, but the power plant. The contraction of muscle itself, towards which this energy is directed, probably resides in some less well understood reactions in which the proteins of the muscle are involved. A small amount of pyruvic acid will be required from time to time to replace oxaloacetic acid which may have inadvertently been consumed on the way; otherwise the machinery is self-perpetuating.

Although pyruvic acid, the product of carbohydrate, would be required to form oxaloacetic acid, other fuel might equally well be burned in the Krebs' combustion device. This indeed seems to be the case. Suppose—and this is a somewhat hypothetical reaction—the two-carbon acetic acid were added to oxaloacetic acid.

Acetic acid + *Oxaloacetic acid* + H − H_2O ⟶ *Cis-aconitic acid*

CH_3-COOH + CH_2-COOH (CO-COOH) + H − H_2O ⟶ CH_2-COOH (HOOC-C=CH_2COOH)

20] $2C+4H+2O$ + $4C+4H+5O$ + H − 2H − O = $6C+7H+6O$

Pyruvic acid ⟶ *Acetic acid*

CH_3-CO-COOH + O − CO_2 ⟶ CH_3-COOH

$3C+4H+3O$ + O − C − 2O = $2C+4H+2O$

By the addition of an atom of H and loss of a molecule of water, it could form the next acid in the cycle, cis-aconitic, and continue the round. At the end the acetic acid would have been burned, leaving oxaloacetic acid again. Although this particular reaction is hypothetical, it is now established that 2-carbon compounds are produced from carbohydrate and are oxidized, presumably over the Krebs' cycle. To carry the hypothetical reaction further, we may picture pyruvic acid as being converted to acetic acid by the addition of one atom of oxygen and the loss of a molecule of CO_2. Acetic acid might be quite suitable as fuel for this combustion device, but quite incapable of replacing oxaloacetic acid, that is keeping up the machinery. Certain work of Hastings with isotopes suggests that this is indeed the case. To replace oxaloacetate, pyruvate is required. I would emphasize again that, although these last reactions serve as an illustration of the facts, they cannot be accepted as a precise representation like that of the Krebs' cycle. From this point on the argument will have to be conducted in such approximate or symbolic terms.

Attention must now be diverted to the metabolism of fat. The structure of fat and fatty acids has already been shown. If the structure of a fat is examined, it is at once apparent that it contains very little oxygen in proportion to carbon and hydrogen. For example, tristearin, a fat composed of the saturated 18-carbon stearic acid, has the formula $C_{57}H_{110}O_6$. To oxidize this completely to CO_2 and H_2O will require the addition of 81.5 molecules of O_2, which yield 57 molecules of CO_2. The respiratory quotient will, therefore be $\frac{57.0}{81.5} = 0.70$. The respiratory quotients of various fats lie between 0.70 and 0.71. Combustion of one gram of fat yields about 9.3 Calories.

If the structures of carbohydrate and fat are compared, it is immediately evident that conversion of carbohydrate to fat must involve loss of oxygen. By comparison of the caloric values of the two foodstuffs it is also apparent that energy must be expended in this conversion.

To provide the carbons of tristearin, $C_{57}H_{110}O_6$, from glucose, $C_6H_{12}O_6$, would require 9.5 molecules of glucose. The total molecular weight of tristearin is 890, that of glucose is 180. In terms of gram molecules, therefore, $9.5 \times 180 = 1710$ gm. of glucose are needed to form 890 gm. of tristearin. The caloric value of 890 gm. of tristearin $= 890 \times 9.3 = 8270$ Calories, while 1710 gm. of glucose yields only $1710 \times 3.8 = 6498$ Calories.* This would leave a deficit of $8270 - 6498 = 1772$ Calories. This means that 1772 extra Calories must be expended in the process of conversion. If this is to be provided by glucose, it will require the combustion of $1772/3.8 = 466.6$ gm. of glucose, equivalent to $\frac{466.6}{180} = 2.6$ mole-

* The caloric value of 1 gm. of glucose is 3.8 Calories. The factor generally used for carbohydrate, 4.1, applies strictly to starch.

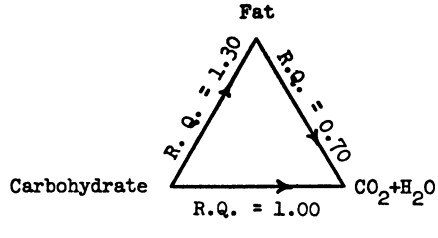
cules. In other words, in addition to the 9.5 molecules of glucose required to provide the structure of the fat, another 2.6 molecules, or almost 30 per cent more, must be burned to effect the conversion. These actual figures are of less importance than the fact that fat cannot be formed from carbohydrate unless some carbohydrate is burned. Because oxygen is given off in this conversion, the respiratory quotient is greater than 1.

As a means of storing fuel, fat has peculiar advantages. A given weight of fat contains $9.3/4.1 = 2.3$ times as many calories as does an equal weight of carbohydrate. The latter, furthermore, must be held in solution. For this purpose about 3 gm. of water are required for each gram of glycogen. Fat, on the other hand, is insoluble, requiring no water. In terms of tissue, therefore, nine Calories of fat occupy the same space or weight as one Calorie of carbohydrate. This constitutes the major problem in the treatment of obesity. In the food, fat conceals itself so effectually; from the body it can be removed so slowly. A single pound of fat represents 4870 Calories. To eliminate this pound in one week an individual must have a dietary deficit of about 700 Calories per day. To lose 50 pounds at this rate will take almost a year.

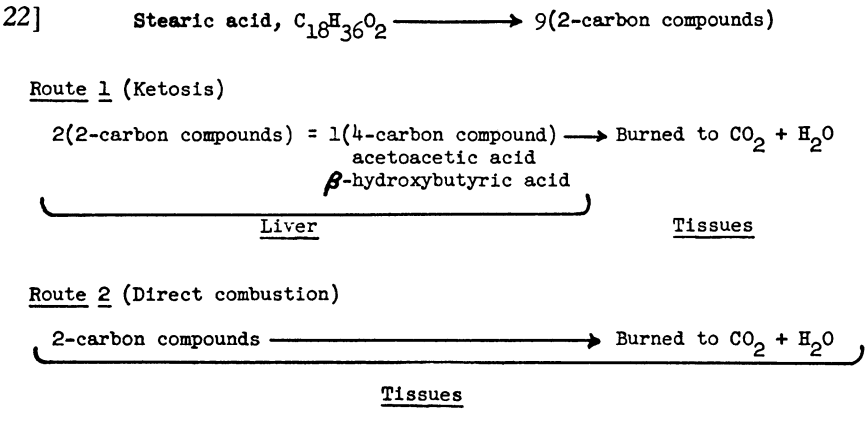
It was long believed that formation of fat from carbohydrate was a relatively rare occurrence, a process that was reserved altogether for the storage of surplus carbohydrate. It is now recognized that it is continuously active. In fact, it is probable that in the course of a day the greater proportion of carbohydrate consumed is converted to fat. Attention has been called to the fact that the amount of glycogen, the only endogenous reserve of carbohydrate, in the body is small. Under ordinary circumstances, moreover, it remains relatively constant. Quite obviously, then, the carbohydrate eaten at meals cannot be stored entirely as glycogen and expended between meals as carbohydrate. The only form in which it can be stored is fat. This fact was missed because respiratory quotients greater than 1.0 were rarely encountered. The point was overlooked that if fat were being burned and formed at the same time, the respiratory quotient would not have to be 1.0, but would be a resultant of the over-all metabolism. The end result of a chemical reaction, both in energy production and in other respects, is the same, regardless of the intermediary reactions that may occur.

The total R.Q., oxygen consumption, CO_2 production and energy expenditure will be the same whether a given amount of carbohydrate is burned directly or is first converted to fat and then burned. This is symbolized thus:

21]



When a fatty acid is burned, it appears first to be broken down into 2-carbon compounds, not stepwise, two carbons at a time, but in an explosive fashion, just as glycogen is broken down to glucose phosphate.



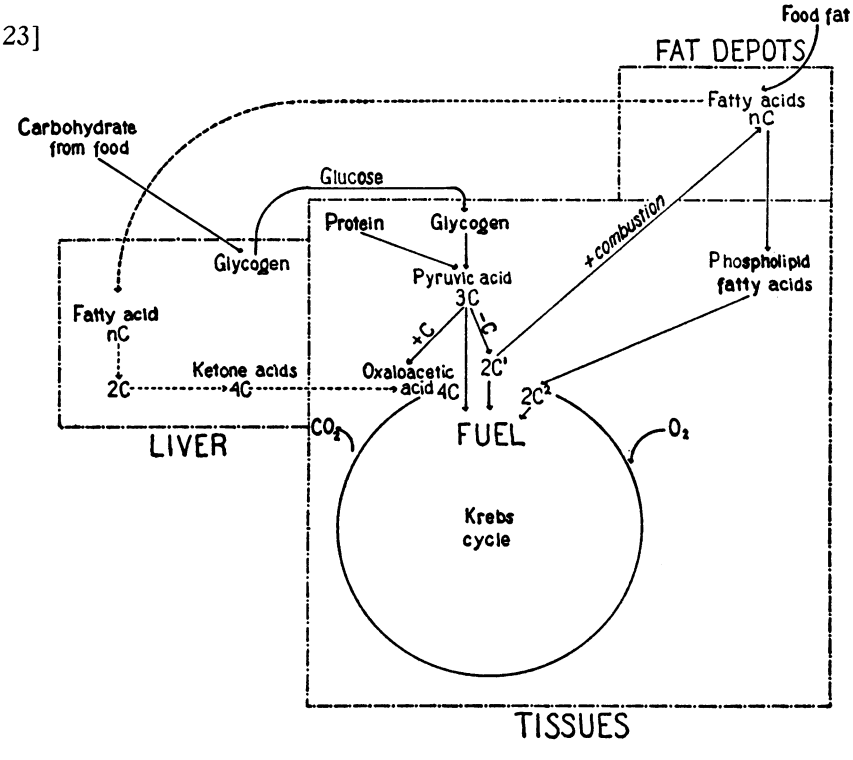
For example, stearic acid with 18 carbons would break down into nine 2-carbon fragments. In the liver these 2-carbon groups may again be coupled to form the 4-carbon compounds, acetoacetic acid and β-hydroxybutyric acid, which are conveyed to the tissues where they are oxidized. This process is exaggerated in starvation and when no exogenous carbohydrate is taken. It is still further exaggerated when the ability to burn carbohydrate is impaired. It was at one time believed that this constituted the sole route for the combustion of fat. It was demonstrated by Stadie that even in the most severely diabetic subject most of the fat is burned by some more direct process. It has been found that isolated muscle tissue from diabetic animals has a respiratory quotient of 0.70, proving that the muscle is burning fat without the intervention of the liver or ketone production. Presumably, then, fatty acids may be broken down in the tissues into 2-carbon compounds that are burned directly.

The formation of 2-carbon compounds from carbohydrate and their combustion over the Krebs' cycle has already been mentioned. It is such 2-carbon groups from carbohydrate that appear to be used for the formation of fat. It is impossible to cite the evidence for each point in this argument. It should be remarked that they have been established chiefly by labeling various compounds with isotopes of carbon or hydrogen and then determining the fate of these isotopes. If acetic acid tagged with the carbon isotope, C^{14}, is given to an animal, the C^{14} is found in all the fatty acids in the body and in the CO_2 of the expired air as well. This is conclusive proof that acetic acid is used for the formation of fat. The C^{14} also appears in the members of the Krebs' cycle, but no appreciable amounts are found in glycogen. It is, therefore, burned over the Krebs' cycle.

It is now necessary to attempt to integrate the functions of the three

foodstuffs. In order to achieve such an integration, certain simplifications are necessary. It must be recognized again that these simplifications involve a certain amount of symbolism. The attempted integration is represented diagrammatically thus:

23]



For our purposes the body is divided into three compartments: the general tissues, the liver, and the fat depots. The functions of the liver in the elaboration of protein, production of urea, rearrangement of lipids, etc. are neglected. The only functions considered are those immediately pertinent to processes of energy production, which are performed by the Krebs' cycle in the tissues. This cycle takes up oxygen and gives off CO_2 in the combustion of appropriate fuel that is fed to it. This fuel is pictured as entering the top of the cycle. In order that the fuel may be burned, however, the machinery of the cycle must be intact, which means that the 4-carbon compound, oxaloacetic acid, must be replenished. Glycogen of the liver is formed from the food and conveyed as glucose to the tissues, where it is again converted to glycogen. This is broken down to the 3-carbon compound, pyruvic acid, which has the capacity both to replenish oxaloacetic acid and to serve as fuel. With an adequate amount of pyruvic acid, therefore, the whole system will run without further assistance. The supply of pyruvic acid is supplemented continuously by protein, the quan-

tity derived from this source depending on the quantity of protein catabolized. The pyruvic acid which is not required to replenish oxaloacetic acid or to supply fuel directly and immediately is represented as forming a 2-carbon group, 2-C^1, which is used for the formation of fatty acids which, together with fat derived from food, are deposited in the fat depots. This formation of fat, however, requires energy which can only be secured by the combustion of a certain proportion of the 2-C^1 in the Krebs' machinery. 2-C^1 can be burned in this machinery, but cannot be used to replenish oxaloacetic acid. From the fat depots fatty acids are conveyed to the phospholipids of the tissues. When they are needed for combustion, these are also broken down to 2-carbon compounds, 2-C^2, which, like 2-C^1, can be burned, but cannot form oxaloacetic acid. There is an alternate, less used path, indicated by broken lines, by which fatty acids are conveyed to the liver. Here they are broken into 2-carbon groups which are immediately coupled to 4-carbon groups, the keto acids, acetoacetic and β-hydroxybutyric, which can be used to replace oxaloacetic acid, thereby enabling the 2-carbon groups from the phospholipids to be burned in the tissues. For this purpose, however, the keto acids cannot be utilized as efficiently as pyruvic acid can. Let me reiterate that the interplay of the foodstuffs cannot yet be defined with such precision as the diagram suggests, but that the principles suggested by the diagram are consistent with known chemical reactions and physiological and pathological phenomena.

If an individual is given large amounts of carbohydrate or carbohydrate and fat, but no protein, the metabolism of protein is, as was remarked before, reduced to a minimum. None is wasted for the formation of pyruvic acid and energy production. If the individual is given no food or no carbohydrate to provide pyruvic acid, the catabolism of protein immediately increases. At the same time fat is mobilized to the liver over the broken line and the formation of ketones increases. This suggests that these substances are being called upon to provide the materials to maintain the Krebs' cycle, which have been represented in this diagram as pyruvic acid and oxaloacetic acid. That they are not being provided for fuel purposes can be easily demonstrated. To reduce the consumption of protein and the formation of ketone acids, so long as the subject has adequate stores of fat in his body, it is not necessary to give enough carbohydrate to meet his fuel requirements, but only a small fraction of this quantity. As little as 100 grams of carbohydrate daily will prevent the previously well-nourished person from wasting excessive amounts of protein and from ketosis. The remainder of his caloric needs will be provided by fat from his adipose tissues. Evidently this carbohydrate is needed for operative purposes, represented here by the formation of pyruvic and oxaloacetic acids.

At this point it becomes apparent that protein is spent with more economy than the other foodstuffs are. In fact, ketosis, the provision from fat of substitutes for operative necessities usually derived from carbo-

hydrate, may be regarded as an expedient to reduce the destruction of protein.

If the pancreas is removed, the ability to burn carbohydrate is reduced to a minimum. Nevertheless, the machinery of the Krebs' cycle continues. Isolated muscle from the depancreatized animal retains the ability to contract, but its respiratory quotient approaches 0.70, indicating that the source of energy is almost entirely fat. Nevertheless, in this muscle all the elements of the Krebs' cycle can be identified and, if proper elements are added, they are utilized. The operating metabolism, therefore, continues, though possibly less efficiently than usual, but the fuel is changed. At just what point or points the continuity of the chains of reactions is broken has not been established with certainty, but the consequences are quite clear. One of these consequences particularly deserves attention because its significance has been too little appreciated. With loss of the ability to burn carbohydrate, formation of fat from carbohydrate is abolished. The implications of this disorder are profoundly important. So long as formation of fat from carbohydrate was regarded as an occasional incident only, little attention was given to it. Controversy instead centered about the question whether fat could form carbohydrate. Those who held the affirmative position in this argument contended that mere interruption of the combustion of carbohydrate could not explain the extreme accumulation of glucose in the blood and the large amounts excreted in the urine by the depancreatized dog and in diabetes mellitus. If, however, formation of fat from carbohydrate is the main pathway for the continuing metabolism of carbohydrate, obstruction of this pathway will immediately cause glucose to back up in the blood and to pour into the urine. Direct evidence that fat forms appreciable quantities of carbohydrate has not been adduced. Studies with isotopic labeled compounds have lent strength to those who have held that conversion of carbohydrate to fat is essentially an irreversible reaction.

In the diabetic the formation of pyruvic acid also is impaired. The source of oxaloacetic acid for the maintenance of the operative machinery of the Krebs' cycle is therefore cut off. In a futile effort to break through this obstruction, the destruction of protein increases to augment the deficient supply of pyruvic acid. Effective relief is afforded by fat, which is mobilized in large quantities from the depots and shipped over the broken-lined path to the liver, where it is broken down to provide ketone acids which can be used to form oxaloacetic acid, thereby enabling the Krebs' cycle to continue and to burn the 2-carbon compounds derived from fat. Under these circumstances, fat, virtually unsupported, sustains energy-production and the operative functions usually carried by carbohydrate.

This substitution, although it enables the subject to survive and subsist for a time, has serious disadvantageous features. In the first place, while the production of ketone bodies may mitigate the destruction of protein, it does not eliminate it. The combination of excessive nitrogenous waste

products and glycosuria provokes a dehydrating diuresis. Secondly, whether because ketone acids cannot be utilized as efficiently as pyruvic acid, or merely because of the overgenerous spirit of the liver, these acids are poured into the blood by this organ so much more rapidly than they are burned by the tissues that they accumulate in the blood, producing acidosis.

The novel position of importance achieved by fat in the continuing metabolism is an intriguing subject for speculation. The foremost point for consideration is that, except for the essential fatty acids, which have a purely operative rôle, fat is not altogether a separate foodstuff; it is equally an intermediary compound in the metabolism of carbohydrate. It is rather banal to state that this is one more good reason why more attention should be given to the metabolism of the lipids, both in physiology and the clinic. It is at least fair to suggest that the metabolism of the two foodstuffs should no longer be categorically separated. The glucose-fed animal, so dear to physiologists and biochemists, is not just what it used to be. Concepts of the rôle of glycogen require modification. It has already fallen far from the high estate it enjoyed in the reign of Hill and Meyerhoff, when it was the sole fuel of muscle. Is it now to be displaced from its position as the chief source of continuing energy? This possibility must at least be given open-minded consideration. An adult male, under ordinary conditions, does not develop ketosis of any important degree until he has starved 48 hours or more, although the glycogen in his body could supply his energy requirements for only a fraction of this time. After an overnight fast three-fifths of the energy production is sustained by fat. If it were not, the glycogen stores would be entirely exhausted.

When there is not a continuous supply of exogenous carbohydrate, the endogenous supply is used with increasing economy as it becomes depleted. What little remains is reserved for essential operative purposes. In this respect the capacity for economy varies from species to species, being most highly developed in carnivores who habitually subsist on diets almost devoid of carbohydrate, compelling them to derive what carbohydrate they need for operating metabolism from protein. Within species similar variations exist. Women and children are more susceptible than men to ketosis, presumably because they are more prodigal with carbohydrate. Pregnancy exaggerates this characteristic of women.

Although muscular exercise is not sustained by carbohydrate alone, it does appear to create a preferential demand for carbohydrate, so long as this is freely available. When Courtice and Douglas walked 10 miles before breakfast, their respiratory quotients rose quite rapidly, to drop as soon as they rested to figures that indicated that 90 per cent of their resting fuel requirements were being supplied by fat. When they resumed walking, the respiratory quotients rose slightly again. If they took carbohydrate while they were walking, this was rapidly expended. This exercise of Courtice and Douglas was not severe enough to induce any appreciable production

of lactic acid. Carbohydrate is not only utilized preferentially for muscular exercise, its utilization is facilitated by exercise. The diabetic patient requires less insulin when he is physically active. This is one of the chief arguments against the common practice of admitting patients to hospitals for regulation of diabetes.

Certain hormones accelerate the expenditure of carbohydrate. Among these epinephrine has a unique action. It appears to inhibit the oxidative metabolism while augmenting the anaerobic breakdown of glycogen. It therefore increases the production of lactic acid and provokes ketosis. Thyroxine seems to behave somewhat like exercise, increasing the total energy-production and promoting the preferential expenditure of carbohydrate. Insulin is, however, the most specific and potent stimulus to the combustion of carbohydrate. Whereas the action of muscular exercise diminishes as the glycogen stores become depleted, the action of insulin is not similarly limited. Moreover, insulin retards the breakdown of protein and therefore impedes the restoration of glycogen from non-carbohydrate sources. It seems also to facilitate the formation of fat from carbohydrate, thereby further dissipating carbohydrate stores. In the absence of an extraneous supply of carbohydrate, therefore, insulin is the most powerful known deglycogenating force.

The reduction of carbohydrate oxidation to a minimum (e.g. in starvation), if it does not involve a complete transformation of the metabolic processes, at least requires a complete revision of their organization or adjustment. The flow through pyruvic acid to oxaloacetate and from 2-C^1 to fat must be greatly retarded, while the channels from fat through 2-C^2 and to ketones in the liver are opened up. The relative speeds of the various enzyme systems have all been changed to meet new conditions. So profound are these changes that they cannot be immediately reversed. If a dose of glucose is given to an adult male who has been starved for 48 hours, the blood sugar curve will resemble that of a diabetic and some sugar is likely to appear in the urine. Some time must elapse before normal utilization of carbohydrate is resumed. A similar state of starvation diabetes can be induced more easily in women or children. Appearance of this state can be accelerated by exercise. In the experiments of Courtice and Douglas cited above, glucose tolerance was distinctly reduced after the morning walks. The glycosuria not uncommonly shown by patients with hyperthyroidism is probably a manifestation of starvation. It usually follows breakfast. Although the blood sugar rises excessively after a dose of glucose before breakfast, sugar is subsequently used with great facility. The hyperthyroid patient, owing to accelerated oxidations and preferential expenditure of carbohydrate, depletes his carbohydrate stores more rapidly than the normal person does and therefore exhibits the phenomena of starvation after a simple overnight fast. Perhaps the simplest way to produce starvation diabetes is by the administration of insulin to a starving individual. Of

course, if carbohydrate is given while the insulin is acting, its combustion will be facilitated. But after a hypoglycemic reaction, if the subject is not immediately given carbohydrate, ketosis will regularly appear. At this time a dose of carbohydrate will provoke excessive and prolonged hyperglycemia, a diabetic type of reaction.

At this point it may be profitable to refer briefly to the bearing of this subject on diabetes. In the first place, it should be clear that in order to keep the stream of metabolism flowing in its normal channels, a small, but steady, supply of carbohydrate should be provided, enough to maintain the operative functions of carbohydrate. Long periods of starvation should be avoided, especially if forces are active, which may accelerate the preferential expenditure of carbohydrate or impair its oxidation. In diabetes, oxidation of carbohydrate is regularly impaired; therefore it becomes doubly important to guard against depletion of carbohydrate stores by starvation or forces that accelerate the expenditure of carbohydrate, of which exercise and insulin are the most important.

The subject cannot be dismissed without some mention of the hormones which regulate combustion of carbohydrate. The peculiar effect of epinephrine has already been mentioned. The rôle played by the hormones of the anterior pituitary and adrenal cortex in the regulation of carbohydrate metabolism is an intricate one, but the purification of the active principles of these glands has in recent years enabled considerable progress to be made in the elucidation of their effects.

As is now well known, the anterior pituitary secretes six hormones. Of these, four are trophic hormones; that is to say, they produce their effects by stimulating the secretion of other endocrine organs. The trophic hormones are the two gonadotrophic agents, follicle stimulating hormone and the luteinizing principle, the thyrotrophic hormone and the adrenocorticotrophic hormone (ACTH). Of these trophic hormones only the last two are of any concern in the regulation of metabolism. In addition to these trophic agents, the anterior pituitary also secretes two other hormones that act directly on tissues that are receptive to their action. These are the lactogenic hormone and the so-called growth hormone, which in reality is a metabolic hormone with a profound effect on protein synthesis. Since the lactogenic hormone probably only plays a significant rôle in the mammal in the special processes related to milk secretion, the only hormones of the anterior pituitary that require consideration as metabolic hormones are the thyrotrophic, the adrenocorticotrophic, and the growth hormone.

Since the thyrotrophic and adrenocorticotrophic hormones merely increase the secretory rate of the thyroid and adrenal cortex respectively, their effects on metabolism are identical with those produced by thyroxine or the adrenal cortical steroids. The effects of the thyroid hormone have been described above.

The adrenal cortical hormones of the type of cortisone, which possess an oxygen atom in the third ring of the steroid nucleus, have the property of accelerating in fasting animals the rate of formation of glucose from the tissue proteins. In fed animals they also inhibit the rate of utilization of carbohydrate. When either ACTH or cortisone is given in excess to normal animals or humans, a temporary diabetic state may be produced. This is also characterized by an extreme degree of resistance to insulin. In partially depancreatized animals, or in individuals with a potential or mild diabetes, these effects on carbohydrate metabolism are much more severe.

The growth hormone, when administered to normal cats or dogs in quite small amounts, will produce in the course of a few days either a very severe temporary diabetes or a permanent one. When the latter occurs, it can be demonstrated that this is a consequence of the widespread degeneration of the islets of Langerhans. It is believed that in susceptible species such degeneration is due to the marked hyperglycemia that is associated with the administration of growth hormone to fed animals.

The effects of growth hormone on carbohydrate metabolism are of a kind that suggest that this hormone inhibits the utilization of glucose by the skeletal muscles. Thus it has been found that the glucose utilization of isolated skeletal muscle is markedly reduced in animals previously treated with this hormone, while its injection is followed by a depression of the respiratory quotient.

It has been suggested by the Coris that both the adrenal cortical steroids and a pituitary hormone (growth?) inhibit the enzyme hexokinase. This enzyme is of central importance in carbohydrate metabolism since it catalyzes the formation of glucose-6-phosphate from glucose, a step that is essential for normal utilization of glucose (see 14]). Although this is a most intriguing suggestion, no definite evidence has been adduced to support it.

It is reasonably certain, however, that it is not the hormones themselves that act as enzymes.

References

It would be clumsy to implement a philosophical article of this kind with a conventional bibliography. In a sense it is a natural consequence of a concept first proposed by the author in an article entitled *A new frame for metabolism,* in 1941. Much of the material is included in *Quantitative clinical chemistry, interpretations,* Vol I, Ed. 2. The chief subsequent references are listed below.

Dr. C. N. H. Long kindly consented to review the paper in order that egregious errors might be avoided. He also generously contributed the last paragraphs on the place of the hormones.

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