A NEW FRAME FOR METABOLISM*

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The last decade has witnessed not only an enormous expansion of technic and factual knowledge but also a revolution in the philosophical concepts of biological chemistry. Emphasis has changed, new relations have been discovered; what were formerly mere factual correlations have been given functional significance.

Concepts of metabolism have, perhaps, undergone the most profound transformation. Early work on metabolism was confined almost entirely to the study of over-all exchanges of calories, oxygen, carbon, hydrogen, nitrogen, and inorganic elements, with little or no attention to intermediary processes. The three major foodstuffs were treated almost independently of one another, as if they were merely different types of fuel. The study of metabolism consisted of analyzing raw products that went into the body and the waste and flue gases that came out. The same method, applied to a manufacturing plant, would yield a rough idea of the economy of the plant; but it would give no valid impression of the purposes of the factory or the complex processes by which these were achieved.

Folin,[10] in his classical paper differentiating exogenous and endogenous metabolism, only partly broke from the preoccupation with fuel and economy. Endogenous metabolism in this system was entirely derived from protein. It was identified with the expenditures or wastage which continued when minimum amounts of protein were fed and practically all the fuel needs of the body were met by carbohydrates and fat. Under these circumstances the excretion of certain nitrogenous compounds, especially urea, diminished; while the excretion of others, such as uric acid, creatinine, and amino acids, remained relatively unchanged. It was inferred that these latter substances represented the products of metabolic reactions that were not primarily directed toward energy production, but were essential

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for the maintenance of bodily functions. They found their way into the urine through inescapable leakage, or were end-products of specialized metabolic processes which the body was unable to oxidize completely. Under normal conditions of diet sufficient protein was continuously taken from the exogenous supply to replace the endogenous detritus, but the great bulk of exogenous protein and all carbohydrate and fat were oxidized, as soon as they entered the body, to urea, carbon dioxide, and water, to provide energy.

The discovery of essential nutritive elements, such as specific amino acids by Osborne and Mendel, vitamins by Hopkins and others, etc., directed attention to the importance of chemical structure. However, investigations of the action of these substances and of the hormones, which were discovered at about the same time, were at first confined almost entirely to their effects on growth and other gross traits.

In physiology and medicine attention was turned from the mere identification of chemical compounds and their derivatives to the physico-chemical forces and mechanisms involved in physiologic activities, such as membrane equilibria, acid-base equilibria (L. Henderson, and Van Slyke), the internal exchange of respiratory gases by means of hemoglobin (Haldane, Barcroft, and Van Slyke), and the reactions involved in muscular activity (Meyerhof, and Hill). The chemical and physiological world came to be regarded as a series of reversible chemical equilibrium reactions that could be written in series: glucose $\rightleftharpoons$ glycogen, glycogen $\rightleftharpoons$ lactic acid,— even creatine $\rightleftharpoons$ creatinine and urea $\rightleftharpoons$ ammonia. Close behind the physical chemical promotion came a renewed interest in the application of the technics of chemistry and physiology to the differentiated activities of organs; the kidneys under Cushny and Richards, muscle under Meyerhof and his associates, the liver under Mann, etc. Both the equilibrium reactions and the activities of individual organs were treated as if they proceeded independently; in this sense the approach was categorical.

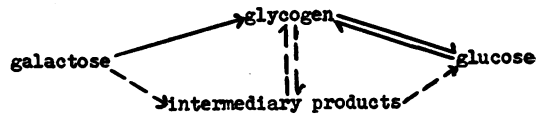
These various interests inevitably impinged upon one another. With the recognition of the importance of chemical structure to function and the realization that hormones, vitamins, and enzymes, formerly vaguely classed as catalysts, were really definite chemical compounds, the isolation and identification of more of these compounds and the discovery of the place and nature of their actions became the preoccupation of both chemists and physiologists. Among

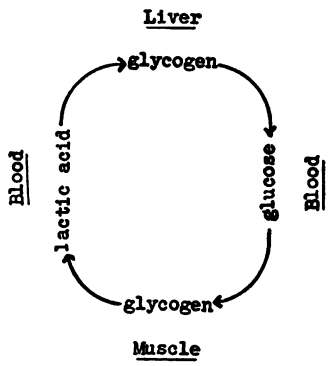
the host of substances that turned up in this analytical fervor may be mentioned Warburg's respiratory enzyme, Fiske and Subbarow's phosphocreatine, Lundsgaard's adenosinetriphosphate, acetylcholine, and some of the components of the vitamin B complex.

These discoveries have evolved new principles of far-reaching importance. Many of the compounds which have been identified have proved to be so unstable that they would have escaped recognition by the method of systematic analysis; some, like adenosinetriphosphate and creatinephosphate, have proved to be aggregations of substances already well known. Vitality is no longer a mere spirit breathed over inert chemical reactions, but something inherent in the orderly arrangement of these reactions. Even the structure of the compounds involved in these reactions will not survive the resolution of the reactions. Hormones are concrete substances of various kinds that probably participate in metabolic processes. Vitamins and essential amino acids are not mystical growth-promoting or life-sustaining principles, but are materials which contain some particular chemical radicle or aggregation which is indispensable for the functional integrity of the animal body, but which must be derived from extraneous sources, usually ultimately from the vegetable kingdom, because they can not be synthesized by animals. Enzymes are not catalysts, in the formerly accepted sense of the term, but complex systems composed of diverse chemical agents which, by a chain of linked interchanges and transportations, implement oxidations, hydrolyses, and other useful reactions, at the same time imparting to them order and direction.

By the time it had become a general habit to express biological reactions as reversible equations, it became apparent that one of the features that distinguishes the orderly processes of life from disorderly autolysis in the test-tube is the unidirectional character of many, if not of most, of its reactions. Creatine $\rightleftharpoons$ creatinine is easily reversible in vitro, but Bloch and Schoenheimer[2] have proved conclusively that it proceeds only to the right in the normal living animal. For glucose the formation and hydrolysis of glycogen in the liver may be defined by the equation glucose $\rightleftharpoons$ glycogen; but this will not serve for any other sugar. Galactose $\rightleftharpoons$ glycogen, if it occurred, could lead only to galactosuria, a metabolic futility. The actual facts are defined by the solid arrows in the following figure, with the reservation that the processes may, at times, be short-circuited through intermediary reactions, as indicated by the

broken arrows. There can not even be certainty that in the reaction, glucose $\rightleftharpoons$ glycogen, hydrolysis retraces the path of synthesis. In muscles, glycogen, though formed from glucose, in the process of degradation breaks down to fructose phosphates. This offers a plausible explanation for the long-recognized fact that muscle glycogen is not utilized to restore the blood sugar in hypoglycemia. Meyerhof [14] and Hill[12] proposed that glycogen was transformed through a series of reactions to lactic acid and was then reconstituted by a reversal of these same reactions with energy derived from combustion of a fraction of the lactic acid. If the particular enzyme systems connected with individual reactions in the chain of transformations from glycogen to lactic acid are isolated, the reactions which they potentiate appear to be reversible.





In the living animal, however, there is increasing evidence that the anaerobic passage of glycogen to lactic acid leads to a dead end, that lactic acid is neither oxidized nor reconverted to glycogen in muscle, but must be carried to the liver before it can return by a devious detour, through the liver. Although any one of these reactions is potentially reversible in an isolated system, in the intact animal direction signs seem to be imposed upon them by other reactions with which they are linked.* The apparent reversibility of many metabolic processes is achieved, not by simple retracement of the original steps, but in a cyclical manner. The most familiar

* There is no chemical obstacle to such a concept. The direction of any equilibrium reaction will depend upon the relative concentrations of reactive substances. For example, the reaction A + B $\rightleftharpoons$ C will move predominantly to the right if there is an excess of A and B. If in the same system there was another component, D, which reacted with C to form E, C + D $\rightleftharpoons$ E, the concentration of D might always be kept so low that the reconversion of C to A and B would be negligible. The prevalence of such coupled reactions and the inevitable loss of free energy which attends all chemical reactions can account for the orientation of chemical processes in intact biological systems.

example is found in the reactions just mentioned. Even the movements of inorganic ions across cellular membranes appear to be oriented and to be linked with energy reactions of one kind or another.[18]

For an indefinite number of substances there appear to be alternative paths of metabolism. Glycogen of muscle may pass to lactic acid or, if oxygen is available, may be broken down without the formation of lactic acid. The products of its degradation may or may not be oxidized to CO_2 and H_2O. Stadie's recent investigations,[24,25] together with his incisive analysis of other evidence, can leave no doubt that fatty acids in the liver are converted to 4-carbon acids which are conveyed to the muscles to be burned. Nevertheless, as long as the liverless animal survives, respiratory quotients indicate that it continues to burn fat.[9, 18]

Over-all concepts of metabolism are no longer adequate; the terms endogenous and exogenous, which base distinctions on the sources of foodstuff are highly unsatisfactory. If they were to be retained they could be applied most realistically to differentiate between those indispensable substances which must be derived from the environment and those chemical compounds which can be synthesized in the body. Proteins can no longer be regarded as stable structural materials, subject to only slight deterioration. Schoenheimer[21] and others, chiefly by the aid of heavy isotopes of hydrogen, nitrogen, and carbon, have proved that there is such a constant and extremely rapidly interchange between food substances and tissues, that the two become indistinguishable in the internal economy almost as soon as the food enters the body. This interaction involves not only the substitution of chemical compounds, but also transfers of elements and small radicles among these compounds. Proteins are continually changing conglomerates; even amino acids are in a state of perpetual flux. Amino acids are separated from their parent proteins to be converted into other compounds, such as creatine, purines, etc. Carbohydrate and fat can no longer be treated as indifferent materials, serving no purpose except the provision of fuel for energy purposes.

A more suitable frame for metabolism must be found, one in which distinctions are drawn according to purpose or function, if these words are not interpreted teleologically. Two broad, but quite distinct, categories of metabolism may be recognized. These may be named *operative* and *energy-producing*. The last requires

little explanation. It consists of those oxidative reactions which have as their principal object the provision of energy for the conduct of biological processes: the combustion of fat and of carbohydrate to carbon dioxide and water, and of protein to carbon dioxide, water, and urea or ammonia.

Operative metabolism includes the specific chemical reactions that enable each cell to perform its specialized functions, irrespective of the source from which it derives its energy. In muscle, during activity, glycogen is broken down. In the absence of oxygen it goes to lactic acid, which may not be oxidized by muscle at all. In the presence of oxygen it is broken down by a more complex series of reactions to other intermediary products, of which at least a part is ultimately oxidized completely to carbon dioxide and water, thereby providing energy. In the muscle of the depancreatized animal this last step does not take place; instead, only fat is burned. Nevertheless, the known reactions involving carbohydrate, except those leading to its oxidation, seem to proceed in diabetic muscle, just as they do in normal muscle. To return to an earlier simile, these reactions may be compared to the spindles that allow a textile factory to make thread rather than stove bolts, whether they are powered by a steam-engine or a hydroelectric plant. In the muscle they are part of the chain of reactions involved in the process of contraction' which must proceed whether carbohydrate is used or not. It follows that carbohydrate participates in these operative reactions, and therefore must be supplied, even when it can provide no energy.

Strictly, there is another category of metabolism that might be termed transformative, to describe the processes by which some compound essential for a particular purpose, in either operative or energy metabolism, is produced from one or more other substances. This is comparable in the biological economy to manufacturing in our factory. It is, however, practically indistinguishable from operative metabolism. For example, creatine may be formed from the amino acids, glycine, adenine, and methionine; as creatine phosphate it participates in the metabolism of muscle. Although it undergoes constant deterioration to creatinine, it is apparently not burned for the production of energy. Nevertheless, it facilitates reactions that are essential for the contraction of muscles and that may be productive of energy. Neither the formation of creatine nor its elimination as creatinine are appreciably influenced by the amount of muscular activity. Its production, therefore, is a transformative metabolic

process; its function in muscle must be purely operative. The mammalian organism is able to supply, from rather simple and indifferent materials, a large proportion of its needs, especially its energy requirements. Certain highly specialized compounds it can elaborate only from prefabricated or processed materials secured from extraneous sources. Among these are the substances known as vitamins and some amino acids. It must be recognized, however, that an amino acid or other compound that can be synthesized within the body is not on that account less essential for the performance of its peculiar functions than one that can not be synthesized; it merely does not have to be provided preformed in the food.

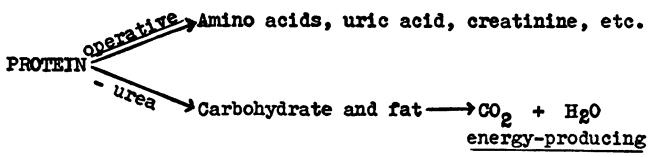
As far as energy-production is concerned, within broad limits the three foodstuffs, protein, carbohydrate, and fat, are interchangeable. However, the operative and transformative functions of protein can not be filled by carbohydrate or fat, because neither contains nitrogen, sulfur, or the complex radicles which are found in protein. It follows that there must at all times be a continuing metabolism of protein. Since fractions of protein can be converted into both carbohydrate and fatty acids, protein can act as a substitute for either of the other foodstuffs. Carbohydrate can also be converted to fat and, consequently, can replace fat in metabolism. On the other hand, no valid evidence has yet been offered that fat can be transformed into carbohydrate.

Of the three foodstuffs, because of its multiformity, protein makes the greatest contribution to both transformative and operative metabolism. It must supply the most diverse materials for the most multifarious purposes. The composition of the protein fed must, therefore, be sufficiently heterogeneous to provide, in appropriate proportions, all the components required for the manufacture of these materials. There are altogether 22 amino acids which, united in innumerable permutations and combinations and series of various numbers, form the structure of proteins in animals. Of these, 10 must be obtained from the diet either because they can not be synthesized in the body or because they include chemical radicals which are essential for the elaboration of some functionally indispensable compound.[19] Any one of these may become a limiting factor for growth or continued existence. No amount of any other substance can compensate for its absence. Without it energy is dissipated to no useful purpose.

If protein in the diet is reduced to a minimum, but large quanti-

ties of carbohydrate are given, excretion of nitrogen may be reduced to as little as the equivalent of 0.25 gm. of protein per kgm. a day, the reduction involving almost entirely the urea fraction of the urinary nitrogen. Urinary amino acid, uric acid, creatinine, and undetermined nitrogen are little altered by such a régime. Urinary nitrogen also diminishes in total starvation, but not to the same extent. At the end of a 31-day fast Benedict's fasting man was still excreting about 1 gm. of protein per kgm.[1] When he broke his fast with a diet containing carbohydrate, nitrogen excretion fell sharply. This same reduction of protein combustion when starvation is interrupted by administration of carbohydrate is evident in Gamble, Ross, and Tisdall's observations on children.[11] When no exogenous carbohydrate is supplied protein is forced to assume the operative burden of carbohydrate in addition to its own. This explains the long-recognized "protein-sparing" action of carbohydrate. It also indicates why, if large amounts of fat are given for energy-production, a small quantity of carbohydrate will hold protein metabolism to a minimum. In starvation, after an interval, Gamble, Ross, and Tisdall[11] found that protein and fat were consumed in approximately constant proportions. Under the prevailing ketogenic-antiketogenic theory which held that "fat burns in the flame of carbohydrate" this was interpreted as an expedient to minimize starvation ketosis. Such a teleological explanation is no longer required; the protein is consumed merely to supply operative carbohydrate, which fat can not provide. Perhaps the clearest example of this phenomenon is found in the experiments of Mirsky,[16] in which the intravenous administration to depancreatized dogs of enough glucose to build up glycogen in the liver reduced the destruction of protein, although presumably the animals burned none of the carbohydrate they received.

The general channels of protein metabolism can, therefore, be depicted in the following manner:



The end-products of protein metabolism consist first of urea, and second of a variety of other compounds, such as amino acids, uric

acid, and creatinine. In the formation of urea, presumably, the amino group of each amino acid is removed and converted to urea, the remainder of the molecule passes through the metabolic channels of carbohydrate and fat. Excesses of protein are utilized chiefly in this manner, as if they were thrown into the hopper to serve as indifferent sources of fuel for energy-production or to substitute in other offices for carbohydrate or fat. Excretory nitrogen-containing products other than urea apparently come from materials that have performed more specific operative and transformative functions. These latter processes continue unabated if protein in the diet is reduced to a minimum, but large amounts of carbohydrate and fat are given. A certain amount of energy may, of course, be derived as a by-product from these incomplete oxidations.

Carbohydrates which can be utilized are universally interchangeable. Indeed, a large number of organic compounds can be converted to carbohydrate in the body. All are transformed to glycogen and thence to glucose as the primary step of metabolism, whatever their ultimate disposition may be. There are minor differences in the nature and speed of this transformation, depending upon the identity of the original carbohydrate or carbohydrate precursor; but, broadly speaking, any one of these substances can serve all the purposes of carbohydrate. In the normal animal receiving adequate amounts of carbohydrate the glycogen stores of the liver are kept well filled. Carbohydrate over and above what can be immediately burned or stored as glycogen is converted to fat to meet future demands for energy. From the glycogen stores of the liver glucose is dispensed to the muscles as it is required. In the muscles it may follow either the anaerobic route to lactic acid or the oxidative route. The latter appears normally to end in combustion to CO_2 and H_2O. Oxidative operative metabolism of carbohydrate is coupled with energy-production.

If no carbohydrate is taken in the food, protein is forced to assume the burden of supplying glycogen. It does not, however, immediately take over the operative functions of carbohydrate; the turnover is initiated only when liver glycogen is seriously depleted. The stimulus which accelerates the breakdown of protein to form liver glycogen seems to be the disappearance of glycogen from the liver, however this may be brought about. The term neoglycogenesis, which is commonly applied to this process, is an unfortunate one. Neoglycogenesis is merely the transfer from a metabolism supported

by a balanced mixture of foodstuffs to one in which protein has assumed the operative responsibilities of carbohydrate. In a sense the dog, on a purely meat diet, lives in a state of continuous neoglycogenesis to which it is by nature adapted. In this state the glycogen stores of the liver are kept amply supplied, but not so abundantly as they are when preformed carbohydrate is eaten. Mirski et al.[15] found that rats which had subsisted upon high carbohydrate diets had less glycogen in their livers after a 24-hour fast than did rats which had subsisted upon protein. Newburger[17] has confirmed Mirski's observations, but has shown that, whether they have received protein or carbohydrate in advance of the fast, rats lose the same quantities of liver glycogen under the influence of ether anesthesia. The glycogen in the livers of the two types of rats, therefore, did not differ in character. It had already been demonstrated that there is more, not less, glycogen in the livers of rats after a fast of 48 or 72 hours than after a fast of 24 hours. Apparently protein destruction does not begin to increase until the glycogen of the liver is greatly reduced. After it has begun it proceeds at a sufficiently rapid rate to maintain an adequate amount of glycogen in the liver. Mirski's rats which had received carbohydrate were examined just at the time when the stores of glycogen derived from carbohydrate were exhausted, but before protein had come to the rescue. The metabolism of the protein-fed rats was already running predominantly on protein; starvation necessitated no shift of machinery, at most a change in the source of supply. The transfer from a diet in which protein predominates to starvation or in the reverse direction appears to have no latent period, no discontinuity. The organism seems to recognize no difference between materials supplied from the external environment and those derived from its own tissues. This is an added reason for abandoning the terms endogenous and exogenous metabolism, which emphasize the sources of materials, for terms that embody more realistic distinctions based upon the nature of materials available and the purposes for which they are utilized.

When there is an abundant supply of exogenous carbohydrate available it is used quite lavishly for energy-production, at least in muscles, especially when these are contracting vigorously. But when carbohydrate become scarce, less and less is burned. When carbohydrate must be supplied by protein, sugar is strictly rationed. When carbohydrate is given to a starved animal its oxidation is not immediately accelerated. Just as protein takes up the operative load of

carbohydrate only after a definite lag and when glycogen is almost exhausted, so carbohydrate does not reassume its responsibilities until a certain reserve of liver glycogen has been built up. Meanwhile combustion of carbohydrate continues to pursue the limited and leisurely pace imposed by niggardly protein. This is the familiar starvation diabetes. It must be recognized that in this state combustion of carbohydrate is not abolished, but only retarded. Respiratory quotients prove that the protein metabolized is completely oxidized; therefore, the moiety of this protein that forms carbohydrate must be oxidized.

Under ordinary circumstances fat seems to have almost no indispensable operative or transformative functions. Animals can apparently subsist on diets entirely devoid of fat, with one exception: a small quantity of unsaturated long-chain fatty acids, either linoleic or arachidonic, must be supplied, presumably because it is needed for some essential purpose and can not be synthesized in the body. It may be inferred that all other necessary fatty acids and glycerine can be formed from carbohydrate, since removal of fat from the diet, provided enough carbohydrate is substituted, does not increase protein metabolism. Fat may be considered as the great energy reserve of the body, a rôle for which it is peculiarly suited because it can be deposited in almost pure form in the tissues and has a caloric value high in proportion to its weight. A gram of either protein or carbohydrate will provide about 4 calories, but can be stored in the body only in conjunction with about 3 grams of water. A gram of fat, which provides about 9 calories, on the other hand, can be stored in practically an anhydrous form. Therefore, as storage material fat is about 9 times as efficient as carbohydrate or protein, in terms of the amount of energy per gram of weight which the body has to carry. It is generally stated that animals have only a limited capacity to store carbohydrate, because the amounts of glycogen which muscles and liver will hold are relatively small. This is, however, only the carbohydrate that is available for operative and transformative purposes. By conversion to fat the energy value of carbohydrate may be accumulated to an unlimited extent.

Stadie's[24, 25] recent elucidation of the phenomena of ketone production and combustion should finally silence the overproductionists who have insisted against all earlier evidence that carbohydrate is formed from fat. Nevertheless, the production of ketones is not an obligatory step in fat-combustion; fatty acids can be burned directly

by the tissues without this preliminary conversion. This is attested by the respiratory quotients of isolated diabetic muscle, of hepatectomized animals, and more directly by Cruickshank's experiments[7] with the aglycemic heart-lung preparation. Stadie, himself, found that in diabetic humans and animals formation and combustion of ketones could account for only a fraction of the energy derived from fat. It may be that a minimal quantity of ketones is essential for the metabolism of tissues and that this comprises the normal operative contribution of fat. For its chief purpose, energy-production, however, fat need not be converted to ketones. If preformed carbohydrate is not available and carbohydrate utilization is consequently diminished, fat is deposited in the liver where it is transformed at an accelerated rate to ketones, which are burned in the tissues. Crandall and his associates[5, 6] have suggested, naturally enough, that this indicates that ketones, under these conditions, are substituted in the oxidative cycle of reactions in muscle for compounds usually derived from carbohydrate. This would mean that fat has a facultative operative metabolism; it is a kind of understudy to carbohydrate, appearing as ketone bodies in the oxidative processes whenever carbohydrate gives out.

In a sense, liver glycogen seems to lead a purely ministrative rôle. It takes carbohydrate when there is a surplus not needed for other purposes. Except in von Gierke's disease it is never permitted to grow too opulent. On the other hand, it is never permitted to become completely extinguished. If it approaches this point protein puts it on the dole. Anything that accelerates the utilization of carbohydrate by the tissues promotes glycogenolysis in the liver; and the tissues are ruthlessly extravagant until protein assumes control. The commonest goad to carbohydrate combustion and the most frequent accelerator of hepatic glycogenolysis is muscular exercise. The most vivid demonstration of its effects can be found in Courtice and Douglas' accounts[4] of the ten-mile walks they took before breakfast. In the course of these walks, conducted at such a leisurely pace that there was no appreciable production of lactic acid, they burned considerable amounts of carbohydrate that must have come from hungry livers. Gradually their respiratory quotients fell as hepatic glycogen became depleted. When they rested, the respiratory quotients slumped sharply, indicating that liver glycogen was also taking time off. Meanwhile, they were forced to subsist largely on fat. But if they resumed their walks

the liver valiantly gave of its remaining glycogen what it could. Subsequently the respiratory quotients fell still further and ketonuria appeared. If they ate carbohydrate on the walk it was quickly spent and the liver got none of it. If, however, they took carbohydrate during the rest, they exhibited the phenomena of starvation diabetes: respiratory quotients did not rise, although blood sugar rose excessively. The carbohydrate all went to the liver to form glycogen, presumably to relieve protein of the necessity of continuing to provide this commodity.

Cessation of carbohydrate combustion appears to be the most potent of all glycogenolytic forces. The necessity for postulating a linkage between the operative and energy-producing functions of carbohydrate has already been mentioned. Although oxidative combustion of sugar becomes greatly retarded during starvation, it does not cease altogether; the glycogen penuriously supplied by protein is burned. In the absence of insulin the muscles apparently lose the power to effect this last step in carbohydrate metabolism. Under these circumstances the liver behaves as if it were under maximum compulsion to provide glucose to the muscles; its glycogen fairly melts away. This it is that has given rise to the opinion that insulin promotes glycogenesis. By restoring the ability of the muscles to burn carbohydrate, insulin does remove the stimulus to glycogenolysis in the diabetic animal, thereby permitting the liver to retain glycogen that is formed. Hepatic glycogenesis, however, is not retarded in the absence of insulin. In fact, it must be extremely active, since every suitable compound is converted to glucose. Even protein is no longer economized; urea excretion increases enormously. With the exception of glucose all these compounds must be converted to glycogen by the liver before they can form glucose. Hepatic glycogen is depleted, not because hepatic glycogenesis is retarded, but because glycogenolysis outstrips it.

When carbohydrate combustion ceases and liver glycogen becomes depleted, protein destruction increases. This continues even when the blood sugar has risen far above normal. In this dilemma protein lends whole-hearted support to carbohydrate, as if the organism was utilizing all its resources in a vain effort to overcome the obstruction to carbohydrate oxidation by sheer force of mass action. Although the predominance of glycogenolysis over glycogenesis in the liver is not checked by spontaneous hyperglycemia, the concentration of glucose in the blood is not altogether without influence upon the

balance between these processes. Ordinarily, as the blood sugar rises excretion of glucose in the urine increases *pari passu,* thus mitigating hyperglycemia. Ingested sugar is absorbed at such a measured rate that it does not surpass the excretory capacity of the kidneys. By intravenous injection of glucose, however, especially when the kidneys were removed, Mirsky[16] was able to raise the blood sugar to such heights that liver glycogen increased in the depancreatized dog. At the same time nitrogen catabolism decreased, further evidence that the response of protein to the call for carbohydrate is implemented by the supply of glycogen in the liver.

The statement that insulin actuates or facilitates the oxidative metabolism of carbohydrate can be regarded only as the hypothesis which, by exclusion, seems most compatible with the facts now available. It has already been pointed out that glycogenesis and glycogenolysis proceed in its absence. The formation of glucose is also preserved. Comparisons of glucose in arterial and venous blood entering and leaving the muscles of diabetic patients or animals suggest that these tissues take up glucose less rapidly than do normal muscles. This has led some to place the break in the chain of reactions at the formation of muscle glycogen. Against this is the fact that muscle glycogen is not greatly depleted in diabetic animals. Furthermore, Bollman, Mann, and Wilhelmj[3] have demonstrated the formation of muscle glycogen in the depancreatized dog. The anaerobic breakdown of glycogen to lactic acid and the reconversion of the latter to glycogen by the liver are not interrupted. It has, however, been established beyond reasonable doubt that oxidation of carbohydrate to CO_2 and H_2O is abolished. The fracture, therefore, would seem, by exclusion, to lie somewhere in the course of the reactions involved in the oxidative degradation of glycogen. But little is known with certainty of the intimacies of these reactions in normal animals; their state has not been explored in diabetic animals other than the pigeon, which does not respond like mammals to pancreatectomy. Inferential evidence suggests that only the initial reactions of the oxidative chain, if any, are reversible. If this is the case, since carbohydrate in the diabetic animal can all be recovered in the urine as glucose, the site of action of insulin would have to be placed in the initial phases of this system. To venture still further into the realm of conjecture, this would afford an explanation for the retardation of the transfer of glucose to muscle glycogen, because it would tend to back up the whole system at or near the glycogen level.

Although the exact site of the action of insulin is a subject of great physiological significance, it is not essential to the argument that has been developed. The phenomena that have been described are not related specifically to the absence of insulin or the inability to burn carbohydrate; they derive from failure to burn carbohydrate. This is implicit in the action of phlorizin. Wierzuchowski[26] established beyond doubt that this drug does not destroy, indeed may not even injure, the capacity of muscles and other organs to utilize carbohydrate. It merely starves these tissues of glucose by abolishing the reabsorptive function of the renal tubules, thus short-circuiting glucose from the blood to the urine before it can reach the tissues. By intravenous injection of enough glucose to maintain the blood sugar at normal levels, or by removal of both kidneys, the phlorizinized animal can be made to utilize carbohydrate in the normal manner. In this respect it differs from the depancreatized animal; but in other respects the two behave alike. In both, hepatic glycogenesis and glycogenolysis are accelerated and protein destruction is increased. This might be expected, since the difference between them is potential, rather than actual. Although the totally phlorizinized animal can burn sugar if it is given the opportunity, it is as effectually prevented from doing so by its renal defect as the depancreatized animal is by lack of insulin.

As protein destruction increases in the diabetic animal the formation of ketone bodies in the liver is also accelerated, greatly surpassing the capacity of the tissues to burn these compounds. Nevertheless, there can be no doubt from Stadie's experiments[24, 25] that diabetic tissues do burn them even more rapidly than do normal tissues. At the same time the tissues continue to burn a certain proportion of fat directly, without preliminary conversion to ketones by the liver. The explosive outburst of ketosis in the diabetic animal lends force to the argument that ketone production is not merely an essential step in the combustion of fat, but that ketones fill a specific operative rôle in the metabolism, which becomes more important when carbohydrate can not be utilized.

The major phases of the metabolism of carbohydrate and fat are outlined in Figure 1. At the left, protein is pictured in the liver as contributing to glycogen or fatty acids after deamination. The glycogen broken down to glucose is conveyed to the muscles to form glycogen again. This may be degraded to lactic acid to become glycogen once more in the liver, a purely operative procedure. On

the other hand, it may pass through the oxidative chain of operative reactions to end in the energy-producing combustion to CO_2 and H_2O. This channel of metabolism is potentiated by insulin. Below, the fat depots are represented, giving off fatty acids to the blood (that is, of course, not an exact representation of the facts, since fatty acids are not found free in blood or tissues). These can pass directly to the muscles, where they are burned to CO_2 and H_2O to provide energy, or to the liver where they are split into ketone acids. It is suggested in the illustration that these acids, when they have been conveyed to the liver are not utilized merely for energy-production, but participate in operative reactions that terminate in oxidative combustion. The parallel arrows on the right indicate that these operative procedures can be served by derivatives of either glycogen or ketones. It is not necessarily implied that both are metabolized by the same chemical reactions; the arrows do not unite. In animals subsisting on mixed diets ketosis is reduced to a minimum because there is enough carbohydrate available to conduct these operative processes unsupported. Fat that is burned passes predominantly over the route of direct combustion to provide energy. When, because insufficient carbohydrate is supplied, protein is forced to take over the functions of carbohydrate, carbohydrate combustion is diminished. To make up the deficit the formation of ketones is accelerated. However, it does not become extreme because ketones are needed only to supplement carbohydrate. Operative metabolism is shared between ketones and carbohydrate derived from protein. Both of the channels indicated by the parallel arrows are flowing together. But, when carbohydrate can no longer be utilized at all, when the right-hand channel is blocked, ketones have to take over the whole load.

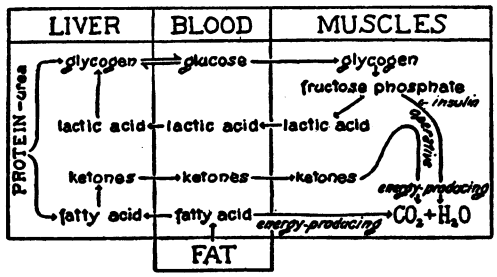


FIG. 1

Apparently the liver overreacts when faced with this unwonted crisis, pouring out ketones faster than they can be utilized. This suggests that ketone production by the liver is not directly attuned to the demands of the tissues. Other evidence would indicate that

it is more closely linked with the glycogen content of the liver or with some function connected with glycogen. Mirsky,[16] in the experiments previously cited, found that when sufficient glucose was injected into depancreatized animals to restore liver glycogen, not only protein destruction but also ketosis were abated. This is especially significant in view of the fact that the administered glucose presumably was not burned. Ketosis can not be linked directly with the formation of glycogen from protein because it does not reach major proportions in animals subsisting predominantly on protein.

The conversion of carbohydrate to fat as an alternative metabolic path for carbohydrate has received little consideration. This process has been treated as a provision for the storage of unusual excesses of carbohydrate which is only occasionally used. Fat has been regarded as a relatively inert material. Schoenheimer[20] has proved that it is undergoing constant and rapid changes. It is possible, if not probable, therefore, that it is being continually formed from carbohydrate and as rapidly burned. It is a self-evident truth that the combustion of carbohydrate with intermediary conversion to fat must have the same respiratory quotient as has the direct combustion of carbohydrate, 1.00. This makes attempts to distinguish the two processes extremely baffling. It has also given rise to the general assertion that the differentiation is of no importance. So long as interest was confined entirely to energy-production and the nature of the fuel consumed, this opinion was tenable. But when metabolism is viewed in its separate steps, the intermediary processes to which any foodstuff is subjected are matters of no little moment. Theoretically it should be possible for the diabetic animal to circumvent some of the incapacity imposed by his disease by burning carbohydrate over this indirect route. Actually, since the depancreatized animal excretes all carbohydrates, given or formed, as glucose in the urine, and has a respiratory quotient of about 0.71, it must be incapable of utilizing this expedient. Drury,[8] on other grounds, has suggested that one of the actions of insulin is to facilitate the conversion of carbohydrate to fat. This is an extreme view. It is impossible, at the present time, to go further than to say that the ability to form fat from carbohydrate is in some manner linked with the capacity to burn carbohydrate. To infer that it involves some one of the reactions included in the oxidative metabolism of carbohydrate is quite unwarranted. It is more probable that the organism will not spare carbohydrate for the formation of fat when it is so

urgently needed for other purposes. This would be only another expression of the fact that when the ability to burn carbohydrate is abrogated all possible reactions in the body are accelerated in the direction of the dead end, glucose.

The total frame of metabolism is outlined in Figure 2. Both protein and carbohydrate can form fat, and protein can also form carbohydrate. The normal function of protein is the conduct of operative and transformative metabolism, of which energy production may be regarded as a by-product. Excesses of protein, after deamination, are oxidized for energy production through the paths of carbohydrate and fat. The chief rôle of carbohydrate also seems to be operative, with energy-production a terminal event. Carbohydrate, like protein, may contribute to energy-production also by intermediate transformation of fat. For the latter, energy-production is the chief purpose of life. Its operative functions appear to be largely facultative and may be represented, for the present, by the dotted line which represents ketones cutting in upon the path of carbohydrate metabolism above the point of energy-production.

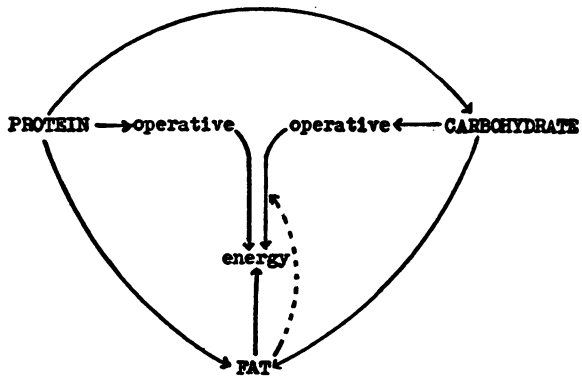


FIG. 2

In presenting this frame it has been necessary to treat the various processes in a discontinuous manner, as if they were separable. To some extent, however, all the reactions mentioned are continuously proceeding simultaneously. Some fat is always burned directly, even when ketosis is maximum. Ketosis itself is not a pathologic phenomenon, but a part of the normal metabolism of fat, its operative metabolism. It becomes pathologic only when it runs riot in diabetic acidosis, and then, not because it is not serving its metabolic purpose usefully and faithfully but because the acidic properties of the ketone acids play havoc with the systems to which acid-base equilibrium, electrolyte equilibrium, and water exchange are

entrusted. Carbohydrate oxdiation is accelerated by exercise; it is reduced to a minimum by lack of insulin. Less insulin is required to burn a given amount of carbohydrate during exercise than during rest.[22] Removal of the hypophysis or the adrenals greatly modifies the effect of pancreatectomy on metabolism. All these are illustrations of a general rule: the fundamental reactions involved in metabolic processes seem neither to cease entirely nor to change their character during life; only the relative rates at which they move vary with circumstances.

References

1 Benedict, F. G.: A study of prolonged fasting. Carnegie Inst., Wash., 1915, Publ. No. 203.

2 Bloch, K., and Schoenheimer, R.: The biological precursors of creatine. J. Biol. Chem., 1941, *138*, 167.

3 Bollman, J. L., Mann, F. C., and Wilhelmj, C. M.: The origin of glucose liberated by epinephrine in depancreatized animals. J. Biol. Chem., 1931, *93*, 83.

4 Courtice, F. C., and Douglas, C. G.: The effects of prolonged muscular exercise on the metabolism. Proc. Roy. Soc., London, 1935-36, B, *119*, 381.

5 Crandall, L. A. Jr.: Hepatic acetone body production in the dog during fasting and fat feeding. Am. J. Physiol., 1940, *131*, 10.

6 Crandall, L. A. Jr.: A comparison of ketosis in man and dog. J. Biol. Chem., 1941, *138*, 123.

7 Cruickshank, E. W., and Kosterlitz, H. W.: The utilization of fat by the aglycaemic mammalian heart. J. Physiol., 1941, *99*, 208.

8 Drury, D. R.: The rôle of insulin in carbohydrate metabolism. Am. J. Physiol., 1941, *131*, 536.

9 Drury, D. R., and McMaster, P. D.: Relation of liver to fat metabolism: I. Effect of liver lack on fat combustion and respiratory-quotient. J. Exper. Med., 1929, *49*, 765.

10 Folin, O.: A theory of protein metabolism. Am. J. Physiol., 1905, *13*, 117.

11 Gamble, J. L., Ross, G. S., and Tisdall, F. F.: The metabolism of fixed base during fasting. J. Biol. Chem., 1923, *57*, 633.

12 Hill, A. V.: *Muscular activity and carbohydrate metabolism.* Mayo Foundation Lectures on Nutrition. W. B. Saunders Co., Philadelphia, 1925.

13 Mann, F. C.: The effects of complete and of partial removal of the liver. Medicine, 1927, *6*, 419.

14 Meyerhof, O.: *Chemical dynamics of life phænomena.* J. B. Lippincott Co., Philadelphia and London, 1924.

15 Mirski, A., Rosenbaum, I., Stein, L., and Wertheimer, E.: On the behaviour of glycogen after diets rich in protein and in carbohydrate. J. Physiol., 1938, *92*, 48.

16 Mirsky, I. A., Heiman, J. D., and Broh-Kahn, R. H.: The anti-ketogenic action of glucose in the absence of insulin. Am. J. Physiol., 1937, *118*, 290.

17 Newburger, Robert: (Personal communication.)
18 Peters, J. P.: Some reactions by which solutes may be differentially concentrated by the kidney. *Chemistry and Medicine.* Univ. of Minnesota Press, Minneapolis, 1940.
19 Rose, W. C.: The nutritive significance of the amino acids. Physiol. Rev., 1938, *18*, 109.
20 Schoenheimer, R., and Rittenberg, D.: Deuterium as an indicator in the study of intermediary metabolism. VI. Synthesis and destruction of fatty acids in the organism. J. Biol. Chem., 1936, *114*, 381.
21 Schoenheimer, R., and Rittenberg, D.: The study of intermediary metabolism of animals with the aid of isotopes. Physiol. Rev., 1940, *20*, 218.
22 Smith, Florence H., and Smith, K. A.: The influence of muscular exercise on blood sugar concentrations. J. Clin. Invest., 1937, *16*, 289.
23 Smith, M.: The minimum endogenous nitrogen metabolism. J. Biol. Chem., 1926, *68*, 15.
24 Stadie, W. C.: Fat metabolism in diabetes mellitus. J. Clin. Invest., 1940, *19*, 843.
25 Stadie, W. C., Zapp, J. A. Jr., and Lukens, F. D. W.: Intermediary metabolism in diabetes mellitus. On the synthesis of carbohydrate from fat in the liver and from acetoacetate in the kidney. J. Biol. Chem., 1941, *137*, 63.
26 Wierzuchowski, M.: Intermediary carbohydrate metabolism. III. Vital action of glucose in phlorhizin diabetes. J. Biol. Chem., 1927, *73*, 445.