Newer Pathways of Carbohydrate Metabolism

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Despite the intensive efforts of biochemists and physiologists in the sixty years since Mering and Minkowski's discovery of the relationship between the pancreas and diabetes, we are still unable to say with any degree of assurance that we understand the defects in sugar metabolism underlying this disease entity. The hope held by biochemists for many years, and thought to have been realized several years ago,[1] that the biochemical lesion in diabetes would be found in a single chemical reaction somewhere along the pathway of glucose metabolism, has not materialized. It appears now that before such a Utopian condition is reached, we shall have to dig more deeply and thoroughly into the complex and devious metabolic routes followed by sugars in their interconversions and in their interactions with other foodstuffs and cell components.

It is in this area, however, that we can strike a more optimistic note. Our knowledge of the pathways of metabolism has broadened so greatly in recent years that in retrospect it seems that we must have been incredibly naïve a few years ago to have expected a clear-cut biochemical answer to the riddle of diabetes on the basis of knowledge available at that time.

I propose to explore with you our present conceptions of the chemical paths of glucose catabolism, and to dwell particularly on what I regard as one of the most exciting of the recent developments in carbohydrate metabolism—a new pathway of glucose dissimilation. I should like to map out those chemical pathways of carbohydrate metabolism which, on the basis of our present knowledge, are of importance in the metabolism of glucose in animal cells.

OUTLINE OF GLUCOSE METABOLISM

Figure 1 shows the over-all outline of glucose metabolism in cells of the higher animals. The first step is an activation by combination with phosphate, following which the hexose phosphate is split to a triose, then converted to pyruvic acid, and ultimately to acetic acid. The molecules of acetic acid mingle with identical acetic acid molecules coming from fatty acids and other substances and are converted to carbon dioxide by means of the so-called citric acid cycle. In this process acetic acid, in the form of an ester of coenzyme A, condenses with a molecule of oxalacetic acid to yield citric acid. This is then successively converted to isocitric, oxalsuccinic, alpha-ketoglutaric, succinic, malic, and oxalacetic acids. In a single revolution of the cycle one acetic acid molecule is completely oxidized to carbon dioxide and regenerates one molecule of oxalacetic acid to carry on the cycle. The regeneration of oxalacetic acid allows the cycle to continue indefinitely, with only trace or catalytic amounts of citric acid cycle components actually present in the cell at any time.

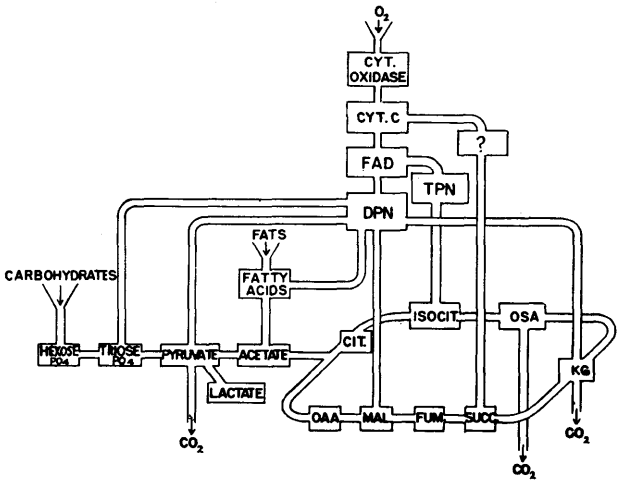

FIG. 1

In the course of the oxidation of a molecule of glucose by these means, twenty-four electrons are lost—twelve for each triose. These are given up at six oxidation steps; namely, triosephosphate, pyruvic acid, isocitric acid, alpha-ketoglutaric acid, succinic acid, and malic acid. These electrons ultimately combine with oxygen to yield water, but before doing so they pass through a series of electron transport agents, which like a bucket brigade, take a pair of electrons from one donor and hand them on to an acceptor, until they ultimately reach

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oxygen. These electron-transferring enzymes, arranged vertically in figure 1, have as coenzymes diphosphopyridine nucleotide (DPN), triphosphopyridine nucleotide (TPN), flavin adenine nucleotide (FAD), and the cytochrome system, including cytochrome c and other cytochromes whose precise function or position in this process is still uncertain. Components of this electron transport system are the well-known vitamins, nicotinamide and riboflavin. Carbon dioxide, which is the ultimate product of the oxidation of carbohydrate, is given off at three places in this process; the conversion of pyruvic to acetic acid, that of oxalosuccinic to alpha-ketoglutaric acid, and that of alpha-ketoglutaric to succinic acid.

As shown, the cycle appears to have only an oxidative function; however, it serves a more important service in the cell as a means of communication between the various foodstuffs and the cell components. For example, the cycle can supply carbon for various amino acids. Alanine can be formed from pyruvic acid, and thence to such other amino acids as serine, glycine, and cysteine. From glutamic acid there are pathways to other amino acids such as proline, hydroxyproline, ornithine and arginine. We can visualize the citric acid cycle as a means of communication between a major foodstuff such as carbohydrate on the one hand and almost any conceivable cell component on the other. All or most of these processes are reversible and thus provide pathways not only for the formation but also for the utilization of the amino acids and other cell components for energy production, or even for the production of sugar.

From the diagram, it is evident that acetic acid is a key substance, a focal point for the merging of the metabolism of both fats and sugar. The actual intermediate is not acetic acid as such but an ester of acetic acid with a new sulfur-containing coenzyme—coenzyme A. The mechanism by which pyruvic acid can be converted to acetyl coenzyme A has been greatly clarified in the past few years. This has emerged as an extremely complicated process, involving no less than five separate cofactors—thiamine pyrophosphate, magnesium, lipoic acid, coenzyme A, and diphosphopyridine nucleotide.[2]

The first step is the decarboxylation of pyruvic acid. This requires diphosphothiamine (DPT) and magnesium, and the products are carbon dioxide and a complex of acetaldehyde and diphosphothiamine. In certain organisms this complex may be oxidized directly to acetic acid, or converted to condensation products such as acetolactic acid or acetoin. In higher animals and in certain other organisms the diphosphothiamine group is exchanged for another new cofactor discovered only in recent years, lipoic acid. This is a new sulfur-containing coenzyme whose structure and function have been cleared up only very recently. This complex of acetaldehyde and lipoic acid then undergoes an intramolecular rearrangement of its electrons, resulting in an oxidation of the acetaldehyde to acetic acid and a reduction of lipoic acid, to yield an acetyl ester of reduced lipoic acid. The acetyl moiety is again transferred—from the reduced form of lipoic acid to coenzyme A yielding acetyl coenzyme A and free reduced lipoic acid. The lipoic acid then becomes oxidized via diphosphopyridine nucleotide back to the oxidized form and is now ready to accept and combine with another acetaldehyde molecule.

Acetyl coenzyme A thus formed has a choice of several pathways. By combining with oxalacetic acid it can be oxidized to carbon dioxide through the citric acid cycle, or it can undergo other types of reaction, such as fatty acid or cholesterol synthesis, or conversion to acetoacetic acid.

The evident complexity of the ostensibly simple reaction, the oxidative decarboxylation of pyruvic acid to form acetyl coenzyme A and carbon dioxide, which has emerged from a thorough study of just one step of the great many involved in sugar metabolism, indicates how superficial is our knowledge of cellular biochemistry.

PATHWAYS OF GLUCOSE CATABOLISM

I shall now take up a still earlier stage; namely, those reactions which give rise to pyruvic acid. Until a few years ago it was generally believed that the main, if not sole route of glucose catabolism was that developed in the previous generation by Embden and Meyerhof. Perhaps the most exciting of recent developments in carbohydrate metabolism is the realization that another pathway of glucose breakdown may play an important role.[3-5] The old and new pathways are shown in figure 2. In the Embden-Meyerhof pathway, on the right, glucose-6-phosphate is converted successively to fructose-6-phosphate, fructose 1, 6-diphosphate, and triose phosphate, ultimately to yield pyruvic acid. In this process each glucose molecule yields two trioses and two pyruvates. The new process has been called the direct oxidative process or the oxidative shunt, but perhaps a better name would be the pentose pathway. Glucose-6-phosphate is oxidized directly to 6-phosphogluconic acid. This is oxidized further, probably to 6-phospho-3-ketogluconic acid, and this is decarboxylated to yield carbon dioxide and a ketopentose, ribulose-5-phosphate. Ribulose-5-phosphate is split to a triose phosphate, which ultimately yields pyruvic acid and a "diose." This term is quoted because

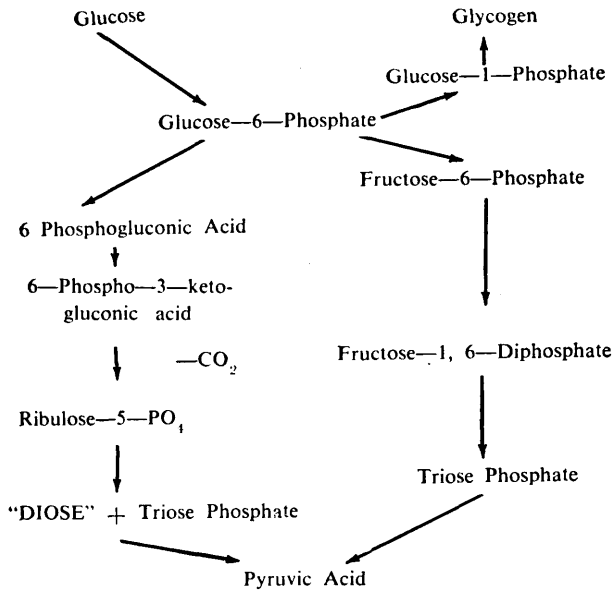


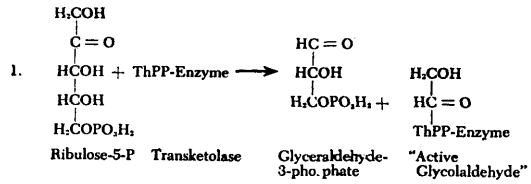
FIG. 2. Embden-Meyerhof and direct oxidative mechanisms of glucose catabolism.

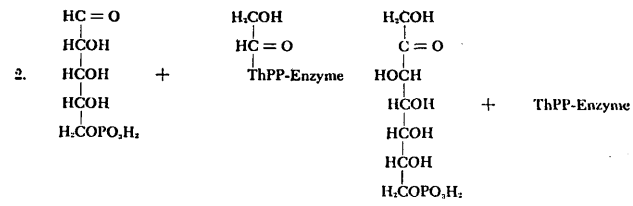
the substance has not yet been isolated and identified. It is presumably glycolaldehyde in the form of an enzyme complex.

The formation of the "diose" from ribulose is actually not as simple as pictured. As shown in equation 1 it appears apparently only in the presence of an acceptor and through the agency of the recently discovered transketolase. It is interesting that this enzyme has a coenzyme diphosphothiamine (or thiamine pyrophosphate shown here as ThPP, the same coenzyme involved in decarboxylation of pyruvic acid. The products are a triose and a "diose"-enzyme complex, the so-called active glycolaldehyde. This as yet elusive substance can be transferred to certain acceptor molecules. One such transfer is shown in equation 2.

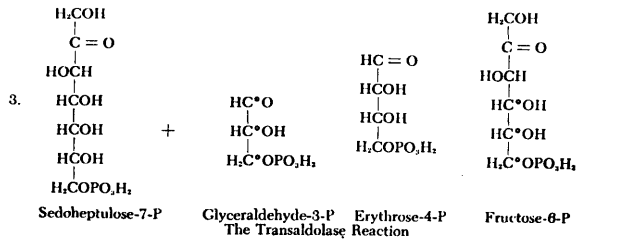
In the presence of an acceptor such as ribose-5-phosphate, which itself can be formed from ribulose phosphate through a specific isomerase, the "diose" group is transferred to yield a 7-carbon sugar phosphate, sedoheptulose-7-phosphate, and the free enzyme, which is now available for splitting another ribulose molecule. This 7-carbon sugar until a few years ago was a rare laboratory curiosity, found only in plants of the sedum family. It is now known to be a widespread intermediate of great importance, capable of being formed and utilized by almost every form of life through the series of reactions now being discussed.

In another similar transfer, shown in equation 3, the transaldolase reaction, a 3-carbon unit representing the first three carbons of the 7-carbon sugar, is transferred to another molecule of triose phosphate to give fructose-6-phosphate. This can be converted to glucose-6-phosphate to re-enter this direct oxidative process, or it may be converted to fructose-1,6-diphosphate, to enter the Embden-Meyerhof process. The other product of this reaction is an as yet incompletely identified tetrose, here designated as erythrose-4-phosphate. Ultimate disposition of this tetrose is not certain. One reaction it can undergo is shown in equation 4. This involves a transfer of a diose molecule from the first two carbons of ribulose phosphate to yield fructose-6-phosphate plus triose-3-phosphate.

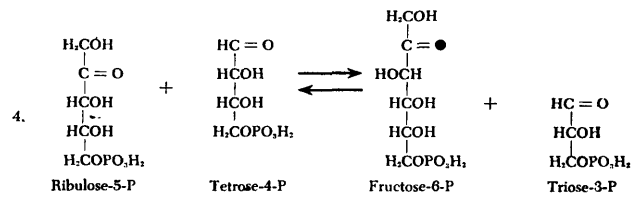




The Formation of Sedoheptulose-7-phosphate in the Transketolase Reaction.



The Transaldolase Reaction



The sum of all of these reactions is shown in figure 3. Three glucose molecules are phosphorylated by means of adenosine triphosphate (ATP), and there are obtained three molecules of carbon dioxide representing the oxidation of half a molecule of glucose. The other half appears as triose phosphate, which can undergo successive conversions to pyruvic acid and acetate. Simultaneously, two molecules of fructose phosphate are regenerated. Thus we see that the occurrence of all of these reactions constitutes a new oxidative cycle in which many intermediates participate, and which ultimately results in the oxidation of glucose.

OXIDATIVE CYCLE BASED ON TRANSKETOLASE AND TRANSALDOLASE REACTIONS

1. 3 Glucose + 3 ATP ⟶ 3 Glucose-6-Phosphate
2. 3 Glucose-6-Phosphate ⟶ 3 6-Phosphogluconic Acid
3. 3 6-Phosphogluconic Acid ⟶ 3 Ribulose-5-Phosphate + 3 CO_2
4. Ribulose-5-Phosphate ⟶ Ribose-5-Phosphate
5. Ribulose-5-Phosphate + Ribose-5-Phosphate ⟶ Sedoheptulose-7-Phosphate + Triose-3-Phosphate
6. Sedoheptulose-7-Phosphate + Triose-3-Phosphate ⟶ Fructose-6-Phosphate + Tetrose-4-Phosphate
7. Tetrose-4-Phosphate + Ribulose-5-Phosphate ⟶ Fructose-6-Phosphate + Triose-3-Phosphate

SUM: 3 Glucose + 3 ATP ⟶ 3 CO_2 + Triose-3-Phosphate + 2 Fructose-6-Phosphate

FIG. 3

We still do not know how important this series of reactions may be or how extensively it occurs in animal tissues generally. Glock and McLean[6] have found that most animal tissues contain glucose-6-phosphate dehydrogenase, and also enzymes for the utilization of 6-phosphogluconic acid and ribose-5-phosphate. The only tissue in which these enzymes are low is muscle. On the reasonable assumption that the presence of enzymes denotes occurrence of a process, it seems probable that this new pathway of glucose metabolism may operate generally in animal tissues. However, there is considerable interest in establishing the extent to which this process *does* operate in animal tissues, and to what extent dietary, hormonal, and other factors might influence its participation in sugar catabolism. Isotopic tracer procedures, using labeled glucose molecules, have been applied to this problem, but much of these data are in a controversial state because of difficulties of interpreting results.[7-9]

In our own laboratory, my associates and I have developed a direct and fairly reliable procedure for determining the relative proportions of the pentose and Embden-Meyerhof processes, and have applied this to a variety of cell types.[10] Time will not permit a description of the principle of this procedure, which also involves isotope tracers. Thus far we have done little more than survey representative cells. Some results obtained are shown in table 1. In liver slices and homogenates the direct oxidative process occurs to a variable degree. In rat liver the Embden-Meyerhof process is preponderant, but in mouse liver about 50 per cent or more of the glucose appears to be metabolized via the oxidative process. On the other hand, kidney, brain, and heart muscle appear to utilize only the Embden-Meyerhof process. One of our motives in carrying out this study was to determine the extent of occurrence of this process in neoplastic tissues. In these tissues a wide range of patterns was observed, ranging from complete Embden-Meyerhof to predominance of the pentose path.

TABLE 1
PATHWAYS OF OXIDATION IN TISSUES

Tissue	Embden-Meyerhof pathway Per cent	Direct oxidative pathway Per cent
Liver, rat and mouse	12-46	54-88
Kidney, rat	100	0
Heart, rat	100	0
Brain, rat	100	0
Mammary carcinoma, mouse	82	18
Rhabdomyosarcoma, mouse	91	9
Ascites TA_3 carcinoma, mouse	32	68
Hepatoma 98/15, mouse	100	0
Sarcoma 37, mouse	57	43

If these studies in vitro reflect occurrences in the intact cell, and if we can assume that the oxidative pattern in heart muscle is similar to that occurring in skeletal muscle, it would seem that the oxidative process does not play an important quantitative role in the over-all metabolism of glucose in the whole animal. Thus far the liver seems to be the only tissue utilizing this process, and this tissue probably represents only a relatively small proportion of the active glucose-metabolizing tissue of an animal. However, much more work will have to be carried out before any generalizations can be made with regard to the significance of this new pathway. The extension of these studies to other specific animal tissues and the study of possible factors which may regulate or control the relative participation of each of these pathways are topics of current interest.

SIGNIFICANCE OF DIRECT OXIDATIVE PATHWAY

Any special significance of the direct oxidative pathway is as yet uncertain, but several interesting possibilities are evident. Of course it probably serves an extremely important function in providing a mechanism for formation of ribose, a constituent of a host of cell components,

including nucleic acids. We have already seen that the net result of the efficient operation of the direct oxidative process results in a cycle of oxidation of hexose. The possibility exists that this cycle may operate in certain tissues as a supplement to or a replacement for the citric acid cycle. Much evidence already available indicates that this process may be of greater importance in the metabolism of plants and micro-organisms than in animals. For example, it is now clear that the process of photosynthesis, whereby green plants synthesize carbohydrate from carbon dioxide and light energy, involves components of the oxidative shunt.[11] The 7-carbon sugar, sedoheptulose, also appears now to be a carbon source of the aromatic amino acids, phenylalanine, tyrosin, and tryptophan.[12]

CONCLUSIONS

Thus far these new findings have complicated rather than clarified the diabetes picture. Our view of the intermediary metabolism of carbohydrate, which formerly extended to a few hexoses and trioses, now must be broadened to include dioses, tetroses, pentoses, and heptoses. We are confronted with the disturbing recognition that many more components, many more enzymes, many more factors are involved in the mosaic of metabolism than was considered possible just a few years ago. These are facts that confront us in our attempt to unravel the forces which control and direct the metabolism of carbohydrate.

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SUMMARIO IN INTERLINGUA

Nove Vias del Processos del Metabolismo de Hydratos de Carbon

Usque pauc annos retro, le conception general esseva que le processo del catabolismo de glucosa in le cellulas sequeva plus o minus exclusivemente le via describite per Embden e Meyerhof con le sequente passos: glucosa → glucosa-6-phosphato → fructosa-6-phosphato → fructosa-1, 6-diphosphato → 2 triosa-phosphato → 2 acido pyruvic. Il deveni de plus in plus obvie que un altere via es possibilemente disponibile al metabolismo de glucosa. Iste via diverge ab illo de Embden e Meyerhof in tanto que illo involve le oxydation de glucosa-6-phosphato in acido 6-phosphogluconic e hinc in ribulosa-5-phosphato. In le presentia de aldosas acceptores, ribulosa-5-phosphato es findite in triosa e glycolaldehydo active. Iste glycolaldehydo active se adde a un acceptor e forma un nove phosphato de sucro. Con ribosa-5-phosphato, iste reaction transcetolasic—como illo ha essite appellate—resulta in sedoheptulosa-7-phosphato. Alora sedoheptulosa-7-phosphato transfere—in le presentia de triosa-phosphato e le enzyma transaldolase—un portion de triosa al triosa-phosphato lo que resulta in tetrosa-phosphato e fructosa-6-phosphato. Assi le processo sequente iste nove via es un processo cyclic de oxydation e regeneration de hexosa. Le signification de iste processo in cellulas animal remane incerte. Provas de su occurrentia a un appreciabile grado ha essite obtenite solmente in le caso del hepate, del glandulas mammari, e de certe tumores, sed le enzymas involvite in illo se trova extensemente in le cellulas animal.