A Newer Pathway of Carbohydrate Metabolism; The Pentose Phosphate Pathway

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In the past thirty years a vast body of information has been compiled relating to carbohydrate metabolism. Nevertheless, it is becoming increasingly evident that the elucidation of the metabolic disturbance in diabetes mellitus requires an even more complete knowledge than is presently available of the complex chemical routes followed by glucose in its conversions and interactions with other cell components. In this area of carbohydrate metabolism most gratifying progress has been made with the demonstration of previously unsuspected pathways of glucose utilization. Until rather recently, it was generally believed that the primary, if not the sole, route of glucose breakdown by mammalian tissue was via the Embden-Meyerhof or anaerobic-glycolytic pathway involving the conversion of glucose to pyruvate and lactate and its subsequent oxidation to CO_2 by the citric acid cycle. Evidence has now accumulated to indicate the existence of at least one new pathway of glucose metabolism, referred to as the pentose phosphate pathway, or the hexose monophosphate oxidative shunt, which may often supplement significantly the anaerobic-glycolytic scheme. Before turning to some of the details and possible significance of this alternate pathway, a brief review of the reactions of glycolysis and the citric acid cycle is desirable.

ANAEROBIC GLYCOLYSIS AND THE CITRIC ACID CYCLE

The exact mechanism of glucose transport across the cell membrane is not well understood.[1] Certain of the reactions of intracellular glycolysis, however, are well established and are summarized in figure 1.[2] The first step in the metabolism of glucose is its conversion to the phosphorylated form, glucose-6-phosphate. This reaction is catalyzed by the enzyme hexokinase and requires as a source of energy and phosphate, adenosine triphosphate (ATP). It is essentially an irreversible reaction.

Presented at the meeting of the Clinical Society of the New York Diabetes Association on Feb. 23, 1956.

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Glucose-6-phosphate has a key position in glycolysis and may have one of several fates.[3] Normally, a small percentage of the glucose-6-phosphate in liver and muscle is converted to glycogen. Liver possesses the enzyme, a phosphatase, necessary for the conversion of glucose-6-phosphate to free glucose. As a result of the presence of this enzyme in liver, this organ is the only major endogenous source of free glucose.

The greatest portion of glucose-6-phosphate, under normal circumstances, is broken down via several intermediates, including fructose-6-phosphate and triose phosphate, to pyruvate and lactate. This is the series of reactions involved in anaerobic glycolysis.

The subsequent metabolism of pyruvate is summarized in figure 1.[4] Pyruvate may be oxidatively decarboxylated to form acetyl coenzyme A which, in turn, condenses with oxalacetate to form citric acid in the tricarboxylic acid cycle. In addition, pyruvate may enter the citric acid cycle directly by a carboxylation reaction to form a dicarboxylic acid precursor of oxalacetate. The major portion of glucose metabolized to pyruvate is converted by means of the reactions of the citric acid cycle to either CO_2 and H_2O or is made available by this cycle for amino acid and ultimately protein synthesis. Acetyl coenzyme A, the so-called activated two-carbon fragment, plays a key role in the crossroads of sugar and fat metabolism. Acetyl coenzyme A is the building block in fatty acid synthesis.[5] The synthesis of fatty acids is a reductive synthesis requiring hydrogen in the form of reduced diphosphopyridine nucleotide (DPNH), which is generated in glucose breakdown by anaerobic glycolysis and the citric acid cycle. Perhaps hydrogen in the form of reduced triphosphopyridine nucleotide (TPNH) may also play a role in fatty acid synthesis. As we shall presently discuss in some detail, TPNH is generated by glucose oxidation via the pentose phosphate pathway.

In experimental diabetes mellitus it has been demonstrated that in addition to the block in glucose utilization at the level of glucose conversion to glucose-6-phosphate, there is a decrease in the synthesis of fatty acids from acetate.[6] The relationship between these two

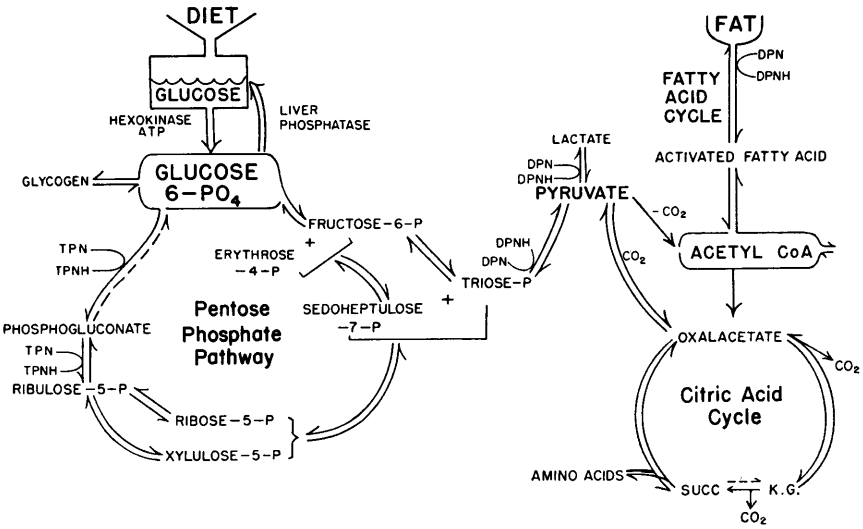


FIGURE 1

metabolic defects is not clear. It may be that in diabetes the block to glycolysis results in an inadequate supply of the hydrogen in the form of DPNH or TPNH necessary to fatty acid synthesis.

PENTOSE PHOSPHATE PATHWAY

Let us now turn our attention to the reactions involved in the alternate pathway of glucose metabolism. The recognition of this newer pathway of glucose utilization, perhaps one of the most exciting of the recent developments in carbohydrate metabolism, is the result of work by many investigators, dating from the studies in the thirties by Warburg,[7] Lipmann,[8] Dickens,[9] and Dische[10] and, more recently, by Horecker,[11] Racker[12] and others. This pathway is referred to as the hexose monophosphate oxidative shunt, because the initial steps involve the direct oxidation of glucose-6-phosphate, or the pentose phosphate pathway, since 5-carbon sugars, i.e., pentoses, are intermediates in this pathway. The individual reactions involved in this cycle are summarized in figures 2-7. They will be discussed briefly, for the details of the reactions are not important to the present purpose. This discussion will primarily indicate certain of the distinctive aspects of this cycle, as well as its possible relationship to the over-all picture of carbohydrate metabolism.

The first step in glucose metabolism by the alternate cycle is identical with that of anaerobic glycolysis, namely, its phosphorylation to glucose-6-phosphate. The oxidation of glucose-6-phosphate results in its conversion to 6-phosphogluconolactone, the ring form of 6-phosphogluconate.[13] 6-phosphogluconate has a carboxy instead of an aldehyde group in position one. This oxidation requires triphosphopyridine nucleotide (TPN) and generates the reduced form of this cofactor, TPNH (figure 2). 6-phosphogluconate is in turn oxidized to the 5-carbon keto sugar, ribulose-5-phosphate.[14] In this oxidative step, CO_2 is formed and, again, TPN is required and TPNH generated (figure 3).

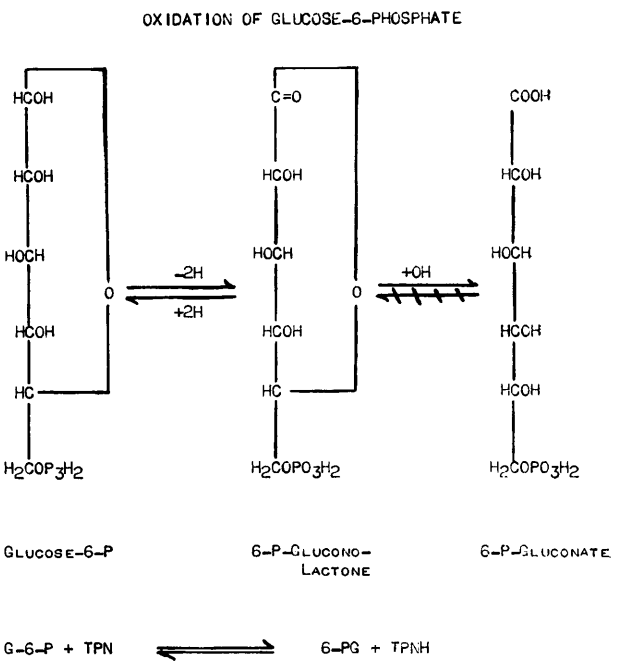
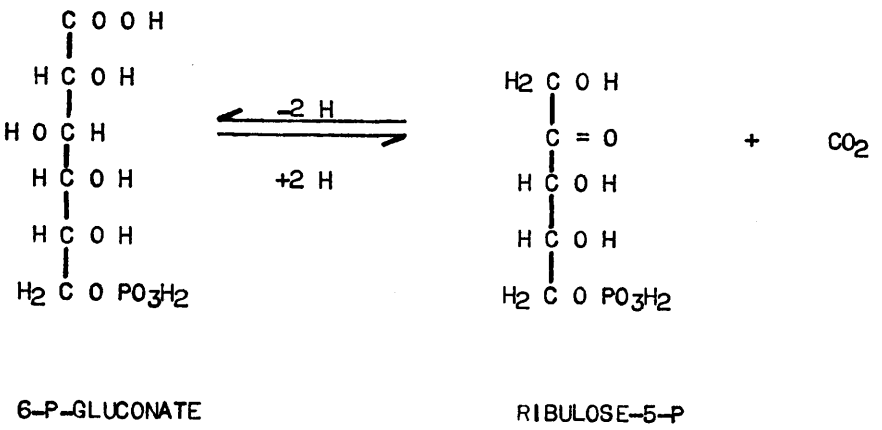


FIGURE 2

OXIDATION OF 6-PHOSPHOGLUCONATE



6-P-GLUCONATE RIBULOSE-5-P

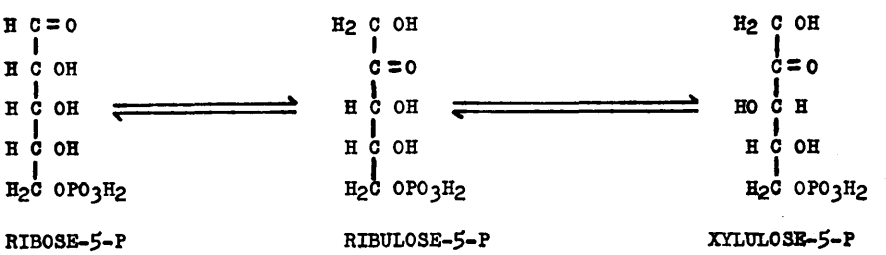
FIGURE 3

$$6\text{-PG} + \text{TPN} \rightleftharpoons \text{Ru-5-P} + \text{TPNH} + CO_2$$

The two steps that have been discussed are oxidative reactions. The subsequent reactions of this pentose phosphate pathway are anaerobic reactions. Recent evidence indicates that the pentoses involved in the next reaction are ribose-5-phosphate and xylulose-5-phosphate.[15, 16] As indicated in figure 4, these 5-carbon sugars are conversion products of ribulose-5-phosphate. In the next reaction (figure 5), catalyzed by the enzyme transketolase,[17, 18] the two top carbons of the keto sugar, xylulose-5-phosphate, are transferred to ribose-5-phosphate with the formation of a 7-carbon sugar, sedoheptulose-7-phosphate and glyceraldehyde-3-phosphate. Until several years ago, sedoheptulose was a rare laboratory curiosity found only in plants of the sedum family. It is now recognized to be widespread in its occurrence, and of great importance in the metabolism of almost every form of plant and animal life. Sedoheptulose-7-phosphate and glyceraldehyde-3-phosphate can be converted, in a reaction catalyzed by the enzyme transaldolase, to the 6-carbon sugar, fructose-6-phosphate and a newly recognized 4-carbon sugar, erythrose-4-phosphate,[19] (figure 6). Fructose-6-phosphate, by reactions identical to those of anaerobic glycolysis, may be converted to fructose-diphosphate and then to two 3-carbon moieties, or converted back to glucose-6-phosphate. The 4-carbon sugar appears to be a highly reactive compound. One reaction which it can undergo is catalyzed by transketolase and involves another molecule of the 5-carbon keto sugar, xylulose-5-phosphate,[15] (figure 7). The products of this reaction are fructose-6-phosphate and glyceraldehyde-3-phosphate, whose possible subsequent fates have been indicated.

The sum of all these reactions is schematically represented in figure 8. For each turn of this complete series of reactions, three glucose molecules are phosphorylated by ATP and there result three molecules of carbon

PENTOSE INTERCONVERSIONS



RIBOSE-5-P RIBULOSE-5-P XYLULOSE-5-P

FIGURE 4

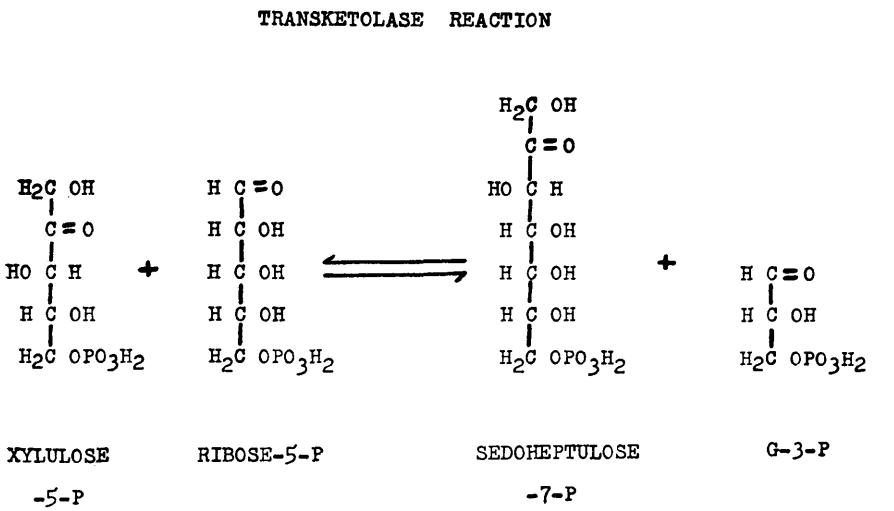


FIGURE 5

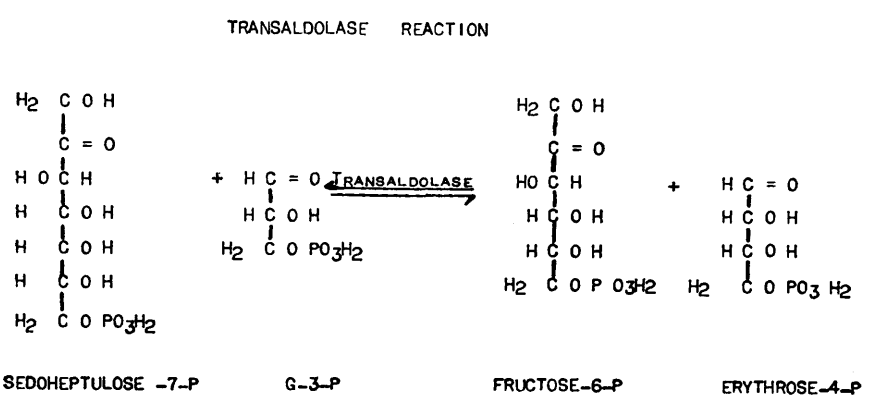


FIGURE 6

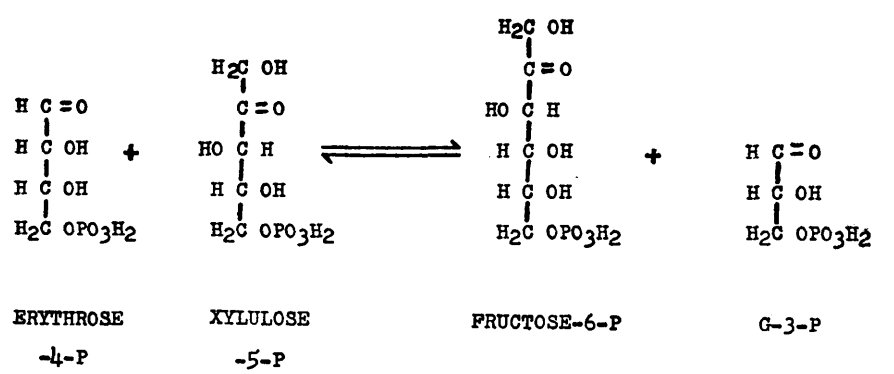


FIGURE 7

dioxide representing the oxidation of half a molecule of glucose. The other half appears as triose phosphate which may either be oxidized to CO_2 via the citric acid cycle or be reconverted to glucose-6-phosphate. In addition, two molecules of a 6-carbon sugar are regenerated. Thus, repeated cycling of this series of reactions, in which several new intermediates participate, can result in the complete oxidation of glucose to CO_2 and H_2O.

The relationship between the reactions of the pentose

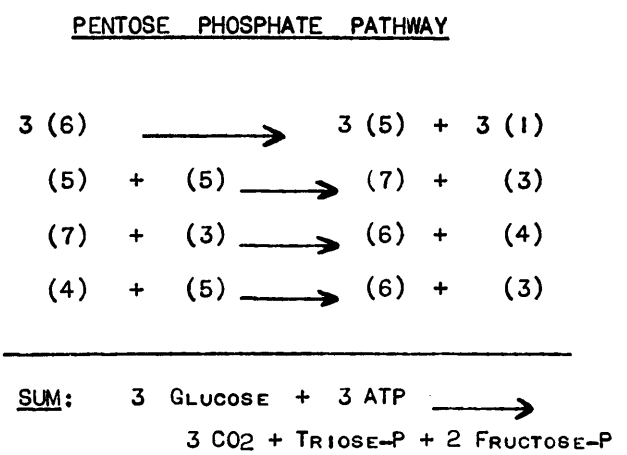


FIGURE 8

phosphate pathway and anaerobic glycolysis can be seen in figure 1. Both pathways share certain intermediates, namely, glucose-6-phosphate, fructose-6-phosphate and triose phosphate. Both schemes, however, represent distinct sets of reactions catalyzed by a separate series of enzymes.

THE ROLE OF PENTOSE PHOSPHATE PATHWAY IN CARBOHYDRATE METABOLISM

Evidence has accumulated to indicate that the enzymes of the alternate cycle, as those of anaerobic glycolysis, are present in most animal tissues.[20] Skeletal muscle alone, of the many normal tissues examined to date, appears to possess pentose pathway enzymes in low concentration. In alloxan diabetic rats, it is reported that the enzymes which catalyze the oxidation of glucose-6-phosphate and 6-phosphogluconate are markedly reduced.[21]

The elucidation of these alternate pathways of glucose metabolism has primarily resulted from work with cell-free systems and frequently isolated enzymes. The investigator is now faced with the task of attempting to assess the relative importance of these various routes of glucose utilization in the intact cell and the total organism. This is an even more difficult, albeit fundamental, problem and one for which there are only the most fragmentary answers at this time.

The availability of isotopic tracer procedures using C^{14}-labeled compounds, has made an approach to this problem possible. In this approach, one may take advantage of the fact that carbon atoms of glucose will have different fates in the two pathways. In the Embden-Meyerhof scheme, glucose-6-phosphate is converted to two molecules of pyruvate in such a fashion that position 1 and 6, 2 and 5, and 3 and 4 give rise to the same carbon atoms of pyruvate (figure 9). Thus, in the subsequent oxidation of pyruvate to CO_2, radioactivity originally in positions 1 and 6 of glucose is assumed to appear in CO_2 at an equal rate. On the other hand, in the pentose phosphate pathway, CO_2 arises from position 1 of glucose, while carbon atoms 2-6 give rise to pentose phosphate. Thus, via the alternate pathway, radioactivity originally present in carbon atom 1 of glucose would presumably appear in CO_2 at a more rapid rate than radioactivity in carbon atom 6 of glucose.

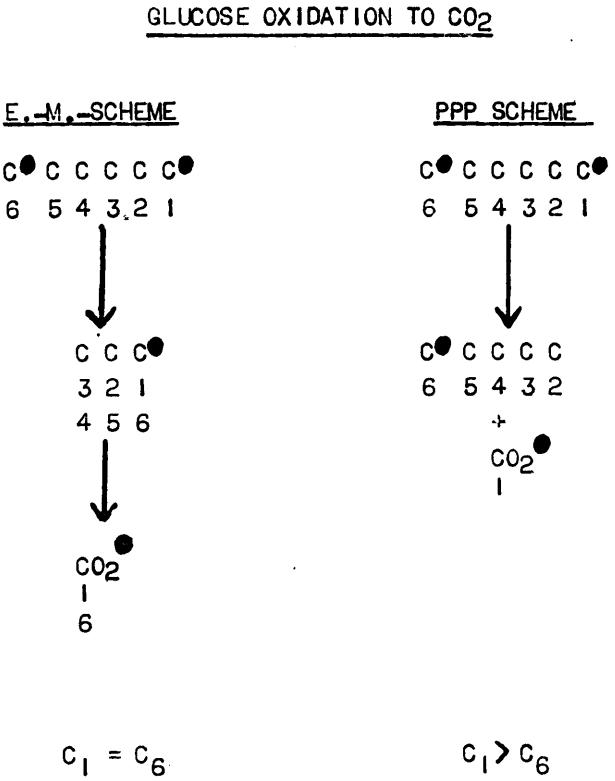


FIGURE 9

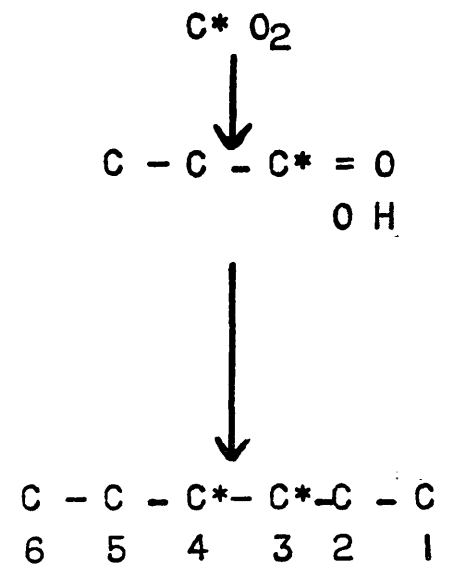
With these theoretical considerations, and a variety of complex mathematical formulations, several investigators using C^{14}-labeled glucose and other specifically labeled intermediates, have attempted to estimate the relative significance of the Embden-Meyerhof and alternate pathways in the oxidation of glucose.[22-28] The results of some of these studies are summarized in table 1. These studies were carried out in vitro with tissue slices from rats or mice. The difficulties encountered

TABLE 1
Pathways of glucose oxidation

Tissue	Embden-Meyerhof Per cent	Pentose phosphate per cent	Reference
Normal			
Liver, rat and mouse	20-98	2-80	22, 23, 24
Kidney, rat	72-100	0-28	22, 23
Heart, rat	100	0	25
Brain, rat	100	0	25
Skeletal muscle, rat	100	0	22, 23
Spleen, mouse	63	37	27
Diabetic			
Liver, rat	51-73	27-49	26
Tumors			
Mammary carcinoma, mouse	82	18	25
Ascites TA_3 carcinoma, mouse	32-77	68	25
Hepatoma 98/15, mouse	100	0	25
Sarcoma 37, mouse	57	43	25
Gardner lymphosarcoma	68	32	27
Ehrlich ascites tumor	77	23	27

in the measurements designed to assess the alternate pathways are complex and it appears that these results must be interpreted with caution.[29] They do give some index of the relative order of magnitude of the two pathways. In normal rat liver, it has been reported that as much as 80 per cent of the glucose oxidized to CO_2 can be metabolized via the pentose phosphate pathway. This figure has been found to vary between 2 and 80 per cent depending somewhat on the method employed in its estimation.[22, 24] On the other hand, brain, heart and skeletal muscle appear to utilize only the Embden-Meyerhof scheme. Diabetic rat liver oxidized glucose to CO_2 via the pentose phosphate pathway to approximately the same extent as normal rat liver.[26] In the several tumor tissues studied, as is indicated in table 1, a rather wide range of patterns has been observed.

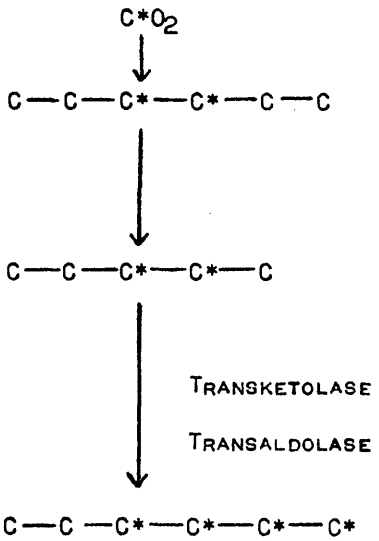
In addition to this effort at evaluation of the relative significance of these pathways in glucose oxidation to CO_2, their role in glycogen synthesis has been investigated. These studies are based upon the predictable differences in the pattern of incorporation of C^{14} into the carbon chain of glucose.[30] Thus, if the Embden-Meyerhof scheme accounts for glycogen synthesis, incubation of liver slices in the presence of $C^{14}O_2$ would be expected to result in incorporation of radioactivity predominantly into carbon atoms 3 and 4 of glucose (figure 10). On the other hand, if the pentose phosphate pathway contributed to glycogen synthesis, C^{14} of CO_2 would be expected to be incorporated into positions 1-4 of glucose (figure 11). In studies with liver slices from both normal and fasted rats, it was found



PREDICTED PATTERN OF $C^{14}O_2$ INCORPORATION IN GLUCOSE VIA EMBDEN-MEYERHOF PATHWAY

FIGURE 10

that radioactivity was incorporated mainly into positions 3 and 4. Less than 5 per cent of the total radioactivity incorporated into glycogen was found in carbon atoms 1 and 2 (table 2).[31] These findings are in agreement with those of Bernstein et al.,[32] who reported that the administration of $C^{14}O_2$ to intact rats yielded glycogen labeled predominantly in positions 3 and 4. It would seem, therefore, that under these conditions glycogen syn-



PREDICTED PATTERN OF $C^{14}O_2$ INCORPORATION IN GLUCOSE VIA PENTOSE PHOSPHATE PATHWAY

FIGURE 11

TABLE 2
Pattern of $C^{14}O_2$ incorporated into rat liver glycogen

	Per cent of total radioactivity					
Carbon atom	1	2	3	4	5	6
Normal rat	2	2	41	52	2	1
Fasted rat	2	1	47	48	1	1

thesis proceeds predominantly via the Embden-Meyerhof scheme.

It is obviously premature to attempt to evaluate the significance of the pentose phosphate pathway in mammalian metabolism. Nevertheless, there are several possible roles of this pathway which may be indicated. It has been demonstrated that the pentose phosphate pathway provides a cycle for glucose oxidation which may operate in certain tissues as a supplement to anaerobic glycolysis and the citric acid cycle. However, the available evidence indicates that this alternate pathway plays a small role, if any, in hepatic glycogen synthesis. The reactions of the pentose phosphate pathway provide a mechanism for the biosynthesis of ribose phosphate.[1] This may prove to be one of its most significant features, since ribose phosphate is a constituent of several cell components, including nucleic acids and various coenzymes. The oxidative steps of this cycle generate hydrogen in the form of TPNH which may play an important role in certain reductive reactions of the body. In addition, recent evidence indicates that the 7-carbon sugar, sedoheptulose, is a carbon source of aromatic amino acids.[33] Perhaps the most well established role of this pathway, and clearly an exceedingly important one, is in the process of photosynthesis. Certain reactions of this alternate cycle provide the mechanism for the generation of the substrate, ribulose-diphosphate, for CO_2 fixation in the synthesis of carbohydrates by green plants.[34]

The discovery of this new pathway of carbohydrate metabolism has perhaps complicated rather than simplified the present efforts at an understanding of the metabolic defect in diabetes. The view of glucose metabolism which recognized a few hexoses and trioses, is now broadened to include 4, 5 and 7-carbon sugars. In addition, it is probable that several pathways differing from the Embden-Meyerhof and citric acid cycle may play a role in mammalian glucose metabolism. Certainly with the discovery of each new pathway, the assessment of the relative significance of these many reactions in the total metabolism of the organism becomes a most difficult task. It is none the less a challenging and fundamental problem which may be solved by newer technics and knowledge.

SUMMARIO IN INTERLINGUA

Un Nove Via del Metabolismo de Hydratos de Carbon; Le Via de Pentosa-Phosphato

Al tempore presente il es un facto ben establite que le metabolismo de glucosa in histos mammalian pote progreder per vias altere que le via del glycolyse anaerobe e del cyclo de acido citric. Recente observationes indica que le reactiones de un tal via alternative—le via de pentosa-phosphato o le derivation oxydative a hexosa-monophosphato—include sucros a quatro, cinque, e septe carbones in ultra del triosas e hexosas. Iste via alternative es catalysate per un serie de enzymas que es distincte ab le enzymas del glycolyse anaerobe o del cyclo de acido citric.

Il es non ancora possibile evalutar le signification del via de pentosa-phosphato in le metabolismo mammalian. Tamen, il existe certe constatationes que indica que sub certe conditiones illo ha un rolo importante in le oxydation de glucosa sed non in le synthese hepatic de glycogeno. In plus, il es possibile que le reactiones del via de pentosa-phosphato provide un mechanismo pro le synthese de ribosa e pro le generation de reducite nucleotidos triphosphopyridinic.

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