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Enzyme relationships in a sorbitol pathway that bypasses glycolysis and pentose phosphates in glucose metabolism

(isozymes/diabetes/alcohol dehydrogenase/reductases/alcoholism)

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ABSTRACT **A pathway from glucose via sorbitol bypasses the control points of hexokinase and phosphofructokinase in glucose metabolism. It also may produce glycerol, linking the bypass to lipid synthesis. Utilization of this bypass is favored by a plentiful supply of glucose—hence, conditions under which glycolysis also is active. The bypass further involves oxidation of NADPH, so the pentose phosphate pathway and the bypass are mutually facilitative. Possible consequences in different organs under normal and pathological, especially diabetic, conditions are detailed. Enzymes with related structures (for example, sorbitol dehydrogenase and alcohol dehydrogenase, and possibly, aldehyde reductase and aldose reductase, respectively) are linked functionally by this scheme. Some enzymes of the bypass also feature in glycolysis (aldolase and alcohol dehydrogenase), and these enzymes, with the reductases involved, are proteins known to occur in different classes or multiple isozyme forms. Two of the enzymes (aldolase and alcohol dehydrogenase) both involve classes with and without a catalytic metal (zinc). The existence of parallel pathways and the occurrence of similar enzymic steps in one pathway may help to explain the abundance and multiplicity of enzymes such as reductases, aldolases, and alcohol dehydrogenases.**

Although alcohol dehydrogenase is widespread in nature, is common in liver, and has been structurally characterized from several sources (1), its exact role in mammalian organs generally has remained unclear. In addition to ethanol oxidation, functions in bile acid formation, fatty acid degradation, vitamin A metabolism, a hydroxysteroid reaction, and other areas of metabolism have been considered (2). Different and multiple functions of alcohol dehydrogenase would be consistent with the considerable species variations and extensive evolutionary changes found.

Recently, another common liver enzyme, sorbitol dehydrogenase, was shown to be structurally, mechanistically, and ancestrally related to alcohol dehydrogenase (3). The substrates of sorbitol dehydrogenase, fructose and sorbitol, are considered important in special organs, such as male sexual organs (4), or special disease states, such as hyperglycemic cataract formation (5) and possibly diabetic neuropathy (6) and glomerulosclerosis (7), but a more general metabolic significance that is compatible with the structural similarity of these enzymes has not been clear.

The structural link between alcohol and polyol dehydrogenases showed a type of parallel or convergent evolution of a second, different enzyme in each case with related specificity (3). Enhancement of alcohol metabolism by fructose gave one association between the substrates for sorbitol and alcohol dehydrogenases. This effect may stem from avoidance of the rate-limiting NADH dissociation off alcohol dehydrogenase by an *in situ* coenzyme reoxidation with glyceraldehyde formed from fructose (8). Increased operation of the glycerol phosphate shuttle could also mediate the effect (9).

The structural relationships, the diverse and unrelated metabolic suggestions, the apparent associations of some intermediate compounds, and the possible convergent relationships motivate a search for unifying explanations, detailing the sorbitol pathway and its consequences.

MATERIALS AND METHODS

Glucose Metabolism via a Five-Step Sorbitol Pathway Bypassing the Regulatory Steps of Glycolysis. In glucose metabolism, flux through the glycolytic and pentose phosphate pathways is regulated at phosphofructokinase (10) and glucose-6-phosphate dehydrogenase (11). Hexokinase is generally also a control point, though the effect in liver is overcome at high glucose concentrations by glucokinase. However, as shown in Fig. 1, a complete pathway similar to glycolysis but fully bypassing the hexokinase and phosphofructokinase steps also is possible. It involves oxidation of one or two molecules of NADPH, thus favoring operation of the pentose phosphate pathway, and is linked to lipid synthesis via glycerol formation (Fig. 1).

The first step of the pathway is conversion of glucose into sorbitol by aldose reductase and NADPH. Oxidation of sorbitol by sorbitol dehydrogenase and NAD^+ then yields fructose. Fructose is not a substrate for glucokinase, but in some circumstances hexokinase and ATP could convert it into fructose 6-phosphate, leading into glycolysis at the phosphofructokinase reaction. This loop via sorbitol then would serve as a transhydrogenase function (reduction of NAD^+ and oxidation of NADPH). Alternatively, fructokinase and ATP could convert fructose into fructose 1-phosphate. This compound can be cleaved by aldolase B to give dihydroxyacetone phosphate and glyceraldehyde. Glyceraldehyde 3-phosphate results from the action of triosephosphate isomerase on dihydroxyacetone phosphate or of triokinase on glyceraldehyde. Entry at this stage into glycolysis via glyceraldehyde 3-phosphate bypasses the hexokinase and phosphofructokinase reactions. Conversion of glyceraldehyde to glycerol is an alternative to phosphorylation. The reduction can be effected by alcohol dehydrogenase and NADH or by aldehyde reductase and NADPH.

Occurrence of the individual enzymes of the pathway is described in relation to the organs mentioned below. The initial steps of the pathway, with the glucose–fructose conversion (sometimes also called the "sorbitol pathway"), have been discussed further in relation to diabetes or insulin effects (12), but usually not the associated, later combination into glycolysis or lipogenesis.

In summary, the essential steps providing the bypass are those leading via sorbitol and fructose 1-phosphate to glyceraldehyde 3-phosphate and linking, among other enzymes, sor-

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bitol dehydrogenase and alcohol dehydrogenase with various reductases and aldolases.

RESULTS AND DISCUSSION

Normal Occurrence. The scheme in Fig. 1 and the enzymes shown demonstrate that the bypass or parts of it may be of functional significance. In this respect, five tissues or organs appear of special interest in normal conditions. They are, on the one hand, the liver, pancreas, and placenta, where some compounds of the scheme may have special roles, and, on the other hand, the brain and the reproductive system, where the bypass appears capable of having a more general metabolic role.

Liver. As the main processor of ingested compounds, the liver might be expected to encounter sorbitol. However, orally ingested sorbitol is poorly absorbed in man and animals, and products of its metabolism by intestinal microorganisms, rather than sorbitol itself, are absorbed (13, 14). Nevertheless, sorbitol entering the liver could be oxidized by hepatic sorbitol dehydrogenase in the same way that ethanol is oxidized by alcohol dehydrogenase.

Most important, liver deals with high postprandial concentrations of glucose. Accumulation of sorbitol formed from glucose within the liver then would be disadvantageous, raising the osmotic pressure of the cells. Significantly, the liver enzymes of the aldose reductase type (that is, NADPH-linked aldehyde reductases) have little (15) activity towards glucose, and much hepatic sorbitol dehydrogenase is present. These aspects suggest that liver may not be the main functional site of the entire bypass for energy production but rather for permitting metabolism of important compounds in any ratios.

Pancreas. Sorbitol is present in pancreas and glucose-induced insulin release evidently requires both NADPH and aldose reductase activity within the β cells (12). Reduction of glucose to sorbitol possibly primes or activates an element in the insulin release mechanism (12). Therefore, it appears possible that in the pancreas, the initial steps of the scheme in Fig. 1 may have special functions. As in the liver, they may not be of energetic value in an entire bypass of glycolysis but serve other functions, which in the case of pancreas may be metabolic regulation via insulin release.

Placenta. Glucose is converted into fructose by the aldose reductase and sorbitol dehydrogenase of normal placenta (16) and umbilical cord (17) in species including man. High concentrations of fructose in the fetal blood of ungulates (for example, sheep and pig) apparently result from nonutilization of fructose by their fetuses (18). Because fructose is not an important fetal energy source in these cases, the loop from glucose to fructose may have an osmoregulatory role or serve a transhydrogenase

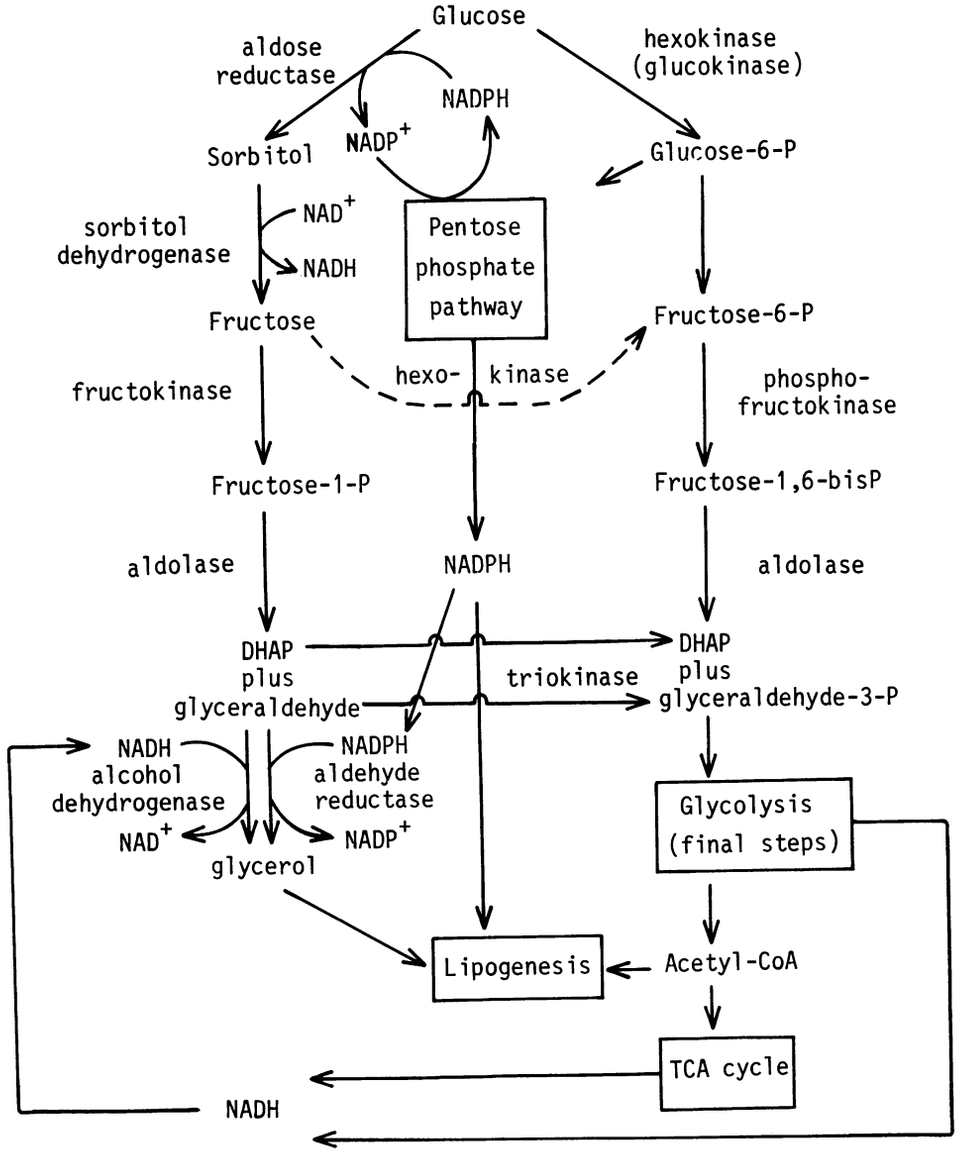

FIG. 1. Schematic representation of three pathways of glucose metabolism. A pathway via sorbitol (left) bypasses the hexokinase and phosphofructokinase steps (usually considered to regulate glucose metabolism through the glycolytic pathway) and leads to the final stages of glycolysis via dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate. Aldolase and alcohol dehydrogenase serve both the bypass and glycolysis. Sorbitol dehydrogenase is homologous to alcohol dehydrogenase, and aldehyde reductase possibly is related to aldose reductase. Links with the pentose phosphate pathway and lipogenesis (via glycerol, acetyl-CoA, and the nicotinamide coenzyme requirements) reveal further consequences of the bypass. Loops and branches of lesser importance in relation to the bypass are omitted, as are all step reversals. TCA, tricarboxylic acid.

function. Such a special role may be compatible with the fact that human and rat fetuses do not have high blood fructose concentrations and that the placentae of these species contain an estradiol-sensitive soluble transhydrogenase (19, 20).

Brain and Male Accessory Sexual Organs. Male accessory sexual organs of most mammals contain both aldose reductase and sorbitol dehydrogenase and provide the fructose and sorbitol of seminal plasma (4). Sperm contain sorbitol dehydrogenase and utilize sorbitol via fructose, which hexokinase converts into fructose 6-phosphate, allowing metabolism by the classical glycolytic pathway (4). In this case, the special circumstance is that absence of glucose allows fructose access to hexokinase. Provision of sorbitol and fructose helps sperm to compete successfully with bacteria, yeasts, and other cells that prefer glucose (4). Therefore, the initial steps of the scheme in Fig. 1, as well as possibly the whole bypass, function here to provide a dependable and competitive energy source.

Possibly the same is true of the brain. It contains aldose reductase (21) and sorbitol dehydrogenase (22) and is a classical source of aldehyde reductases (23). The presence of all of these enzymes may reflect the constant energy demand of the brain, securing all metabolic routes available from glucose.

Pathological Conditions. In the tissues mentioned so far, the scheme in Fig. 1 or part of it is evident under normal conditions. In three other tissues of great importance—lens, nerve, and kidney—inappropriate operation of the pathway accompanies hyperglycemia.

Lens. During continuously high glucose levels, the aldose reductase of animal lens (24) can produce high enough sorbitol concentrations to cause cataract formation (5). Human lens has less aldose reductase and more sorbitol dehydrogenase, so the situation is less unfavorable (25). The decisive factor allowing accumulation in human lens (26) may be decrease in hexokinase activity.

Nerve. In sciatic nerve, reduction of glucose to sorbitol, particularly in the Schwann cells, leads to sorbitol accumulation in diabetes mellitus, causing cellular damage consistent with a segmental demyelinating process (27). Sorbitol dehydrogenase activity is associated with the axons. The sorbitol and fructose in the cerebrospinal fluid of diabetic subjects (28) probably come from spinal nerves composing the cauda equina (27). Neuropathy at different locations is a common diabetic complication, and the metabolic explanations have been little studied. It does not appear impossible that the availability of the sorbitol bypass in the neural system secures better glucose utilization under normal conditions but causes the neuronal localization of the complications when pathologically high glucose concentrations persist.

Kidney. An increased sorbitol concentration in the kidneys of chronic diabetics is the consequence of the properties and location of aldose reductase and sorbitol dehydrogenase within the kidney (29). Here, these enzymes may relate to the development of diabetic glomerulosclerosis (7), interstitial nephritis, and papillary atrophy (30). In this context and in regard to the scheme in Fig. 1, it also may be noticed that the kidney is one of the internal organs where considerable alcohol dehydrogenase activity is present.

Alcoholism. All of the above examples show the importance in normal and pathological conditions of different parts of the bypass or of the entire pathway to glycerol or glyceraldehyde 3-phosphate via sorbitol. Functions appear to range from the special cases discussed to the provision of alternative energy sources in some tissues.

Alcoholism with its metabolic changes is another pathological condition in which some of the enzymes in the pathway are of special interest. Mammalian alcohol dehydrogenase is polymorphic and exhibits multiple isozymes. Three loci, with polymorphism at the third locus, correspond to the protein chains that explain the presence of the more cathodic isozymes (cf. ref. 1); variable alleles at the second locus can explain the "atypical" enzyme (cf. ref. 1), the "Indianapolis" enzyme (31), and the "oriental" enzyme (32); further loci are required to explain alcohol dehydrogenases π and χ (33, 34) and sorbitol dehydrogenase. The possible importance of this isozyme complexity has been discussed in relation to alcoholism, but it is difficult to discern which substrate specificity may be of special relevance to alcoholism or its secondary disorders.

In this regard, it is noticed that alcohol dehydrogenase also works on glyceraldehyde to form glycerol, which is a precursor in lipogenesis (Fig. 1). Increased utilization of this part of the scheme would be a very effective and direct response to handle increased ethanol metabolism. It also would fit with fatty infiltration in the liver as a consequence of increased alcohol metabolism. Therefore, if alcohol dehydrogenase isozymes are important in alcoholism, the specificities of particular interest, as evidenced from the present scheme, should include glyceraldehyde reduction. This specificity often is not looked for and it is important to check for that activity in future characterizations.

Enzyme Relationships. Apart from the metabolic and functional links shown in Fig. 1 and discussed above, the participating enzymes also suggest further, structural relationships. In some cases, clear ancestral connections have been established; in others, isozyme relationships can be well documented.

Reductases. The NADPH-linked reduction of glucose to sorbitol is catalyzed by certain members of a group of aldehyde reductases. Different activities have been described—aldehyde reductase (EC 1.1.1.2), aldose reductase (EC 1.1.1.21), D-glucuronate reductase (EC 1.1.1.19), mevaldate reductase (EC 1.1.1.33), and reductases that work, for example, on L-hexonate and daunorubicin (35). However, these activities need not all correspond to separate enzymes, neither needs each activity be represented by just one protein form. Relevant members of this group are monomeric proteins of M_r 35,000–40,000, which catalyze *re* attack on the aldehyde carbonyl by the 4-*pro*-(*R*) hydrogen of NADPH (35). Thus, the glucose/sorbitol and glyceraldehyde/glycerol steps (Fig. 1) can be catalyzed by closely similar reductases, not excluding something like isozyme relationships.

Dehydrogenases. The glyceraldehyde/glycerol conversion (Fig. 1) is effected not only by the reductases but also by NADH-linked alcohol dehydrogenase (EC 1.1.1.1). This mammalian enzyme has a similar polypeptide chain length to the reductases but is oligomeric (dimeric in liver, though tetrameric in yeast). Unlike aldehyde reductase, which contains no metal (36), liver alcohol dehydrogenase has active-site zinc (1), although nonmetal dehydrogenases with this activity also occur in nature (3). Liver alcohol dehydrogenase is known to be strictly homologous with the second enzyme of the present pathway, sorbitol dehydrogenase (3). The structural identities place the characterized sorbitol dehydrogenase (from sheep) exactly in between the two best-studied alcohol dehydrogenases (from horse and yeast). Furthermore, the enzymatic mechanisms of sorbitol dehydrogenase and these alcohol dehydrogenases are identical, as demonstrated by a zinc content and active site zinc ligands (3) in both.

Aldolase. This fourth enzyme of the pathway via sorbitol is essentially the same enzyme as the aldolase of glycolysis and gluconeogenesis, though isozymes B and C rather than A of the class I aldolases serve the bypass (37).

Aldolases are of two types, which are distributed differently.

Class I aldolases, mentioned above, function without metal [classically in animals and higher plants, although such aldolases with other specificities also have been described in prokaryotes (38)]. Class II aldolases (typically in fungi, yeast, and bacteria) contain active site zinc (39).

Interestingly, the same kind of relationship exists among alcohol dehydrogenases though the distribution of the two enzyme types is different. Here, the "classical" enzyme (in mammals and yeast) contains active site zinc, whereas the other type (in *Drosophila*) does not and apparently has evolved a similar activity by convergence, although ancient ancestral connections probably also exist between building units of dehydrogenases (3).

Thus, aldolases and alcohol dehydrogenases demonstrate a similar separation, each into two enzyme types. Furthermore, they form parts of both the present scheme (Fig. 1) and glycolysis. Interestingly, they also are enzymes for which limited structural similarities have already been noticed (40). Thus, assuming a one-residue gap, an NH_2-terminal part (residues 6–27) of rabbit muscle aldolase (class I) and a similar part (residues 12–32) of horse liver steroid-active alcohol dehydrogenases have 11 of 22 residues identical (40).

Possible Control. Aldose reductases have high K_m values for glucose (20–200 mM) (21, 41). ATP stimulates and ADP inhibits the enzyme by about ±20% over the physiological concentration range of these nucleotides (41). Sorbitol dehydrogenase forms an unproductive complex with sorbitol and NADH (42). Therefore, conversion of glucose into fructose is favored by high glucose and high ATP concentrations and by low ADP and low NADH concentrations, which implies that it accompanies efficient glycolysis and respiration when there is an ample supply of glucose.

After glyceraldehyde 3-phosphate, the steps are common to both pathways (Fig. 1). Interestingly, these include the steps that differ between organisms. Pyruvate reduction is linked to NADH oxidation in mammals and many other organisms by direct reduction to lactate (lactate dehydrogenase), in yeast by decarboxylation and reduction of the acetaldehyde (pyruvate decarboxylase and alcohol dehydrogenase), and in various marine invertebrates by ketoxime formation and reduction (alanopine dehydrogenase, strombine dehydrogenase, or octopine dehydrogenase). The complex kinetic behavior of glyceraldehyde 3-phosphate dehydrogenase from several sources, of a few lactate dehydrogenases (43), and of octopine dehydrogenase (44) affords possibilities for regulation.

Interrelationships. Most of the similarities mentioned, in structure, metabolic pathways, and splitting of enzyme types, might result individually from random changes in unrelated structures. However, the clear relationship between alcohol and sorbitol dehydrogenases could not be explained in such a way. Also, the multiple links mentioned more likely seem to indicate very old and profound connections. In particular, the reductases of the sorbitol bypass, as noted above, have subunits of the same size classes as dehydrogenases and other oligomeric enzymes of glycolysis. Therefore, further functional or ancestral links between all three metabolic pathways of glucose metabolism are possible.

The question remains open whether regulated steps via hexokinase and phosphofructokinase in glycolysis evolved as improvements over a parallel primordial pathway via sorbitol or whether evolution of a route through sorbitol improved upon classical glycolysis. Relationships between the proteins extend across the well-known pathways and indicate possible new approaches to clinically important metabolic problems. In addition, they place alcohol dehydrogenase and several of the otherwise scattered enzymes in a pathway with a general function compatible with the widespread abundance of the corresponding proteins. Finally, the use of identical or similar enzymes in subsequent steps or parallel pathways may be the functionally guided evolutionary explanation why isozymes and class splittings are common. This applies to alcohol dehydrogenases, where the isozyme substrate specificities at the end of the presently discussed pathway may influence metabolism in alcoholism.

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