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Research Article

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The rate of fructose uptake from the blood, as described by other investigators, can be based on the activity of ketohexokinase reported in the present paper. In human liver, ketohexokinase is present in a four-fold activity of glucokinase and hexokinase. This result may explain the well-known fact that fructose is metabolized faster than glucose.

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Enzymes of Fructose Metabolism in Human Liver

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ABSTRACT The enzyme activities involved in fructose metabolism were measured in samples of human liver. On the basis of U/g of wet-weight the following results were found: ketohexokinase, 1.23; aldolase (substrate, fructose-1-phosphate), 2.08; aldolase (substrate, fructose-1,6-diphosphate), 3.46; triokinase, 2.07; aldehyde dehydrogenase (substrate, D-glyceraldehyde), 1.04; D-glycerate kinase, 0.13; alcohol dehydrogenase (nicotinamide adenine dinucleotide [NAD]) (substrate, D-glyceraldehyde), 3.1; alcohol dehydrogenase (nicotinamide adenine dinucleotide phosphate [NADP]) (substrate, D-glyceraldehyde), 3.6; and glycerol kinase, 0.62. Sorbitol dehydrogenases (25.0 U/g), hexosediphosphatase (4.06 U/g), hexokinase (0.23 U/g), and glucokinase (0.08 U/g) were also measured. Comparing these results with those of the rat liver it becomes clear that the activities of alcohol dehydrogenases (NAD and NADP) in rat liver are higher than those in human liver, and that the values of ketohexokinase, sorbitol dehydrogenases, and hexosediphosphatase in human liver are lower than those values found in rat liver. Human liver contains only traces of glycerate kinase.

The rate of fructose uptake from the blood, as described by other investigators, can be based on the activity of ketohexokinase reported in the present paper. In human liver, ketohexokinase is present in a four-fold activity of glucokinase and hexokinase. This result may explain the well-known fact that fructose is metabolized faster than glucose.

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INTRODUCTION

Our present knowledge of the metabolism of fructose in human liver has been derived from data obtained from animals. In rat liver we found a potent enzyme system for fructose metabolism (1). This system corresponds to pathways postulated by Hers (2), Leuthardt and Stuhlfauth (3), and Heinz, Bartelsen, and Lamprecht (4) (Fig. 1).

Fructose is phosphorylated by ketohexokinase and adenosine triphosphate (ATP) to fructose-1-phosphate (5–10), which is converted to dihydroxyacetone phosphate and D-glyceraldehyde by liver aldolase (11–13), the enzyme Rutter called "aldolase 1-B" (14). Dihydroxyacetone phosphate may enter the glycolytic pathway. Metabolism of D-glyceraldehyde, however, is catalyzed by four different enzymes. Triokinase, described by Hers (2, 11) and studied in more detail by our group (15), effects phosphorylation of D-glyceraldehyde to D-glyceraldehyde 3-phosphate by ATP. Aldehyde dehydrogenase (NAD) described by Racker (16), oxidizes D-glyceraldehyde to D-glyceric acid (17, 18), which is then phosphorylated by ATP and D-glycerate kinase of liver to 2-phospho-D-glyceric acid (19, 20), a compound that is also an intermediate of the Embden-Meyerhoff pathway. Furthermore, reduction of glyceraldehyde to glycerol can be performed by two alcohol dehydrogenases, one operating with NADH (the so-called "normal" alcohol dehydrogenase) and the other

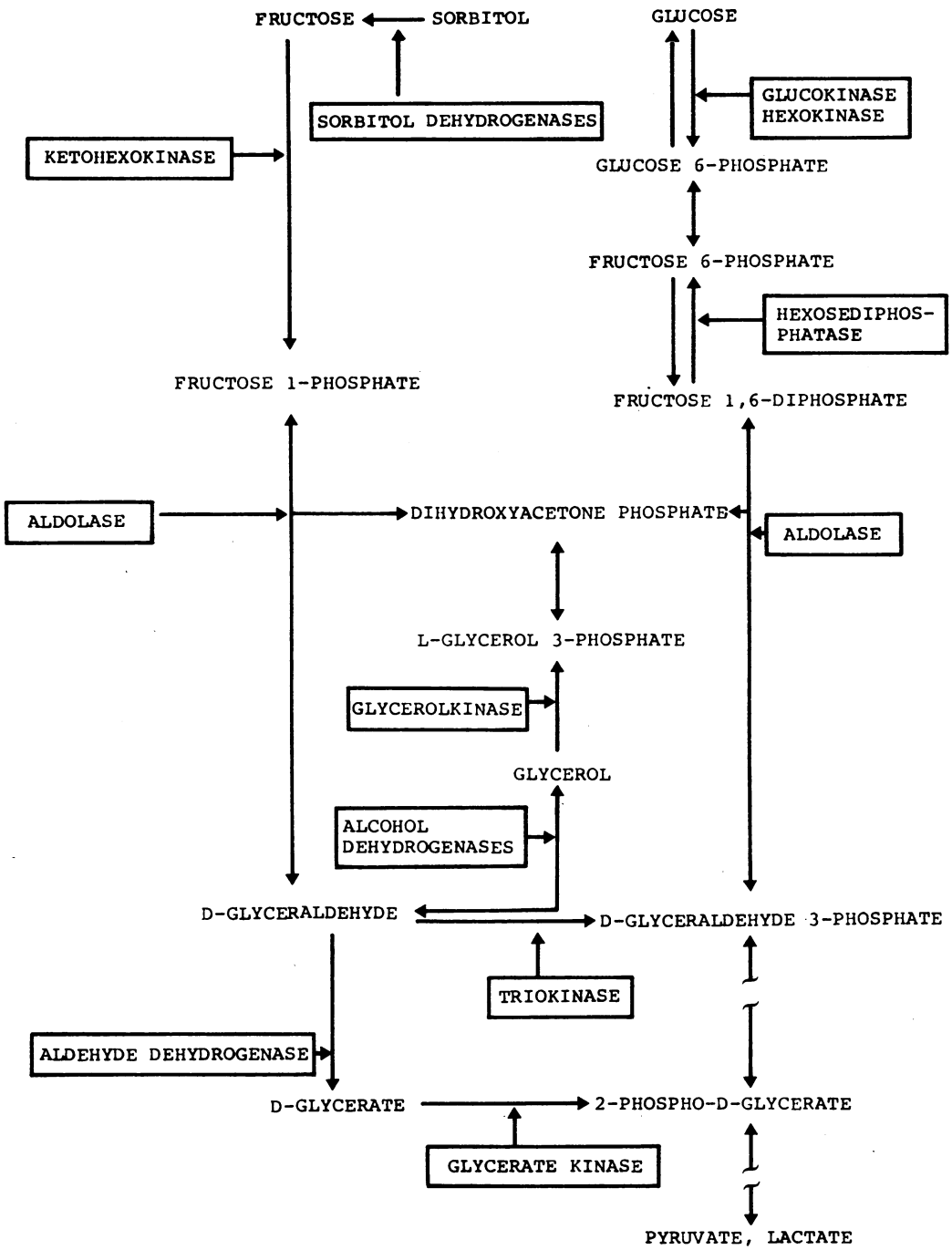

FIGURE 1 The metabolism of fructose in liver.

operating with NADPH, isolated by Moore (21) (formerly called aldehyde reductase or glycerol dehydrogenase). Glycerol is phosphorylated by means of glycerol kinase, first isolated from liver by Bublitz and Kennedy (22) and crystallized by Wieland and Suyter (23). The reduction of D-glyceraldehyde to glycerol is at variance with isotope studies on rat liver, reported by Hers (24) and by Rauschenbach and Lamprecht (25) using fructose-6-^{14}C. These authors have shown

that glucose, which derives from liver glycogen, exhibits radioactivity in carbon atoms 1 and 6 only. Glycerol formed from fructose-6-^{14}C is phosphorylated stereospecifically by glycerol kinase, as shown by Bublitz and Kennedy (26). This glycerol phosphate should introduce the label to carbon atoms 3 and 4 of the glucose molecule. However, such labeling was not detected in glucose from glycogen of animals treated with fructose-6-^{14}C. Besides these results and the high K_M of 3×10^{-2} moles/liter for glyceraldehyde, as measured by Holzer and Schneider (27, 28) with alcohol dehydrogenase from bovine liver, the reduction of glyceraldehyde will be discussed together with the oxidation of ethanol on the surface of the enzyme.

In this paper, activities of enzymes involved in fructose metabolism in the human liver will be described and compared with enzyme levels found in rat liver (1). The activities of the sorbitol dehydrogenases will also be stated, since sorbitol is oxidized to fructose by these enzymes. The levels of hexose diphosphatase were measured, because of the rate-limiting function of this enzyme in glycogen or glucose synthesis from fructose.

METHODS

Enzymes, coenzymes, and substrates were obtained from C. F. Boehringer and Sons, Mannheim, W. Germany; D-glyceraldehyde from Fluka, Buchs, Switzerland. D-glyceric acid was prepared as described by Wohl and Schellenberg (29) and the crude product was purified as previously described (30). Enzymes were extracted from liver samples by the following procedure: one part of liver was homogenized with nine parts (w/v) of triethanolamine buffer, pH 7.5, 0.05 mole/liter in a homogenizer (Buehler, Tuebingen, W. Germany) for 1 min at 0°C. The mixture was centrifuged for 30 min at 45,000 rpm in a Spinco L-2 rotor 50 at 0°C. The pellet was discarded and the supernatant stored in ice. Estimation of enzyme activities was carried out within 2 hr by the methods previously described (1). For estimation of glucokinase and hexokinase the method of Viñuela, Salas, and Sols (31) was modified as follows: an excess of 6-phosphogluconate dehydrogenase was added to the reaction mixture, in addition to the enzyme already present in the liver extract. Thus 2 moles of NADPH were formed during the phosphorylation of 1 mole of glucose by hexokinase or glucokinase. Therefore the optical density was divided by 2 instead of 1.5 [according to Viñuela et al. (31)] for the calculation of the activities.

The enzyme activities are expressed as international units (U), but the measurements were made at 25°C.

Source of human liver samples. Liver samples were obtained during abdominal operations; no attempt was made to select patients on the basis of age, sex, or nutritional state. Histological examination of all samples was carried out. Although mild or serious liver disease was obvious from the histological picture in several cases, no correlation could be found between enzyme activities and the degree of liver disease. Because of the small number of samples and the wide range of diseases, a clear-cut correlation was not to be expected.

RESULTS AND DISCUSSION

Table I shows the activities of the enzymes involved in fructose metabolism in human and rat liver. Data are given on a protein base or per gram of wet weight. For human liver, the range of enzyme activities is indicated.

Human liver contains 1.23 U/g of ketohexokinase as compared to 2.2 U/g found in rat liver. This latter activity amounts to 3.0 U/g when measured at 37°C. The extraction rate of fructose during liver perfusion agrees well with the enzyme activities measured under the above conditions. Livers were perfused by Schimasek's (32) method. An analogous extrapolation for human liver would give a phosphorylation rate of 1.7 μM fructose per g/min under optimal conditions. Thus the capacity for fructose phosphorylation of the whole human liver amounts to 0.45 g/min (weight, 1500 g). An extraction rate of 1.5–2.3 μM fructose per g/min or an equivalent rate of 0.41–0.55 g/min for the whole liver can be calculated from the results reported by Tygstrup, Winkler, and Lundquist (33) and Craig, Drucker, Miller, Owens, Woodward, Brofman, and Pritchard (34), which are in excellent agreement with the results we obtained at 37°C under optimum conditions in vitro. The phosphorylation rate of glucose is 1.98 μM per g/min under optimal conditions in rat liver, a figure which corresponds to activities of 1.83 U/g of glucokinase with a K_m of 1×10^{-2} (31) and of 0.15 U/g of hexokinase, respectively (Table II). In human liver 0.08 U/g of glucokinase and 0.23 U/g of hexokinase were found, thus confirming results reported by Brown (35). We believe that the data for glucokinase are lower than they would be normally, since the patients fasted for 12–24 hr before the operation. Pérez, Clark-Turri, Rabaijlle, and Niemeyer (36) have shown that glucokinase activity in rats depends on the amount of glucose in their food. In contrast to glucokinase, fasting has no effect on ketohexokinase activity and other enzymes of fructose metabolism, if the activities are given on a wet-

TABLE I

Enzymes of Fructose Metabolism in Human and Rat Liver (1)

Enzyme	Rat liver		Human liver		Range
	wet-weight	Specific activity	wet-weight	Specific activity	wet-weight
	U/g		*U/g*		*U/g*
Ketohexokinase	2.20 n = 8 SD = 0.39	0.018	1.23 n = 8 SD = 0.37	0.012 n = 4 SD = 0.51	0.87–1.97
Aldolase	4.34	0.035	3.46	0.041	2.54–4.27
S: fructose-1, 6-diphosphate	n = 5 SD = 0.20		n = 11 SD = 0.75	n = 11 SD = 0.009	
S: fructose-1-phosphate	1.63 n = 5 SD = 0.11	0.012	2.08 n = 11 SD = 0.49	0.025 n = 11 SD = 0.006	1.36–2.96
Triokinase	1.65 n = 8 SD = 0.37	0.013	2.07 n = 8 SD = 0.21	0.028 n = 8 SD = 0.006	1.32–2.63
Alcohol dehydrogenase (NAD)	1.64	0.013	3.1	0.040	2.24–4.55
S: D-glyceraldehyde	N = 4 SD = 0.27		n = 10 SD = 1.5	n = 10 SD = 0.02	
Alcohol dehydrogenase (NADP)	0.38	0.003	3.6	0.046	2.46–5.80
S: D-glyceraldehyde	n = 10 SD = 0.2		n = 9 SD = 1.1	n = 9 SD = 0.018	
Aldehyde dehydrogenase	1.4	0.011	1.04	0.013	0.67–1.50
S: D-glyceraldehyde	n = 6 SD = 0.04		n = 7 SD = 0.32	n = 7 SD = 0.006	
D-Glycerate kinase	3.19	0.026	0.13	0.0007	0.0–0.49
S: D-glyceric acid	n = 5 SD = 0.51		n = 6 SD = 0.12	n = 5 SD = 0.05	
Glycerol kinase	2.08 n = 7 SD = 0.27	0.016	0.62 n = 7 SD = 0.01	0.0072 n = 7 SD = 0.0004	0.46–0.72
Sorbitol dehydrogenases	18.9 n = 8 SD = 1.1	0.15	25.0 n = 8 SD = 6.2	0.30 n = 8 SD = 0.097	16.2–33.2
Hexosediphosphatase	6.49 n = 7 SD = 0.48	0.053	4.06 n = 7 SD = 0.62	0.048 n = 7 SD = 0.015	3.35–4.85

Enzyme activities are given in International Units (U). Temperature, 25°C; S, substrate; n, number of samples; SD, standard deviation.

weight base. These results were received by Adelman, Spolter, and Weinhouse (37) for rat liver.

Although glucokinase activity is somewhat enhanced, this enzyme, together with hexokinase, can not compare with the activity of ketohexokinase. Thus fructose can be phosphorylated faster than glucose in human liver, a fact that has also been found to hold good for rat liver.

Fructose-1-phosphate formed by the ketohexokinase reaction is split to dihydroxyacetone

TABLE II

Glucokinase and Hexokinase in Human and Rat Liver

Enzyme	Rat liver		Human liver		Range
	wet-weight	Specific activity	wet-weight	Specific activity	wet-weight
	U/g		*U/g*		
Glucokinase	1.35 n = 7 SD = 0.25	0.011	0.08 n = 5 SD = 0.06	0.0009 n = 5 SD = 0.00001	0.003–0.17
Hexokinase	0.25 n = 7 SD = 0.06	0.002	0.23 n = 5 SD = 0.08	0.18 n = 5 SD = 0.08	0.15–0.35

Enzyme activities are given in International Units (U). Temperature, 25°C; n, number of samples; SD, standard deviation

phosphate and D-glyceraldehyde by liver aldolase [aldolase type 1-B (14)], the same enzyme that catalyzes the conversion of fructose-1,6-diphosphate to dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate. With fructose-1-phosphate, aldolase activity measures 1.63 U/g in rat liver and 2.08 U/g in human liver. With fructose-1,6-diphosphate, 4.34 and 3.46 U/g were found, respectively. The activity of aldolase, as measured with fructose-1-phosphate, would be high enough for quantitative splitting of fructose-1-phosphate formed from fructose in vivo.

The ratio of aldolase activity measured with fructose-1,6-diphosphate and with fructose-1-phosphate is 2.62 (n = 5, SD = 0.24) for rat liver and 1.67 (n = 11, SD = 0.16) for human liver. These figures point to a significant difference between the aldolases of rat and human liver. Our results agree with those reported by Anstall, Lapp, and Trujillo who reported (38) differences of aldolase isozymes in human and rat liver.

In human liver the activity of triokinase is 2.7 U/g, but in rat liver it was found to be only 1.65 U/g. Alcohol dehydrogenase (3.1 U/g) and alcohol dehydrogenase (NADP) (1.36 U/g) are higher in human liver than they are in rat liver (1.64 and 0.62 U/g, respectively). In contrast, glycerolkinase activity in human liver is only 0.62, compared with 2.08 U/g in rat liver.

If it is assumed that with D-glyceraldehyde the K_m of alcohol dehydrogenase of human liver is the same as reported by Holzer and Schneider (27, 28) for bovine liver, the concentration of glycerol formed from D-glyceraldehyde will not be high enough to saturate the glycerolkinase (K_M, 4×10^{-4}) (23). If D-glyceraldehyde were reduced predominantly by NADP-dependent alcohol dehydrogenase, the activity of glycerolkinase would be insufficient for complete phosphorylation; this would result in accumulation of glycerol in human liver and its escape into the blood. However, reduction by alcohol dehydrogenase (NADP) can occur only in the presence of high NADPH levels. In human liver an activity of 1.04 U/g of aldehyde dehydrogenase was found. That in rat liver is 1.4; 60% of the activity was localized in the hyaloplasm, compared with 40% in the mitochondrial fraction. The K_m for the enzyme isolated from rat liver is 2.0×10^{-4} (17). Therefore, oxidation of glyceraldehyde to D-glyceric acid is likely to take place. However, in human liver D-glycerate kinase, which phosphorylates D-glyceric acid to 2-phospho-D-glyceric acid, was found in traces only (0.13 U/g); in rat liver 3.19 U/g was measured.

We believe that in human liver the capacity of the kinase is too low to play any physiological role. If D-glyceraldehyde were oxidized to D-glyceric acid, this substrate would accumulate in human liver and escape into blood.

The activity of sorbitol dehydrogenases in human liver, i.e. the total of iditol dehydrogenase localized in the hyaloplasm and of xylitol dehydrogenase in the mitochondria, was 25 U/g; this is higher than that found in rat liver (18.9 U/g). The activity of hexosediphosphatase (4.06 U/g) is sufficient for the transformation of fructose either to glucose or glycogen in human liver.

Summarizing the above results, it must be emphasized that there are some pronounced differences in fructose metabolism between human and

rat liver. Whereas ketohexokinase is identical in man and rat, human liver contains another type of aldolase, one not present in rat liver. Triokinase is higher in human liver than it is in rat. Therefore, direct phosphorylation of D-glyceraldehyde, as postulated by Hers (2), seems likely to occur in human liver. In contrast to rat liver the alcohol dehydrogenases levels in human liver are high. Thus there is a possibility that D-glyceraldehyde might be reduced to glycerol. But a clear-cut decision can be drawn only from experiments with fructose-6-^{14}C. The activity of aldehyde dehydrogenase is three-quarters of that found in rat liver. The glycerate kinase level in human liver is very low and it certainly does not play a physiological role. Therefore, any oxidation of D-glyceraldehyde to D-glyceric acid, should result in accumulation of the latter.

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