Enzymes of Glucose Metabolism in Liver of Subjects with Adult-onset Diabetes

Francesco Belfiore, M.D., Francesco Romeo, M.D., Elena Napoli, M.D., and Luigi Lo Vecchio, M.D., Catania, Italy

SUMMARY

An enzyme study was made on needle biopsy specimens of liver from thirty-two subjects with adult-onset diabetes and normal body weight and thirty-two controls. The enzyme pattern in the patients with diabetes was different from that seen with alloxan diabetes.

The activities of the two glucose phosphorylating enzymes tested were changed in opposite directions, hexokinase being enhanced and glucokinase moderately decreased. Total glucose phosphotransferase activity remained unchanged.

Phosphofructokinase had a reduced activity, which suggested depressed glycolysis, especially if considered together with the enhanced activity of the opposing enzyme, fructose-1, 6-diphosphatase. Normal activity was found for most other glycolytic enzymes, as well as for key gluconeogenic enzymes, including glutamic oxalacetic and glutamic pyruvic transaminases, phosphoenolpyruvate carboxykinase and glucose-6-phosphatase. The finding suggests normal glucose release.

Glucose-6-phosphate- and 6-phosphogluconate dehydrogenase activity was elevated. This would indicate an increased metabolism of glucose through the oxidative pathway and, therefore, increased formation of NADPH. This metabolic condition, which is known to favor fatty acid synthesis, might contribute to fatty liver changes. On the other hand, NADP-isocitrate dehydrogenase, which does not provide NADPH for fatty acid synthesis, was little changed. DIABETES *23:* 293-301, April, 1974.

Several studies of enzyme activities occurring with hepatic glucose metabolism in rats with alloxan diabetes have shown well-defined changes, which consist primarily of a decrease in the activity of glucokinase*[1-4] (with a normal value of hexokinase[2,3]), a reduction in the activity of glucose-6-phosphate metabolizing enzymes, such as

* Nonstandard names and abbreviations used:
*Hexokinase (HK) is ATP:D-hexose-6-phosphotransferase, E.C. 2.7.1.1;
*Glucokinase (GK) is ATP:D-glucose-6-phosphotransferase, E.C. 2.7.1.2;
*Phosphoglucomutase is alpha-D-glucose-1,6-diphosphate:alpha-D-glucose-1-phosphate phosphotransferase, E.C. 2.7.5.1;
*Phosphohexose isomerase (PHI) is D-glucose-6-phosphate ketol isomerase, E.C. 5.3.1.9;
*Phosphofructokinase (PFK) is ATP:D-fructose-6-phosphate 1-phosphotransferase, E.C. 2.7.1.11;
*Aldolase (ALD) is D-fructose-1,6-bisphosphate D-glyceraldehyde-3-phosphate-lyase, E.C. 4.1.2.13;
*Phosphotriose isomerase is D-glyceraldehyde-3-phosphate ketol-isomerase, E.C. 5.3.1.1;
*Phosphoglycerate kinase (PGK) is ATP-3-phospho-D-glycerate 1-phosphotransferase, E.C. 2.7.2.3;
*Phosphoglycerate mutase (PGM) is 2,3-diphospho-D-glycerate:2-phospho-D-glycerate phosphotransferase, E.C. 2.7.5.3;
*Enolase (ENO) is 2-phospho-D-glycerate hydro-lyase, E.C. 4.2.1.11;
*Pyruvate kinase (PK) is ATP:pyruvate phosphotransferase, E.C. 2.7.1.40;
*Lactate dehydrogenase (LDH) is L-lactate:NAD oxidoreductase, E.C. 1.1.1.27;
*Glucose-6-phosphatase (G6Pase) is D-glucose-6-phosphate phosphohydrolase, E.C. 3.1.3.9;
*Fructose-1,6-diphosphatase (F16Pase) is D-fructose-1,6-diphosphate 1-phosphohydrolase, E.C. 3.1.3.11;
*Phosphoenolpyruvate carboxykinase (PEPC) is GTP:oxaloacetate carboxy-lyase (transphosphorylating), E.C. 4.1.1.32;
*Pyruvate carboxylase is Pyruvate:CO_2 ligase (ADP), E.C. 6.4.1.1;
*Glutamic oxalacetic transaminase (GOT) is L-aspartate: 2-oxoglutarate aminotransferase, E.C. 2.6.1.1;
*Glutamic pyruvic transaminase (GPT) is L-alanine: 2-oxoglutarate aninotransferase, E.C. 2.6.1.2;
*Glucose-6-phosphate dehydrogenase (G6PDH) is D-glucose-6-phosphate: NADP oxidoreductase, E.C. 1.1.1.49;
*6-phosphogluconate dehydrogenase (6PGDH) is 6-phospho-D-gluconate: NADP oxidoreductase (decarboxylating), E.C. 1.1.1.44;
*Malate dehydrogenase (MDH) is L-malate:NAD oxidoreductase, E.C. 1.1.1.37;
*Isocitrate dehydrogenase (ICDH) is Ls-isocitrate:NADP oxidoreductase (decarboxylating), E.C. 1.1.1.42;
*Malic enzyme is L-malate:NADP oxidoreductase (decarboxylating), E.C. 1.1.1.40.

From the I° Clinica Medica, University of Catania Medical School, Ospedale Garibaldi, 95123 Catania, Italy.

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glucose-6-phosphate dehydrogenase,[5-7] 6-phosphogluconate dehydrogenase,[5,6,8] phosphohexose isomerase[8,9] and lactate dehydrogenase,[10] and increase in the activity of key gluconeogenic enzymes, including glucose-6-phosphatase,[8,11-13] fructose-1, 6-diphosphatase,[13,14] phosphoenolpyruvate carboxykinase,[13,15] pyruvate carboxylase,[13,16,17] glutamic oxalacetic transaminase[10,18,19] and glutamic pyruvic transaminase.[10,20] Increased levels for the glucose-phosphorylating activity attributed to glucose-6-phosphatase have also been reported.[21]

Phosphohexose isomerase has been found to be diminished only in diabetic rats feeding on a 60 per cent glucose or fructose diet[8] or when values are expressed on a DNA basis.[9] A normal level of pyruvate carboxylase was observed in some experiments.[22] However, the available data seem to suggest that in liver of diabetic rats uptake of glucose and its utilization, especially through the oxidative pathway, are decreased, while gluconeogenesis and release of glucose into the blood are enhanced. In contrast to these relatively clear-cut data, information regarding liver enzyme activities in human beings with diabetes is slight and conflicting. It can be summarized as follows:

1. As concerns glucose phosphotransferases, studies conducted prior to the discovery of the glucokinase, the enzyme with the high Km for glucose, uncovered an apparent increase of the capacity to phosphorylate glucose in persons with untreated diabetes.[23] Enhanced activity has been found also in a second study[24] in which assays were made at a high concentration of glucose (50 mM); at this concentration both hexokinase and glucokinase are active; consequently, results obtained concerned total glucose phosphotransferase activity. Thus, a study considering hexokinase and glucokinase activities separately in human diabetic liver is still lacking. Even with regard to normal liver, some disagreements exist. The high Km enzyme, glucokinase, was found by some workers[25-27] but could not be detected by others,[28] probably because of its sensitivity to nutritional state.[25]
2. Of the enzymes of the oxidative pathway, glucose-6-phosphate dehydrogenase has been found normal in some studies,[23,29] but in others[24] a diminished activity has been observed for this enzyme as well as for 6-phosphogluconate dehydrogenase.
3. Of the glycolytic enzymes, decreased activity has been reported for phosphofructokinase,[23] enhanced activity for phosphotriose isomerase[23] and pyruvate kinase[23,24] and normal or slightly changed values for other enzymes.[23,24]
4. Of gluconeogenic enzymes, glucose-6-phosphatase has been found increased by several workers,[23,29-31] but in an extensive study on forty-one diabetics,[32] normal activity was observed. Glutamic oxalacetic transaminase was normal in some studies,[23,24] in contrast with other observations[33] giving increased values in untreated diabetics compared to levels after treatment. Slightly lowered activity of glutamic pyruvic transaminase has been noted.[23,24]

From these data it is apparent that liver enzymes behave differently in human beings with diabetes than in rats with alloxan diabetes and that discrepancies occur between reported activities for some enzymes. Therefore, we undertook the present research regarding several enzyme activities associated with glucose metabolism in the liver of subjects with adult-onset diabetes, studied under well-defined conditions, and tried to find an explanation for the enzyme pattern observed.

MATERIAL AND METHODS

The study was carried out on thirty-two hospitalized patients with diabetes mellitus of the adult-onset type. The group comprised eighteen males and fourteen females, aged forty-two to sixty-eight years (mean 53 ± 6). Thirty-two control subjects, hospitalized for minor illnesses, included eighteen males and fourteen females, aged thirty-nine to sixty-six years (mean 50 ± 5.5). Body weight of all but two subjects, both diabetic and control, was either normal or exceeded the ideal value by no more than 10 per cent. The caloric intake was about 1800, of which 50 per cent was derived from carbohydrates, 20 per cent from proteins and 30 per cent from fats. Age, body weight and caloric intake were similar in diabetic and control subjects. The diabetes in each case was of moderate severity; the subjects required insulin at a daily dose ranging between 20 and 72 U. (mean 41 ± 13). Insulin was withheld for forty-eight hours before studies were made to avoid its metabolic effects and to induce moderate metabolic decompensation. At the time of study, elevation of glycemia was revealed by a fasting level of 160 to 256 mg./100 ml. (mean 198 ± 25).

Liver biopsies were made by means of a Menghini needle. Consent was obtained from all subjects before biopsy. Hepatic tissue specimens were washed in cold (+4° C.) saline, homogenized in distilled water by means of a blade homogenizer, and centrifuged in a refrigerated centrifuge for five minutes at 3,000 r.p.m. The supernatant was analyzed for protein con-

centration by the method of Lowry et al.[34] and for enzyme activities. Enzyme determinations were always carried out on paired normal and diabetic subjects, and never on control or diabetic subjects alone. In twenty-five diabetics a portion of the liver tissue specimen was reserved for histologic examination. Staining with hematoxylin eosin was used. In the remaining seven cases the specimens obtained were smaller, and no histologic study was made.

Hexokinase and glucokinase were assayed according to Viñuela et al.,[2] except that a double amount of glucose-6-phosphate dehydrogenase was added to the reaction mixture. Phosphoglycerate kinase was measured according to Bücher,[38] by utilizing the reaction from 3-phosphoglycerate to 1, 3-diphosphoglycerate. Other methods used are indicated by the reference number after each enzyme: phosphohexose isomerase,[35] phosphofructokinase,[36] aldolase,[37] phosphotriose mutase and enolase,[39] pyruvate kinase,[40] lactate dehydrogenase,[41] isocitrate dehydrogenase,[42] malate dehydrogenase,[43] glucose-6-phosphate dehydrogenase,[44] 6-phosphogluconate dehydrogenase,[45] glutamic oxalacetic transaminase,[46] glutamic pyruvic transaminase,[47] phosphoenolpyruvate carboxykinase,[48,49] fructose-1, 6-diphosphatase[50] and glucose-6-phosphatase.[51]

In measuring enzyme activities care was taken to make appropriate controls, mainly to test for (1) the possible presence of small amounts of enzymes contaminating the enzyme preparations used as reagents; (2) the possible contamination of substrates used with trace amount of the compound to be measured, usually the product of the enzymatic reaction under study; and (3) the presence of interfering endogenous compounds preformed in the homogenate. For all the enzymes studied activity was expressed in international units (U); 1 U. is defined as the amount of activity that converts 1 μmol of substrate in one minute under standard conditions, according to the International Union of Biochemistry.[52] The number of patients studied for each enzyme is given in table 1. Data were statistically analyzed with a calculating machine according to methods outlined by Cavalli-Sforza.[53]

Reagents used in the enzyme assays were of the finest available type. N-acetyl-D-glucosamine and dithiothreitol were obtained from Calbiochem, San Diego, Calif.; cystein, EDTA and the buffer substances glycine and sodium cacodylate from C. Erba, Milan, Italy; molybdate and amino-naphtol-solfonic acid from Chemetron, Milan, Italy; 2-mercaptoaethanol and KBO_4 from Fluka, Buchs, Switzerland; glucose from Merck, Darmstadt, West Germany; and all other compounds, including substrates, ATP, ADP, co-enzymes and enzymes from Boehringer, Mannheim, West Germany.

RESULTS

The results are listed in table 1 and may be summarized as follows: (1) The activities of the glucose phosphorylating enzymes measured were changed in the opposite direction, hexokinase being increased and glucokinase moderately decreased, while total glucose phosphotransferase activity remained unchanged. (2) Of the glycolytic enzymes, phosphofructokinase was significantly decreased, phosphohexose isomerase increased and other enzymes showed variations that did not reach statistical significance. (3) Both enzymes of the oxidative pathway that were studied, glucose-6-phosphate- and 6-phosphogluconate dehydrogenases, showed a marked and statistically significant increase in activity. (4) Of the gluconeogenic enzymes, fructose-1, 6-diphosphatase had an increased activity, while other enzymes considered —glucose-6-phosphatase, phosphoenolpyruvate carboxykinase, glutamic oxalacetic and glutamic pyruvic transaminases—showed normal activity. (5) The activity of malate dehydrogenase was enhanced, while that of NADP-linked isocitrate dehydrogenase was not significantly changed.

The enzyme pattern resulting from the changes we have observed is shown in figure 1.

In eighteen of the twenty-five cases (72 per cent) in which a histologic study was made, a more or less marked fatty degeneration of the liver was found. In ten patients nuclear vacuolization was present. In two instances an infiltration of mononuclear cells, of a moderate degree, was seen in the portal spaces.

DISCUSSION

Glucose phosphotransferases. The increase of hexokinase activity found in the present research is in agreement with the only comparable data we know in the literature.[23] As regards total glucose phosphotransferase activity, our findings showing a normal level are somewhat different from those reported by other workers,[24] who observed increased values. Diabetes in human beings is not accompanied by the marked decrease of glucokinase that occurs in the liver with alloxan diabetes[1-4] as a result of depressed

TABLE 1

Enzyme activities of glucose metabolism in liver tissue from normal subjects and patients with adult-onset diabetes

Enzymes	Normal		Diabetics			
	No. of cases	Mean ± S.D. U./gm. protein	No. of cases	Mean ± S.D. U./gm. protein	Per cent variation	P
Glucose phospho-transferases						
HK	18	0.84 ± 0.26	18	1.37 ± 0.14	+63	<0.05
GK	18	3.78 ± 1.41	18	3.15 ± 1.06	−17	= 0.05
Total	18	4.62 ± 1.22	18	4.52 ± 1.01	−2	>0.80
Glycolytic						
PHI	10	1.99 ± 29	10	2.30 ± 27	+15	<0.05
PFK	16	11.98 ± 5.61	16	7.90 ± 5.25	−33	<0.05
ALD	6	1.39 ± 0.67	6	1.55 ± 0.70	+11	>0.20
PGK	12	101 ± 41	12	105 ± 50	+4	>0.80
PTM	6	119 ± 49	6	141 ± 46	+18	>0.10
ENO	6	2.47 ± 1.09	6	2.98 ± 1.44	+20	>0.10
PK	10	20.37 ± 3.60	10	16.96 ± 3.65	−17	>0.05
LDH	13	108 ± 40	13	129 ± 41	+19	>0.05
Gluconeogenic						
G6Pase	14	12.53 ± 5.47	14	12.46 ± 6.87	−1	>0.90
F16Pase	16	3.36 ± 1.59	16	4.54 ± 1.91	+35	<0.05
PEPC	4	5.18 ± 2.42	4	4.89 ± 2.57	−6	>0.80
GOT	14	217 ± 101	14	225 ± 113	+3	>0.60
GPT	14	44 ± 17	14	42 ± 20	−5	>0.60
Oxidative shunt						
G6PDH	14	1.51 ± 0.82	14	2.33 ± 0.76	+54	<0.02
6PGDH	14	2.53 ± 1.05	14	3.47 ± 1.08	+37	<0.05
Other						
MDH	13	299 ± 76	13	369 ± 81	+23	<0.05
ICDH	11	111 ± 48	11	131 ± 53	+18	>0.10

synthesis[54] or accelerated inactivation,[55] and that seems to contribute largely to decrease glucose tolerance.[56] Differences between man and the rat seem to occur also in the fasting state, which in some aspects is similar to diabetes. In fact, while glucokinase is decreased[1,3] and hexokinase unchanged[3] in fasting rats, data regarding poorly nourished human beings show a decrease in glucokinase activity accompanied by a marked elevation of hexokinase, from 1.56 ± 0.19 mU./mg. protein to 2.66 ± 0.22.[25] Therefore, the regulatory mechanism of hepatic glucose phosphotransferases in man appears to be different from that in the rat,[1-4,54,55,57-60] and remains open to further research. It is noteworthy that even in rats, a shift in favor of hexokinase, without significant change in total glucose phosphotransferase activity, has been observed in some instances, and has been regarded as an expression of a regulatory mechanism.[61]

It must be pointed out that, in accordance with some studies,[24,27] we assayed both hexokinase and glucokinase activities on total homogenate, while other workers[1-4,25,26,28] have assayed these enzyme activities in homogenates after high speed centrifugation, in order to remove microsomes and, with them, glucose-6-phosphatase, which may interfere with glucose phosphotransferase determination.[62] However, recent observations[63] have shown that about 50 per cent of hepatic glucokinase is bound to microsomes, and, therefore, removal of these particles leads to a significant loss of enzyme activity. Actually, on the grounds of this observation, data obtained with homogenates after high-speed centrifugation should be re-evaluated. On the other hand, taking into account the specific activity of glucose-6-phosphatase that we found in human liver, we calculated that in the reaction mixture the ratio between activity of added glucose-6-phosphate dehydrogenase and that of endogenous glucose-6-phosphatase was above 25. If we consider that the pH at which glucose phosphotransferases were assayed is quite different from the pH optimum of glucose-6-phosphatase, this ratio was probably much higher. Therefore, influence of the latter enzyme was small. Moreover, because

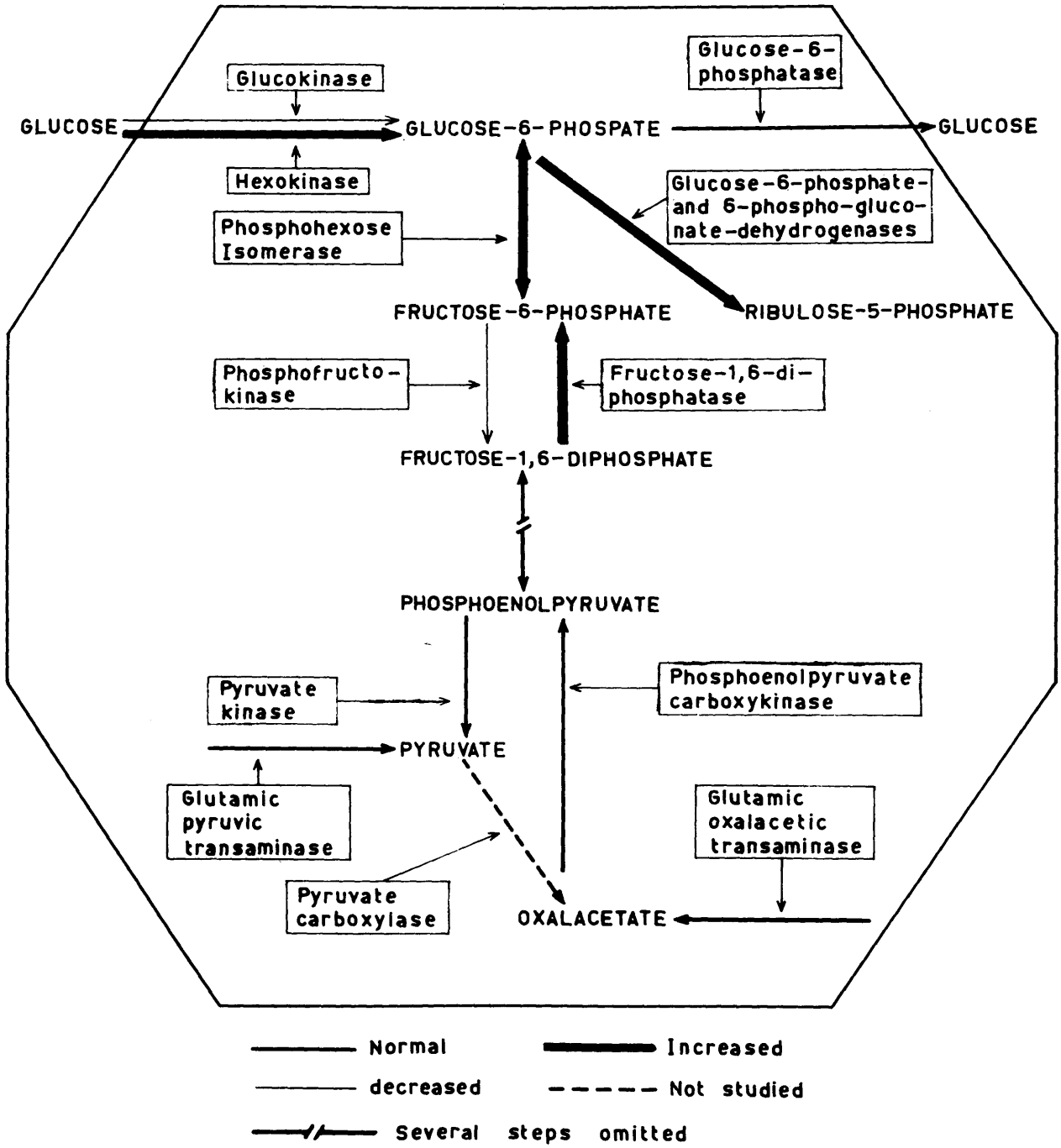

FIG. 1. Enzyme pattern of human diabetic liver according to results obtained in the present research.

glucose-6-phosphatase in this study had a similar activity in normal subjects and diabetics, its possible interference was equal in both groups of subjects. Finally, removal of microsomal fraction inevitably results in loss of material, which is undesirable, considering the small amount of liver tissue obtainable by needle biopsy.

Glycolytic enzymes. The decrease of phosphofructokinase activity, especially if considered together with the enhanced activity of the opposing enzyme

fructose-1,6-diphosphatase, would indicate that at this step the metabolic flux through the glycolytic pathway is slowed down. This would lead, other things being equal, to increased concentration of fructose-6-phosphate and, therefore, through the action of phosphohexose isomerase, to increased concentration of glucose-6-phosphate.

Gluconeogenic enzymes. The normal value found for most gluconeogenic enzymes studied—glucose-6-phosphatase, phosphoenolpyruvate carboxykinase, glutamic oxalacetic and glutamic pyruvic transaminases—is in agreement with some data previously reported for human liver,[23,24,32] but is in contrast with others.[29-31,33] The reason for this contrast is not clear. It is probable, however, that it is due, at least in part, to the sensitivity of gluconeogenic enzymes to hormonal, metabolic, nutritional and dietary factors, which may have been different in the various series of patients studied.

The increase of fructose-1,6-diphosphatase shows that the behavior of this enzyme was dissociated from that of other gluconeogenic enzymes. Lack of correlation of changes in fructose-1,6-diphosphatase with other gluconeogenic enzymes has been reported in rats during starvation.[13]

Our data indicate that in human beings with diabetes the behavior of liver gluconeogenic enzymes is quite different from that occurring with alloxan diabetes, where all key gluconeogenic enzymes are increased. The enhanced activity of fructose-1,6-diphosphatase would imply an increased formation of fructose-6-phosphate, a metabolite that is in equilibrium with glucose-6-phosphate. As concerns the metabolic fate of this last compound, the normal activity of glucose-6-phosphatase indicates that its splitting to free glucose and release into the blood are probably not much altered. The activity of phosphoglucomutase, which channels glucose-6-phosphate toward glycogen synthesis and glucuronic acid pathway, was not studied in this research.

Enzymes of the oxidative pathway. The elevated activity found for both dehydrogenases of oxidative shunt makes it probable that an amount of glucose-6-phosphate higher than normal is metabolized through this pathway. Since metabolism of glucose via oxidative shunt leads to the formation of NADPH, availability of this compound would be increased in the liver of patients with adult-onset diabetes. The normal[23,29] or decreased[24] activity reported by other workers for these dehydrogenases cannot be confirmed by our data.

Conclusion. The liver enzyme pattern of patients with adult-onset diabetes, as shown by this research (figure 1), is different from that in rats with alloxan diabetes (figure 2). This difference might be linked either to species or type of diabetes. In determining these two enzyme patterns a primary role might be played by two pairs of enzymes, each pair including two separate enzymes with opposite activities, which regulate key steps of glucose metabolism. One pair consists of glucose phosphotransferases and glucose-6-phosphatase, the second pair of phosphofructokinase and fructose-1,6-diphosphatase. In fact, it might be tentatively suggested that, because of difference in the severity of insulin deficiency or other reasons, these two pairs of enzymes are affected differently by adult-onset diabetes and alloxan diabetes. In adult-onset diabetes changes would concern mainly the phosphofructokinase/fructose-1,6-diphosphatase system, while in alloxan diabetes, and perhaps in growth-onset diabetes in human beings, also the glucose phosphotransferase/glucose-6-phosphatase system would be involved, together with other enzymes of gluconeogenesis.

It is relevant that with another form of diabetes that differs from alloxan diabetes, namely diabetes of Chinese hamsters, the liver enzyme pattern was distinct from that of alloxan diabetes of rats, the significant changes being an increase of phosphoenolpyruvate carboxykinase and, in three month old animals, of glucose-6-phosphatase, while hexokinase, glucokinase, pyruvate kinase, lactate dehydrogenase, fructose-1,6-diphosphatase and 6-phosphogluconate dehydrogenase showed normal activity.[64]

Fatty degeneration of the liver is common with diabetes mellitus,[65,66] especially the adult-onset type, even in the early stage of the disease[67] and in newly diagnosed and untreated cases.[68] The high frequency of this histologic change was confirmed by our results, as stated above. The increased activity of the two dehydrogenases of the oxidative shunt, and perhaps that of malate dehydrogenase, which coupled with malic enzyme in the malate transhydrogenation cycle participates in the NADPH formation,[69] would suggest an enhanced availability of NADPH. This metabolic condition favors fatty acid synthesis,[69] and, therefore, might contribute to the development of fatty liver in persons with adult-onset diabetes. It is of interest that NADP-isocitrate dehydrogenase, which does not provide NADPH for fatty acid synthesis,[69] was little changed in our patients.

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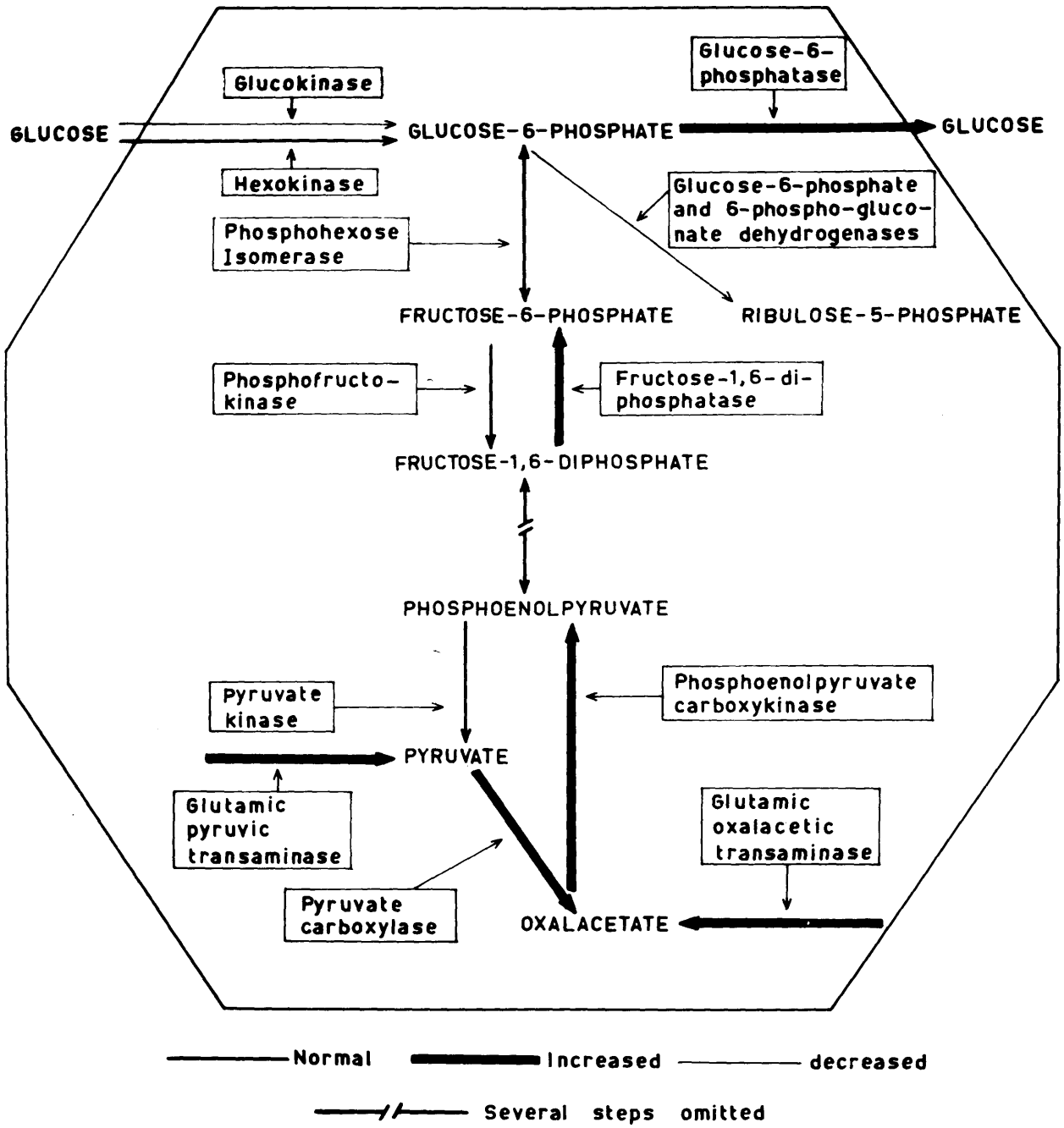

FIG. 2. Enzyme pattern of liver in rats with alloxan diabetes, according to data reported in the literature.[1-20]

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