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*THE EFFECT OF DIABETES AND FASTING ON LIVER GLUCOSE-6-PHOSPHATASE**

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Studies on the metabolism of C^{14}-labeled fructose, glycerol, and pyruvate by rat liver slices[1] have shown that livers from diabetic rats exhibit an increased production of glucose and a decreased production of glycogen from these substrates, as well as a diminished uptake of glucose. Shipley and Humel,[2] using nonisotopic substrates, have previously observed that liver slices from diabetic rats produce more glucose than slices from normal rat liver. Stetten *et al.*[3] have estimated serum glucose production by isotopic dilution in intact normal and diabetic rats during the constant infusion of C^{14}-labeled glucose. Their data suggest an increased glucose synthesis in the diabetic over the normal. These findings led us to determine whether the activity of glucose-6-phosphatase was altered in diabetic liver tissue.

Drabkin and Marsh[4] have found liver acid and alkaline phosphatases elevated in alloxan-diabetic rats. Cori and Cori[5] have reported that liver glucose-6-phosphatase is drastically lowered in patients suffering from glycogen storage disease. We have determined the activity of this enzyme in liver from fed and fasted normal and diabetic rats. Two methods of assay have been used: (*a*) measuring the release of inorganic phosphate on incubation of G-6-P[6] with liver homogenate, as previously described by Swanson[7] and De Duve *et al.*,[8] and (*b*) measuring the oxygen uptake in a coupled glucose-6-phosphatase, glucose oxidase plus catalase system.

MATERIAL AND METHODS

Albino rats of the Wistar strain, weighing between 200 and 300 gm., were used. Alloxan-diabetic animals were produced by intravenous injection of 40 mg/kg of alloxan monohydrate (Eastman). These animals were not used until at least 15 days after alloxan administration, and only when their blood sugar was greater than 300 mg. per cent. All animals were maintained on water and Purina Laboratory Chow ad lib. To determine the effect of insulin, alloxan-diabetic rats were injected with 5 units of protamine Zn insulin at 12-hour intervals, beginning 48 hours prior to sacrifice of the animals.

Animals were killed either by a blow on the head or by exsanguination under sodium amytal anesthesia. The liver was removed, and 0.50 gm. was homogenized

in 6 ml. of water or isotonic KCl, using a Potter-Elvehjem glass homogenizer. Neither the method of sacrifice nor the solution used for homogenization was found to influence liver phosphatase activity. The homogenate was centrifuged at room temperature for 5 minutes at 1,800 rpm in an International No. 2 centrifuge.

Phosphate Release Assay.—Aliquots of the liver homogenate were assayed according to the method of Swanson.[7] A pH activity curve (Fig. 1) was plotted for the various preparations studied. In this assay our data on optimum pH and relative rates of hydrolysis of other hexose phosphates by liver homogenates are in agreement with those of others.[5, 7, 8] The failure of Be ion at 10^{-3} *M* to inhibit glucose-6-phosphatase activity of our preparations is in accord with similar observations of Cochran *et al.*[9] and constitutes further evidence that this enzyme is quite different from other phosphatases, alkaline phosphatase (serum and tissue), ATP-ase, and phosphoglucomutase, which are strongly inhibited by this ion.

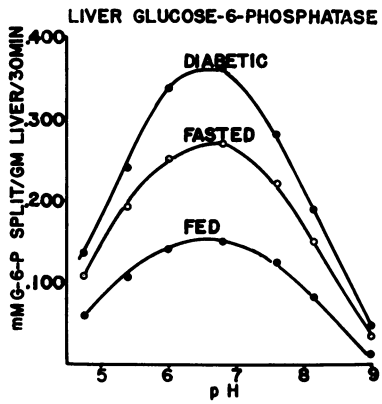

FIG. 1.—pH activity curve of liver glucose-6-phosphatase from fed and fasted normal and diabetic rats. Citrate buffer (0.1 *M*) was used for the pH range 4.76–6.88, and 0.1 *M* borate buffer for pH 7.6–9.0. Each curve represents the mean of six or more animals.

Glucose Release Assay.—This method depends upon the measurement of the glucose released from G-6-P. The glucose liberated was converted to gluconic acid by glucose oxidase in the presence of catalase, and the oxygen consumed was measured manometrically.

One hundred milligrams of glucose oxidase (Sigma) and 0.1 mg. of crystalline catalase (Worthington) were suspended in 5 ml. of 0.154 *M* acetate buffer, pH 5.7. One milliliter of this suspension was mixed with 0.5 ml. of liver homogenate (0.50 gm liver wet weight/6 ml of water) and 0.5 ml. of 0.2 *M* G-6-P, and the oxygen uptake was measured in a Scholander respirometer[10] at 30° C. Appropriate controls on substrate and homogenate, with and without glucose oxidase present, were used.

Keilin and Hartree[11] have reported that glucose oxidase is highly specific for glucose and does not act on any of the closely related carbohydrates, including hexose-6-phosphate. We have confirmed these observations. Incubation of G-6-P as a substrate with liver homogenate and glucose oxidase plus catalase therefore results in oxygen uptake only after liver glucose-6-phosphatase has split G-6-P to glucose and inorganic phosphate. If the phosphatase was the rate-limiting step and all other components were present in excess, the activity of the phosphatase could be measured. From Figure 2 it can be seen (curve *1*) that when glucose was the substrate, the oxygen uptake was three times as great per unit time as with G-6-P (curve *2*). Thus the glucose oxidase–catalase system was present in excess and was not rate-limiting. Likewise, the homogenate without added G-6-P (curve *4*) showed little oxygen consumption, and the substrate (G-6-P) plus glucose oxidase without homogenate (curve *5*) even less. The fact that the oxygen-uptake curves (*2* and *3*) were linear over the time of the assay indicates that the substrate (G-6-P) was present in excess and did not become rate-limiting during the course of the assay. Also, if, as others have shown,[5, 7, 12] glucose is produced by

the liver homogenate only as a result of the action of glucose-6-phosphatase on G-6-P, then the small oxygen uptake in curve *4* (homogenate plus glucose oxidase without added G-6-P) is probably the result of endogenous production of G-6-P by the liver homogenate. This would result in increasing the amount of substrate (G-6-P). which was already present in excess. The oxygen uptake of curve *4* has therefore not been subtracted from the assay determination. Liver homogenates plus G-6-P alone resulted in no consumption of oxygen.

The two assays are compared in Table 1. It can be seen that the glucose-6-phosphatase activity was approximately the same by either assay, the average deviation being ±12 per cent.

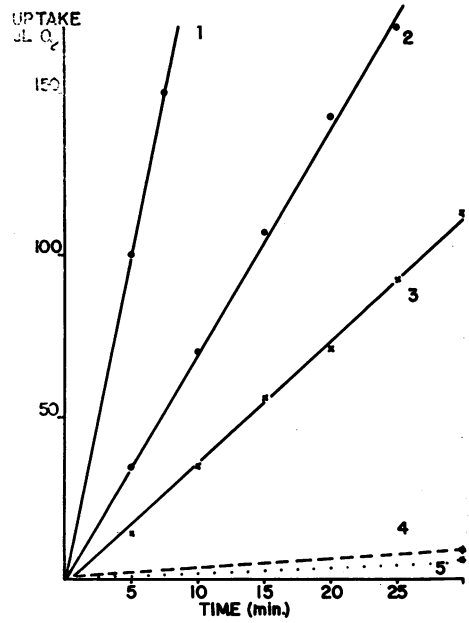

FIG. 2.—Oxygen-uptake curves in the assay for glucose-6-phosphatase. *1*, Glucose control: 0.5 ml. liver homogenate plus 1 ml. glucose oxidase solution in 0.154 *M* acetate buffer (pH 5.7) plus 0.5 ml. 0.2 *M* glucose. *2*, Diabetic liver: 0.5 ml. diabetic liver homogenate plus 1 ml. glucose oxidase solution plus 0.5 ml. 0.2 *M* G-6-P. *3*, Normal liver: same as *2*, except normal liver homogenate was used instead of diabetic. *4*, Control without substrate: 0.5 ml. homogenate plus glucose oxidase. *5*, Control without homogenate: G-6-P plus glucose oxidase.

RESULTS

In Table 2, glucose-6-phosphatase activity in rat livers under the several experimental conditions examined is presented in two ways—activity per gram of wet liver and activity per total liver. The results obtained by both methods of assay show that liver glucose-6-phosphatase was elevated in the fasting and alloxandiabetic rat, when expressed per gram of liver.

Although fasting and alloxan diabetes resulted in an increased concentration of glucose-6-phosphatase, the total quantity of this enzyme present in the liver was elevated only in the fed alloxan-diabetic rat. Fasted normal rats had twice the glucose-6-phosphatase activity per gram of liver that fed normal rats had, but, since fasting resulted in a 50 per cent decrease in liver weight, the total phosphatase per liver remained the same. It would therefore appear that fasting in the normal rat resulted in an increased concentration of this enzyme rather than an accelerated synthesis or an activation process. Fasting in the diabetic rat resulted in a net decrease in total liver glucose-6-phosphatase, although the amount of enzyme per gram of liver was unchanged. Fasted normal and alloxan-diabetic rats had the same concentrations of glucose-6-phosphatase per gram of liver, as well as the same total liver glucose-6-phosphatase.

When equal quantities of liver homogenate from fed and fasted normal rats were mixed, the resulting phosphatase activity was the expected arithmetic mean of the component homogenates (Table 3). This again suggests that one was not dealing with a process of activation or inhibition. In the case of the fed diabetic rat, there

was an increase in the total liver glucose-6-phosphatase, as well as in the concentration of the enzyme in the liver (Table 2). Again, a mixture of equal quantities of homogenate from normal and diabetic livers resulted in an activity approaching the mean of the components (Table 3).

TABLE 1

A COMPARISON OF GLUCOSE-6-PHOSPHATASE ACTIVITY IN RAT LIVER HOMOGENATE BY TWO METHODS OF ASSAY*

Rat No.	Phosphate Release	Glucose Release	d
1	0.320	0.345	+0.025
2	.475	.408	− .067
3	.336	.330	− .006
4	.362	.362	± .000
5	.156	.180	+ .024
6	.168	.180	+ .012
7	.188	.258	+ .070
8	.462	.350	− .112
9	.336	.408	+ .072
10	.308	.312	+ .004
11	.368	.337	− .031
12	.424	.365	− .059
13	0.346	0.365	+0.019
Mean†	0.327	0.323	±0.039

* Values expressed as millimoles of G-6-P split per gram of liver per 30 minutes at 30° C.
† Average deviation, 11.9 per cent.

The effect of inhibitors that combine reversibly with enzymes can be partially overcome by dilution,[13] which results in dissociation of the enzyme inhibitor complexes. Thus the presence of natural inhibitors can be detected by measuring enzyme activity at various dilutions,[14] an increase in activity on dilution suggesting the presence of an inhibitor. Applying this criterion, we have observed no increase in activity on two and threefold dilution of this enzyme. These observations suggested that there was, in normal rat liver tissue, no inhibitor present which was removed by fasting or diabetes.

The in vivo injection of insulin returned the glucose-6-phosphatase activity of diabetic rats toward normal (Table 2). This suggested that the enzyme was elevated in diabetic rat liver, due to conditions arising from the lack of insulin. No in vitro action of insulin on rat liver glucose-6-phosphatase was observed.

CONCLUSIONS

A manometric method has been developed for the assay of glucose-6-phosphatase in rat liver homogenates. This method differed from the phosphate-release method previously used[5, 7, 8] in that the glucose released from G-6-P was measured, instead of inorganic phosphate. Glucose was determined by oxygen uptake in the presence of glucose oxidase and catalase. Both methods gave comparable values for glucose-6-phosphatase when applied to the same homogenate.

Rat liver glucose-6-phosphatase, as previously demonstrated by others,[5, 7, 8] has an optimum pH from 6 to 7 and is not inactivated by Be ion at 10^{-3} M. By these criteria, this enzyme is markedly different from nonspecific organic phosphatases of liver.

The concentration of glucose-6-phosphatase in rat liver was elevated by fasting and alloxan diabetes. That the same enzyme was elevated under these conditions

is suggested by the identity of the pH optima shown by the pH activity curves (Fig. 1). The presence of activators or inhibitors that might account for alterations

TABLE 2

RAT LIVER GLUCOSE-6-PHOSPHATASE*

	No. Obs.	Mean	S.E.	P
ANIMALS FED:				
Normal:				
Phosphate release assay:				
mM G-6-P per gm. liver	13	0.147	0.011	...
mM G-6-P per total liver	6	2.37	.140	...
Glucose release assay:				
mM G-6-P per gm. liver	6	0.195	.019	...
Diabetic:				
Phosphate release assay:				
mM G-6-P per gm. liver	11	0.336	.019	<0.01†
mM G-6-P per total liver	6	5.25	.468	< .01†
Glucose release assay:				
mM G-6-P per gm. liver	3	0.358	.005	< .01†
Diabetic and insulin (in vivo):				
Phosphate release assay:				
mM G-6-P per gm. liver	6	0.158	.008	< .01‡
ANIMALS FASTED:				
Normal:				
Phosphate release assay:				
mM G-6-P per gm. liver	13	0.268	.008	< .01†
mM G-6-P per total liver	7	2.60	.142	> .05§
Glucose release assay:				
mM G-6-P per gm. liver	5	0.362	.026	< .01†
Diabetic:				
Phosphate release assay:				
mM G-6-P per gm. liver	7	0.288	.026	> .05‡‖
mM G-6-P per total liver	4	3.00	.196	> .05#
Glucose release assay:				
mM G-6-P per gm. liver	6	0.362	0.016	>0.05‡‖

* Values expressed as millimoles of G-6-P split per 30 minutes. Calculations per gram of liver (wet weight) and per total liver weight. Phosphate release assays were incubated in 0.1 *M* citrate buffer (pH 6.2) and the glucose release assays in 0.154 *M* acetate buffer (pH 5.7). All assays were at 30° C.
† Calculated *P*-value as compared with normal fed animals.
‡ Calculated *P*-value as compared with diabetic fed animals.
§ Difference not statistically significant as compared with normal fed animals.
‖ Difference not statistically significant as compared with diabetic fed animals.
Difference not statistically significant as compared with normal fasted animals.

TABLE 3

GLUCOSE-6-PHOSPHATASE ACTIVITY RESULTING FROM MIXTURES OF HOMOGENATES OF LIVER FROM FED AND FASTING NORMAL AND DIABETIC RATS*

FED NORMAL AND DIABETIC RATS (MIXED 1:1)		FED AND FASTED NORMAL RATS (MIXED 1:1)	
Obs.	Calc.	Obs.	Calc.
0.350	0.342	0.212	0.209
.356	.313	.243	.246
.284	.268	.265	.228
.272	.268	.281	.265
0.237	0.212	.243	.246
		0.236	0.215
Mean 0.300	0.281	Mean 0.247	0.235

* Values expressed as millimoles of G-6-P split per gram of liver per 30 minutes. Incubations were carried out at 30° C. in 0.1 *M* citrate buffer, pH 6.2.

in enzyme activity was not detected. It is therefore suggested that the increased activity of this enzyme in fasting and diabetes was due to an actual increase in concentration of enzyme.

Although fasting increased the concentration of glucose-6-phosphatase in the normal rat liver, it caused a net decrease in the total glucose-6-phosphatase in the diabetic liver but did not alter the concentration of the enzyme in the diabetic. The similarity of the glucose-6-phosphatase contents of livers from fasted normal and diabetic rats was striking. Indeed, in so far as this enzyme was concerned, fasting tended to make the normal and the diabetic states indistinguishable.

The increased concentration of glucose-6-phosphatase in diabetic liver could be returned toward normal by the in vivo injection of insulin. In vitro insulin was without effect. This suggests that liver glucose-6-phosphatase was elevated in the diabetic liver due to general metabolic changes that occur in diabetes.

The nature of the changes in glucose-6-phosphatase that occur in diabetes suggests that this enzyme adapts in response to a decreased ability of the peripheral tissues to utilize glucose, thus bringing about an increase in the concentration of blood sugar. In fasting, glucose-6-phosphatase acts to release glucose arising from both glycogenolytic and gluconeogenic processes. In diabetes the increase in this enzyme offers evidence for the overproduction of glucose believed to occur in this disease.

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† Schering Fellow of the Endocrine Society for 1953–1954.

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