LIVER GLUCOSE-6-PHOSPHATASE AND PYROPHOSPHATE-GLUCOSE PHOSPHOTRANSFERASE: EFFECTS OF FASTING*

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In addition to its well-known hydrolytic activity (Reaction 1), rat liver microsomal glucose-6-phosphatase also catalyzes the transfer of a phosphoryl group from inorganic pyrophosphate (PP_i) to the sixth position of glucose (Reaction 2) (Nordlie and Arion, 1964; Arion and Nordlie, 1964; Stetten and Taft, 1964).

$$\text{Glucose-6-phosphate} + H_2O \longrightarrow \text{Glucose} + \text{orthophosphate } (P_i) \quad (1)$$

$$PP_i + \text{glucose} \longrightarrow \text{Glucose-6-phosphate} + P_i \quad (2)$$

On the basis of kinetic studies which showed that the maximal reaction velocities of both activities are equal, we (Arion and Nordlie, 1964; Nordlie and Arion, 1965a) suggested the possibility that catalysis of the phosphotransferase reaction might be the principal function of this enzyme under certain physiological conditions (e.g., experimental diabetes), while under other circumstances involving hypoglycemia the enzyme would function primarily as a phosphohydrolase. To investigate this possibility further,

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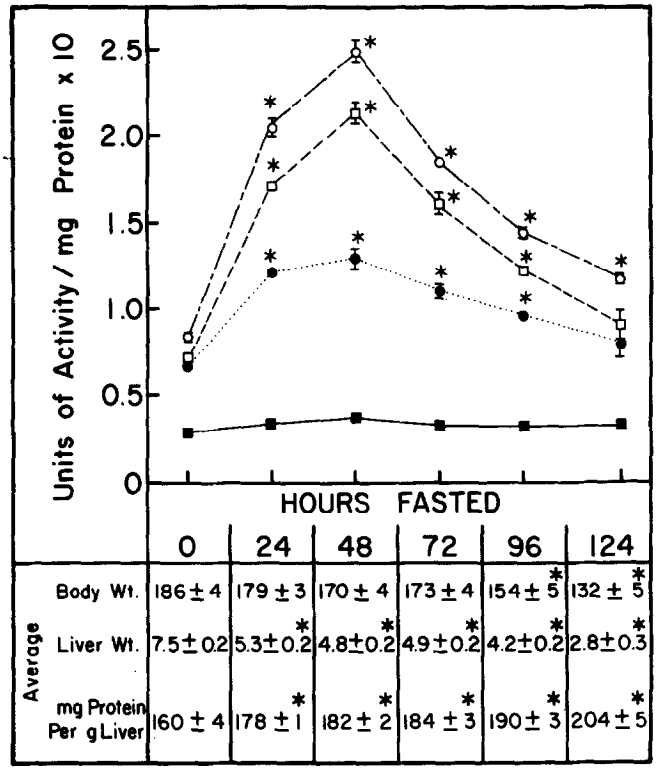
we have undertaken studies of the effects of alterations in the hormonal and nutritional states of test animals on the tissue levels of the two activities. The effects of alloxan diabetes, insulin administration, adrenalectomy and cortisone therapy have been described elsewhere (Nordlie and Arion, 1965a and b). In this report the responses of phosphohydrolase and phosphotransferase activities catalyzed by liver glucose-6-phosphatase to fasting are presented. An apparent selective increase in the former was observed when activities were assayed in the absence, but not presence, of deoxycholate.

Sources of chemicals and analytical procedures were as described previously (Nordlie and Arion, 1964 and 1965a; Arion and Nordlie, 1964). Fresh liver homogenates were prepared and supplemented either with distilled water or neutral sodium deoxycholate solution (to a final concentration of 0.2 percent, w/v) also as described earlier. Young, male albino rats of Sprague-Dawley origin, obtained from the Badger Research Corp., Madison, Wisconsin, were employed. Assays for both enzymic activities were performed simultaneously with freshly prepared homogenates. Glucose-6-phosphatase assay mixtures, pH 6.5, contained 40 mM sodium cacodylate buffer, 20 mM glucose-6-phosphate, and homogenate in a volume of 1.5 ml. Phosphotransferase assay mixtures, pH 5.5, contained 40 mM sodium cacodylate buffer, 20 mM sodium pyrophosphate, 180 mM D-glucose, and homogenate in 1.5 ml. Incubations were carried out for 10 minutes at 30° in a shaking, thermostatically regulated water bath. Linearity of reactions with protein concentrations and with time for at least 15 minutes under these conditions was demonstrated in supplementary experiments.

The results of a fasting experiment are presented in Fig. 1. One unit of enzymic activity is that amount catalyzing 1 μmole of glucose-6-phosphate production (phosphotransferase) or hydrolysis (phosphohydrolase) per minute. Results are presented in the graph in terms of specific activity; average values for liver and body weights, and liver protein concentrations,



Average	0	24	48	72	96	124
Body Wt.	186 ± 4	179 ± 3	170 ± 4	173 ± 4	154 ± 5*	132 ± 5*
Liver Wt.	7.5 ± 0.2	5.3 ± 0.2*	4.8 ± 0.2*	4.9 ± 0.2*	4.2 ± 0.2*	2.8 ± 0.3*
mg Protein Per g Liver	160 ± 4	178 ± 1*	182 ± 2*	184 ± 3*	190 ± 3*	204 ± 5*

Fig. 1. Effects of fasting on rat liver pyrophosphate-glucose phosphotransferase (squares) and glucose-6-phosphate phosphohydrolase (circles) activities, body and liver weights, and liver protein concentrations.

Animals were fasted for the periods indicated. Assays were carried out in the presence (open symbols) and absence (closed symbols) of deoxycholate. Definition of units of enzymic activity, as well as additional experimental details, are given in the text. Each point on the graph, or tabulated number, represents the average of values obtained with four experimental animals. All results were subjected to statistical analysis (Dixon and Massey, 1957); standard deviations of means are indicated either by vertical bars on the graph, or are given immediately after "±" in the tabulation in the lower portion of the figure. (In certain instances, e.g., phosphotransferase activity values obtained in the absence of deoxycholate, the vertical lines lie within the symbols). Asterisks (*) indicate that differences between indicated and control group average values are statistically highly significant ($p < 0.05$).

tabulated at the bottom of Fig. 1, permit calculations of activity on other bases if desired. The conclusions presented below hold regardless of the mode of expression of data, however. When assays were carried out in the presence of deoxycholate, a statistically significant increase ($p < 0.01$) in both activities was observed within 24 hours. Maximal activation, to 300

percent of control levels, was reached after 48 hours of fasting; activity then decreased progressively with time toward control levels during the remainder of the experimental period. In all instances under these conditions, the ratio of phosphotransferase to phosphohydrolase activity remained constant. These findings are interpreted to substantiate further the catalysis of both activities by a single enzyme. Moreover, these observations, as well as the similar pattern of stimulation of glucose-6-phosphate hydrolysis assayed in the absence of deoxycholate, are in agreement with the earlier findings of Weber et al (1965) who measured only the latter activity in the absence of detergent. Clearly, elevation of activity produced by fasting involves an actual increase in enzyme formation. In this respect, the effects of fasting are similar to those of alloxan diabetes (Nordlie and Arion, 1965a), but differ from the cortisone-effected response which involves an apparent stimulation of activity that persists only in the absence (and not in the presence) of deoxycholate (Nordlie and Arion, 1965b).

Interestingly, however, phosphotransferase specific activity* is not significantly stimulated ($p > 0.05$ in all instances) by starvation, when assays are carried out in the absence of added detergent. This unexpected finding is consistent with our earlier contention (Nordlie and Arion, 1965b) that factors other than synthesis of new protein also are involved in the regulation of enzymic activities associated with liver microsomal glucose-6-phosphatase. According to Ernster, Siekevitz and Palade (1962), deoxycholate both disrupts and "solubilizes" the microsomal vesicles which form as a result of fragmentation of the endoplasmic reticulum during homogenization of the tissue. It would seem certain that the activity values obtained

* A significant increase is observed in the phosphotransferase activity levels, based on assays performed in the absence of deoxycholate, if activity is expressed as units per g liver. However, this increase is due to the marked loss of weight of liver (see Fig. 1) caused by depletion of glycogen. No significant change in total phosphotransferase activity per liver is apparent.

using tissue preparations supplemented with optimal concentrations (0.2 percent, w/v) of deoxycholate provide a valid measurement of the total enzymic activity present in liver microsomes. Consequently, it may be concluded from the data in Fig. 1 that enzyme newly formed in response to fasting is principally a phosphohydrolase possessing latent phosphotransferase activity.

Such a selective enzymic response to fasting is in accord with the possible physiological regulatory role previously suggested (Arion and Nordlie, 1964; Nordlie and Arion, 1965a) for this multifunctional enzyme. Under these conditions the enzyme would be expected to act as a hydrolase functioning to compensate for lowered blood sugar levels (Weber, et al, 1965) by releasing glucose through hydrolysis of the hexose phosphate formed by gluconeogenic pathways in the liver. Also, under such conditions glucose-6-phosphate synthesis via the phosphotransferase reaction would be contraindicated. The masking of this latter activity of enzyme synthesized in response to fasting thus is consistent with a role for this catalyst as a part of a homeostatic mechanism involved in the regulation of carbohydrate metabolism.

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