The progressive effects of fasting on glucose phosphorylation by isolated rat hepatocytes: The involvement of a high $K_{0.5}$ enzyme

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The effects of varied durations of food deprivation on the rates and kinetics of glucose phosphorylation by isolated rat hepatocytes have been examined. Glucokinase activity was measured concurrently in extracts from these cells prepared from livers of rats which had fasted for 0, 24, 48 and 72 h. Significant levels of hepatocyte glucose phosphorylation were noted even when glucokinase levels were extrapolated to zero. The $K_{0.5}$-glucose value of 33 mM in cells from fed rats increased to 48 mM after a 72-h fast. It is concluded that a high $K_{0.5}$ glucose-phosphorylating enzyme or enzymes compensatory to insulin-dependent glucokinase is/are involved in rat liver glucose phosphorylation.

Glucose phosphorylation *Hepatocytes* *Fasting* *Glucokinase* *Glucose-6-phosphatase* *Rat liver*

1. INTRODUCTION

Bontemps et al. [1] described glucose phosphorylation in hepatocytes from livers of fed, 48-h starved, diabetic, and newborn rats. They also measured glucokinase (EC 2.7.1.2) levels in extracts from these same hepatocytes. On the basis of an apparent direct correlation between these glucose phosphorylation rates and glucokinase activity levels in cells from these various experimental subjects grouped together, they concluded that glucokinase plays an exclusive role in hepatocyte glucose phosphorylation. Further, they rejected the possibility that synthetic activities of glucose-6-phosphatase (EC 3.1.3.9) may take over the role of glucokinase when the activity of the latter is diminished.

Our own feeling is that fasting, diabetes, and the stage of pre- or post-natal development of the hepatocyte donor should be considered as individual variables. A heterogeneity of factors, some unique for each individual experimental model, may be involved.

Accordingly, we have carried out a series of systematic studies on the effects of varying duration of food deprivation on rates and kinetics of glucose phosphorylation and on glucokinase levels in isolated hepatocytes from young adult male rats. Results of these studies, which indicate clearly rates of glucose phosphorylation beyond those explainable by glucokinase, are described here.

2. MATERIALS AND METHODS

All fine chemicals (reagent grade) were purchased from Sigma Chemical Co. (St. Louis, MO). Sources of all other chemicals were as in [2]. Young adult male albino rats from ARS/Sprague–Dawley (Madison, WI) weighing 200–250 g, were routinely used for the preparation of hepatocytes. These animals were maintained on Purina Lab Chow and tap water, ad libitum, and were deprived of food for the specified periods of

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Abbreviation: HEPES, 4-(2-hydroxyethyl)-piperazine-ethanesulfonic acid

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time before killing. Hepatocytes were isolated as in [2].

The rate of glucose phosphorylation was estimated from the detritiation of D-[2-^{3}H]glucose [3,4]. Hepatocytes were incubated in Krebs-Henseleit buffer containing, in a final volume of 2 ml, 1.5% bovine serum albumin, the indicated concentration of glucose, 0.05 μCi/μmol D-[2-^{3}H]glucose, and hepatocytes equivalent to 0.10–0.15 g wet wt of liver. Incubations were carried out in an atmosphere of 95% O_2 and 5% CO_2 at 37°C for 30 min and terminated by the addition of 1 ml of 12% (w/v) perchloric acid. The deproteinized supernate was neutralized with KOH and 0.5-ml aliquots were transferred to columns containing borate-form anion exchange resin. The recovery of [^{3}H]water was carried out as in [2]. All values were adjusted for a partial retention of ^{3}H as in [3,4]. Glucose phosphorylation was a linear function of incubation time under these conditions. Cells were counted and protein determined as in [2]. Kinetic analysis of glucose phosphorylation by isolated hepatocytes was as in section 3.

Cell extracts for glucokinase assay were prepared by homogenizing a 1-ml aliquot of the hepatocyte preparation in 5 ml of mercaptoethanol buffer (pH 7.3) containing 150 mM KCl, 5 mM EDTA, 5 mM $MgCl_2$, and 10 mM 2-mercaptoethanol. This homogenate was centrifuged at 79 500 × g for 38 min and the supernate, which contained all the glucokinase, was retained for assay. Glucokinase activity was measured at pH 7.4 and 37°C by the method of [5]. Incubation mixtures contained in 1.5 ml, 40 mM HEPES, 100 mM D-glucose, 5 mM ATP, 7.5 mM $MgCl_2$, and 1–2 mg of cytosolic protein.

All rates are expressed as μmol · min^{-1} · g liver^{-1} as in [2].

3. RESULTS

Observed rates of glucose phosphorylation, determined at 5 concentrations of glucose with hepatocytes from fed animals and from rats which had fasted for 24, 48, and 72 h, are shown in fig. 1. A progressive decrease in glucose phosphorylation rate is seen after 24 or 48 h, but not after 72 h of fasting. In no case was the system saturated with glucose even at the highest level of this hexose studied: 60 mM. A slight sigmoidicity as also seen in [1,4] is apparent at low levels of glucose.

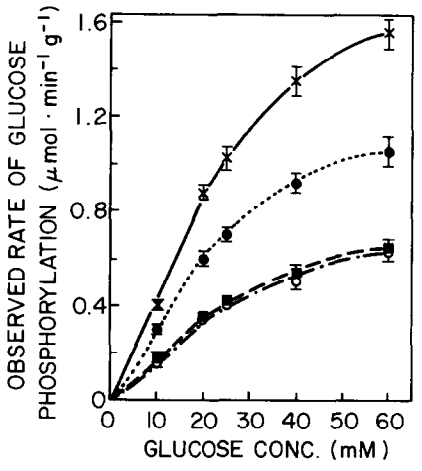

Fig. 1. Rate of phosphorylation of D-[2-^{3}H]glucose by isolated hepatic parenchymal cells, studied as a function of glucose concentration. Hepatocytes from fed (×), 24-h (●), 48-h (■), and 72-h (○) fasted rats were employed. Cell preparations from 5 fed rats, and 3 rats in each fasted category were studied in duplicate. Vertical bars indicate ± SE-values; where not shown, SE-values lie within the symbols.

Table 1

Effects of various periods of fasting on rat hepatocyte glucokinase levels

Duration of fasting	Glucokinase activity (μmol . min^{-1} . g liver^{-1})
Fed ad lib.	0.83 ± 0.11
24 h	0.35 ± 0.09
48 h	0.27 ± 0.05
72 h	0.14 ± 0.03

Mean ± SE-values for duplicate determinations with cells from 5 fed and 3 fasted rats in each category are presented. Differences between fasted and fed values are in all cases highly significant ($p < 0.01$)

Glucokinase activities measured in extracts of hepatocytes from these same preparations are given in table 1. These levels declined progressively with the duration of fasting to a value only 17% that for the fed animal after 72 h of food deprivation. Hexokinase was not detected in these hepatic parenchymal cell preparations.

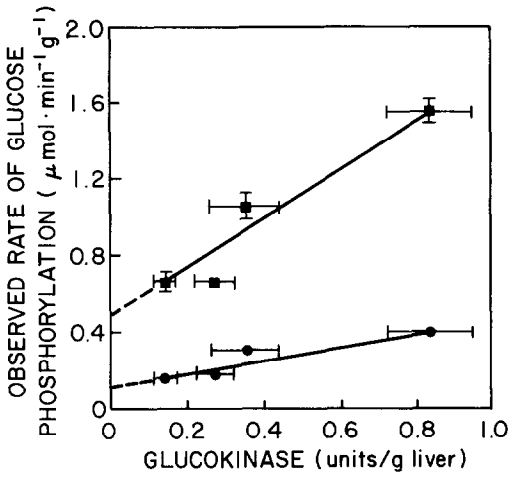

Fig. 2. Correlation between observed rates of glucose phosphorylation by isolated hepatocytes from rats fed ad lib. or fasted for 24, 48, or 72 h and their levels of glucokinase activity. Glucokinase values are from table 1; fasting is progressive from right to left. Phosphorylation rates observed with 10 mM (○) or 60 mM (■) D-[2-^{3}H]glucose are from fig. 1. Correlation coefficients are, respectively, 0.86 and 0.92 with 10 and 60 mM glucose. Horizontal and vertical bars indicate ± SE-values.

In fig. 2, rates of glucose phosphorylation observed with hepatocytes from fed, 24-, 48-, and 72-h fasted rats are plotted as a function of glucokinase levels in these cells. Rates of phosphorylation with relatively low (10 mM) and high (60 mM) glucose concentrations are presented. A correlation between these two parameters (i.e., glucose-phosphorylation rates and glucokinase levels) is apparent in both cases. However, examination of the data by linear regression analysis indicates clearly that significant amounts of cellular glucose phosphorylation persist even when glucokinase activity is extrapolated to the zero level (i.e., to the y-axis). This residual glucose phosphorylative capacity at zero glucokinase levels amounts to 27% and 32% of the phosphorylative rate in cells from fed, control rats assayed with 10 mM and 60 mM glucose, respectively.

Kinetic analysis of data in fig. 1 by the method in [6] was performed with the computer-based program in [7]. Data obtained with 20, 25, 40 and 60 mM glucose were used to avoid complications due to the slight sigmoidicity at low glucose levels. Direct graphical analysis, as well as 'per cent fit' values from computer analysis, indicated linearity under these conditions. The V_{max} values obtained in this way for the cellular glucose-phosphorylative process are presented in table 2. A significant decrease in V_{max} from the fed value was noted on fasting. V_{max}-values decreased progressively up to 24 and 48 h, but not further after 72 h, of fasting. $K_{0.5}$-values were determined by direct inspection of plots for individual experiments of the type illustrated generally in fig. 1, using V_{max}-values obtained by extrapolation in each case. $K_{0.5}$ = that concentration of glucose for which $v = ½\ V_{max}$. The $K_{0.5}$ values obtained in all instances were 3.5–5 times higher than the value of 10 mM usually accepted for rat liver glucokinase [8]. The $K_{0.5}$-value increased somewhat after 48 h of fasting and was significantly elevated (to 142% of fed control value) after 72 h of fasting.

Table 2

Effects of various periods of fasting on $K_{0.5}$ and V_{max}-values for glucose phosphorylation by isolated hepatocytes

Duration of fasting	$K_{0.5}$ (mM)	V_{max} (μmol · min^{-1} · g liver^{-1})
Fed ad lib.	33.4 ± 2.3	2.50 ± 0.10
24 h	33.3 ± 3.1	1.68 ± 0.09[a]
48 h	41.2 ± 1.4	1.10 ± 0.05[a]
72 h	47.5 ± 0.9[a]	1.16 ± 0.12[a]

[a]Differences between indicated and fed control groups are highly significant ($p < 0.05$)

Glucose phosphorylation was measured by the release of ^{3}H from D-[2-^{3}H]glucose. Mean ± SE-values are presented; assays were in duplicate with cells from 5 fed controls, and 3 rats in each fasted category

4. DISCUSSION

These studies demonstrate that glucose phosphorylation measured by the release of ^{3}H from D-[2-^{3}H]glucose in hepatic parenchymal cells from fed rats or rats fasted for varying amounts of time is highly concentration-dependent, and that the system(s) responsible are not saturated even at

60 mM glucose (see fig. 1). The $K_{0.5}$-values for cellular glucose phosphorylation (table 2) further bear out quantitatively the fact that a system or systems with a $K_{0.5}$ considerably larger than that accepted for glucokinase is/are involved in this process. Fig. 2 dramatically illustrates that hepatic parenchymal cells must contain functional system(s) for glucose phosphorylation acting additionally to glucokinase.

That this supplemental system has a $K_{0.5}$ considerably higher than that of glucokinase is apparent from the kinetic analysis and is also indicated by the higher value for the ratio, glucose phosphorylation for zero glucokinase/glucose phosphorylation with fed glucokinase levels, noted with 60 mM glucose as compared with 10 mM glucose (see above).

The identity of the system involved, which would appear from the high $K_{0.5}$-values (table 2) to be functioning to a degree in the fed as well as the fasted state, can not be deduced unambiguously from the present studies. We would point out, however, that the present results do contradict the conclusion in [1] that phosphotransferase activity of glucose-6-phosphatase may safely be eliminated from further consideration. Indeed, the high $K_{0.5}$-values for glucose observed, as well as the apparent selective further emergence of this activity as fasting progresses (see table 2), are both consistent with the involvement of this type of activity. Calculations with the kinetic parameters in [9] and the steady-state glucose-6-P levels determined in [10] with livers of 48-h fasted rats, indicate that with 60 mM glucose, glucose-6-P: [2-^{3}H]-glucose phosphotransferase activity of glucose-6-phosphatase [5,11] could account, maximally, for no more than ½ the observed rate of residual hepatocyte glucose phosphorylation extrapolated to the zero glucokinase level (fig. 2).

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