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EFFECT OF GLUCOSE AND GLUCONEOGENIC SUBSTRATES ON FASTING-INDUCED SUPPRESSION OF ACETAMINOPHEN GLUCURONIDATION IN THE RAT*

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Abstract—Previous studies in rats have shown that an acute fast decreases the apparent rate constant for glucuronidation of hepatotoxic doses of acetaminophen which results in a prolongation of the mean residence time of the drug in the animals and, hence, increased acetaminophen reactive metabolite formation and liver injury. Since acetaminophen glucuronidation under these conditions is limited by UDPGA formation, we have attempted to reverse the potentiating effects of fasting by administering glucose or gluconeogenic substrates. Histological and pharmacokinetic studies revealed that glucose (2 g/kg, i.p.) given 0.25 and 1.5 hr after acetaminophen (700 mg/kg, i.p.) did not protect the rats from liver injury or enhance acetaminophen glucuronidation. The administered glucose did not increase hepatic levels of UDP-glucose or UDPGA either basally or following administration of a hepatotoxic dose of acetaminophen. Administration of the gluconeogenic substrates, lactate, alanine, fructose and galactose, raised blood glucose levels, but did not protect the rats from liver injury or enhance glucuronidation, suggesting that the glucose-6-phosphate formed from these compounds was not available for UDPGA production for acetaminophen glucuronidation. Collectively, these studies indicate that administration of glucose and these gluconeogenic substrates does not reverse the fasting-induced potentiation of acetaminophen hepatotoxicity, and that the rate-determining step for UDPGA synthesis for glucuronidation of hepatotoxic doses of acetaminophen is prior to UDP-glucose formation.

Previous studies in rats have shown that the potentiation of acetaminophen hepatotoxicity induced by an acute fast is associated with a depressed glucuronidation capacity [1], secondary to a decreased capacity to form UDP-glucuronic acid (UDPGA) [2, 3]. This effect was in addition to the previously reported fasting-induced depression of hepatic levels of reduced glutathione [4–6]. Fasting decreases the apparent rate constant for acetaminophen glucuronication by approximately 40% as compared to fed rats [1]. The fasting-induced decrease in clearance of the drug by the nontoxic pathway results in a significant increase in the proportion of dose converted to the toxic metabolite and, hence, a potentiation of liver injury [1].

Examination of the mechanism by which fasting decreases the glucuronidation capacity revealed that fasting lowers hepatic levels of UDP-glucose, the precursor of the glucuronidation cosubstrate, UDPGA, by approximately 40% both basally and following administration of a hepatotoxic dose of acetaminophen [3]. Since glycogen is virtually depleted from the fasted liver, it follows that the glucose units made available for UDPGA synthesis must be derived from either gluconeogenesis or blood glucose. It was thus of interest to determine whether the rate of glucuronidation of hepatotoxic doses of acetaminophen could be enhanced by administration of either glucose or gluconeogenic substrates. This study reports the effects of administration of glucose and the gluconeogenic substrates, lactate, alanine, fructose and galactose, on fasting-induced modulation of acetaminophen hepatotoxicity and acetaminophen glucuronidation capacity.

METHODS

Animal treatments. Male Long Evans rats (75–125 g) were purchased from Charles Rivers (Wilmington, MA). For 1 week after their delivery, the animals were maintained under a 12-hr light:12-hr dark cycle and were allowed Wayne Lab Blox (Allied Mills, Inc., Chicago, IL) and water *ad lib.* Where indicated, food but not water, was removed from the fasted animals 24 hr prior to administration of acetaminophen (700 mg/kg, i.p., dissolved in 20% Tween 80 in normal saline) or vehicle. All experiments were initiated at 11:00 a.m. Glucose, alanine (pH 6.0), fructose, or galactose (2 g/kg, i.p.) was given 0.25 and 1.5 hr after acetaminophen or vehicle. Lactate (2 g/kg, pH 7.3, i.p.) was given only once, 0.25 hr after acetaminophen or vehicle, because a second dose was usually fatal.

Assessment of liver necrosis. Groups of fed and fasted rats received acetaminophen plus carbohydrate, carbohydrate precursor or saline. Approximately 24 hr later a blood sample was withdrawn

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from the orbital sinus of each animal with a heparinized capillary tube (75 μl). After centrifugation the plasma was analyzed for glutamic-oxaloacetic transaminase (GOT) activity using a kit (Gilford Diagnostics, Cleveland, OH). Approximately 46 hr after receiving acetaminophen, the animals were decapitated, and slices of their livers were prepared for light microscopy by the method of Culling [7] as previously described [8]. Quantitative analysis was carried out by the method of Chalkley [9] as described by Mitchell *et al.* [10].

Metabolism studies. Animals received a single i.p. injection of [^{3}H]acetaminophen (Amersham Corp., Arlington Height, IL) (700 mg/kg, 200 μCi/kg). Each animal was immediately placed in a separate metabolic cage. At the designated times, saline, glucose or gluconeogenic substrate was administered. Serial blood and total urine samples were collected from each animal and used to calculate the kinetic parameters of acetaminophen metabolism, as described previously [8]. The apparent rate constants for formation of each metabolite [acetaminophen-glucuronide (K'_G), -sulfate (K'_S), -mercapturate (K'_{MA}), and methylthioacetaminophen-glucuronide and -sulfoxide ($K'_{MTAG+SOX}$)] and the renal elimination rate constant (K_E), were calculated as the product of the urinary metabolite fraction times the overall elimination rate constant, β [1, 11].

Determinations of hepatic levels of UDPGA, UDP-glucose, glycogen and glucose. Animals were killed at various times after administration of acetaminophen plus glucose or saline. Livers were quickly excised and immediately frozen with liquid nitrogen. Levels of UDPGA were measured by the method of Watkins and Klaassen [12] using [^{3}H]diethylstilbestrol (DES) (Amersham Corp.) as a substrate and guinea pig liver microsomes as a source of glucuronyl transferase. Levels of UDP-glucose were estimated by a modification [11] of the method of Watkins and Klaassen [12] as the difference between the amount of [^{3}H]DES-glucuronide formed in the presence and absence of UDP-glucose dehydrogenase (Sigma Chemical Co., St. Louis, MO) (0.2 units) and NAD^+ (1.5 mM). Levels of glycogen were estimated as the differences between total hepatic levels of non-protein anthrone-positive sugars (measured by the method of Carroll *et al.* [13]) and liver glucose levels (measured in zinc sulfate–barium chloride supernatant fractions by the glucose oxidase method), as described previously [1, 3].

Measurements of blood glucose levels. Blood samples were collected from the orbital sinus with a 75-μl heparinized capillary tube. After centrifugation, the plasma was analyzed for glucose levels by the glucose oxidase method [3].

Statistical analyses. Statistically significant differences were assessed using either Student's *t*-test of correlated means for small groups or two-way analysis of variance. The means were then compared by using Duncan's Multiple Range Test. Significant differences were judged to have P values <0.05.

RESULTS

Effect of glucose on fasting-induced potentiation of acetaminophen hepatotoxicity. The extents of hepatic necrosis in fed, fasted, and fasted plus glucose-treated rats killed 46 hr after acetaminophen were compared (Table 1). In agreement with previous studies [1, 4], fasted rats were more susceptible to acetaminophen hepatotoxicity than were fed rats, as judged by an increase in both the incidence and severity of liver necrosis, and by a statistically significant elevation of GOT levels. Administration of glucose did not protect the fasted rats from liver injury, as evidenced by lack of reduction in the incidence and severity of liver necrosis and by lack of significant decrease in GOT levels, as compared to saline-treated fasted rats (Table 1).

Effect of glucose on the kinetic parameters of acetaminophen metabolism. In agreement with previous studies [1], fasting reduced the apparent rate constant for glucuronidation by about 40% and the apparent rate constant for sulfation by about 30% as compared to fed rats (Table 2). However, administration of glucose did not increase the clearance of acetaminophen by glucuronidation or significantly

Table 1. Effect of glucose on acetaminophen-induced hepatotoxicity in rats

Animal treatments*	No. of rats	Extent of necrosis† (% of animals) 0	1+	2+	3+	4+	GOT‡ (units/l)
Fed + saline	16	75	25				310 ± 40§
Fasted + saline	13	15	31	31	23		892 ± 127‖
Fasted + glucose	12	8	25	33	33		1119 ± 575‖

* Animals were allowed food *ad lib.* or fasted for 24 hr prior to receiving acetaminophen (700 mg/kg, i.p.). At 0.25 and 1.5 hr after administration of acetaminophen the animals also received either glucose (2 g/kg) or saline vehicle, i.p.

† Extent of hepatic necrosis was scored in livers from rats killed 46 hr after administration of acetaminophen: 0 = no necrosis; 1+ = necrosis of 5% or less of hepatocytes; 2+ = necrosis of 6–25% of hepatocytes; 3+ = necrosis of 26–50% of hepatocytes; and 4+ = greater than 50% of hepatocytes.

‡ Glutamic-oxaloacetic transaminase levels were determined in plasma samples taken at 24 hr after administration of acetaminophen. Values are means ± SE.

§, ‖ Values with different superscripts are significantly different, $P < 0.05$.

Table 2. Effect of glucose on the kinetic parameters of acetaminophen metabolism in rats

Animal treatments*	β (hr^{-1})	Apparent rate constants for metabolite formation†				Renal elimination rate constant
		K'_G	K'_S	K'_{MA}	$K'_{MTAG+SOX}$	K_E
		(hr^{-1})				(hr^{-1})
Fed + saline	0.494 ± 0.009‡	0.367 ± 0.007‡	0.089 ± 0.001‡	0.021 ± 0.001‡	0.007 ± 0.001‡	0.025 ± 0.005‡
Fasted + saline	0.346 ± 0.021§	0.226 ± 0.018§	0.065 ± 0.006§	0.020 ± 0.002‡	0.008 ± 0.001‡	0.027 ± 0.004‡
Fasted + glucose	0.336 ± 0.017§	0.216 ± 0.013§	0.066 ± 0.005§	0.028 ± 0.002‡	0.009 ± 0.001‡	0.016 ± 0.002‡

* Animal treatments were identical to those described under Table 1 except that [^{3}H]acetaminophen (200 μCi/k.g.) was included in the injection solution.

† Each animal was placed in a separate metabolic cage. Sequential blood samples and total urinary collection were obtained for each animal and were used to calculate the kinetic parameters as described previously [8]. The apparent rate constants for each metabolite [acetaminophen-glucuronide (K'_G), -sulfate (K'_S), -mercapturate (K'_{MA}), and methylthiometabolites ($K'_{MTAG+SOX}$)] were calculated as $\beta \times$ urinary metabolite fraction [1, 11]. Values are means ± SE, N = 12.

‡, § Values with different superscripts are significantly different from each other for each parameter, $P < 0.05$.

alter the other kinetic parameters of acetaminophen metabolism.

Effect of glucose on hepatic levels of UDPGA and UDP-glucose in fasted rats. To investigate the lack of effect of glucose administration on acetaminophen glucuronidation, hepatic levels of the cosubstrate, UDPGA, and its precursor, UDP-glucose, were measured. As shown in Fig. 1, administration of glucose neither prevented nor slowed the acetaminophen-induced depletions of UDPGA and UDP-glucose. Further, glucose administration did not enhance the rate of their repletion. Overall, glucose did not increase significantly the levels of UDPGA or UDP-glucose at any time after acetaminophen, indicating that the administered glucose did not enhance the synthesis of these compounds.

Effect of glucose on hepatic levels of glycogen and glucose and on blood levels of glucose. Since the administered glucose did not increase the hepatic levels of UDPGA or UDP-glucose, it was of interest to determine the fate of the administered glucose. In agreement with previous studies [1, 3, 10, 14, 15], administration of acetaminophen to fed rats induced a marked decrease in glycogen levels (from 28.9 ± 1.04 to 3.60 ± 1.21 mg/g liver) as measured at the nadir, 2 hr after the drug was given (Table 3). In further agreement with earlier studies [1, 3], administration of acetaminophen had no significant

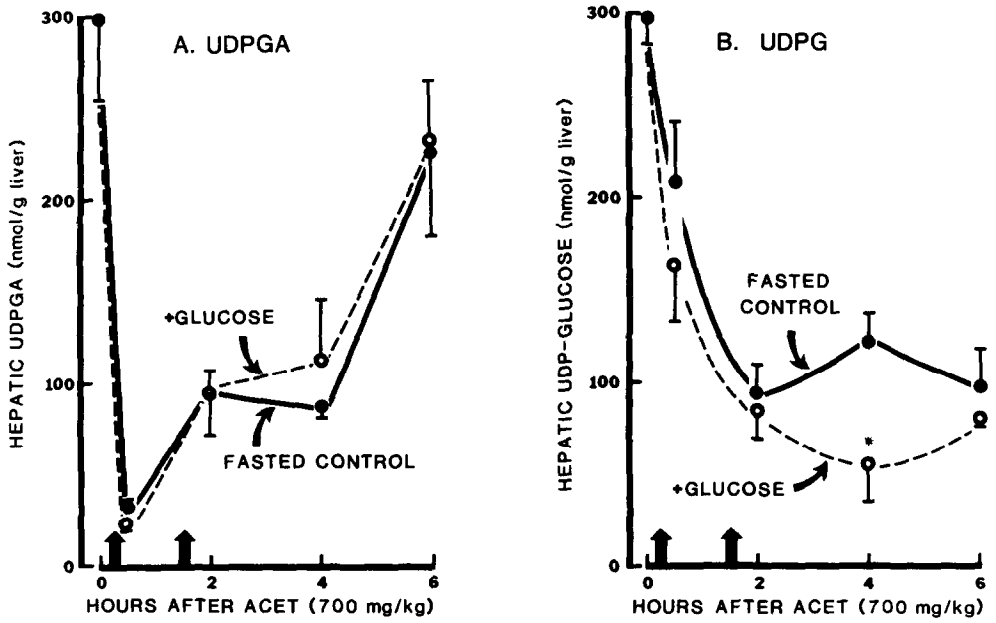

Fig. 1. Effect of administration of glucose on hepatic levels of UDPGA (A) and UDP-glucose (B) in fasted rats. Animals were fasted 24 hr prior to receiving vehicle or acetaminophen (700 mg/kg, i.p.). Glucose (2 g/kg, i.p.) or saline was given at the times indicated by the arrows (0.25 and 1.5 hr after administration of acetaminophen). Levels of UDPGA and UDP-glucose were determined as described under Methods. Values are means ± SE, N = 4, and are representative of two separate experiments. Key: *significantly different from fasted control rats, $P < 0.05$.

Table 3. Effect of glucose on hepatic levels of glycogen

Animal treatments*	Acetaminophen (700 mg/kg)	Glycogen† (mg/g liver)
Fed + saline	−	28.9 ± 1.04‡
	+	3.60 ± 1.21§
Fasted + saline	−	0.37 ± 0.10‖
	+	0.23 ± 0.06‖
Fasted + glucose	−	16.0 ± 1.90¶
	+	5.01 ± 0.45§

* Animals were allowed food *ad lib* or fasted for 24 hr prior to receiving vehicle or acetaminophen (700 mg/kg, i.p.). Saline or glucose (2 g/kg, i.p.) was given 0.25 and 1.5 hr after acetaminophen or vehicle.

† Glycogen levels were estimated 2 hr after acetaminophen or vehicle as described under Methods. Values are means ± SE, N = 4, and are representative of two separate experiments.

‡–¶ Values with different superscripts are significantly different from each other, $P < 0.05$.

effect on the depleted glycogen levels of fasted rats. Administration of glucose to fasted rats resulted in a significant repletion of glycogen (to a maximum at 2 hr post-acetaminophen of *ca.* 16 mg/g liver), as compared to fasted control rats. This repletion of glycogen per total liver (*ca.* 6 g liver/150 g rat) represents approximately 107 mg of glucose and hence accounts for less than 20% of the dose of glucose administered (600 mg/150 g rat). However, co-administration of acetaminophen to glucose-treated fasted rats resulted in glycogen levels that were significantly lower (*ca.* 5 mg/g liver), as compared to fasted rats that received only glucose. These glycogen levels represent approximately 33 mg of glucose per total liver and account for about only 5% of the dose of glucose administered (600 mg/150 g rat). These data indicate that either glycogen synthesis or deposition was reduced by coadministration of acetaminophen with the glucose.

Administration of glucose significantly increased free glucose levels in the livers of fasted rats to a maximum at 2 hr post-acetaminophen (1.66 ± 0.22 mg/g liver, fasted controls, vs 4.36 ± 0.17 mg/g liver, glucose-treated fasted rats, $P < 0.05$). However, the elevation per total liver (*ca.* 6 g liver/150 g rat) was approximately 30 mg and, hence, cannot account for more than 5% of the dose of glucose administered (*ca.* 600 mg/150 g rat). These data suggest that hepatic free glucose does not represent a significant fate of the administered glucose. Collectively, the data suggest that the major fate of the administered glucose is not hepatic glycogen or free glucose.

To explore the fate of the glucose administered to acetaminophen-treated rats, blood levels of glucose were examined. As shown in Fig. 2A, in the absence of acetaminophen, blood glucose levels were increased modestly in glucose-treated fasted rats over those in fasted control rats. The elevation was statistically significant though the values were still within a normal range. These data indicate that the major portion of the administered glucose is removed from the blood by entry into tissues, including the liver. In agreement with previous studies [3], acetaminophen induced a significant elevation of blood glucose levels in fasted control rats (Fig. 2B). Administration of glucose to acetaminophen-treated fasted rats resulted in a dramatic elevation of blood glucose levels of approximately 4-fold over that of

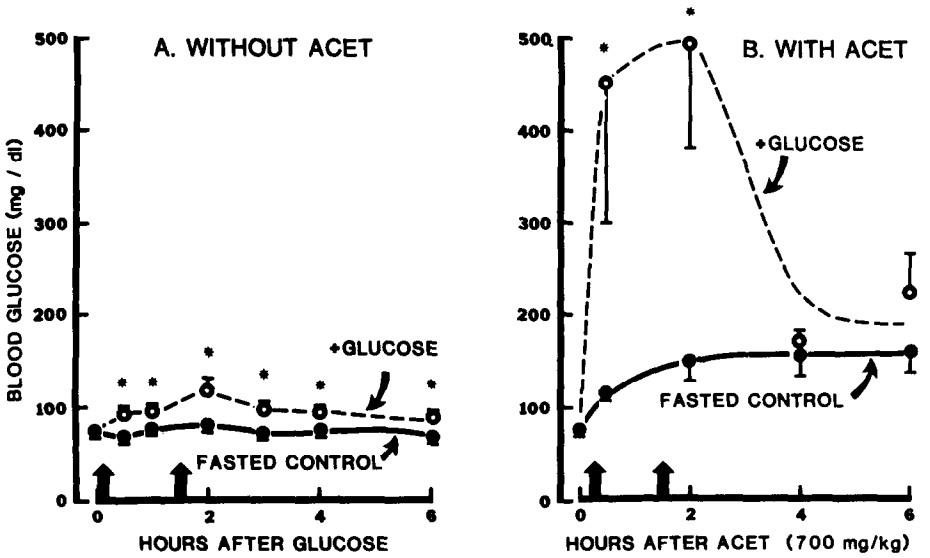

Fig. 2. Effect of administration of glucose on blood levels of glucose in fasted rats in the absence or presence of acetaminophen. Animals were fasted for 24 hr prior to receiving vehicle (A) or acetaminophen (700 mg/kg, i.p.) (B). Glucose (2 g/kg, i.p.) or saline was given at the times indicated by the arrows. Blood glucose levels were determined by the glucose oxidase method as described under Methods. Values are means ± SE, N = 4, and are representative of three separate experiments. Key: * significantly different from fasted control rats, $P < 0.05$.

Table 4. Effects of lactate, alanine, fructose or galactose on acetaminophen hepatotoxicity in fasted rats

Animal treatments*	No. of rats	Extent of necrosis† (% of animals) 0	1+	2+	3+	4+	GOT‡ (units/l)
Fasted + saline	21	0	42	57			1462 ± 450§
Fasted + lactate	9	0	57	29	14		1346 ± 749§
Fasted + alanine	9	11	22	33	33		1719 ± 668§
Fasted + fructose	8	0	0	75	25		4318 ± 668‖
Fasted + galactose	8	13	37	13	37		1575 ± 287§

* Rats were fasted for 24 hr and given lactate, alanine, fructose, galactose or saline vehicle after administration of acetaminophen (700 mg/kg, i.p.) as described under Methods.

†, ‡ Extent of necrosis and transaminase levels were determined as desdcribed under Table 1.

§, ‖ Values with different superscripts are significantly different from each other, $P < 0.05$.

fasted controls. Since the dose of glucose administered was the same in non-acetaminophen-treated (Fig. 2A) and acetaminophen-treated (Fig. 2B) rats, these data suggest that acetaminophen suppresses the uptake of the administered glucose into tissues. Of major importance, blood glucose levels were elevated markedly during the period in which acetaminophen glucuronidation is limited by a decreased capacity to form UDPGA. Collectively, these data suggest that fasting-induced suppression of glucuronidation is not due to lack of glucose *per se* but to the inability of the hepatocyte to convert glucose to UDPGA.

Effects of lactate, alanine, fructose and galactose on fasting-induced acetaminophen hepatotoxicity. To explore the possibility that UDPGA synthesis in the fasted liver is limited by the availability of glucose units produced by gluconeogenesis, lactate, alanine, fructose or galactose was administered to fasted rats, and their effects on acetaminophen liver injury and glucuronidation were examined. It is well known that the fixations of glucose, fructose and galactose in the liver cell occur by different mechanisms: glucose by glucokinase, fructose by fructokinase and galactose by galactokinase. Lactate and alanine are both known to enter the liver readily and to be converted rapidly to pyruvate which, in turn, can be oxidized via the Krebs cycle or be converted to glucose via the pyruvate–malate shuttle and gluconeogenesis. These mechanisms and enzyme levels are very disparate and may well support UDPGA formation at very different rates. As shown in Table 4, none of the compounds tested protected the fasted rats from liver injury as judged by the lack of decrease in the severity of liver necrosis and lack of significant reduction in GOT elevations. In contrast, fructose potentiated liver injury as judged by enhancement in the severity of liver necrosis and by a significant increase in GOT elevations. The potentiating effect of fructose may be associated with the previously reported ATP-depleting effect of fructose [16, 17].

Effects of lactate, alanine, fructose and galactose on the kinetic parameters of acetaminophen metabolism. As shown in Table 5, none of the compounds tested significantly altered the apparent rate constant for glucuronidation or any kinetic parameter of acetaminophen metabolism. Collectively, these data suggest that the glucose-6-phosphate formed from these gluconeogenic substrates did not enhance the

Table 5. Effects of lactate, alanine, fructose or galactose on the kinetic parameters of acetaminophen metabolism in fasted rats

Animal treatments*	β (hr^{-1})	Apparent rate constants for metabolite formation† (hr^{-1}) K'_G	K'_S	K'_{MA}	$K'_{MTAG+SOX}$	Renal elimination rate constant K_E (hr^{-1})
Fasted + saline	0.310 ± 0.015	0.154 ± 0.010	0.064 ± 0.005	0.022 ± 0.003	0.024 ± 0.003	0.033 ± 0.004
Fasted + lactate	0.303 ± 0.039	0.160 ± 0.019	0.065 ± 0.005	0.022 ± 0.001	0.022 ± 0.004	0.035 ± 0.005
Fasted + alanine	0.294 ± 0.007	0.155 ± 0.018	0.058 ± 0.004	0.024 ± 0.001	0.023 ± 0.001	0.034 ± 0.006
Fasted + fructose	0.296 ± 0.034	0.175 ± 0.016	0.061 ± 0.009	0.024 ± 0.004	0.016 ± 0.002	0.021 ± 0.005
Fasted + galactose	0.260 ± 0.025	0.138 ± 0.006	0.054 ± 0.005	0.021 ± 0.002	0.025 ± 0.003	0.022 ± 0.004

* Animal treatments were identical to those described under Table 4 except that [^{3}H]acetaminophen (200 μCi/kg) was included in the injection solution.

† Designations and calculations of the apparent rate constants for metabolite formation were identical to those described under Table 2. Values are means ± SE, N = 8.

Table 6. Effect of lactate, alanine, fructose, or galactose on hepatic levels of glycogen

Animals*	Treatments* Acetaminophen	Carbohydrate	Glycogen† (mg/g liver)
Fed	−	−	50.9 ± 4.04‡
	+	−	2.25 ± 1.01§
Fasted	−	−	2.18 ± 0.40§
	+	−	0.35 ± 0.10‖
Fasted	−	+ Lactate	3.02 ± 0.39§
	+	+ Lactate	0.45 ± 0.07‖
Fasted	−	+ Alanine	4.31 ± 0.78§
	+	+ Alanine	0.06 ± 0.06‖
Fasted	−	+ Fructose	18.3 ± 1.30¶
	+	+ Fructose	4.78 ± 1.75§
Fasted	−	+ Galactose	13.1 ± 0.84¶
	+	+ Galactose	3.19 ± 0.55§

* Animals were allowed food *ad lib.* or fasted for 24 hr prior to receiving vehicle or acetaminophen (700 mg/kg, i.p.). Saline or gluconeogenic substrate was administered as described under Methods.

† Glycogen levels were determined 2 hr after acetaminophen administration as described under Methods. Values are means ± SE, N = 4, and are representative of two separate experiments.

‡–¶ Values with different superscripts are significantly different from each other, $P < 0.05$.

synthesis of UDP-glucose or UDPGA and hence was unable to stimulate acetaminophen glucuronidation or reverse the fasting-induced potentiation of acetaminophen liver injury (Table 4).

Effects of lactate, alanine, fructose and galactose on hepatic levels of glycogen and on blood levels of glucose. Since the enhancement of gluconeogenesis in the liver by lactate, alanine, fructose or galactose did not enhance acetaminophen glucuronidation, it was of interest to examine the fate of the glucose units. As shown in Table 6, lactate and alanine did not increase markedly the accumulation of glycogen in the liver at the 2-hr time point in either the absence or presence of acetaminophen. Fructose and galactose caused significant glycogen synthesis and deposition in the absence of acetaminophen.

Examination of blood glucose levels revealed that in the absence of acetaminophen all four compounds

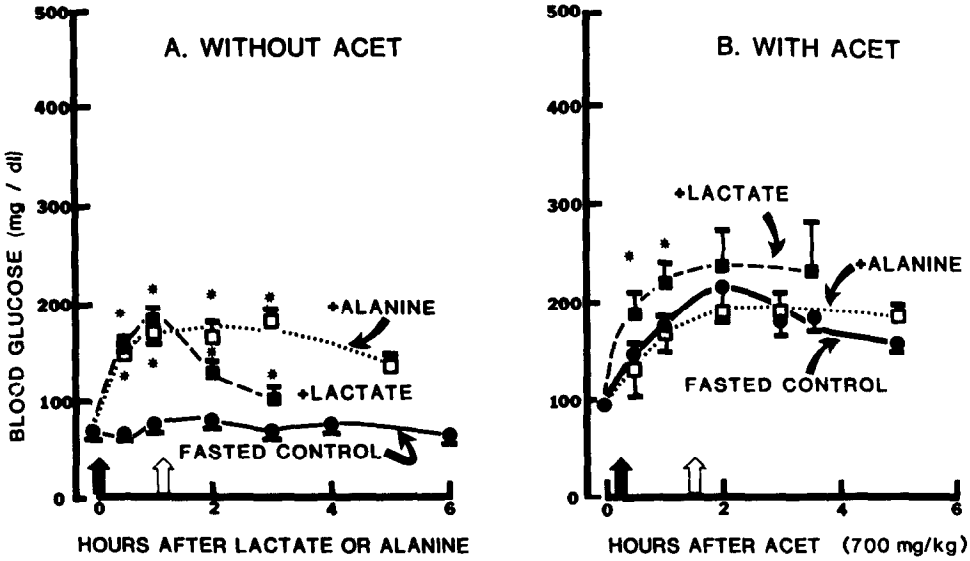

Fig. 3. Effect of administration of lactate or alanine on blood levels of glucose in fasted rats in the absence or presence of acetaminophen. Animals were fasted for 24 hr prior to receiving vehicle (A) or acetaminophen (700 mg/kg, i.p.) (B). Lactate or alanine was given at the times indicated by the arrows. Blood glucose levels were determined by the glucose oxidase method as described under Methods. Values are means ± SE, N = 4, and are representative of three separate experiments. Key: * significantly different from fasted control rats, $P < 0.05$.

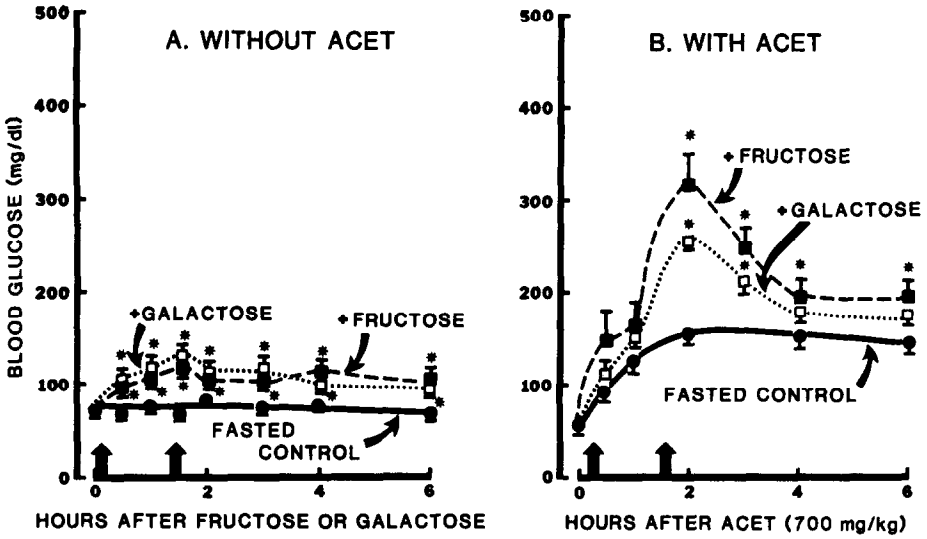

Fig. 4. Effect of administration of fructose or galactose on blood glucose levels in fasted rats in the absence or presence of acetaminophen. Animals were fasted for 24 hr prior to receiving vehicle (A) or acetaminophen (700 mg/kg, i.p. (B). Fructose or galactose was given at the times indicated by the arrows. Blood glucose levels were determined by the glucose oxidase method as described under Methods. Values are means ± SE, N = 4, and are representative of three separate experiments. Key: * significantly different from fasted control rats, $P < 0.05$.

significantly increased blood glucose levels above the normal range (Figs. 3A and 4A), confirming the well known fact that they are gluconeogenic. As noted above, administration of acetaminophen to the fasted rats induced a marked rise in blood glucose levels. Co-administration of lactate led to a further increase in this acetaminophen-induced hyperglycemia, whereas alanine administration did not (Fig. 3B). The hyperglycemia induced by acetaminophen was further enhanced by fructose and galactose to approximately twice that of fasted controls (Fig. 4B). The data suggest that the glucose-6-phosphate formed in the liver from galactose, fructose and lactate is dephosphorylated and enters the bloodstream. The reason that alanine did not similarly increase blood glucose levels in the presence of acetaminophen is unclear. Overall, the data are consistent with the hypothesis that the activity of glucose-6-phosphatase is higher relative to that of the enzymes involved in UDP-glucose formation, i.e. phosphoglucomutase and/or UDP-glucose pyrophosphorylase.

DISCUSSION

Previous studies in rats have shown that the fasting-induced depression of acetaminophen glucuronidation is a major factor contributing to the potentiation of acetaminophen liver injury [1] and that the rate-determining step in the glucuronidation of acetaminophen is the formation of UDPGA [2, 3]. We thus postulated that, if the formation of UDPGA could be increased by administration of glucose or gluconeogenic substrates, acetaminophen glucuronidation would be enhanced, the proportion of the dose converted to the toxic metabolite would be decreased, and the fasting-induced potentiation of liver injury would be reversed. From a clinical viewpoint, recognition of a new opportunity for development of therapy for acetaminophen overdosage is beneficial. It is known that patients with the longest half-life of acetaminophen are at the greatest risk of subsequently developing liver injury [18]. Since the lengthening of the half-life reflects a deficiency in glucuronidation in both humans [19] and animals [1, 8, 11], it follows that, if we could enhance the glucuronidation capacity of the overdosed patient, the potential for liver injury would be reduced.

The present study has examined the effects of administration of glucose *per se* and of gluconeogenic substrates on the hepatotoxicity and metabolism of acetaminophen in fasted rats. The results indicate that none of the compounds tested reversed the fasting-induced potentiation of liver injury. Indeed, fructose markedly potentiated the liver injury (Table 4). Similarly, administration of large doses of glucose and the other carbohydrate precursors failed to enhance acetaminophen glucuronidation (Tables 2 and 5) and failed to increase hepatic levels of either UDPGA or UDP-glucose at any time after acetaminophen (Fig. 1).

The mechanism underlying the failure of the large doses of glucose and of the various gluconeogenic substrates to override the relative deficiency of UDPGA production in the livers of fasted rats is unclear. The treatments of glucose and carbohydrate precursors used (2–4 g/kg) were sufficient to raise total body glucose units, as evidenced by increased blood glucose levels (Figs. 2A, 3A and 4A) and by increased glycogen deposition in the case of glucose,

fructose and galactose (Tables 3 and 6). It follows that the inability to enhance the rate of UDPGA formation in the liver is not due to the shortage of glucose units *per se*. Therefore, the rate-limiting factor(s) must reside in one or more steps in the UDPGA synthetic pathway.

Perhaps the most striking observation of the present studies is the profound hyperglycemia seen in the glucose-supplemented fasted rats when acetaminophen was co-administered. It is of interest that this extreme elevation of blood glucose (*ca.* 500 mg/dl) was similar to that seen in our diabetic rats who show enhanced acetaminophen glucuronidation capacity and resistance to acetaminophen hepatotoxicity [8, 11]. This similarity in blood glucose levels in the two situations and the disparity in their glucuronication capacity emphasize clearly that factors other than the availability of glucose units, *per se*, determine the ability of the liver to produce UDPGA in response to a high metabolic demand. Equally striking is the observation that co-administration of acetaminophen resulted in an outpouring from the liver of the glucose units synthesized from lactate, fructose and galactose over that seen in acetaminophen-treated fasted controls (Figs. 2B, 3B, and 4B). Thus, glucose units formed within the intact liver were directed away from the glucuronic acid pathway. Collectively, these data suggest that, in the presence of acetaminophen, the activity of glucose-6-phosphatase is higher relative to that of glucokinase (in the case of glucose administration) and of phosphoglucomutase and/or UDP-glucose pyrophosphorylase (in the case of lactate, alanine or fructose and galactose treatments).

The effects of fasting on the activity of the enzymes of intermediary carbohydrate metabolism have been studied extensively (for review, see Ref. 20). Fasting lowers blood glucose levels which, in turn, lowers insulin levels, which reduces glucokinase activity. Fasting also triggers release of glucagon and glucocorticoid which promotes glycogenolysis and gluconeogenesis. The breakdown of glycogen and the resultant higher cellular levels of glucose-6-phosphate lead to higher free glucose cellular levels via glucose-6-phosphatase. The equilibrium constant for phosphoglucomutase favors glucose-6-phosphate rather than glucose-1-phosphate formation. Clearly, the effects of fasting on glucose homeostasis are the result of a complex interplay between numerous factors.

There are additional problems in understanding the effects of fasting on glucose homeostasis *in vivo* because much of our current information has been obtained from studies using isolated livers and hepatocytes. Extrapolation from the isolated hepatocytes to the *in vivo* situation is difficult, as reviewed by Thurman and Kauffman [21], and as illustrated, for example, by studies demonstrating that addition of glucose or other carbohydrates to isolated cell suspensions increases UDPGA levels and glucuronidation [22–28]. The results of the present study clearly indicate that administration of glucose does not have the same effects *in vivo*.

In addition to their gluconeogenic potential to provide glucose units for UDPGA formation, lactate and alanine may be used to examine the role of NAD^+/NADH ratio in the control of UDPGA production at the level of UDP-glucose dehydrogenase, as has been suggested on the basis of *in vitro* studies [29, 30]. It is well known that the metabolism of lactate results in a net increase in cytoplasmic NADH due to the initial conversion of lactate to pyruvate, whereas alanine, which lacks this initial oxidative step, does not. Thus, these two compounds have the same metabolic fate yet differ in their effect on NADH levels. However, as shown in Table 5, lactate and alanine had similar effects on acetaminophen glucuronidation capacity which suggests that their effects on UDPGA production are also similar. Thus, in agreement with previous *in vivo* studies [3], a major role of NADH levels in the inhibition of UDPGA synthesis *in vivo* seems unlikely.

The combined effects of fasting and acetaminophen on intermediary carbohydrate metabolism *in vivo* are almost completely unknown. Hinson *et al.* [15, 31] have shown that the administration of large doses of acetaminophen to fed mice induces a massive release of insulin and mobilization of liver glycogen. If a similar release of insulin occurred in fasted rats, the insulin might be expected to increase glucokinase and decrease glucose-6-phosphatase activities [20]. This response, however, is relatively slow; maximal effects of insulin secretion on glucokinase levels are not seen for approximately 24 hr [32]. However, Seitz *et al.* [33] suggest that rapid changes in the insulin/glucagon ratio can rapidly switch hepatic intermediary metabolism from glucose production to glucose utilization within minutes. The present studies suggest that acetaminophen activates either glucose-6-phosphatase and/or the gluconeogenic pathway. However, effects of acetaminophen to decrease glucokinase and/or UDP-glucose pyrophosphorylase activities are also possible. Measurement of glucose-6-phosphate and glucose-1-phosphate concentrations in the liver might differentiate between these possible mechanisms of fasting-induced decreased capacity to form UDP-glucose for UDPGA synthesis and acetaminophen glucuronidation.

Collectively, our data indicate that the fasting-induced lengthening of the half-life of hepatotoxic doses of acetaminophen, the decrease in hepatic UDPGA synthesis, and the enhancement of acetaminophen hepatotoxicity seen in the rat are not offset by administration of large doses of glucose or gluconeogenic substrates. The suppression of UDPGA formation in the fasted liver is clearly not due to deficiency of glucose units in the animal but appears to result either from an imbalance in glucose fixation in the liver (glucose-6-phosphatase/glucokinase ratio) or a decrease in the activity of UDP-glucose pyrophosphorylase activity. Identification of the rate-limiting step in hepatic UDPGA synthesis in the fasted liver is essential if enhancement of glucuronidation is to become a useful therapeutic adjunct for the patient severely intoxicated with acetaminophen.

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