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MECHANISM OF DECREASED ACETAMINOPHEN GLUCURONIDATION IN THE FASTED RAT*

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Abstract—The mechanism by which an acute fast decreases the glucuronidation of hepatotoxic doses of acetaminophen in the rat was examined. Fasting did not depress the level of the enzyme, glucuronyl transferase, or the basal level of the co-substrate, UDP-glucuronic acid (UDPGA). Administration of a hepatotoxic dose of acetaminophen rapidly depleted UDPGA levels in both fed and fasted rats to the same nadir. Fed and fasted rats differed in that the rate of repletion of UDPGA levels was markedly slower in fasted rats. The total hepatic levels of UDP-glucose dehydrogenase and its cofactor, NAD^+, were not decreased by fasting. In fasted rats, hepatic levels of the UDPGA precursor, UDP-glucose, were approximately 60% those of fed rats both before and after a hepatotoxic dose of acetaminophen. In fed rats, acetaminophen induced a marked depletion of hepatic glycogen levels and a dramatic increase in blood glucose levels. Acetaminophen induced a similar marked increase in blood glucose levels in fasted rats in spite of the fact that they lacked hepatic glycogen. It is concluded that the fasting-induced decrease in the glucuronidation of hepatotoxic doses of acetaminophen results from decreased production of UDPGA. The decreased synthetic capacity for UDPGA does not appear to be due to the inability of the liver to produce glucose units *per se*, but rather to the fasting-induced altered activities of the enzymes of carbohydrate metabolism which, in turn, alter the fate of glucose-6-phosphate derived from gluconeogenesis.

It is known that an acute fast potentiates acetaminophen-induced hepatotoxicity in the rat [1]. This effect has been attributed to a decrease in the glutathione protective capacity of the liver [1, 2]. We have observed recently that, in addition, the potentiation is also associated with a decreased clearance of the drug by glucuronidation and sulfation [3]. At hepatotoxic dose levels of acetaminophen, fasting decreases the apparent rate constants for formation of glucuronide and sulfate conjugates by approximately 40 and 30% respectively [3]. The capacity for formation of the toxic metabolite, as estimated by the apparent rate constant for mercapturate formation, is not altered by fasting [3]. Thus, since the activity of the toxic pathway is not changed by fasting, whereas the activities of the nontoxic pathways are depressed, the proportion of the dose converted to the toxic metabolite is enhanced significantly [3].

Of the two nontoxic pathways, glucuronidation is quantitatively more important in the clearance of high doses of acetaminophen than is sulfation [4–6]. However, at these high dose levels, glucuronidation of acetaminophen shows evidence of capacity limitation [6, 7], associated with a limitation in the capacity of the liver cell to synthesize the cosubstrate, UDP-glucuronic acid (UDPGA)‡ [7]. The rate-limiting step(s) in the formation of UDPGA in the intact liver is not known.

The present study examines the mechanism underlying the fasting-induced decrease in glucuronidation capacity. This was accomplished by assessing the rate-limiting role of: (a) glucuronyl transferase activity, (b) hepatic levels of UDPGA, and (c) various factors involved in production of UDPGA during metabolism of hepatotoxic doses of acetaminophen.

METHODS

Animal treatments. Male Long Evans rats (75–125 g) were purchased from Charles Rivers (Wilmington, MA). The animals were maintained under a 12-hr light: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 at 11:00 a.m. 24 hr prior to administration of acetaminophen. All experiments were initiated at 11:00 a.m.

Determination of microsomal acetaminophen glucuronyl transferase activity. Enzyme activity toward acetaminophen was measured in microsomal fractions that were freshly isolated from livers of fed and fasted rats according to a modification of the method of Bolanowska and Gessner [8]. In a total reaction volume of 0.5 ml, approximately 2.5 mg of microsomal protein was incubated with various concentrations of [^{3}H]acetaminophen (Amersham Corp., Arlington Heights, IL) (1–10 mM; 0.5 μCi/tube), UDPGA ammonium salt (Sigma Chemical

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‡ Abbreviations: UDPGA, UDP-glucuronic acid; G6P, glucose-6-phosphate; and G1P, glucose-1-phosphate.

Co., St. Louis, MO) (10 mM), UDP-*N*-acetylglucosamine (Sigma) (2 mM), $MgCl_2$ (10 mM), Tris (100 mM, pH 7.4) and the detergent Brij 58 (Sigma) (0.05%) for 45 min. The amount of acetaminophen glucuronide formed was determined by liquid scintillation spectrometry following removal of unconjugated acetaminophen by two extractions with ethyl acetate. Microsomal protein concentration was determined by the method of Lowry *et al.* [9] using bovine serum albumin as a standard.

Determinations of hepatic levels of UDPGA, UDP-glucose and glycogen. Animals were killed by decapitation at various times after administration of acetaminophen (700 mg/kg, i.p.). Livers were quickly excised and frozen with liquid nitrogen. Subsequently, levels of UDPGA were measured by the method of Watkins and Klaassen [10] using [^{3}H]diethylstilboestrol (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 previously described modification [11] of the method of Watkins and Klaassen [10] as the difference between the amount of [^{3}H]DES-glucuronide formed in the presence and absence of both UDP-glucose dehydrogenase (Sigma) (0.2 units/ml) and NAD^+ (1.5 mM). In addition, portions of the livers were homogenized in 0.05 M sodium phosphate buffer (pH 7.4) with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) after which sulfosalicylic acid supernatant fractions were prepared and used for measurement of glycogen levels. Glycogen was estimated as the difference between total hepatic non-protein anthrone-positive sugars [12] and liver glucose measured by the glucose oxidase method using a kit from the Sigma Chemical Co.

Determination of the blood half-life of acetaminophen and the apparent rate constant for acetaminophen glucuronide formation. Animals received a single i.p. injection of [^{3}H]acetaminophen (20–1000 mg/kg, 200 μCi/kg). Each animal was then immediately placed in a separate metabolic cage. Serial blood samples were taken from the orbital sinus of each animal using 75-μl heparinized capillary tubes. Blood half-life ($T_{\frac{1}{2}}$) and the overall elimination rate constant (β) were determined from concentrations of acetaminophen in serial blood samples as previously described [6]. For each animal, urine was collected over dry ice for 24 hr. The urinary metabolites were separated by thin-layer chromatography and quantitated as previously described [6]. The apparent rate constant for formation of acetaminophen-glucuronide (K'_G) was calculated as the product of the urinary metabolite fraction times β [11].

Determination of UDP-glucose dehydrogenase activity. Liver cytosolic fractions were freshly prepared in 0.15 M KCl [11]. In a total reaction volume of 0.5 ml, approximately 200 μg of cytosol protein were incubated in the presence of UDP-glucose (Sigma) (0.3 mM) and NAD^+ (1.5 mM) for 0–20 min as described previously [11]. The reaction was stopped by boiling and the amount of UDPGA formed was measured by the method of Watkins and Klaassen [10]. Cytosolic protein concentration was determined by the method of Lowry *et al.* [9].

Measurement of hepatic levels of NAD^+ and NADH. From tissue that had been frozen with liquid nitrogen, liver extracts were prepared by the method of Burch *et al.* [13]. Total hepatic levels of NAD^+ and NADH were estimated with the cycling assay of Nisselbaum and Green [14].

Measurement of blood glucose levels. Blood samples were collected from the orbital sinus with a 75-μl heparinized capillary tube. Following centrifugation, glucose concentrations were determined in the plasma samples by the glucose oxidase method using a kit from the Sigma Chemical Co.

Statistical analyses. Levels of statistical significance were assessed using either Student's *t*-test of correlated means for small groups, or two-way analysis of variance. Significant differences were judged to have P values < 0.05.

RESULTS

Effect of fasting on glucuronyl transferase levels. To determine whether fasting decreased the amount of glucuronyl transferase enzyme, microsomal fractions were isolated from the livers of fed and fasted rats, and their glucuronyl transferase activities with respect to acetaminophen were determined. As shown in Fig. 1, hepatic microsomes from fed and fasted rats showed similar V_{max} values, indicating similar levels of the enzyme.

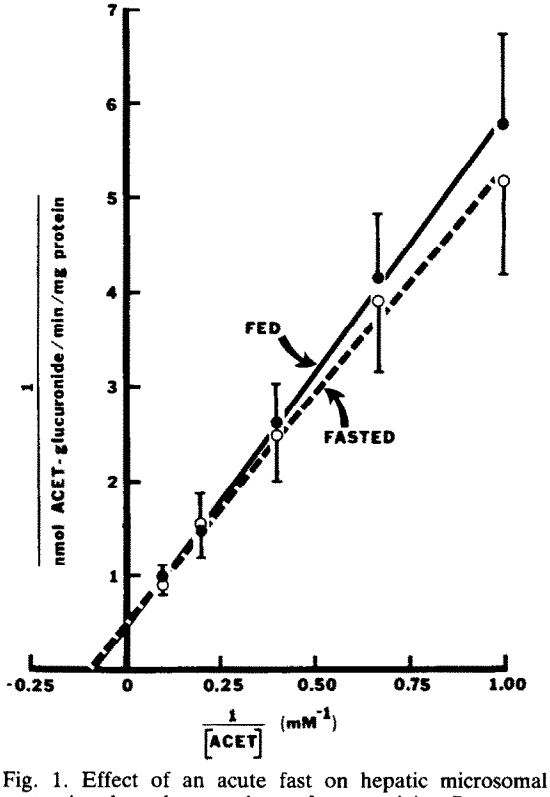

Fig. 1. Effect of an acute fast on hepatic microsomal acetaminophen glucuronyl transferase activity. Rats were allowed food *ad lib.* (●) or were fasted for 24 hr (○) prior to isolation of hepatic microsomal fractions. Values are means ± SE, N = 7.

Table 1. Effect of fasting on hepatic levels of UDPGA

Animals*	UDPGA† (nmol/g liver)	UDPGA† (μmol/total liver/kg rat)
Fed	483 ± 37	22.5 ± 1.7
Fasted	511 ± 45	18.5 ± 1.6

* Animals were allowed food *ad lib.* or were fasted 24 hr prior to being killed.

† UDPGA was measured by the method of Watkins and Klaassen [10]. Values are means ± SE, N = 4, and are representative of three separate experiments.

Effect of fasting on hepatic levels of UDPGA. To examine whether the lower rate of glucuronidation of hepatotoxic doses of acetaminophen in fasted rats was due to a lower availability of the cosubstrate, hepatic levels of UDPGA were determined in both fed and fasted animals. Basal levels of UDGPA were not significantly different between fed and fasted rats, whether calculated per g liver or per total liver per kg rat (Table 1).

After administration of a hepatotoxic dose of acetaminophen (700 mg/kg), hepatic UDPGA levels were rapidly depleted in both fed and fasted rats (Fig. 2). Both the rate of depletion (data not shown) and the nadir of hepatic UDPGA were similar. In contrast, the rate of recovery was markedly slower in fasted rats, suggesting that the fasted rat had a lower capacity to synthesize UDPGA in response to the metabolic demand.

To explore this possibility, the initial rate of UDPGA synthesis required for glucuronidation during the first blood half-life of elimination of acetaminophen in fed and fasted rats was calculated [11] for doses of acetaminophen ranging from nontoxic to toxic. The amount of UDPGA needed for the glucuronidation of acetaminophen during the first elimination half-life of the drug (equivalent to the metabolism of 50% of the dose) was calculated by dividing the total amount of acetaminophen glucuronide recovered in the urine 24 hr after drug administration by two. The "synthetic rate" was then estimated by dividing this amount by the number of hours equivalent to one half-life period of the drug. The data for each dose are expressed as micromoles of UDPGA synthesized per hour per kg rat. In fed rats (Fig. 3A), the initial UDPGA synthetic rate increased markedly with increasing doses of acetaminophen, reaching a plateau of approximately 600 μmol/hr/kg. Also shown in Fig. 3A are values of the apparent rate constant for glucuronide formation (K'_G) for each dose. Of importance, K'_G values were constant throughout the acetaminophen dose range where the UDPGA synthetic rate was increasing (20–400 mg/kg), and fell significantly in the acetaminophen dose range where the UDPGA synthetic rate plateaued (400–1000 mg/kg). These data provide clear evidence that glucuronidation of acetaminophen is capacity limited by UDPGA production, and that this occurs at doses above 400 mg/kg in fed rats.

In fasted rats (Fig. 3B), the initial UDPGA synthetic rate also increased with increasing dose of acetaminophen (20–300 mg/kg), reaching a lower plateau (approximately 400 μmol/hr/kg) as compared to fed rats. Values of K'_G for fasted rats declined significantly at doses of 300 mg/kg and greater. These data indicate that the fasted rat shows the same pattern of capacity limitation of acetaminophen glucuronidation due to limitation of UDPGA production as seen in fed rats, except that the maximum capacity for UDPGA production is markedly lower.

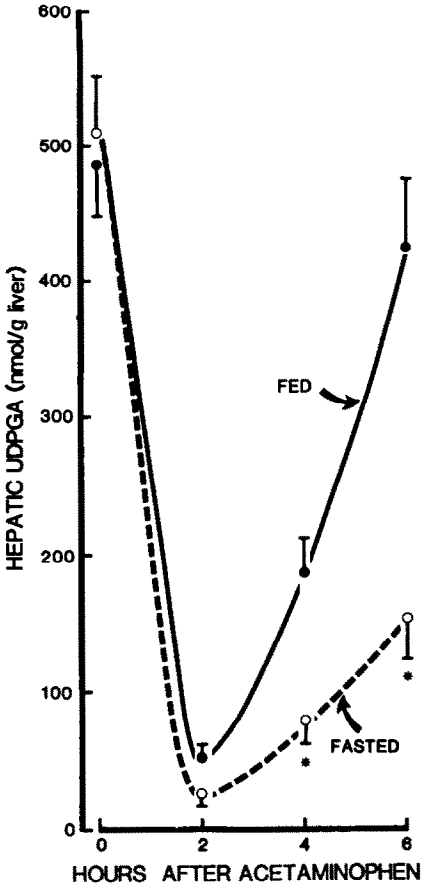

Fig. 2. Effect of acetaminophen on hepatic levels of UDPGA in fed (●) and fasted (○) rats. UDPGA levels were determined before and at various times after administration of acetaminophen (700 mg/kg, i.p.) by the method of Watkins and Klaassen [10]. Values are means ± SE, N = 4, and are representative of three separate experiments. Key: (*) significantly different from fed rats, $P < 0.05$.

Effect of fasting on the activity of UDP-glucose dehydrogenase. UDP-glucose dehydrogenase levels were measured in hepatic cytosolic fractions isolated from fed and fasted rats (Table 2). No significant differences were noted between the two groups, suggesting that the lower rate of UDPGA synthesis seen in fasted rats was not due to decreased levels of the enzyme responsible for its formation.

Effect of fasting on total hepatic levels of NAD^+ and NADH. Since *in vitro* studies have suggested that UDP-glucose dehydrogenase activity may be regulated by cellular levels of NAD^+ and/or NADH

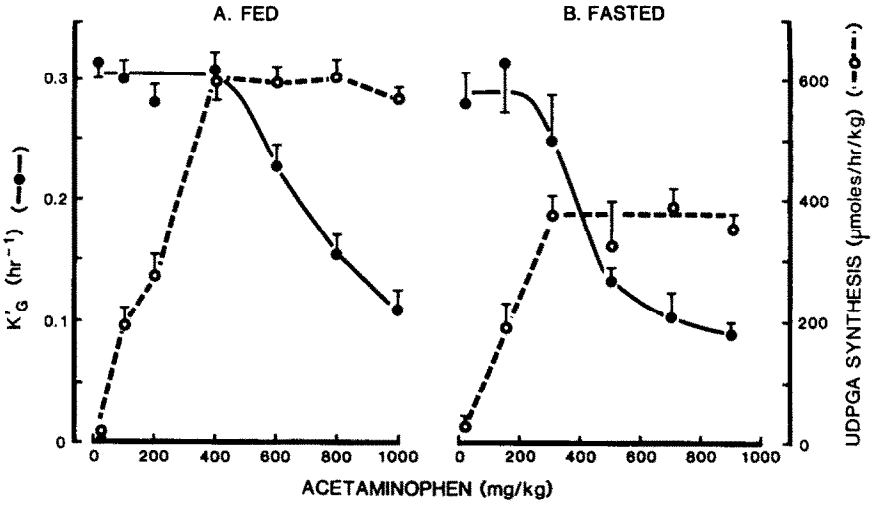

Fig. 3. Effect of dose of acetaminophen on the apparent rate constant for glucuronidation (K'_G) (●) and on acetaminophen-induced initial UDPGA synthesis (○) in the livers of fed (A) and fasted (B) rats. Values of K'_G were determined as described under Methods. Initial UDPGA synthesis was calculated as the total amount of acetaminophen glucuronide in 24-hr urine divided by 2; this amount was divided by one half-life period in hours. Values are means ± SE, N = 4, and are representative of three separate experiments at each dose level.

[15, 16], it is possible that a change in the levels or ratio of these nucleotides could alter the rate of UDPGA synthesis. In agreement with previous workers [17], the total basal levels of NADH in the present study prior to acetaminophen were modestly but significantly increased by fasting, as indicated by the T_o of Fig. 4. Fasting had no effect on basal levels of NAD^+. After administration of acetaminophen (700 mg/kg), NAD^+ levels were not changed significantly in fed and fasted rats, either from the T_o values or from each other. Levels of NADH modestly decreased in both fed and fasted rats. Overall, the changes seen were modest for both NAD^+ and NADH levels in both fed and fasted rats. Collectively, these data suggest that, although the higher initial levels of NADH may contribute to the slower rate of UDPGA synthesis seen in fasted rats, the contribution would probably be minor and transient.

Effect of fasting on hepatic levels of UDP-glucose. Since the activity of UDP-glucose dehydrogenase in the intact liver may be determined by the supply of substrate, hepatic levels of UDP-glucose were measured. Basal levels of UDP-glucose in fasted rats (461 ± 22 nmol/g liver) were only about 60% those

Table 2. Effect of fasting on hepatic levels of UDP-glucose dehydrogenase

Animals*	UDP-glucose dehydrogenase† (nmol/min/g liver)
Fed	390 ± 6
Fasted	370 ± 15

* Animals were allowed food *ad lib.* or were fasted for 24 hr prior to being killed.

† Activity was measured as described under Methods. Values are means ± SE, N = 4, and are representative of two separate experiments.

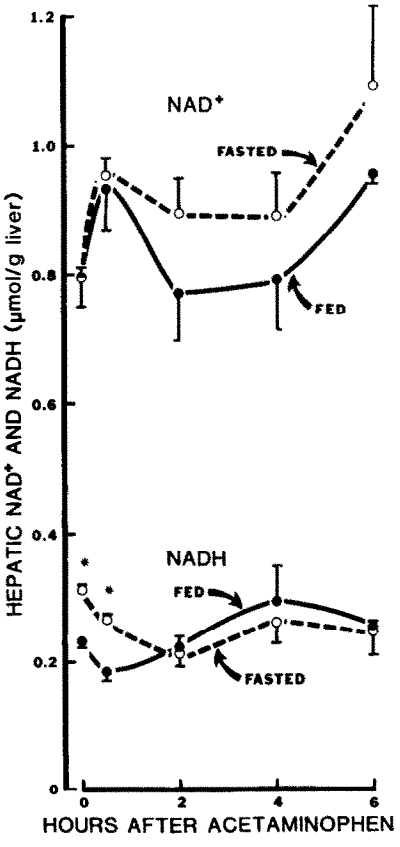

Fig. 4. Effect of acetaminophen on total hepatic levels of NAD^+ and NADH in fed (●) and fasted (○) rats. Nicotinamide adenine nucleotide levels were determined before and at various times after administration of acetaminophen (700 mg/kg, i.p.) by the method of Nisselbaum and Green [14]. Values are means ± SE, N = 4, and are representative of two separate experiments. Key: (*) significantly different from fed rats, $P < 0.05$.

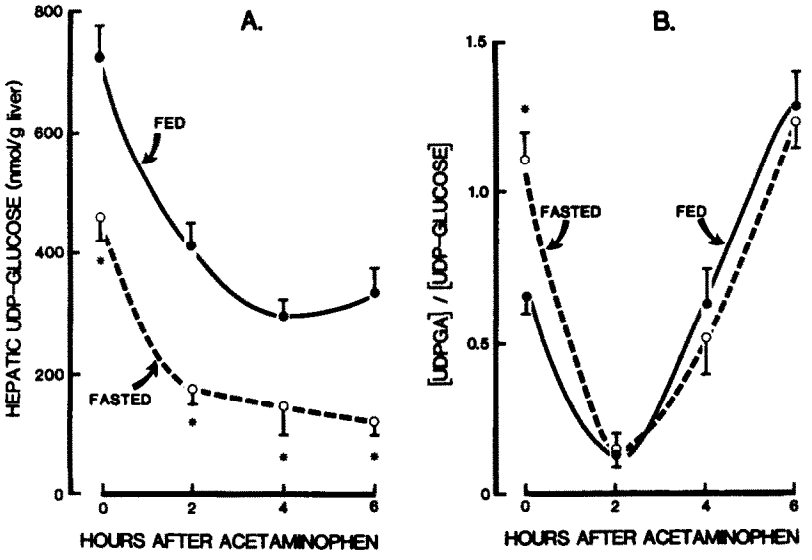

Fig. 5. Effect of acetaminophen on hepatic levels of UDP-glucose (A) and on the ratio of hepatic levels of UDPGA/UDP-glucose (B) in fed (●) and fasted (○) rats. UDP-glucose levels were determined before and at various times after administration of acetaminophen (700 mg/kg, i.p.) as described under Methods. Values are means ± SE, N = 4, and are representative of two separate experiments. Key: (*) significantly different from fed rats, $P < 0.05$.

of fed rats (725 ± 34 nmol/g liver) ($P < 0.05$). After administration of acetaminophen (700 mg/kg), UDP-glucose levels fell rapidly in both groups to approximately 30–40% of initial levels and remained low throughout the observation period (6 hr after drug administration) (Fig. 5A). At all times studied, UDP-glucose levels were significantly lower in fasted than in fed rats, raising the possibility that the slower rate of UDPGA synthesis in fasted rats was the result of lower availability of the precursor, UDP-glucose.

Of interest, the ratio of hepatic levels of UDPGA/UDP-glucose after administration of a hepatotoxic dose of acetaminophen was calculated for fed and fasted rats. This ratio can be considered as an estimate of the *in vivo* activity of UDP-glucose dehydrogenase during the acetaminophen-induced high metabolic demand for UDPGA. As shown in Fig. 5B, at the 2-hr time point after acetaminophen overdose, there was a marked decrease in the ratio in both fed and fasted rats, indicating that at this time period there was a marked limitation at the level of UDP-glucose dehydrogenase activity. However, at later times (4–6 hr after acetaminophen) the ratio returned to normal in both fed and fasted rats, implying that the rate-limiting step for UDPGA synthesis had shifted upstream of the dehydrogenase.

Effect of fasting on hepatic levels of glycogen. UDP-glucose is a common precursor for the formation of both UDPGA and glycogen. Since hepatic glycogen depletion has been reported to occur following hepatotoxic doses of acetaminophen in mice and rats [18–20] and Thurman and colleagues [21, 22] have suggested that availability of glucose from glycogen may be a major determinant of UDPGA formation, we examined the effect of acetaminophen on hepatic levels of glycogen in fed and fasted rats. As shown in Fig. 6, basal levels of glycogen in fasted rats were only 2% of those in fed rats. In agreement with previous workers [18–20], administration of acetaminophen (700 mg/kg) to fed rats induced a rapid depletion of hepatic glycogen to approximately 10% of the initial levels. In fasted rats, acetamino-

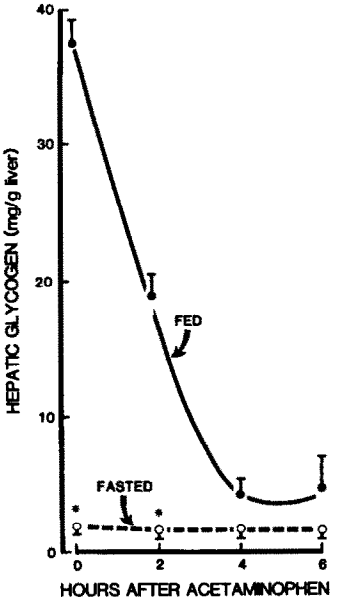

Fig. 6. Effect of acetaminophen on hepatic levels of glycogen in fed (●) and fasted (○) rats. Glycogen levels were estimated before and at various times after administration of acetaminophen (700 mg/kg, i.p.) as the differences between total hepatic nonprotein anthrone positive sugars [12] and liver glucose measured by the glucose oxidase method using a kit from the Sigma Chemical Co. Values are means ± SE, N = 4, and are representative of three separate experiments. Key: (*) significantly different from fed rats, $P < 0.05$.

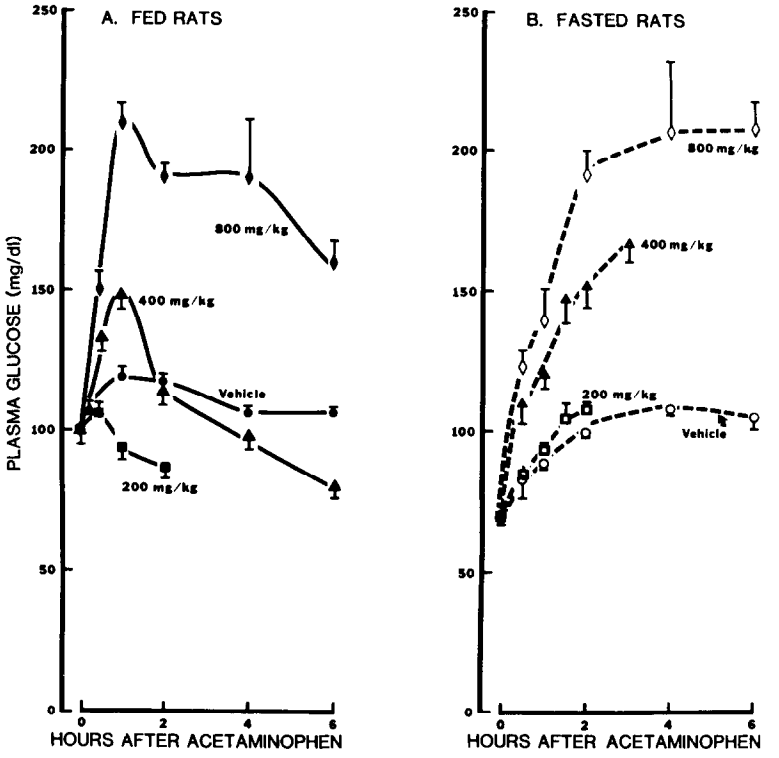

Fig. 7. Effect of acetaminophen on plasma glucose levels in (A) fed (closed symbols) and (B) fasted (open symbols) rats. Blood was collected from the orbital sinus before and at various times after administration of various doses of acetaminophen or vehicle. After centrifugation, glucose levels were determined in plasma by the glucose oxidase method using a kit from the Sigma Chemical Co. Values are means ± SE, N = 4.

phen had no additional effect on the already extensively depleted glycogen levels. Since previous studies have shown that the rate of glucuronidation in fasted rats is approximately 60% of that in fed rats [3], the low glycogen levels in fasted rats suggest that the glucuronidation capacity is not directly dependent on glycogen levels *per se*. These data also imply that the glucose units required for UDP-glucose and UDPGA synthesis in fasted rats must be derived from gluconeogenesis.

Effect of fasting and blood glucose levels. Hinson *et al.* [20, 23] have shown previously that acetaminophen induces a significant increase in blood glucose levels in fed mice. In agreement, administration of acetaminophen to fed rats induced a marked dose-dependent increase in blood glucose levels (Fig. 7A). These data clearly indicate that the glucose-1-phosphate (G1P) formed by the acetaminophen-enhanced breakdown of glycogen is available not only for UDP-glucose and UDPGA formation, but also for conversion to glucose-6-phosphate (G6P), which, in turn, is dephosphorylated and transported out of the liver.

As shown in Fig. 7B, basal levels of blood glucose were significantly lower in fasted rats than in fed rats (70 ± 2 vs 100 ± 5 mg/dl) ($P < 0.05$). Surprisingly, administration of acetaminophen also induced a time- and dose-dependent increase in blood glucose levels in fasted rats. The time–course of the increase in blood glucose levels after acetaminophen appeared to be slightly slower in fasted than in the fed animals although the extent of elevation was similar and the duration was prolonged slightly. These data suggest that, since the glucose reservoir, glycogen, is essentially depleted by fasting, the increased blood glucose levels must be derived from hepatic gluconeogenesis.

DISCUSSION

Previous studies have shown that the fasting-induced potentiation of acetaminophen hepatotoxicity in rats is associated with a decreased glucuronidation capacity [3]. Conceptually, two major factors determine the rate of glucuronidation in the high-dose situation: (a) the activity of the enzyme, glucuronyl transferase, and (b) the availability of the co-substrate, UDPGA. The present data indicate that the fasting effect was not due to depression of the amount of glucuronyl transferase enzyme in the liver, as determined by measurement of the V_{max} value in isolated microsomes (Fig. 1). These findings are in agreement with previous observations *in vivo* that both fed and fasted rats had similar values of the apparent rate constant for glucuronidation (K'_G) after therapeutic doses of acetaminophen [3]. At these low doses, glucuronidation is not limited by the availability of UDPGA [7], and K'_G may be taken to reflect the enzymatic first-order rate constant (V_{max}/K_m) for glucuronyl transferase *in vivo* [11].

Since it is likely that the K_m values are generally similar in fed and fasted rats, it would follow that the lack of difference in K'_G at these low doses reflects the lack of difference in V_{max} of the enzymes in the intact livers. Collectively, the data do not support the hypothesis that the lower maximal glucuronidation capacity for acetaminophen observed in fasted rats is due to lower levels of the glucuronyl transferase enzyme.

In initial studies on the possible role of UDPGA availability in limiting the glucuronidation of high doses of acetaminophen, similar basal (predrug) levels of UDPGA were observed in fed and fasted rats (Table 1, Fig. 2), indicating that basal levels *per se* do not determine the glucuronidation capacity of the fasted animals. This conclusion is in agreement with previous observations in fed animals [7], which indicated that the amount of UDPGA required for the glucuronidation of a high dose of acetaminophen (800 mg/kg) may exceed basal levels by a hundred-fold or more As shown in Fig. 2, fasted animals given a hepatotoxic dose of acetaminophen resynthesized UDPGA at a slower rate, suggesting that the fasted rat had a lesser capacity to respond to the metabolic demand for UDPGA. Estimation of the rate of UDPGA synthesis during the first half-life of acetaminophen indicated that, in fed rats, the maximal capacity to synthesize UDPGA was approximately 600 μmol/hr/kg rat. In contrast, fasted rats had a significantly lower maximal capacity for UDPGA production of approximately 400 μmol/hr/kg rat (Fig. 3). Thus, while in both fed and fasted animals the glucuronidation of high doses of acetaminophen appears to be capacity limited by UDPGA production, this limitation is significantly greater in fasted animals.

Although the effect of fasting on intermediary carbohydrate metabolism has been studied extensively, relatively little is known about the effect of fasting on the regulation of UDPGA production. As illustrated in Fig. 8, there are numerous interrelationships between UDPGA formation and the rest of intermediary carbohydrate metabolism. The complexity of the system indicates that regulation of UDPGA synthesis could occur at a variety of sites. In the present study, the data indicate that fasting does not decrease the amount of the enzyme, UDP-glucose dehydrogenase (Table 2) or cause major perturbation in the level of the cofactor, NAD^+ (Fig. 4). Further, administration of an hepatotoxic dose of acetaminophen did not alter significantly hepatocellular NAD^+ levels in either fed or fasted animals. Thus, changes in cellular amounts of the enzyme or the cofactor do not appear to be responsible for the greater limitation of UDPGA production seen in the fasted animals given acetaminophen.

The situation in regard to NADH levels, however, is more complex. NADH levels could control UDP-glucose dehydrogenase activity directly by feedback inhibition as has been suggested by *in vitro* studies or by changes in cellular redox state [15, 16]. Experimentally, fasting induced a modest but statistically significant increase in hepatocellular NADH levels over that of fed animals and this elevation was maintained for at least 30 min after administration of

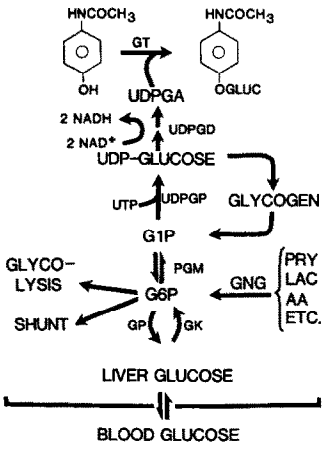

Fig. 8. Interrelationships between acetaminophen glucuronidation and intermediary metabolism of glucose in the liver. Abbreviations: GT, glucuronyl transferase; UDPGD, UDP-glucose dehydrogenase; UDPGP, UDP-glucose pyrophosphorylase, PGM, phosphoglucomutase; GNG, gluconeogenesis; GP, glucose-6-phosphatase; GK, glucokinase; PYR, pyruvate; LAC, lactate; and AA, amino acids.

a hepatotoxic dose of acetaminophen (Fig. 4). In agreement, calculation of the ratio of UDPGA to UDP-glucose (Fig. 5B) indicated that, at early time points during the metabolism of a high dose of acetaminophen, the UDPGA levels fell more than the UDP-glucose levels, suggesting that during this early phase the dehydrogenase activity was rate-limiting in fasted animals. However, the fall in the ratio of UDPGA to UDP-glucose also occurred in fed animals, indicating that the fed animals experienced a similar early transient phase in which UDPGA formation was limited by UDP-glucose dehydrogenase activity. Since NADH levels were not elevated during this time period in fed animals, it seems unlikely that NADH levels *per se* are a major factor determining the activity of the overall pathway. However, it should be noted that the NADH levels reported here are total liver levels and it remains possible that the cytoplasmic compartment within the liver may experience more profound changes and hence be more important in control of cytoplasmic enzymes.

Of importance, the ratio of hepatic UDPGA to UDP-glucose later returned to pre-drug values in both groups of animals (Fig. 5B), indicating that, post 2 hr, the dehydrogenase was not rate-limiting and that the overall activity of the pathway was being determined by steps prior to UDP-glucose formation. Of particular interest, fasting significantly decreased levels of UDP-glucose, both before and after administration of acetaminophen (Fig. 5A). This observation suggests that fasted rats have an overall lower capacity to produce UDP-glucose, both basally and during metabolic demand.

In agreement with earlier workers [20, 23], administration of acetaminophen to fed rats induced a marked depletion of hepatic glycogen levels and a significant increase in blood glucose levels (Figs. 6 and 7). Hence, the source of glucose units for UDP-

glucose formation in fed rats is probably glycogen. Fasted rats, in contrast, were extensively depleted of basal hepatic glycogen, and acetaminophen had no further effect on glycogen levels (Fig. 6). However acetaminophen still induced a major and dose-dependent increase in blood glucose levels, similar in extent to that seen in fed rats (Fig. 7). Clearly, the fasted animals were capable of producing glucose units in amounts not greatly dissimilar to those available in the livers of fed animals. The source of the increased blood glucose in these fasted rats is presumably gluconeogenesis, which is known to operate at a higher rate in the fasted state [24, 25]. Thus, the difference in UDPGA production in fasted versus fed rats appears to be due not to a deficiency of glucose units *per se*, but rather to the fate of G6P equivalents derived from gluconeogenesis.

As shown in Fig. 8, G6P has many possible fates. Of importance for acetaminophen glucuronidation, G6P can be converted to G1P by the action of phosphoglucomutase, which can then be converted to UDP-glucose by the action of UDP-glucose pyrophosphorylase and UTP. Aw and Jones [26, 27] have shown that, under hypoxic conditions, the production of UDPGA in isolated hepatocytes is determined by UDP-glucose pyrophosphorylase activity. However, extrapolation from the isolated cells to the *in vivo* situation is known to be difficult, as illustrated for example by studies indicating that glucose, which increases glucuronidation in intact hepatocytes [16, 21, 28–31], did not have the same effect *in vivo*.* The role of UDP-glucose pyrophosphorylase and/or UTP in the regulation of UDPGA formation *in vivo* is presently unknown.

In addition to conversion to UDP-glucose, G6P may be utilized in glycolysis or the hexose monophosphate shunt (Fig. 8). However, the activities of both of these pathways are known to be reduced by fasting [24, 32]. On the other hand, hepatic glucose-6-phosphatase catalyzed hydrolysis of G6P to glucose is known to be enhanced by fasting [33]. Further, the complexity of the system has been indicated by the studies of Herrera *et al.* [34] and McGarry *et al.* [35] who suggest that liberation of gluconeogenically-derived glucose units into the blood of fasted animals occurs mainly by way of glycogen formation and breakdown. The complexity of the present situation is magnified because acetaminophen itself causes major perturbations of intermediary carbohydrate metabolism, as shown by the marked depletion of glycogen levels and elevation of blood glucose levels [20, 23]; (Fig. 7) and the marked elevation of insulin levels [23]. While the present studies do not allow precise identification of the rate-limiting step for UDPGA synthesis, it is possible that, in the fasted animal, the ratio of glucose-6-phosphatase to phosphoglucomutase and/or UDP-glucose pyrophosphorylase is higher than it is in fed animals such that a greater proportion of glucose phosphate units formed in the fasted liver are hydrolyzed and released into the bloodstream. That is, the fate of G1P and G6P units formed via glycogen degradation and gluconeogenesis depends on the relative activities of the enzymes of intermediary carbohydrate metabolism utilizing those substrates.

Collectively, the present data suggest that UDPGA formation, hence acetaminophen glucuronidation, is dependent upon the rate and direction of flux of glucose equivalents through the pathways of intermediary carbohydrate metabolism, and that these are different in fasted animals. It is of interest that those acetaminophen-overdosed patients who show the greatest prolongation of acetaminophen blood half-lives are at greatest risk of developing liver injury [36]. Since glucuronidation is the major pathway of clearance in these overdosed patients [37] as in animals [5, 6, 38], and hence the major determinant of prolonged elimination half-lives and subsequent toxicity, it is possible that the glucuronidation capacity of these patients is similarly dependent on their capacity to synthesize UDPGA and that this is, in turn, dependent on their metabolic poise of intermediary carbohydrate metabolism. Of particular interest, since suicide-prone individuals may have inadequate nutrition immediately prior to suicide attempts [39, 40], fasting-induced alterations in intermediary carbohydrate metabolism may play a similar role as it does in fasted animals.

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