Biochemical Pharmacology, Vol. 36, No. 4, pp. 427–433, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00
Pergamon Journals Ltd.

MECHANISMS OF FASTING-INDUCED POTENTIATION OF ACETAMINOPHEN HEPATOTOXICITY IN THE RAT*

VERONICA F. PRICE†, MARION G. MILLER‡ and DAVID J. JOLLOW
Department of Pharmacology, Medical University of South Carolina, Charleston, SC 29425, U.S.A.

(*Received* 10 *February* 1986; *accepted* 26 *June* 1986)

Abstract—The effects of an acute fast on acetaminophen metabolism and hepatotoxicity were investigated in male Long Evans Hooded rats. Histologic studies confirmed that fasting potentiated acetaminophen-induced hepatic necrosis. The previous known fasting-induced decrease in hepatic levels of glutathione and depletion of glycogen levels were also confirmed. Pharmacokinetic studies revealed that, at high dose levels of acetaminophen, fasting decreased the overall rate of elimination as evidence by a longer blood half-life of the drug. The decreased clearance was largely the result of decreases in the apparent rate constants for glucuronidation (*ca.* 40%) and for sulfation (*ca.* 30%). Fasting had no significant effects on the apparent rate constants for formation of either acetaminophen mercapturate or the methylthio derivatives. The depression of the nontoxic glucuronidation and sulfation pathways resulted in an increased proportion of the dose converted to the toxic metabolite and, hence, contributed to the potentiation of liver injury in fasted rats. In addition, these studies demonstrated that significant glucuronidation capacity (*ca.* 60% of that in fed rats) was maintained in fasted rats, indicating that: (a) the glucuronidation capacity was not directly correlated with glycogen levels; and (b) in fasted rats the glucose required for UDP-glucuronic acid formation for acetaminophen glucuronidation was supplied from sources other than glycogen.

It is well known that fasting increases the susceptibility of rats [1, 2] and mice [3–6] to acetaminophen-induced liver necrosis. It is also well established that fasting is associated with decreases in liver levels of reduced glutathione in these species [7–9]. Since the ability of liver microsomes isolated from fasted rats to convert acetaminophen to its reactive metabolite was not different from that from fed rats [1], it was suggested that the potentiation of hepatic necrosis was due to the lower levels of glutathione available to detoxify the reactive metabolite, rather than to an enhanced formation of the reactive metabolite *in vivo* [1, 2].

Fasting is also well known to deplete liver levels of glycogen [10] and has been reported to lower the hepatic level of the glucuronidation co-substrate, UDP-glucuronic acid (UDPGA) [11]. Since hepatotoxic doses of acetaminophen are cleared predominantly by glucuronide conjugate formation [12, 13], which in turn is dependent on the rate of UDPGA synthesis [14], we postulated that fasting may decrease the clearance of acetaminophen by glucuronidation. A decrease in the nontoxic clearance by glucuronidation would result in an increase in the half-life of the drug and hence an increase in the proportion of the dose converted to the reactive metabolite [15]. The purpose of the present study was to determine whether an acute fast alters acetaminophen glucuronidation and, if so, to evaluate the role of this effect in the fasting-induced potentiation of acetaminophen hepatotoxicity.

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: 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 at 11:00 a.m., 24 hr prior to administration of acetaminophen. All experiments were initiated at 11:00 a.m.

Assessment of liver necrosis. Groups of fed and fasted rats received various doses of acetaminophen (150–1000 mg/kg, i.p., dissolved in 20% Tween 80 in normal saline). Approximately 24 hr later, a blood sample was withdrawn 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 48 hr after receiving acetaminophen, the animals were decapitated, and slices of their livers were prepared for light microscopy by the method of Culling [16] as previously described [13]. Quantitative analysis was carried out by the method of Chalkley [17] as described by Mitchell *et al.* [18].

Metabolism studies. Animals received a single i.p. injection of [^{3}H]acetaminophen (Amersham Corp., Arlington Heights, IL) (20, 400, or 700 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

* A portion of this work was presented at the FASEB Annual Meeting, Chicago, IL, U.S.A. (1983).

† Correspondence should be addressed to: Dr. V. F. Price, Department of Pharmacology, Medical University of South Carolina, 171 Ashley Ave., Charleston, SC 29425, U.S.A.

‡ Present address: Department of Environmental Toxicology, College of Agricultural and Environmental Science, University of California, Davis, CA 95626, U.S.A.

animal using 75-μl heparinized capillary tubes. Blood half-life ($T_{\frac{1}{2}}$) and overall elimination rate constant (β) were determined from concentrations of acetaminophen in serial blood samples as previously described [13]. For each animal urine was collected over dry ice for 24 hr. The urinary metabolites were separated by thin-layer chromatography and quantitated by liquid scintillation spectrometry as previously described [13]. The apparent rate constants for formation of each metabolite (acetaminophen-glucuronide, K'_G, -sulphate, K'_S, -mercapturate, K'_{MA}, 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 [19, 20].

Determinations of hepatic levels of glutathione, glutathione dilsulfide and glycogen. Animals were decapitated at various times after administration of acetaminophen (700 mg/kg, i.p.). Livers were quickly excised and immediately frozen with liquid nitrogen. Subsequently, portions of the livers were homogenized in 4.5 vol. of ice-cold 0.05 M sodium phosphate buffer (pH 7.4) with a Polytron (Brinkmann Instruments, Westbury, NY) after which sulfosalicylic acid supernatant fractions were prepared [18]. Levels of glutathione and glutathione disulfide were determined by the methods of Mitchell *et al.* [18] and Tietze [21] as previously described [13]. Levels of glycogen were estimated as the differences between total hepatic levels of nonprotein anthrone-positive sugars measured by the method of Carroll *et al.* [10], and liver glucose levels were measured by the glucose oxidase method using a kit from the Sigma Chemical Co. (St. Louis, MO).

Statistical analyses. The TD_{50} (dose at which hepatotoxicity was observed in 50% of the animals) values for, and the relative susceptibilities to, acetaminophen-induced hepatic necrosis (with 95% confidence limits) were estimated by the method of Litchfield and Wilcoxon [22] using the percentage of animals showing necrosis (1+ and greater lesions). 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 acetaminophen-induced hepatic necrosis. The extents of hepatic necrosis in fed and fasted rats 48 hr after various doses of acetaminophen were compared (Table 1, Fig. 1). In agreement with previous workers [1, 2], fasted rats were more susceptible to acetaminophen hepatotoxicity than fed rats. The potentiating effects of fasting was seen at all doses greater than 150 mg/kg by both an increased incidence and severity of necrosis. Plasma levels of GOT obtained at 24 hr after acetaminophen were significantly greater in fasted rats at all doses above 400 mg/kg (Table 1). To analyze the statistical significance of the potentiating effect of fasting on acetaminophen hepatotoxicity, the percentage of animals in each dose group showing necrosis (1+ and greater lesions) was plotted against the log of the dose of the drug (Fig. 1). The statistical approach of Litchfield and Wilcoxon [22] was used to calculate the TD_{50} values with 95% confidence limits and the relative susceptibility to liver injury by the fed and fasted animals. The TD_{50} values for fed and fasted rats were 750 (636–885) and 430 (374–495) mg/kg respectively. Fasted rats were significantly more susceptible to hepatotoxicity [1.74 (1.40 to 2.18) times] than were fed rats ($P < 0.05$).

Effect of fasting on hepatic glutathione levels. In agreement with earlier work [7–9], fasting significantly decreased basal glutathione levels by approximately 30% as measured by the specific assay of Teitze [21] (Fig. 2) (6.77 ± 0.83 μmoles/g liver in fed rats vs 4.68 ± 0.58 μmoles/g liver in fasted rats, $P < 0.05$). Administration of acetaminophen (700 mg/kg, i.p.) caused depletion of glutathione in both groups of rats. The nadir of glutathione depletion was significantly lower in the fasted rats (0.77 ± 0.12 μmoles/g liver) as compared with the fed rats (1.74 ± 0.19 μmoles/g liver) ($P < 0.05$). Glutathione disulfide levels remained low (<0.1 μmoles/g liver) at all times in both fed and fasted rats (data not shown).

Fasting is well known to decrease liver and body weights of rats, associated with a loss of glycogen and water content. In these rats an acute fast decreased the liver weight to body weight ratio by approximately 20% (0.046 ± 0.001 in fed vs 0.036 ± 0.001 in fasted rats). Thus, in addition to decreased levels of glutathione in their livers (Fig. 2), fasted rats had smaller livers. Hence total hepatic glutathione was decreased even more markedly than is indicated by the concentration data in Fig. 2. Calculations on a per kg rat basis indicated that fasting significantly decreased basal total hepatic glutathione by approximately 45% (314 ± 38 μmoles/kg fed rat vs 170 ± 21 μmoles/kg fasted rat). The nadir of glutathione depletion after acetaminophen was similarly markedly lower in fasted rats (81 ± 9 μmoles/kg fed rat vs 28 ± 4 μmoles/kg fasted rat).

Effect of fasting on hepatic glycogen levels. In agreement with previous workers [10], an acute fast depleted basal glycogen levels to less than 2% of the levels in fed rats (Fig. 3). Administration of acetaminophen (700 mg/kg) induced a dramatic fall in glycogen levels in fed rats, similar to that seen in fed mice by Hinson *et al.* [6]. Acetaminophen had no significant effect on residual glycogen levels in fasted rats.

Effect of fasting on blood half-life of acetaminophen. To examine the effect of fasting on the rate of elimination of acetaminophen, the disappearance of the drug from blood was followed for 6 hr after drug administration. As shown in Fig. 4, a dose of 700 mg/kg was eliminated at a significantly slower rate by fasted rats than by fed rats. Rates of elimination of additional doses are shown in Table 2. After a therapeutic dose (20 mg/kg), the half-life was similar in fed and fasted rats, whereas after a threshold toxic dose (400 kg/kg) the drug half-life was significantly longer in fasted rats. The peak concentrations of acetaminophen at the earliest times blood was sampled (15 and 30 min) were essentially equal for fed and fasted rats, indicating equal rates of drug

Table 1. Effect of an acute fast on acetaminophen-induced hepatic necrosis in rats

Acetaminophen dose (mg/kg)	No. of rats	Mortality* (% of rats)	Extent of necrosis† (% of survivors) 0	1+	2+	3+	4+	GOT‡ (units/l)
Fed								
150	10	0	100					130 ± 13
300	10	0	100					74 ± 16
400	12	0	88	12				211 ± 27
500	12	0	88	12				280 ± 87
600	10	0	75	13	12			343 ± 108
700	16	0	62	38				471 ± 40
800	10	10	43	29	25	4		1232 ± 318
1000	12	33	0	75	13	12		2174 ± 468
Fasted								
150	10	0	100					292 ± 25
300	12	0	83	17				185 ± 36
400	17	0	47	29	24			479 ± 111
500	8	0	38	25	25	12		1323 ± 795§
600	17	0	35	18	29	18		2062 ± 551§
700	26	4	4	44	52			2482 ± 375§
800	30	10	4	7	37	48	4	3183 ± 494§
1000	10	40	0	0	17	50	33	3858 ± 590§

* Death occurred between 24 and 48 hr after administration of acetaminophen.

† Extent of hepatic necrosis was scored in livers from rats killed 48 hr after administration of acetaminophen: 0 = no necrosis; 1+ = necrosis of 5% or less of hepatocytes; 2+ = necrosis of 6–25% of hepatocytes; 3+ = 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 as described in Methods. Values are means ± SE.

§ Significantly different from fed rats at same dose level, $P < 0.05$.

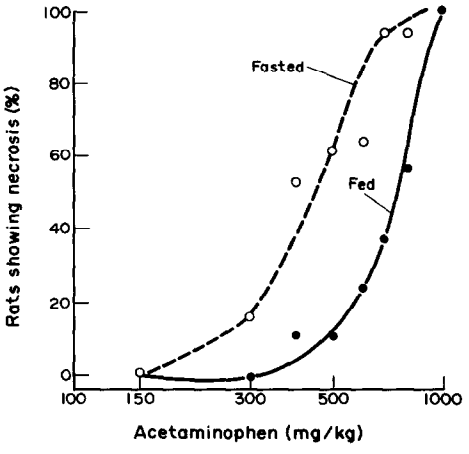

Fig. 1. Effect of dose of acetaminophen on the percentage of fed (●) and fasted (○) rats showing hepatic necrosis 48 hr after receiving various doses of acetaminophen.

absorption. In agreement, the volume of distribution of acetaminophen was constant for fed and fasted rats at all doses of the drug.

Effect of fasting on the metabolic disposition of acetaminophen. To examine the mechanism by which fasting decreased the rate of elimination of the higher doses of acetaminophen, the urinary metabolite compositions were determined (Table 2). In agreement with earlier studies [13], sulfation was the major route of elimination after a low dose of acetaminophen (20 mg/kg). As the dose was increased to 400 and 700 mg/kg, the proportion of metabolites

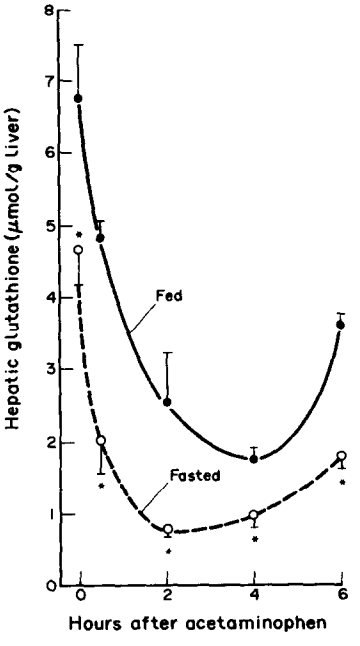

Fig. 2. Effect of acetaminophen on hepatic levels of glutathione in fed (●) and fasted (○) rats. Glutathione levels were measured before and at various times after administration of acetaminophen (700 mg/kg, i.p.) as described under Methods. Values are means ± SE, N = 4, representative of three separate experiments. (*) Significantly different from fed rats ($P < 0.05$).

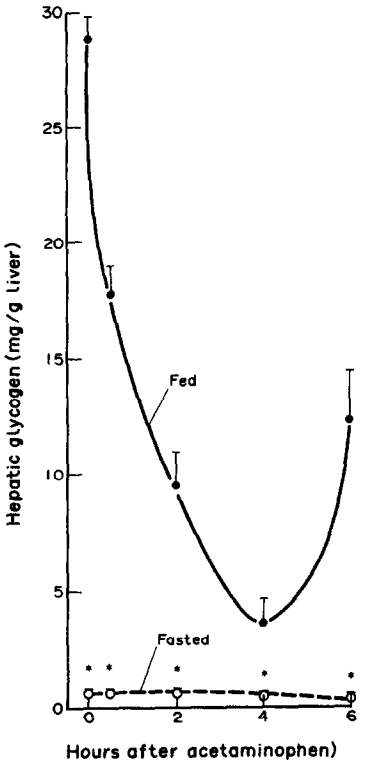

Fig. 3. Effect of acetaminophen on hepatic levels of glycogen in fed (●) and fasted (○) rats. Glycogen levels were estimated before and at various times after administration of acetaminophen (700 mg/kg, i.p.) as described under Methods. Values are means ± SE, N = 4, representative of three separate experiments. (*) Significantly different from fed rats ($P < 0.05$).

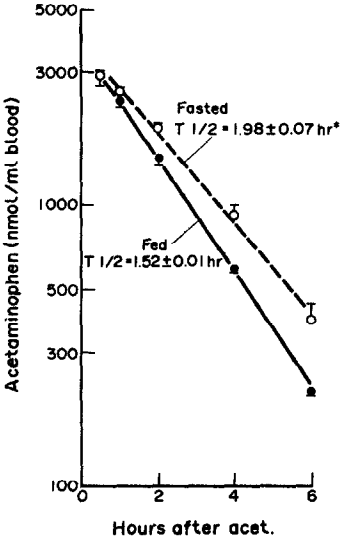

Fig. 4. Effect of fasting on the blood half-life of acetaminophen. Concentrations of acetaminophen were determined at various times after administration of [^{3}H]acetaminophen (700 mg/kg, i.p.) as previously described [13]. Values are means ± SE, N = 4, representative of three separate experiments. Lines of best fit were determined by linear regression analysis ($r > 0.99$). (*) Significantly different from fed rats, $P < 0.05$.

excreted as the sulfate conjugate decreased and the proportion of the glucuronide conjugate increased. Acetaminophen mercapturate and methylthio derivatives were minor metabolites at all doses.

The urinary metabolite composition in fasted rats was generally similar to that seen in fed rats. After administration of a therapeutic dose (20 mg/kg), fasting had no significant effect on any metabolite fraction. As the dose was increased to 400 and 700 mg/kg, the percentage of acetaminophen mercapturate excreted by fasted rats was significantly greater than that by fed rats.

The calculated apparent rate constants for metabolite formation are shown in Table 3. The apparent rate constants for glucuronidation (K'_G) were generally similar to those previously reported [13]. In further agreement to the earlier studies [13], the apparent rate constant for sulfation (K'_S) decreased as the dose of acetaminophen was increased; this pattern is consistent with the well known capacity limitation of this pathway [12, 23] as well as with Michaelis–Menten–Henri kinetic relationships [15]. The apparent rate constant for acetaminophen mercapturate formation (K'_{MA}) also decreased as the dose of acetaminophen was increased.

The effect of dose of acetaminophen on the pattern of apparent rate constants for metabolite formation was generally similar in fasted rats as in fed rats. Both fed and fasted rats had similar glucuronidation capacities at the therapeutic dose (20 mg/kg, Table 3). However, as the dose was increased to 400 and 700 mg/kg, fasted rats showed significantly lower capacity for glucuronidation (*ca.* 77 and 61% that of fed rats respectively). At the therapeutic dose, K'_S values were not significantly different in fed and fasted rats. Increasing the dose to 400 and 700 mg/kg resulted in a significant reduction of the sulfation capacity in fasted rats (*ca.* 62 and 71% that of fed rats respectively). The 24-hr fast had no significant effect on the apparent rate constant for mercapturate formation at any dose of acetaminophen. Since the rate of formation of the reactive metabolite *in vivo* can be estimated by determining K'_{MA} at nonhepatotoxic subthreshold doses [15], the similar values of K'_{MA} suggest that the potentiating effect of fasting on liver injury cannot be explained by an increase in the apparent rate constant for formation of the reactive metabolite. Similarly, fasting did not alter the apparent rate constant for formation of methylthio metabolites ($K'_{MTAG+SOX}$) throughout the dose range of acetaminophen. The renal elimination rate constant (K_E) values were generally similar for fed and fasted rats and constant throughout the dose range (Table 3), as expected for excretion by nonsaturable renal filtration.

DISCUSSION

Previous studies in rats and mice have demonstrated that fasting potentiates acetaminophen-induced hepatotoxicity [1–6]. Potentiation was associated with a decrease in hepatic levels of glutathione, hence a decreased capacity to detoxify the reactive metabolite of acetaminophen [1, 2]. The

Table 2. Effect of an acute fast on acetaminophen half-life and urinary metabolite composition

Dose	Half life (hrs)	Glucuronide (% of urinary metabolites)	Sulfate	Mercapturate	Methylthio derivatives	Free acetaminophen
20 mg/kg						
Fed	0.34 ± 0.01	14.5 ± 1.5	73.7 ± 2.3	5.60 ± 0.56	3.83 ± 0.53	2.37 ± 0.10
Fasted	0.36 ± 0.02	13.9 ± 0.8	72.9 ± 0.6	6.20 ± 0.45	4.18 ± 0.42	2.90 ± 0.23
400 mg/kg						
Fed	0.94 ± 0.05	46.9 ± 4.1	39.2 ± 3.1	4.78 ± 0.50	1.00 ± 0.10	7.60 ± 0.72
Fasted	1.25 ± 0.02*	49.7 ± 3.8	34.2 ± 3.0	7.95 ± 0.81*	1.60 ± 0.20	6.58 ± 0.09
700 mg/kg						
Fed	1.52 ± 0.01	65.3 ± 1.0	23.6 ± 1.4	3.46 ± 0.06	1.19 ± 0.16	6.60 ± 0.85
Fasted	1.98 ± 0.07*	60.2 ± 1.9*	26.2 ± 1.7	6.40 ± 0.80*	1.47 ± 0.15	5.85 ± 1.11

Animals received various doses of [^{3}H]acetaminophen, i.p., and were placed in individual metabolic cages. Blood and urine was collected and used to determine the half-life and metabolite composition as described previously [13]. Recovery of radioactivity was ≥85%. Values are means ± SE, N = 4, representative of three separate experiments at each dose level.

* Significantly different from fed animals at same dose level, $P < 0.05$.

Table 3. Effect of an acute fast on the kinetic parameters of acetaminophen metabolism in rats

Dose	β (hr^{-1})	Apparent rate constants for metabolite formation*				Renal elimination rate constant K_E (hr^{-1})
		K'_G	K'_S	K'_{MA}	$K'_{MTAG+SOX}$ (hr^{-1})	
20 mg/kg						
Fed	2.037 ± 0.074	0.294 ± 0.030	1.51 ± 0.09	0.114 ± 0.012	0.0078 ± 0.0010	0.048 ± 0.003
Fasted	1.974 ± 0.097	0.272 ± 0.018	1.44 ± 0.07	0.123 ± 0.012	0.0082 ± 0.0008	0.058 ± 0.007
400 mg/kg						
Fed	0.772 ± 0.038	0.358 ± 0.012	0.306 ± 0.037	0.039 ± 0.006	0.0080 ± 0.0004	0.057 ± 0.008
Fasted	0.555 ± 0.009†	0.277 ± 0.024†	0.190 ± 0.016†	0.044 ± 0.004	0.0090 ± 0.0005	0.037 ± 0.001†
700 mg/kg						
Fed	0.457 ± 0.002	0.298 ± 0.004	0.108 ± 0.007	0.016 ± 0.001	0.0066 ± 0.0008	0.030 ± 0.004
Fasted	0.344 ± 0.013†	0.180 ± 0.018†	0.077 ± 0.006†	0.016 ± 0.001	0.0059 ± 0.0006	0.020 ± 0.002

Animals received [^{3}H]acetaminophen (20, 400 or 700 mg/kg, i.p.) and were placed in individual metabolic cages. Sequential blood samples and total urinary collections were obtained for each animal and used to calculate the kinetic parameters as described previously [13]. The apparent rate constant for formation of each metabolite was calculated as $\beta \times$ urinary metabolite fraction [19, 20]. Values are means ± SE, N = 4, representative of three separate experiments at each dose level.

* Apparent rate constants for formation of acetaminophen-glucuronide (K'_G); -sulfate (K'_S); -mercapturate (K'_{MA}); and -methylthio derivatives ($K'_{MTAG+SOX}$).

† Significantly different from fed animals at the same dose level, $P < 0.05$.

results of the present study confirm the fasting-induced decreased hepatic glutathione levels and are in complete agreement with the earlier reports. Of interest, fasted rats had less basal hepatic glutathione expressed in terms of body weight (μmoles/kg rat, approximately 55% of levels in fed rats) than when expressed in terms of liver weight (μmoles/g liver, approximately 70% of levels in fed rats).

However, fasting also has numerous effects on intermediary carbohydrate metabolism [10, 11, 24], and from a theoretical viewpoint fasting could potentiate acetaminophen hepatotoxicity by a second mechanism; that is, by decreasing metabolic clearance through the nontoxic glucuronidation pathway. Decreased clearance by glucuronidation would result in an increase in the half-life of the drug and, hence, an increase in the proportion of the dose converted to the reactive metabolite. Thus, a second major effect of fasting may contribute to potentiation of liver injury.

To examine this postulate, the present study focused on the effects of fasting on the metabolism of acetaminophen. The results indicate that the overall elimination rate constant (β) for a threshold toxic dose (400 mg/kg) and a hepatotoxic dose (700 mg/kg) was lower in fasted rats. The decreased metabolic clearance was largely the result of decreases in the apparent rate constants for glucuronidation and sulfation. Of these two pathways, the contribution of sulfation to β at threshold and toxic dose levels was smaller than that of glucuronidation [12, 13] and, therefore, changes in sulfation made correspondingly small changes in β. The fasting-induced lengthening of the half-life was due largely to a decreased glucuronidation capacity.

The rate of formation of the reactive metabolite (estimated from the apparent rate constant for formation of acetaminophen mercapturate) was not affected by fasting. This result is in agreement with the reports of Pessayre *et al.* [1] that the ability of liver microsomes isolated from fasted rats to convert acetaminophen to its reactive metabolite is not different than that of fed rats. The fraction of the dose of acetaminophen converted to the reactive

metabolite (K'_{MA}/β) was increased significantly after the threshold and toxic doses in fasted rats (Table 4) due to the reduction in β (Table 3). That is, due to the decreased capacity of the liver to clear acetaminophen by the nontoxic glucuronidation and sulfation pathways, a greater amount of the reactive metabolite was formed in cells that had a decreased capacity to detoxify it, due to the lower glutathione levels.

The mechanism by which fasting decreased the glucuronidation capacity could theoretically be due either to decreased glucuronyl transferase activity, or to decreased availability of the cosubstrate, UDPGA. The first possibility seems unlikely because both fed and fasted rats had similar values of K'_G after the therapeutic dose of acetaminophen. At this low dose, glucuronidation was not limited by the availability of UDPGA, and K'_G may be taken to reflect the enzymatic first-order rate constant (V_{max}/K_m) for glucuronyl transferase *in vivo*. Assuming that the K_m values are generally similar in fed and fasted rats, it would follow that differences in K'_G at this dose level would reflect differences in V_{max} of the enzyme in the two groups of animals. Since fed and fasted rats had similar values of K'_G at the therapeutic dose, these data suggest that the amount of enzyme present in their livers was similar. Thus, the data do not support the hypothesis that lower glucuronidation capacity in fasted rats is due to lower levels of glucuronyl transferase.

In parallel studies in fed rats, we have observed that, as the dose of acetaminophen is increased to hepatotoxic levels, the rate of glucuronidation becomes capacity limited [13, 15] due to a decreased ability to synthesize UDPGA, which in turn is dependent on the rate of glucose flux through the glucuronic acid pathway [14]. Thurman and colleagues [24, 25] have used the isolated perfused rat liver to examine the control of glucuronidation of *p*-nitrophenol. They concluded that in the perfused liver system the rate of glucuronidation is determined largely by the supply of glucose from glycogen for UDPAG synthesis [24, 25] rather than by the activity of UDP-glucose dehydrogenase, cellular levels of NAD^+, or the availability of UTP [26]. However, in our fasted rats, liver glycogen was essentially depleted, yet the glucuronidation rate was still approximately 60–70% of that in fed rats. These data indicate that in the intact fasted animal (a) the glucuronidation rate was not directly related to the hepatic glycogen levels; and (b) the glucose required for UDPGA formation for acetaminophen glucuronidation was supplied from sources other than glycogen, namely gluconeogenesis.

Table 4. Effect of fasting on the reactive metabolite fraction (K'_{MA}/β)

Acetaminophen (mg/kg)	K'_{MA}/β	
	Fed	Fasted
20	0.056 ± 0.006	0.062 ± 0.005
400	0.048 ± 0.005	0.080 ± 0.008*
700	0.035 ± 0.001	0.064 ± 0.008*

Rats received various doses of [^{3}H]acetaminophen. Serial blood samples were taken for the estimation of β; the amount of acetaminophen mercapturate in urine was determined and used to calculate K'_{MA}, as previously described [13, 19, 20]. Values are means ± SE, N = 4, representative of three separate experiments at each dose level.

* Significantly different from fed rats at same dose level, $P < 0.05$.

Collectively, the present data indicate that fasting had profound effects on acetaminophen metabolism and hepatotoxicity. Fasted animals have been used in numerous studies to evaluate the metabolism and/or toxicity of other drugs and xenobiotics, particularly with regard to cytochrome P-450-dependent metabolism. Although fasting may not affect the activity of cytochrome P-450 isozymes *per se*, the products of many of these reactions undergo subsequent phase II conjugative reactions with glutathione, UDPGA, and/or phosphoadenosine phosphosulfate. The present studies demonstrate that these phase II reactions may be decreased significantly by fasting, particularly when the substrate is studied in the high dose range where cosubstrate formation may become capacity limiting in clearance. Depression of non-toxic clearance reactions could lead to potentiation of toxicity, as was seen with acetaminophen. Thus, the selection of a fasted versus fed animal for toxicity testing could have profound effects on the severity of the lesion unrelated to the effects on cytochrome P-450 *per se*. It follows that alteration of feeding habits during chronic toxicity testing might alter significantly the sensitivity of the animals to toxicity.

Acknowledgements—This study was supported by a grant from the U.S. Public Health Service (GM 30546) and a Medical University of South Caroline Biomedical Research Grant. The authors thank Jennifer Schulte, Mischelle Johnston and John Peters for excellent technical assistance.

REFERENCES

1. D. Pessayre, A. Dolder, J-Y. Artigon, J-C- Wandscheer, V. Descatoire, C. Degott and J-P. Benhamou, *Gastoeneterology* **77**, 264 (1979).
2. D. Pessayre, J-C. Wandscheer, B. Cobert, R. Level, C. Degott, A. M. Batt, N. Martin and J. P. Benhanou, *Biochem. Pharmac.* **29**, 2219 (1980).
3. A. Wendel, S. Feuerstein and K-H. Konz, *Biochem. Pharmac.* **28**, 2051 (1979).
4. O. Strubelt, E. Dost-Kempf, C-P. Seigers, M. Younes, N. Völpel, U. Preuss and J. G. Dreckmann, *Toxic. appl. Pharmac.* **60**, 66 (1981).
5. R. M. Walker, T. E. Massey, T. F. McElligott and W. J. Racz, *Can. J. Physiol. Pharmac.* **60**, 399 (1982).
6. J. A. Hinson, J. B. Mayo and A. M. Cameron, *Biochem. Pharmac.* **32**, 1979 (1983).
7. G. Leaf and A. Neuberger, *Biochem. J.* **41**, 280 (1947).
8. L. V. Beck, V. D. Rieck and B. Duncan, *Proc. Soc. exp. Biol. Med.* **97**, 229 (1958).
9. E. Maruyama, K. Kojima, T. Higashi and Y. Sakamoto, *J. Biochem., Tokyo* **63**, 398 (1968).
10. N. V. Carroll, R. W. Longley and J. H. Roe, *J. biol. Chem.* **220**, 583 (1956).
11. B. F. Felsher, N. M. Carpio and K. Van-Couvering, *J. Lab. clin. Med.* **93**, 414 (1979).
12. D. J. Jollow, S. S. Thorgeirsson, W. Z. Potter, M. Hashimoto and J. R. Mitchell, *Pharmacology* **12**, 251 (1974).

13. V. F. Price and D. J. Jollow, *J. Pharmac. exp. Ther.* **220**, 504 (1982).
14. V. F. Price and D. J. Jollow, *Xenobiotica* **14**, 553 (1984).
15. D. J. Jollow, S. Roberts, V. Price, S. Longacre and C. Smith, *Drug Metab. Rev.* **13**, 983 (1982).
16. C. F. A. Culling, *Handbook of Histological Techniques*, 2nd Edn. Butterworth, London (1963).
17. H. W. Chalkley, *J. Natn. Cancer Inst.* **4**, 47 (1943).
18. J. R. Mitchell, D. J. Jollow, W. Z. Potter, D. C. Davis, J. R. Gillette and B. B. Brodie, *J. Pharm. exp. Ther.* **187**, 185 (1973).
19. V. F. Price and D. J. Jollow, *Biochem. Pharmac.* **35**, 687 (1986).
20. M. G. Miller, V. F. Price and D. J. Jollow, *Biochem. Pharmac.* **35**, 817 (1986).
21. F. Tietze, *Analyt. Biochem.* **27**, 502 (1969).
22. J. T. Litchfield and F. Wilcoxon, *J. Pharmac. exp. Ther.* **96**, 99 (1949).
23. G. Levy and H. Yamada, *J. pharm. Sci.* **60**, 215 (1971).
24. L. A. Reinke, S. A. Belinsky, R. K. Evans, F. C. Kauffman and R. G. Thurman, *J. Pharmac. exp. Ther.* **217**, 863 (1981).
25. R. G. Thurman, L. A. Reinke, S. Belinsky, R. K. Evans and F. C. Kauffman, *Archs Biochem. Biophys.* **208**, 137 (1981).
26. R. G. Thurman and F. C. Kauffman, *Pharmac. Rev.* **31**, 229 (1980).