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ANOMALOUS SUSCEPTIBILITY OF THE FASTED HAMSTER TO ACETAMINOPHEN HEPATOTOXICITY*

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Abstract—The effect of an acute fast on susceptibility to acetaminophen-induced hepatotoxicity was investigated in male Golden Syrian hamsters. Overnight starvation markedly elevated hepatic levels of glutathione throughout the diurnal cycle (peak concentration: 10.6 ± 0.06 mM vs 7.3 ± 0.3 mM in controls). However, despite this apparent increase in the glutathione protective capacity of the liver, acetaminophen-induced hepatic necrosis was modestly potentiated by fasting, as judged by liver histology and elevation of serum transaminase (SGOT) activity. Parallel pharmacokinetic studies indicated that the overall elimination rate constant for acetaminophen was decreased in fasted animals, due largely to decreases in the apparent rate constants for formation of acetaminophen glucuronide and acetaminophen mercapturate. Formation of acetaminophen sulfate was not affected by fasting. Since the major nontoxic pathway (glucuronide) and the toxic pathway (as measured by mercapturate) decreased to a similar extent, the data indicate that the anomalous lack of protection cannot be explained on the basis of altered metabolic disposition of the drug. Measurement of hepatic glutathione levels revealed that, despite the higher initial level of glutathione in the fasted animals, the nadir to which liver glutathione levels fell after acetaminophen was the same in fed and fasted animals. Comparison of the amount of acetaminophen mercapturate in the urine with the amount of glutathione which disappeared from the liver showed close agreement for fed animals, but a major discrepancy for fasted hamsters. These data indicate that a major fraction of glutathione in the liver of the fasted hamsters is not utilized for detoxification of the acetaminophen reactive metabolite and hence does not contribute to the glutathione protective capacity.

It is well known that acetaminophen overdose causes hepatic necrosis in both man [1] and experimental animals [2]. Mechanistic studies in animals have demonstrated that acetaminophen-induced liver injury is associated with cytochrome P-450-dependent formation of a toxic chemically reactive metabolite [3–5]. At low doses of acetaminophen this reactive metabolite is detoxified by conjugation with hepatic glutathione and, after further metabolism, appears in the urine as the mercapturate derivative [6–8]. However, in the overdose situation, hepatic glutathione stores become depleted and, in the absence of glutathione, the reactive metabolite can covalently bind to hepatocellular macromolecules. This binding reaction has been associated with the development of liver injury [4, 7].

Previous studies have shown that susceptibility to liver injury can be modulated by interventions which affect the glutathione protective capacity of the liver [6, 7]. For example, pretreatment with diethyl maleate depletes glutathione and markedly potentiates hepatic necrosis. It has also been demonstrated that fasting of rats and mice decreases hepatic glutathione levels [9–15] and increases the susceptibility of these species to acetaminophen-induced liver injury [13–18]. From these observations it has been proposed that fasting-induced potentiation of acetaminophen hepatotoxicity is the result of decreased availability of glutathione for conjugation with, and hence detoxification of, the acetaminophen reactive metabolite.

This paper reports that, in contrast to the mouse and rat, the fasted hamster shows a marked elevation of hepatic glutathione levels. Since enhancement of hepatic glutathione should lead to decreased susceptibility to acetaminophen-induced hepatic necrosis [6–19], we examined the effect of fasting on acetaminophen hepatotoxicity in hamsters. We report that contrary to expectations, an acute fast did not protect hamsters from liver injury. The additional glutathione is apparently not available for conjugation with the reactive metabolite of the drug.

MATERIALS AND METHODS

Materials. Unlabeled acetaminophen was obtained from Eastman Organic Chemicals (Rochester, NY). [^{3}H]Acetaminophen was purchased from the New England Nuclear Corp. (Boston, MA). The radiolabeled acetaminophen was checked for purity and, if less than 99.5% pure, impurities were removed by thin-layer chromatography using silica gel thin-layer plates and a chloroform–ethylacetate–acetic acid (6:3:1) solvent mixture. All other chemicals were of reagent grade.

Animals. Male Golden Syrian hamsters (Charles Rivers, Lakeview, NJ) weighing 90–110 g were used

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in all experiments. The animals were maintained under a 12-hr light, 12-hr darkness cycle. Control animals were allowed Wayne Lab Blox (Allied Mills, Chicago, IL) and water *ad lib*. Food was removed from the fasted animals in the evening approximately 12 hr prior to experimentation. All studies were begun at 7:00 a.m. unless indicated otherwise.

Measurement of hepatic glutathione. Fed and fasted hamsters were killed by decapitation. Livers were excised and homogenized in 4 vol. of ice-cold phosphate buffer, 0.05 M, pH 7.0, and after protein precipitation, reduced glutathione was measured spectrophotometrically using Ellman's reagent [6] and by the glutathione-specific assay of Tietze [20]. Glutathione disulfide was measured as previously described [21].

Histological assessment of liver necrosis. Groups of fasted and fed animals received various doses of acetaminophen (i.p.) dissolved in 20% Tween 80 in normal saline. After injection, both groups were deprived of food for 40 hr and then killed by decapitation. The livers were excised, sectioned and fixed in 10% phosphate-buffered formalin. Paraffin sections were prepared and stained with hematoxylin and eosin [22]. The extent of necrosis was quantitated by the method of Chalkley [23] as described by Mitchell *et al.* [3]: 0 = no necrosis, 1+ = necrosis of 1–5% of hepatocytes, 2+ = necrosis of 6–25% of hepatocytes, 3+ = 26–50% of hepatocytes and 4+ = greater than 50% of hepatocytes.

Measurement of serum enzymes. Serum glutamate oxaloacetic transaminase (SGOT) activity was measured to assess liver damage after acetaminophen. Serum was collected from animals used in histology studies and analyzed for GOT activity using a Worthington/Gilford diagnostic kit (Millipore Corp., Freehold, NJ).

Acetaminophen metabolism studies. Fed and fasted animals received intraperitoneal injections of various doses of acetaminophen (20–350 mg/kg) including 200 μCi/kg of [^{3}H]acetaminophen after which each animal was placed in a separate metabolic cage. The concentration of acetaminophen in serial blood samples, the composition of urinary metabolites, and the following kinetic parameters of acetaminophen metabolism: the overall elimination rate constant (β); apparent rate constants for formation of acetaminophen-glucuronide (K'_G), -sulfate (K'_S), -mercapturate (K'_{MA}), methylthioacetaminophen-glucuronide and -sulfoxide ($K'_{MTAG+SO}$); and the renal elimination rate constant (K_E) were determined as previously described [21].

Statistics. Levels of statistical significance were assessed using Student's *t*-test of correlated means for small groups. Significant differences were judged as P values < 0.05.

RESULTS

Effect of fasting on hepatic glutathione levels. The glutathione content of the liver was measured at 6-hr intervals over a 48-hr time period in normal fed hamsters and in hamsters that were deprived of food (Fig. 1). Both groups of animals exhibited a diurnal rhythm in the concentration of hepatic glutathione. Glutathione disulfide (GSSG) levels remained low (<0.1 mM) at all time intervals. In the fed hamster, glutathione levels rose throughout the evening to a peak of approximately 8 mM around midnight and then fell to a nadir of about 6.5 mM at noon. Fasted animals showed a similar cycling; however, the glutathione content of the liver was elevated markedly by food deprivation. These enhanced levels of glutathione were maintained throughout the 48-hr

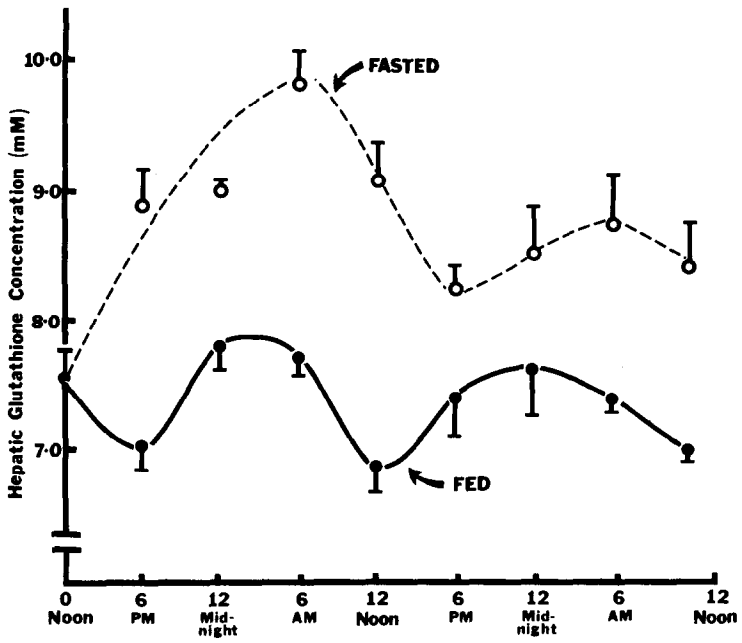

Fig. 1. Diurnal variation of hepatic glutathione levels in fed (●) and fasted (○) hamsters. Food was removed from fasted animals at noon. Glutathione levels were determined as described in Materials and Methods. Values are means ± S.E., N = 4.

Table 1. Effect of an overnight fast on acetaminophen-induced hepatic necrosis in hamsters

Acetaminophen treatment (mg/kg)	No. of animals	Extent of necrosis*					Mortality†
		0	1+	2+	3+	4+	
		% Animals					
Fed							
150	10	90	10				0
175	10	70	30				0
200	22	45	32	14	9		0
250	10	30	30	30	10		0
300	10			30	70		0
Fasted							
150	10	80	20				0
175	10	60	30	10			0
200	22	23	23	27	18	9	0
250	10			20	50		30
300	10			10	30	10	50

* Extent of hepatic necrosis was scored in liver from hamsters killed 40 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.

† Death occurring between 24 and 40 hr after acetaminophen administration.

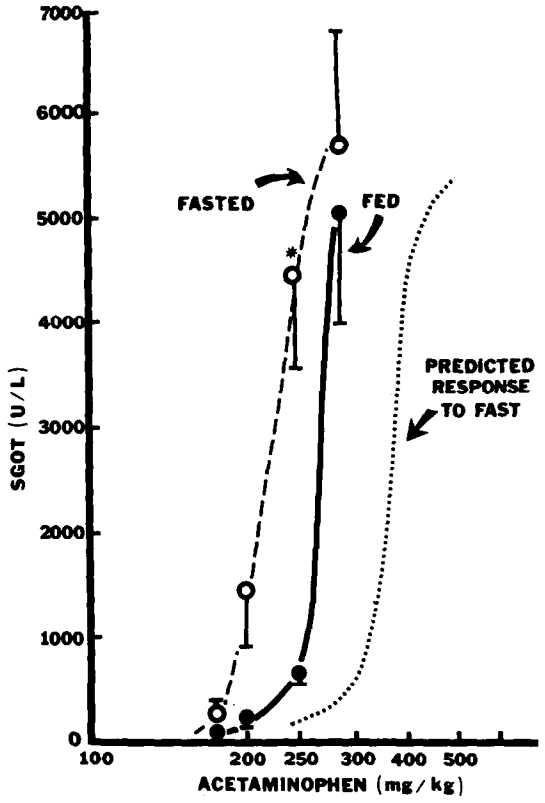

Fig. 2. Effect of fasting on SGOT levels in hamsters. Animals were allowed food *ad lib.* (●) or fasted for 12 hr (○) prior to receiving various doses of acetaminophen (i.p.). Approximately 40 hr later serum was obtained and SGOT levels were determined as described in Materials and Methods. Values are means ± S.E., N ≥ 10. An asterisk (*) indicates significantly different from fed hamsters at same dose level, $P < 0.05$. The position of the predicted dose–response curve for fasted hamsters was estimated by calculating the additional amount of acetaminophen required to deplete the extra glutathione present in their livers (see text).

experimental period but were more pronounced between 12 and 24 hr after removal of food. Since the effect on glutathione was greatest at these times, the fasted animals in all subsequent studies were subjected to a 12-hr overnight fast prior to experimentation at 7:00 a.m. This overnight 12-hr fast had no significant effects on either total body weight or liver wet weight of the hamsters.

Effect of fasting on acetaminophen-induced hepatic necrosis. Acetaminophen was administered to fed hamsters and to hamsters that had been fasted overnight. Histological examination of the livers revealed the characteristic centrilobular lesion previously described after large doses of acetaminophen [3]. This lesion increased both in incidence and severity with increasing dose (Table 1). Food deprivation for 12 hr prior to administration of acetaminophen did not protect the hamsters against acetaminophen-induced hepatic necrosis. As judged by both the percentage of animals showing the lesion and the severity of the lesion, hepatotoxicity was modestly enhanced (Table 1). In addition, a significant number of the fasted animals died. Since these deaths occurred 24–40 hr after administration of the drug, when liver injury would have developed, it is likely that death was associated with hepatotoxicity.

Hepatic necrosis was also assessed by serum transaminase elevation (SGOT). As shown in Fig. 2, both fed and fasted hamsters showed a dose-dependent rise in SGOT levels. The elevation in fasted hamsters occurred at lower doses than in the fed animals as indicated by the statistical difference in the values after 250 mg acetaminophen/kg. For comparison purposes, Fig. 2 also shows a predicted dose-response for fasted hamsters based on the extra glutathione content of their livers; the shift in this curve relative to that of the fed animals was estimated by calculating the additional amount of acetaminophen required to deplete the extra glutathione present in the liver of fasted animals (Fig. 1).

Effect of fasting on the rate of acetaminophen elim-

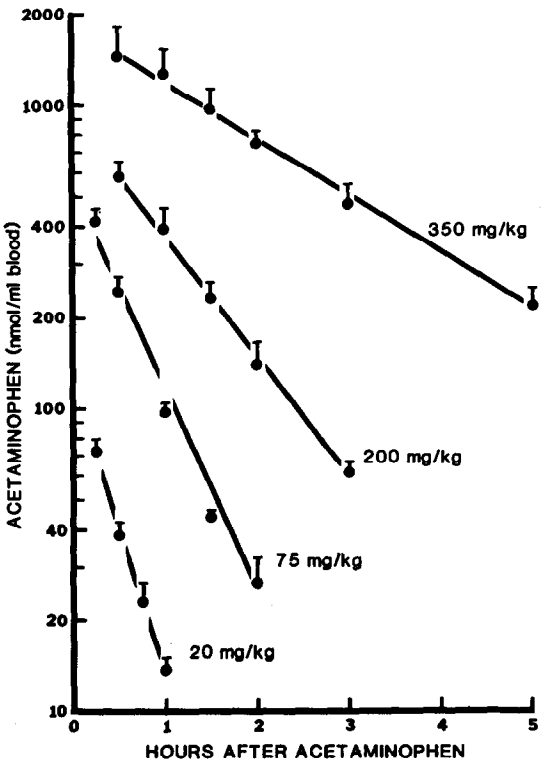

Fig. 3. Effect of dose of acetaminophen in the blood half-life in fed hamsters. Concentrations of acetaminophen were determined as previously described [21]. Values are means ± S.E., N = 4. The lines of best fit were determined by linear regression analysis ($r > 0.99$).

ination. The disappearance of acetaminophen from the blood was followed for a period of about four half-lives, corresponding to the elimination of over 90% of each dose of acetaminophen. In agreement with earlier reports [21, 24, 25], the elimination of acetaminophen was linear on semi-log plots throughout the time period of observation, at all doses of the drug used in this study (Fig. 3). The slope of the lines, however, decreased with increase in dose reflecting a progressive decrease in the overall elimination constant (β, Table 2), indicating capacity limitation of the major pathways of clearance [25]. Fasted hamsters showed similar linearity and a similar decrease in β as the dose was increased (Table 2). However, at dose levels above 20 mg/kg, β was significantly less in fasted animals as compared with the corresponding fed animals. In addition, peak concentrations of acetaminophen at the earliest time blood was sampled (15 and 30 min) were essentially equal for fed and fasted hamsters, indicating equal rates of drug absorption. In agreement, the volume of distribution of acetaminophen was constant for fed and fasted hamsters at all doses of the drug.

Effect of fasting on the metabolic disposition of acetaminophen. To investigate the mechanism by which fasting slows the overall elimination of acetaminophen, the apparent rate constants for the formation of each metabolite (K') were calculated. Since in this situation acetaminophen shows first-order, single-compartment kinetics and its metabolites are eliminated by renal excretion, the apparent rate constants can be estimated as the product of the overall elimination rate constant and the urinary metabolite fraction [21, 25, 26].

Figure 4 shows the effect of dose on the apparent rate constants for formation of each metabolic pathway of acetaminophen in both fed and fasted animals; the effect of dose on K_E is also shown. Fasting decreased the apparent rate constant for glucuronidation at all doses of the drug (Fig. 4A). The reduction was statistically significant at the lower doses. In contrast, the apparent rate constant for sulfation (Fig. 4B) and the renal elimination rate constant (Fig. 4C) were not decreased in fasted hamsters. Generally, mercapturate formation (Fig. 4D) and methylthio-derivative formation (Fig. 4E) were depressed by fasting; the decrease in mercapturate formation was significant at doses of 75, 200, and 350 mg/kg, whereas methylthio derivative formation was significant at only 75 and 350 mg/kg.

Collectively, these data indicate that the reduction in the overall elimination rate constant (β) was largely due to a decrease in the apparent rate constant for glucuronidation with lesser but significant contribution from depression of the P-450-dependent mercapturate pathway.

Effect of fasting on acetaminophen-dependent depletion of hepatic glutathione. Hepatic glutathione levels were measured in fed and fasted hamsters after administration of 20, 75, 150, 200 and 350 mg/kg of acetaminophen (Fig. 5). All values are presented

Table 2. Effect of dose of acetaminophen and an overnight fast on the overall elimination rate constant (β), and the reactive metabolite fraction K'_{MA}/β

Acetaminophen (mg/kg)	β (hr^{-1}) Fed	β (hr^{-1}) Fasted	K'_{MA}/β Fed	K'_{MA}/β Fasted
20	2.13 ± 0.10	1.90 ± 0.08	0.159 ± 0.011	0.132 ± 0.018
75	1.91 ± 0.08	1.44 ± 0.12*	0.160 ± 0.023	0.134 ± 0.016
150	1.07 ± 0.02	0.92 ± 0.04*	0.129 ± 0.006	0.110 ± 0.013
200	0.88 ± 0.02	0.75 ± 0.04*	0.136 ± 0.010	0.109 ± 0.014
350	0.42 ± 0.03	0.33 ± 0.01*	0.114 ± 0.008	0.082 ± 0.017

Hamsters were allowed food *ad lib.* or fasted for 12 hr prior to receiving various doses of 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 [21]. Values are means ± S.E., N = 4.

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

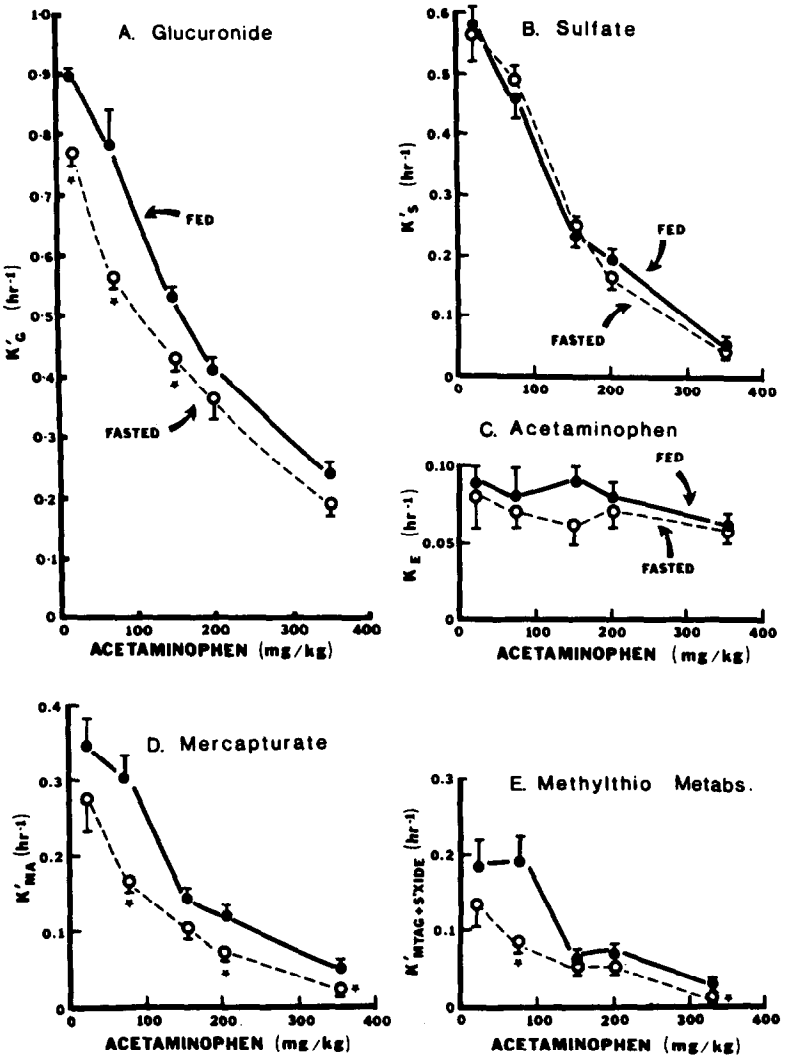

Fig. 4. Effect of fasting on the kinetic parameters of acetaminophen metabolism. Hamsters were allowed food *ad lib.* (●) or fasted for 12 hr (○) prior to receiving various doses of [^{3}H]acetaminophen, i.p. Sequential blood samples and total urinary collection were obtained for each animal and used to calculate the apparent rate constants for formation of acetaminophen (A) -glucuronide; (B) -sulfation; (D) -mercapturate; and (E) -methylthio derivatives; and (C) the renal elimination rate constant, K_E. Values are means ± S.E., N = 4. An asterisk (*) indicates significantly different from fed hamsters, $P < 0.05$.

as a percentage of levels in fed hamsters prior to administration of acetaminophen. As previously reported [8, 19], the nadir of glutathione depletion in the fed animals decreased with increasing dose from approximately 10% depletion at 20 mg/kg to over 90% depletion at 350 mg/kg (Fig. 5A). A generally similar dose-dependent depletion of hepatic glutathione was observed in the fasted hamsters (Fig. 5B). In agreement with the diurnal variation study (Fig. 1), the initial glutathione concentration in these fasted hamsters was 140% of the glutathione concentration in the liver of fed hamsters. Direct comparison of the maximal extent of glutathione depleted in fed and fasted animal (Fig 5, A and B) showed that, despite the marked difference in the initial concentrations of hepatic glutathione, the nadirs were not markedly different. However, since the initial concentration of glutathione was markedly higher in fasted hamsters, the total amount of glutathione which disappeared from their livers was markedly greater than that which disappeared from the livers of fed animals. This is illustrated for the 200 mg/kg dose in Fig. 6. These data raise the possibility that the protective glutathione capacity of the liver may not always be directly related to initial levels of hepatic glutathione.

Comparison of experimentally observed glutathione depletion and acetaminophen-mercapturate formation. Previous studies [8] have demonstrated that there is a reasonably close agreement between the amount of glutathione depleted from hamster livers after acetaminophen and the amount of glutathione required for the synthesis of acetaminophen mercapturate found in 24-hr urine samples. In view of the apparent greater utilization of glutathione in fasted hamsters (Figs. 5 and 6), we have performed

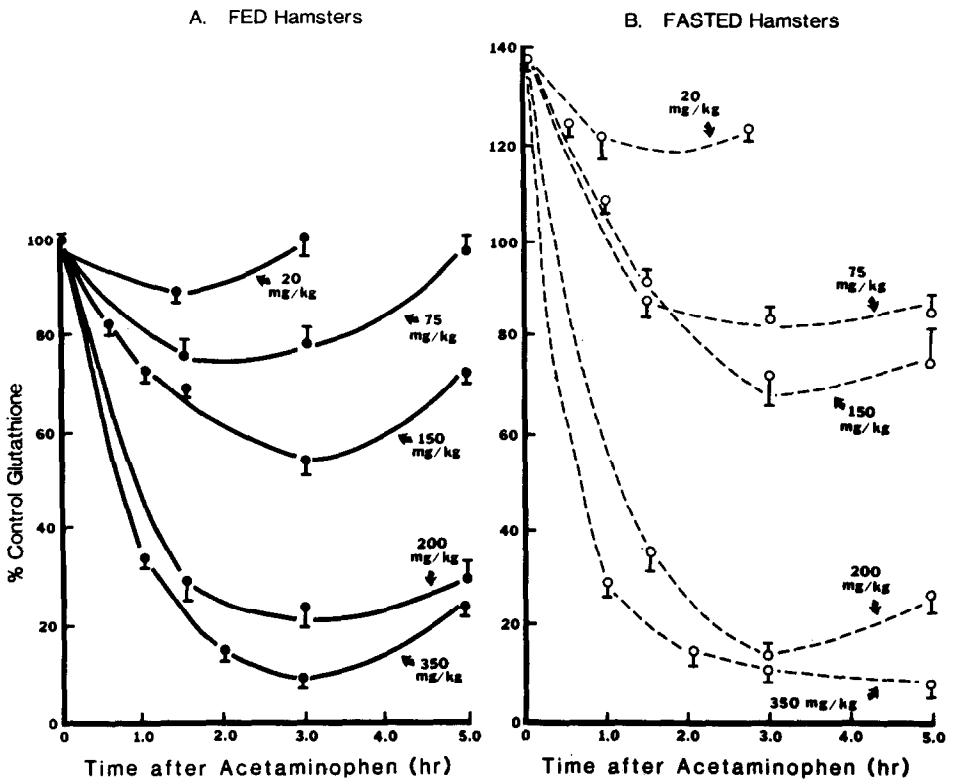

Fig. 5. Effect of fasting on hepatic glutathione levels after various doses of acetaminophen. Hamsters were (A) fed (●) or (B) fasted for 12 hr (○) prior to administration of acetaminophen, i.p. Glutathione levels were determined as described under Materials and Methods. All data are expressed as percentages of concentration in fed hamsters prior to acetaminophen administration. Values are means ± S.E., N = 4.

a similar calculation using the data from our glutathione depletion and metabolism studies. The amount of glutathione depleted after various doses of acetaminophen (Fig. 7) was estimated as the difference between the initial value (T_0) and the nadir occurring after administration of the drug (Fig. 5). The values are calculated per 100 g hamster. As shown in Fig. 7, in the fed hamster, the amount of glutathione depleted from the liver was in good agreement with the amount of acetaminophen mercapturate recovered in the urine at all doses of acetaminophen. These data suggest that the disappearance of glutathione from the liver is largely due to its utilization for the conjugation of glutathione with the reactive metabolite and hence subsequent mercapturate formation. However, in the fasted hamster there was a marked discrepancy between the amount of hepatic glutathione depleted from the livers and the amount of acetaminophen mercapturate recovered in the urine. Throughout the dose range, approximately one-half of the glutathione disappearing from the liver of fasted hamsters cannot be accounted for by mercapturate formation.

DISCUSSION

Previous studies in rats and mice have demonstrated that hepatic levels of nonprotein sulfhydryls exhibit a circadian rhythm [11, 27–29]. The nonprotein sulfhydryl content of liver fell by approximately 30% throughout the day and rose again during the night [11, 29]. These investigators also reported a circadian variation in susceptibility to the toxic effects of acetaminophen [29] and 1,1-dichloroethylene [11]. It was noted that the lethality of those hepatotoxins was greatest in animals that had been injected at times when liver levels of nonprotein sulfhydryls were lowest. Further studies reported that fasted rats and mice had significantly lower levels of hepatic nonprotein sulfhydryls compared to fed animals [9–15], and also that fasting increased susceptibility to acetaminophen-induced hepatic necrosis [13–18]. From these data, it has been proposed that both nutritional and normal daily changes in the glutathione content of the liver could greatly influence susceptibility to the hepatotoxic effects of certain chemicals.

The present studies have examined the effect of an overnight fast on hepatic glutathione levels and susceptibility to acetaminophen-induced hepatic necrosis in the hamster. Fed hamsters showed a circadian rhythm in hepatic glutathione content (Fig. 1). Peak glutathione levels occurred in the early morning hours and the nadir in the afternoon. In fasted hamsters, the circadian rhythm was generally similar except that the liver levels of glutathione were elevated markedly. The elevation was most pronounced in the 24 hr immediately after food dep-

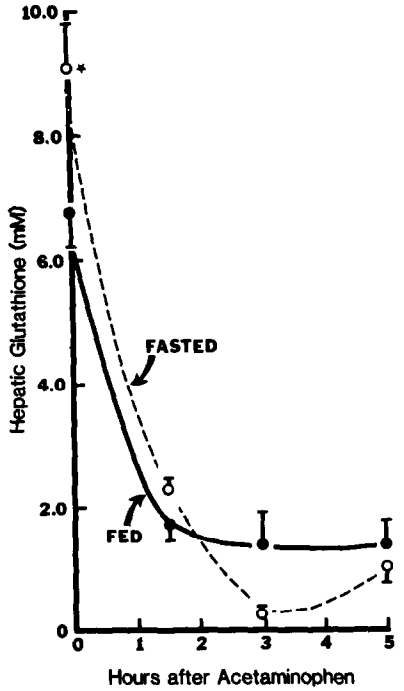

Fig. 6. Effect of fasting on hepatic glutathione levels after 200 mg/kg acetaminophen. Hamsters were fed (●) or fasted (○) for 12 hr prior to receiving acetaminophen, i.p. Glutathione levels were determined by the method of Tietze [20]. Values are means ± S.E., N = 4. An asterisk (*) indicates significantly different from fed hamsters, $P < 0.05$.

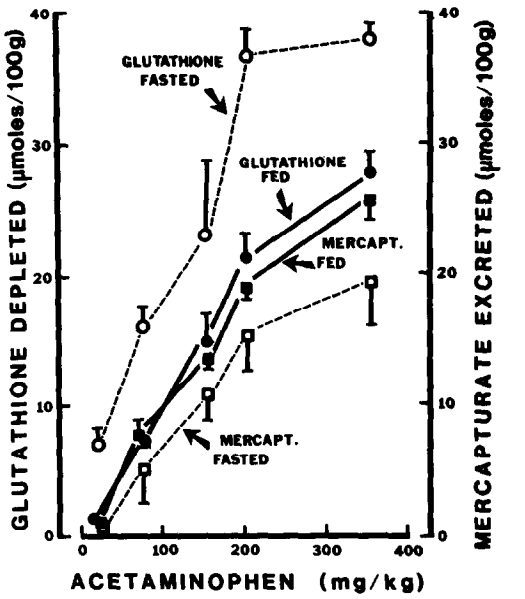

Fig. 7. Comparison of the extent of hepatic glutathione depleted and the amount of acetaminophen-mercapturate excreted in the urine of fed (closed symbols) and fasted (open symbols) hamsters. The extent of glutathione depleted from the liver per 100 g hamster (circles) was estimated as the difference between the initial value (T_0) and the nadir occuring after administration of various doses of acetaminophen (Fig. 5). The amount of urinary mercapturate per 100 g hamster (squares) was determined as previously described [21]. Values are means ± S.E., N = 4.

rivation and was maintained throughout the subsequent 24 hr (Fig. 1).

In view of the known relationship between glutathione content of the liver and susceptibility to acetaminophen hepatotoxicity [6, 19], the fasted hamster would be predicted to show significantly decreased susceptibility to hepatotoxicity (Fig. 2). However, the data demonstrated that an overnight fast modestly potentiated rather than diminished acetaminophen-induced hepatic necrosis in the hamster, as judged by both histological examination of liver sections (Table 1) and serum enzyme elevation (SGOT) (Fig. 2).

To investigate this anomalous potentiation of acetaminophen hepatotoxicity in the face of elevated liver glutathione levels, we first examined the effect of fasting on the metabolic disposition of acetaminophen. It is well known that the half-life of acetaminophen is a major determinant of the proportion of the dose converted to the reactive metabolite and that the activity of the conjugative enzyme systems (especially glucuronidation) is an important determinant of the half-life [8, 25]. In a comparison study [30], we have observed that the glucuronidation capacity of the fasted rat is decreased and that the resultant increased production of the reactive metabolite is an important contributor to the enhanced hepatotoxicity in this situation. To determine whether a similar reduction in glucuronidation capacity could account for the lack of protection in the hamster, the relative contributions of the individual pathways of acetaminophen were estimated in terms of their apparent rate constants. These studies indicated that both the overall elimination rate constant (β) of acetaminophen (Table 2) and the apparent rate constant for glucuronide formation K'_G (Fig. 4A) were decreased by fasting. However, fasting also decreased the activity of the toxic cytochrome P-450 pathway (as measured by mercapturic acid formation) to a similar extent (Fig. 4D). Thus, the potentiating effect of decreased glucuronidation was offset by the decrease in the toxic pathway, resulting in little change in the toxic metabolite fraction (K'_{MA}/β) (Table 2). It follows that the anomalous lack of protection by fasting against acetaminophen hepatotoxicity in the hamster cannot be explained on the basis of change in the metabolic disposition of the drug such that the fasted hamsters formed more toxic metabolite to match the greater amount of glutathione in their livers.

Previous studies have indicated that the depletion of glutathione from the liver after acetaminophen reflects its conjugation with the reactive metabolite of the drug [8]. In the present study, the initial levels of glutathione were approximately 3 mM higher in fasted animals. However, administration of various doses of acetaminophen to fed and fasted hamsters rapidly depleted hepatic glutathione to similar levels (Figs. 5 and 6). These data, which suggest that reactive metabolite formation had been greatly increased in the livers of the fasted animals, are thus at variance with the metabolite disposition data discussed above. To examine this discrepancy, we compared the amount of glutathione disappearing from the liver

after administration of various doses of acetaminophen with the amount of urinary acetaminophen mercapturate for both fed and fasted hamsters (Fig. 7). In agreement with previous results [8], a good correlation was observed for fed animals. In contrast, the correlation was poor for fasted hamsters. The data suggest that a substantial amount of the glutathione which disappears from the livers of fasted hamsters is not due to its conjugation with the reactive metabolite of acetaminophen.

The contribution of methylthio-metabolite formation to the depletion of hepatic glutathione in this situation is uncertain. Two different pathways have been proposed to lead to methylthio-metabolites [31]. The first uses methionine as donor of the methylthio group and involves its direct conjugation with chemically reactive metabolites such as the quinoneimide structure believed to act as the toxic metabolite of acetaminophen. In the second pathway, glutathione conjugates of xenobiotics are converted to their cysteine conjugates, which may act as substrates for a cysteine conjugate β-lyase. The thio-metabolites thus formed are then methylated by a *S*-methyltransferase. Clearly, if the methylthio-metabolites of acetaminophen arose via this second pathway, they should be considered to contribute to hepatic glutathione depletion, whereas if they were formed via the first pathway, they would not. In the present studies, however, this uncertainty is moot, in that the amount of methylthio-metabolites formed at the high dose levels was relatively small (*ca.* 2% of the dose) and not sufficient to explain the glutathione discrepancy observed in fasted hamsters (Fig. 7).

Earlier studies have suggested that the glutathione protective capacity of the liver against acetaminophen- and bromobenzene-induced toxicities is the sum of reduced and oxidized glutathione plus any glutathione synthesized after administration of the hepatotoxins [19]. The failure of this relationship to hold for the fasted hamster points to a possible major inadequacy of this concept in that it suggests that, under some conditions, a portion of the total hepatic glutathione is in a kinetic pool that is unavailable for detoxification of reactive metabolites. Of particular interest, this "unavailable" glutathione did not remain in the liver, but was rapidly lost, presumably into plasma or bile, after administration of the drug.

The nature and significance of this labile pool of glutathione are unknown. Since it occurs in fasted rather than fed hamsters, it is of interest to speculate that it may reflect changes in the hepatic glutathione cycle for methionine–cysteine interconversion. As described by Reed and colleagues [32], methionine released from extrahepatic tissues of rats is taken up by the liver and converted to cysteine which is then incorporated into glutathione. Subsequent release of the glutathione from the liver allows extrahepatic uptake and hydrolysis yielding extrahepatic cysteine. In the situation of an acute fast, utilization of protein-derived amino acids for energy production in extrahepatic tissues may be expected to lead to an excess of methionine in extrahepatic tissues, increased methionine transport to the liver, and glutathione synthesis. Enhanced turnover of hepatic glutathione in fasted as compared with fed rats has been reported by Mitchell and colleagues [33, 34]. The activity of the methionine–cysteine–glutathione pathway would correspondingly increase. The increased glutathione content of the fasted hamster liver could thus be explained if it is hypothesized that transport of glutathione out of the hamster liver is the rate-limiting step of the overall methionine–cysteine cycle under these experimental conditions. This assumption could also explain the liability of this pool in that glutathione destined for transport may be closely associated with plasma or biliary membranes. Alteration in membrane function secondary to metabolite effects (change in levels of ATP, UTP, etc.) caused by the high dose of acetaminophen could trigger rapid release of this glutathione.

In summary, these studies have demonstrated that an overnight fast of hamsters markedly elevated hepatic levels of glutathione. However, despite this apparent increase in the glutathione protective capacity of the liver, fasted hamsters showed a modestly increased susceptibility to acetaminophen-induced hepatic necrosis. The potentiation of hepatotoxicity by fasting could not be explained by a marked decrease in glucuronidation capacity with consequent increase in the reactive metabolite fraction. More importantly, the amount of glutathione disappearing from the hamster liver after administration of acetaminophen could not be correlated with the amount of acetaminophen mercapturate which appeared in the urine. We conclude that, in this situation, the initial levels of hepatic glutathione are not a reliable indicator of susceptibility to acetaminophen-induced hepatic necrosis. Apparently, a major fraction of the glutatione in the fasted hamster liver is readily transported out of the liver and is not available for conjugation with the acetaminophen reactive metabolite.

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REFERENCES

1. L. F. Prescott, N. Wright, P. Roscoe and S. S. Brown, *Lancet* **1**, 519 (1971).
2. E. M. Boyd and G. H. Bereczky, *Br. J. Pharmac.* **26**, 606 (1966).
3. J. R. Mitchell, D. J. Jollow, W. Z. Potter, D. C. Davis, J. R. Gillette and B. B. Brodie, *J. Pharmac. exp. Ther.* **187**, 185 (1973).
4. D. J. Jollow, J. R. Mitchell, W. Z. Potter, D. C. Davis, J. R. Gillette and B. B. Brodie, *J. Pharmac. exp. Ther.* **187**, 195 (1973).
5. W. Z. Potter, D. C. Davis, J. R. Mitchell, D. J. Jollow, J. R. Gillette and B. B. Brodie, *J. Pharmac. exp. Ther.* **187**, 203 (1973).
6. J. R. Mitchell, D. J. Jollow, W. Z. Potter, J. R. Gillette and B. B. Brodie, *J. Pharmac. exp. Ther.* **187**, 211 (1973).
7. W. Z. Potter, S. S. Thorgeirsson, D. J. Jollow and J. R. Mitchell, *Pharmacology* **12**, 129 (1974).
8. D. J. Jollow, S. S. Thorgeirsson, W. Z. Potter, M. Hashimoto and J. R. Mitchell, *Pharmacology* **12**, 251 (1974).
9. G. Leaf and A. Neuberger, *Biochem. J.* **41**, 280 (1947).

10. E. Maruyama, K. Kojima, T. Higashi and Y. Sakamoto, *J. Biochem., Tokyo* **63**, 398 (1968).
11. R. J. Jaeger, R. B. Conolly and S. D. Murphy, *Res. Commun. Chem. Path. Pharmac.* **6**, 465 (1973).
12. N. Tateishi, T. Higashi, S. Shinya, A. Naruse and Y. Sakamoto, *J. Biochem., Tokyo* **75**, 93 (1974).
13. A. Wendel, S. Feuerstein and K. H. Konz, *Biochem. Pharmac.* **28**, 2051 (1979).
14. O. Strubelt, E. Dost-Kempf, C. P. Siegers, M. Younes, M. Volpel, U. Preuss and J. G. Dreckmann, *Toxic. appl. Pharmac.* **60**, 66 (1981).
15. R. M. Walker, T. E. Massey, T. F. McElligott and W. J. Racz, *Can. J. Physiol. Pharmac.* **60**, 399 (1982).
16. D. Pessayre, A. Dolder, J. Antigou, J. Wandscheer, V. Descatoire, C. Degott and J. Benhamou, *Gastroenterology* **77**, 264 (1979).
17. D. Pessayre, J. C. Wandscheer, B. Cobert, R. Level, C. Degott, A. M. Batt, N. Martin and J. P. Benhamou, *Biochem. Pharmac.* **29**, 2219 (1980).
18. A. E. M. McLean and P. Day, *Biochem. Soc. Trans.* **2**, 317 (1974).
19. D. J. Jollow, *Archs Toxic.* Suppl. 3, 95 (1980).
20. F. Tietze, *Analyt. Biochem.* **27**, 502 (1969).
21. V. F. Price and D. J. Jollow, *J. Pharmac. exp. Ther.* **220**, 504 (1982).
22. C. F. A. Culling, *Handbook of Histological Techniques*, 2nd Edn. Butterworth, London (1963).
23. H. W. Chalkley, *J. natn. Cancer Inst.* **4**, 47 (1943).
24. R. E. Galinsky and G. Levy, *J. Pharmac. exp. Ther.* **219**, 14 (1981).
25. D. J. Jollow, S. Roberts, V. F. Price, S. Longacre and C. Smith, *Drug Metab. Rev.* **13**, 983 (1982).
26. A. J. Cummings, M. L. King and B. K. Martin, *Br. J. Pharmac.* **29**, 150 (1967).
27. L. V. Beck, V. D. Rieck and B. Duncan, *Proc. Soc. exp. Biol. Med.* **97**, 229 (1958).
28. G. Calcutt and M. Ting, *Naturwissenchaften* **56**, 419 (1969).
29. R. C. Schnell, H. P. Bozigian, M. H. Davies, B. A. Merrick and K. L. Johnson, *Toxic. appl. Pharmac.* **71**, 353 (1983).
30. V. F. Price and D. J. Jollow, *Fedn Proc.* **42**, 889 (1983).
31. M. Tateishi and H. Shimizu, *Enzymatic Basis of Detoxification* (Ed. W. B. Jakoby), Vol. II, pp. 121–30. Academic Press, New York (1980).
32. D. J. Reed, A. E. Brodie and M. J. Meredith in *Functions of Glutathione* (Eds. A. Larsson, S. Orrenius, A. Holmgren and B. Mannervik), pp. 39–49. Raven Press, New York (1983).
33. B. H. Lauterburg and J. R. Mitchell, *Adv. exp. Med. Biol.* **136A**, 453 (1982).
34. B. H. Lauterburg, J. D. Adams and J. R. Mitchell, *Hepatology* **4**, 586 (1984).