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STRAIN DIFFERENCES IN SUSCEPTIBILITY OF NORMAL AND DIABETIC RATS TO ACETAMINOPHEN HEPATOTOXICITY*

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Abstract—The effects of streptozotocin (STZ)-induced diabetes on acetaminophen metabolism and hepatotoxicity in male Sprague–Dawley (SD) and Long Evans Hooded (LEH) rats were compared. In agreement with earlier studies, normal SD rats were more resistant to acetaminophen-induced hepatic necrosis than normal LEH rats. In contrast to LEH rats, the diabetic state did not protect SD rats from liver injury. Pharmacokinetic studies revealed that normal SD rats eliminated acetaminophen faster than normal LEH rats, and that the diabetic state further enhanced elimination in both strains of rats; however, the effect was much greater in LEH rats. Normal SD rats had a greater capacity to metabolize acetaminophen to nontoxic glucuronide and sulfate conjugates than normal LEH rats. In LEH rats, the diabetic state enhanced acetaminophen glucuronidation and sulfation, whereas in SD rats the diabetic state increased only sulfation; glucuronidation was unaffected. Additional studies revealed that the difference in the glucuronidation capacities between normal LEH and normal SD rats was not due to differences in either the amount of the enzyme, glucuronyl transferase, or basal hepatic levels of the cofactor, UDPGA. Similarly, the diabetes-induced enhancement of glucuronidation in LEH rats was not due to differences in predrug levels of either glucuronyl transferase or UDPGA. Thus, the major difference in susceptibility of the two strains of normal rats to acetaminophen hepatotoxicity appears to be due to the capacity to clear the drug through nontoxic pathways. The greater glucuronidation capacity seen in diabetic LEH rats and in normal and diabetic SD rats as compared to normal LEH rats, appears to be due to a greater ability to produce UDPGA in response to the metabolic demand.

Recent studies have shown that, when male Long Evans Hooded (LEH) rats are made diabetic with streptozotocin (STZ), they become less susceptible to acetaminophen-induced liver necrosis [1]. This increased resistance was found to be associated with an enhancement in the capacity of the animals to clear the drug by conversion to the nontoxic metabolites, acetaminophen-glucuronide and -sulfate, and with an enhancement of hepatic levels of glutathione (GSH) [1]. Of these effects, the enhancement of glucuronidation appeared to be the major component contributing to the decreased susceptibility to liver injury.

In the course of these studies, we observed that male Sprague–Dawley (SD) rats, which are normally more resistant to acetaminophen hepatotoxicity [2, 3], were also more resistant to the diabetes effect; that is, when SD rats were made diabetic with STZ, they did not become less susceptible to acetaminophen hepatotoxicity. This paper reports the comparison of the metabolic handling of acetaminophen in normal LEH and SD rats and the effects of STZ-diabetes in each strain.

METHODS

Animals. Both SD and LEH strains of rats were purchased from the Department of Laboratory Animal Medicine, Medical University of South Carolina, Charleston, SC. The animals (75–125 g) were fasted overnight prior to receiving STZ (120 mg/kg, i.p.) as previously described [1]. Following the STZ injection, the rats were allowed Wayne Lab Blox (Allied Mills, Inc., Chicago, IL) and water *ad lib.* throughout the course of the experiments. Animals with blood glucose levels of greater than 200 mg/dl were judged to be diabetic. Blood glucose levels were measured by the glucose oxidase method using a kit from the Sigma Chemical Co. (St. Louis, MO).

Liver necrosis studies. Six days after receiving STZ or vehicle, the rats were given various doses of acetaminophen (400–1200 mg/kg, i.p., dissolved in 20% Tween 80 in normal saline). Approximately 24 hr later, blood samples were taken from the orbital sinus for measurement of glutamate oxaloacetic transaminase (GOT) activity using a kit from Worthington Diagnostics (Freehold, NJ). Approximately 48 hr after administration of acetaminophen, the animals were killed by decapitation. Livers were quickly removed, sliced and fixed in 10% buffered formalin phosphate. Paraffin sections were prepared and stained with hematoxylin and eosin as described by Culling [4]. Quantitative analysis of liver injury was carried out by the method of Chalkley [5] as described by Mitchell *et al.* [2]. Extent of necrosis was quantitated as: 0 = no necrosis, 1+ = necrosis of 1–5% or less of hepatocytes, 2+ = necrosis of 6–25% of hepatocytes, 3+ = 26–50% of hepatocytes and 4+ = greater than 50% of hepatocytes.

Metabolism studies. Six days after administration of STZ or vehicle, the rats received [^{3}H]acetaminophen (Amersham Corp., Arlington Heights,

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IL) (20 or 600 mg/kg, 200 μCi/kg, i.p.). Each animal was then immediately placed in a separate metabolic cage. Blood and urine samples were collected and used to calculate the kinetic parameters of acetaminophen metabolism, as previously described [1]. The apparent rate constants for each metabolite were calculated as $\beta \times$ urinary metabolite fraction.

Determination of microsomal glucuronyl transferase activity. Six days after administration of STZ or vehicle, hepatic microsomes were isolated by the method of Gessner [6]. Glucuronyl transferase activity toward acetaminophen was measured by a modification of the method of Bolanowska and Gessner [7]. 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 (1–10 mM; 0.5 μCi/tube), UDPGA ammonium salt (Sigma) (10 mM), UDP-*N*-acetylglucosamine (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. Protein concentration was determined by the method of Lowry *et al.* [8] using bovine serum albumin as a standard.

Determination of hepatic levels of UDPGA, UDP-glucose and UDP-glucose dehydrogenase activity. Six days after receiving STZ or vehicle the rats were killed by decapitation. Liver extracts were prepared and levels of UDPGA measured by the method of Watkins and Klaassen [9] 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 modification of the method of Watkins and Klaassen [9] as the differences between the amount of [^{3}H]DES-glucuronide formed in the presence and absence of UDP-glucose dehydrogenase (Sigma) (0.2 units/ml) and NAD^+ (1.5 mM).

Endogenous UDP-glucose dehydrogenase activity was measured by an additional modification of the method of Watkins and Klaassen [9]. In a total reaction volume of 0.5 ml, approximately 200 μg of hepatic cytosolic protein was incubated in the presence of UDP-glucose (Sigma) (0.3 mM) and NAD^+ (1.5 mM) for 0–20 min. The reaction was stopped by boiling and the amount of UDPGA formed was measured as described above. Protein concentration was determined by the method of Lowry *et al.* [8].

Measurement of hepatic levels of NAD^+ and NADH. Six days after administration of STZ or vehicle, liver extracts were prepared by the method of Burch *et al.* [10]. Total hepatic levels of NAD^+ and NADH were estimated with the cycling assay of Nisselbaum and Green [11].

Statistics. The TD_{50} values for, and the relative resistance to, acetaminophen hepatotoxicity (with 95% confidence limits) were calculated by the method of Litchfield and Wilcoxon [12] using the percentage of animals showing necrosis. Levels of statistical significance were assessed using either Student's *t*-test of correlated means for small groups and two-way analysis of variance. Significant differences were judged as P values < 0.05.

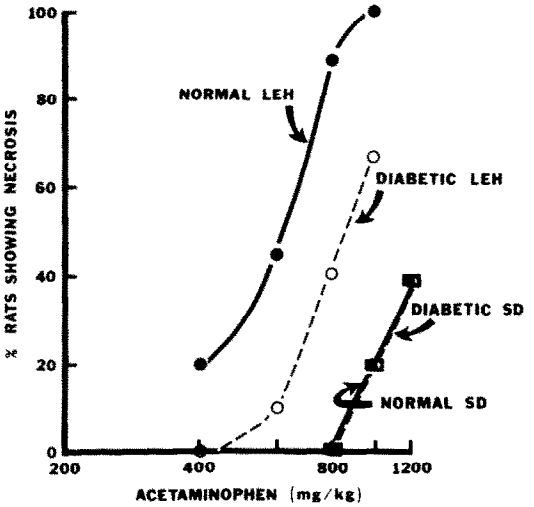

Fig. 1. Effect of dose of acetaminophen on the percentage of normal LEH (●), diabetic LEH (○), normal SD (■) and diabetic SD (□) rats showing hepatic necrosis 48 hr after receiving acetaminophen.

RESULTS

Effect of strain and the diabetic state on acetaminophen-induced hepatotoxicity. The hepatic morphology of normal LEH and SD rats and of diabetic LEH and SD rats 48 hr after various doses of acetaminophen were compared (Table 1, Fig. 1). In agreement with earlier studies [2, 3], normal SD rats were more resistant to acetaminophen hepatotoxicity than normal LEH rats. After a dose of 800 mg/kg, SD rats showed no evidence of liver injury, whereas about 90% of LEH rats were susceptible.

Liver necrosis was also seen in diabetic rats. In agreement with previous studies [1], diabetic LEH rats were less susceptible to necrosis than normal LEH rats. However, they were still more susceptible than normal SD rats (Table 1, Fig. 1). In SD rats, the diabetic state did not further protect the animals from liver injury as evidenced by a similar incidence and severity of necrosis as that seen in normal SD rats.

To determine whether the difference between the two strains with respect to the diabetes-induced protective effect was due to differences in the severity of the diabetic state, blood glucose levels were compared. Both normal LEH and normal SD rats had blood glucose levels of 147 ± 6 mg/dl. Six days after induction of the diabetic state with STZ, LEH rats had blood glucose levels of 645 ± 28 mg/dl and SD rats had blood glucose levels of 644 ± 46 mg/dl. These data indicate that STZ caused the same extent of elevation of blood glucose levels in both strains of rats, suggesting that the severity of the diabetic state was similar. Thus, varying degrees of the diabetic state do not appear to account for differences in acetaminophen hepatotoxicity seen between the two strains of rats.

Effect of strain and the diabetic state on blood half-life of acetaminophen. To examine the mechanism by which SD rats are more resistant to acet-

Table 1. Effect of strain and the diabetic state on extent of hepatic necrosis 48 hr after various doses of acetaminophen in LEH and SD rats

Animals*	Acetaminophen (mg/kg)	No. of rats	Acute mortality†	Extent of necrosis‡ 0	1+	2+	3+
			% of rats	% of survivors			
LEH							
Normal	400	10	0	80	20		
	600	20	0	55	30	15	
	800	10	0	10	70	20	
	1000	12	33	0	75	13	12
Diabetic	400	14	0	100			
	600	30	0	90	10		
	800	12	0	58	42		
	1000	10	40	33	50	17	
SD							
Normal	800	17	0	100			
	1000	22	0	77	18	5	
	1200	22	27	62	25	13	
Diabetic	800	11	18	100			
	1000	19	21	73	27		
	1200	23	30	56	31	13	

* Animals received acetaminophen 6 days after STZ (120 mg/kg, i.p.) or vehicle.
† Death occurred less than 12 hr after administration of acetaminophen.
‡ Extent of hepatic necrosis was scored according to the criteria described under Methods.

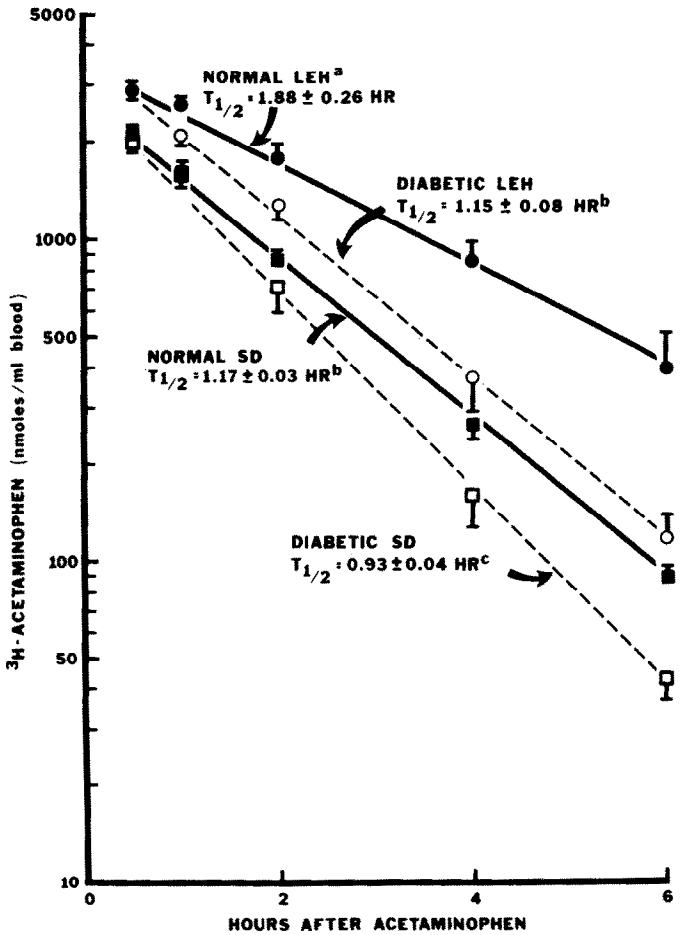

Fig. 2. Effect of strain and the diabetic state on blood half-life of acetaminophen. Concentrations of acetaminophen were determined at the indicated times after administration of [^{3}H]acetaminophen (600 mg/kg, i.p.) as described previously [1]. Values are means ± S.E., N = 4; representative of three separate experiments. Lines of best fit were determined by linear regression analysis ($r > 0.99$). Key: (●) normal LEH; (○) diabetic LEH; (■) normal SD; and (□) diabetic SD. Values with different superscripts are significantly different, $P < 0.05$.

Table 2. Effect of strain and the diabetic state on acetaminophen urinary metabolite composition in LEH and SD rats

Animals*	Percent of urinary metabolites†				
	Glucuronide	Sulfate	Mercapturate	Methylthio-derivatives	Free acetaminophen
LEH					
Normal	52.3 ± 4.4‡	27.3 ± 5.4‡	7.6 ± 1.5‡	2.3 ± 0.3‡	7.1 ± 0.5‡
Diabetic	46.7 ± 1.9‡	31.5 ± 2.0‡	3.3 ± 0.6§	2.4 ± 0.7‡	16.2 ± 1.4§
SD					
Normal	52.5 ± 4.3‡	30.9 ± 3.8‡	4.7 ± 0.3§	2.5 ± 0.7‡	9.5 ± 2.8‡
Diabetic	44.1 ± 4.1‡	31.4 ± 3.1‡	3.6 ± 0.9§	2.1 ± 0.8‡	18.8 ± 1.2§

* Animals received [^{3}H]acetaminophen (600 mg/kg, i.p.) 6 days after STZ or vehicle.

† Metabolite composition of 24-hr urine was determined as described previously [1]. Values are means ± S.E., N = 4; representative of three separate experiments.

‡,§ For each metabolite, values with different superscripts are significantly different ($P < 0.05$).

aminophen liver injury than are normal LEH rats, the disappearance of [^{3}H]acetaminophen from blood was followed for a period of 6 hr (Fig. 2). After a 600 mg/kg dose of acetaminophen, normal LEH rats had a half-life of 1.88 ± 0.26 hr. After the same dose of acetaminophen, normal SD rats had a half-life of 1.17 ± 0.03 hr. Thus, the SD strain of rats cleared the drug at a significantly faster rate than the LEH rats.

In agreement with earlier studies [1], induction of diabetes in LEH rats shortened the blood half-life of acetaminophen from about 1.9 hr to approximately 1.1 hr (Fig. 2). The half-life of the drug was also reduced significantly in diabetic SD rats to approximately 0.9 hr. However, in view of the initial shorter half-life (1.2 hr), the extent of the diabetes-induced reduction was less in SD rats (*ca.* 25%) than in LEH rats (*ca.* 40%).

Table 3. Effect of strain and the diabetic state on kinetic parameters of acetaminophen metabolism in LEH and SD rats

Animals*	β (hr^{-1})	Apparent rate constant for metabolite formation† (hr^{-1})				Renal elimination rate constant K_E (hr^{-1})
		K'_G	K'_S	K'_{MA}	$K'_{MTAG+SO}$	
LEH						
Normal	0.402 ± 0.042‡	0.210 ± 0.016‡	0.109 ± 0.005‡	0.030 ± 0.007‡	0.011 ± 0.001‡	0.030 ± 0.001‡
Diabetic	0.612 ± 0.044§	0.286 ± 0.023§	0.192 ± 0.018§	0.022 ± 0.003‡	0.014 ± 0.002‡	0.101 ± 0.014§
SD						
Normal	0.596 ± 0.015§	0.312 ± 0.024§	0.184 ± 0.022§	0.028 ± 0.002‡	0.015 ± 0.001‡	0.050 ± 0.022‡
Diabetic	0.748 ± 0.034‖	0.327 ± 0.015§	0.237 ± 0.035‖	0.028 ± 0.008‡	0.016 ± 0.004‡	0.141 ± 0.009§

* Animals received [^{3}H]acetaminophen (600 mg/kg, i.p.) 6 days after STZ or vehicle.

† Kinetic parameters were calculated as described previously [1]. Values are means ± S.E., N = 4; representative of three separate experiments.

‡–‖ For each kinetic parameter, values with different superscripts are significantly different ($P < 0.05$).

Table 4. Effect of strain and the diabatic state on kinetic parameters of the metabolism of a low dose of acetaminophen in LEH and SD rats

Animals*	β (hr^{-1})	Apparent rate constant for metabolite formation† (hr^{-1})				Renal elimination rate constant K_E (hr^{-1})
		K'_G	K'_S	K'_{MA}	$K'_{MTAG+SO}$	
LEH						
Normal	1.700 ± 0.058	0.292 ± 0.009	1.145 ± 0.064	0.110 ± 0.011	0.074 ± 0.008	0.075 ± 0.007
Diabetic	1.710 ± 0.046	0.262 ± 0.042	1.096 ± 0.056	0.132 ± 0.010	0.101 ± 0.016	0.123 ± 0.022
SD						
Normal	1.643 ± 0.007	0.326 ± 0.036	1.040 ± 0.054	0.070 ± 0.008	0.071 ± 0.008	0.169 ± 0.042
Diabetic	2.384 ± 0.285	0.344 ± 0.057	1.420 ± 0.142	0.105 ± 0.014	0.064 ± 0.010	0.179 ± 0.010

* Animals received [^{3}H]acetaminophen (20 mg/kg, i.p.) 6 days after STZ or vehicle.

† Kinetic parameters were calculated as described previously [1]. Values are means ± S.E., N = 4; representative of two separate experiments.

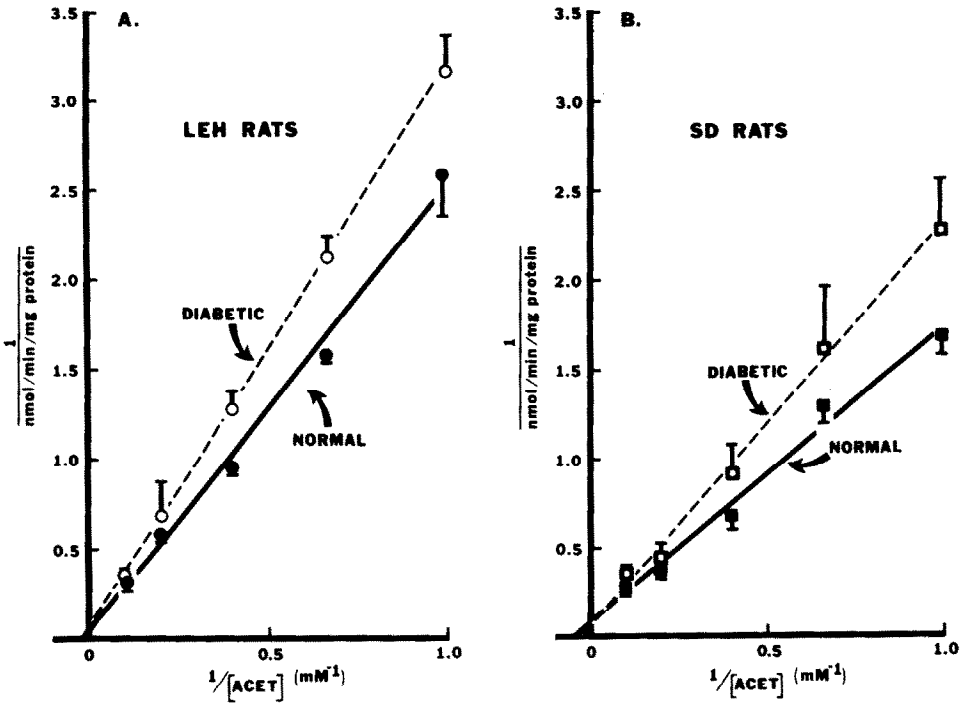

Fig. 3. Effect of strain and the diabetic state on microsomal glucuronyl transferase activity. Rats were given STZ or vehicle 6 days prior to the isolation of hepatic microsomal fractions. Various concentrations of [^{3}H]acetaminophen were incubated with the microsomes, UDPGA and the detergent Brij for 45 min as described in Methods. Lineweaver–Burk plots were constructed to determine V_{max} values. (A) LEH rats: normal (●) and diabetic (○); (B) SD rats: normal (■) and diabetic (□). Values are means ± S.E., N = 5.

Effect of strain and the diabetic state on the metabolic disposition of acetaminophen. To examine the mechanism by which the elimination of acetaminophen was greater in the normal SD rats, the urinary metabolite composition (Table 2) and apparent rate constants for metabolite formation (Table 3) were determined for normal LEH and SD rats. As shown in Table 2, the percentages of glucuronide, sulfate, methylthio-derivatives and free acetaminophen were similar in normal LEH and SD rats. The only differential effect observed between the two strains of normal rats was that the percentage of mercapturate excreted by normal SD rats was significantly lower than the percentage of mercapturate excreted by normal LEH rats.

Using the urinary metabolite composition (Table 2) and the overall elimination rate constant (β), the individual apparent rate constants for glucuronidation (K'_G), sulfation (K'_S), mercapturate formation (K'_{MA}) and methylthio-acetaminophen-glucuronide plus sulfoxide formation ($K'_{MTAG+SO}$) and the renal elimination rate constant (K_E) were calculated. As shown in Table 3, K'_G and K'_S were significantly greater in normal SD rats as compared to normal LEH rats. However, there was no significant difference in K'_{MA} or $K'_{MTAG+SO}$ values between the two strains of normal rats.

The urinary metabolite composition and apparent rate constants for metabolite formation were also determined in diabetic LEH and SD rats. As shown in Table 2, in LEH rats diabetes had no significant effect on the percentages of urinary glucuronide, sulfate, or methylthio-derivatives; diabetes significantly decreased the percentage of mercapturate and increased the percentage of free acetaminophen excreted. In SD diabetic rats, the only significant effect was a doubling of the percentage of free acetaminophen excreted.

Calculation of the apparent rate constants for the diabetic animals (Table 3) indicated that in LEH rats diabetes significantly increased K'_G, K'_S and K_E but had no effect on K'_{MA} or $K'_{MTAG+SO}$. In SD rats, diabetes significantly increased K'_S and K_E but had no effect on K'_G, K'_{MA}, $K'_{MTAG+SO}$. Thus, the major differential effect of diabetes between the two strains of rats was that the diabetic SD rats did not show an enhanced glucuronidation capacity similar to that seen in the diabetic LEH rats.

Effect of strain and the diabetic state on glucuronyl transferase activity. To examine the mechanisms by which K'_G is greater in normal SD rats than in normal LEH, and by which the diabetic state enhances K'_G in LEH rats but not in SD rats, the level of glucuronyl transferase was estimated by two methods. First, the value of K'_G was determined after a low dose (20 mg/kg) of acetaminophen. Since at this dose of acetaminophen, UDPGA is not rate limiting for glucuronidation [13], 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 the two strains, it would follow that differences in K'_G would reflect differences in V_{max} of the enzyme in the two strains. As shown in Table 4, there was

Table 5. Effect of strain and the diabetic state on basal levels of hepatic UDPGA

Animals	UDPGA (nmoles/g liver)
LEH	
Normal	350 ± 52
Diabetic	333 ± 66
SD	
Normal	295 ± 25
Diabetic	226 ± 29

Animals received STZ or vehicle 6 days prior to being killed. Hepatic levels of UDPGA were determined by the method of Watkins and Klaassen [9]. Values are means ± S.E., N = 7.

no significant difference in K'_G between normal LEH and SD rats, suggesting that the amounts of glucuronyl transferase enzyme present in their livers were generally similar. In addition, there were no significant differences in other kinetic parameters between normal LEH and SD rats.

Table 6. Effect of strain and the diabetic state on the calculated average rate of UDPGA synthesis during three half-lives of acetaminophen metabolism

Animals*	Calculated average rates of UDPGA synthesis during three half-lives of acetaminophen metabolism† (μmoles/kg rat/hr)
LEH	
Normal	364 ± 23‡
Diabetic	518 ± 10§
SD	
Normal	524 ± 40§
Diabetic	550 ± 26§

* Animals received [^{3}H]acetaminophen (600 mg/kg, i.p.) 6 days after STZ or vehicle.

† Calculated from the total amount of [^{3}H]acetaminophen glucuronide found in urine 24 hr after drug administration, multiplied by 0.875, divided by three half-life periods in hours. Values are means ± S.E., N = 4; representative of three separate experiments.

‡,§ Values with different superscripts are significantly different ($P < 0.05$).

The effect of the diabetic state on the *in vivo* metabolism of 20 mg/kg acetaminophen in both strain of rats is also shown in Table 4. Diabetes did not enhance significantly the K'_G values or alter the other kinetic parameters in either strain of rat. The lack of effect on K'_G at this low dose level argues that the diabetic effect does not occur by increasing the amount of glucuronyl transferase enzyme in the liver.

The second method used to estimate the level of glucuronyl transferase was the determination of *in vitro* glucuronidation of acetaminophen in isolated detergent-treated liver microsomes. As shown in Fig. 3, both normal LEH and SD rats showed similar V_{max} values (13.9 ± 4.1 vs 16.5 ± 2.9 nmoles/min/mg protein), indicating similar total levels of the enzyme. In addition, diabetes did not increase significantly V_{max} in either strain of rat (11.5 ± 2.0 in LEH vs 17.4 ± 2.2 in SD). The apparent K_m values for the microsomal catalyzed reactions (30–40 mM) were similar to those reported by previous workers [7] and were not significantly different either between the two strains of rats or between the normal and diabetic state of each strain. Collectively, the data do not support the hypothesis that the differences in glucuronidation in either diabetic LEH rats or in normal SD rats are due to higher levels of glucuronyl transferase.

Effect of strain and the diabetic state on hepatic levels of UDPGA. To examine whether the differences in glucuronidation in either diabetic LEH rats or in normal SD rats were due to differences in the availability of the cosubstrate, hepatic levels of UDPGA were determined in the four groups of animals. As shown in Table 5, there were no significant differences in basal levels of UDPGA between normal LEH and SD rats. Similarly, the diabetic state did not increase basal levels of UDPGA in either strain of rat.

An alternate hypothesis to explain the differences in glucuronidation may be that the ability of the animals to produce hepatic UDPGA in response to the metabolic demand is different. To explore this possibility, the average rate of UDPGA synthesis required for glucuronidation of acetaminophen (600 mg/kg) was calculated for the four groups of

Table 7. Effect of strain and the diabetic state on basal levels of hepatic UDP-glucose, UDP-glucose dehydrogenase and the ratio NADH/NAD$^+$

Animals*	UDP-glucose† (nmoles/g liver)	UDP-glucose dehydrogenase† (nmoles/min/g liver)	$\frac{\text{NADH}‡}{\text{NAD}^+}$
LEH			
Normal	434 ± 50§,‖	409 ± 15§	0.26 ± 0.03§
Diabetic	432 ± 17§,‖	344 ± 17§,‖	0.49 ± 0.02‖
SD			
Normal	487 ± 29§	300 ± 32§,‖	0.29 ± 0.02§
Diabetic	343 ± 55‖	249 ± 28‖	0.45 ± 0.02‖

* Rats received STZ or vehicle 6 days prior to being killed.

† Basal levels of hepatic UDP-glucose and UDP-glucose dehydrogenase were determined as described in Methods. Values are means ± S.E., N = 7.

‡ Basal levels of hepatic NADH and NAD$^+$ were determined by the method of Nisselbaum and Green [11]. Values are means ± S.E., N = 4.

§,‖ For each parameter, values with different superscripts are significantly different ($P < 0.05$).

animals. The UDPGA demand over three blood half-lives of the drug (equivalent to the metabolism of 87.5% of the dose) was calculated from the total amount of acetaminophen glucuronide found in urine 24 hr after drug administration. The data are expressed as μmoles of UDPGA required per kg rat per hr. As shown in Table 6, the calculated average rate of hepatic UDPGA synthesis during the first three half-lives of acetaminophen metabolic clearance was significantly higher in diabetic LEH rats than in normal LEH rats. This diabetes-induced elevation was, however, not seen in the SD rats. Of particular interest, the calculated average rate of UDPGA synthesis in normal SD rats was similar to that seen in the diabetic elevated state of LEH rats, raising the possibility that an average synthetic rate of about 500 μmoles/kg/hr may be close to maximal for these animals.

Effect of strain and the diabetic state on basal levels of hepatic UDP-glucose and UDP-glucose dehydrogenase and the ratio of NADH/NAD⁺. To examine the mechanism by which UDPGA was synthesized at a faster rate in diabetic LEH rats and normal and diabetic SD rats, hepatic levels of the precursor, UDP-glucose, and the synthetic enzyme, UDP-glucose dehydrogenase, were determined in the four groups of animals. As shown in Table 7, there were no significant differences in predrug levels of UDP-glucose among normal and diabetic LEH rats and normal SD rats; UDP-glucose levels in diabetic SD rats were modestly lower than in normal SD rats. There were no significant differences in the basal levels of UDP-glucose dehydrogenase between normal LEH and SD rats or between normal and diabetic rats of each strain.

Since *in vitro* studies have suggested that UDP-glucose dehydrogenase activity may be inhibited by either increased levels of NADH [14] or limitation of NAD^+ [15], a change in the ratio of NADH/NAD^+ may control the rate of UDPGA synthesis. In agreement with previous workers [16], the ratio of NADH/NAD^+ was increased by the diabetic state (Table 7). The occurrence and magnitude were similar in both strains of rats, indicating that the apparent increase in UDPGA synthesis in diabetic LEH rats was not associated with a change in this ratio.

DISCUSSION

The present study was undertaken to determine why STZ-induced diabetes does not confer protection from acetaminophen hepatotoxicity in SD rats as it does in LEH rats [1]. In the course of these studies, however, it became apparent that there was a need to first consider the mechanism by which normal SD rats are more resistant to acetaminophen liver injury than normal LEH rats [2, 3].

Comparison of the metabolic handling of acetaminophen in the two strains of normal rats (Table 3) indicated that the SD rats cleared the drug significantly faster than the LEH rats, and that this enhanced clearance was due to their relatively greater activities of the nontoxic pathways, glucuronidation and sulfation, as measured by their apparent rate constants (K'_G and K'_S). In contrast, the activities of the cytochrome P-450-dependent pathways, acetaminophen-mercapturate (K'_{MA}) and -methylthio metabolites ($K'_{MTAG+SO}$) formation, were not different. Since at subthreshold doses, the rate of acetaminophen mercapturate formation is believed to reflect the activity of the toxic pathway [17], these data imply that the SD animals would clear a smaller proportion of the dose of acetaminophen via the toxic pathway. This is illustrated in Table 8 which compares the calculated toxic metabolite fraction (K'_{MA}/β) for each strain after 600 mg acetaminophen/kg with an index of their severity of liver injury after 800 mg of drug/kg. Clearly, the toxic metabolite fraction for normal LEH rats was significantly greater than that of normal SD rats given the same dose of drug, notwithstanding the fact that since the LEH rats showed some liver injury at the 600 mg/kg dose (Table 1, Fig. 1), their K'_{MA}/β value (0.076 ± 0.015) is likely to be an underestimation. Collectively, the data indicate that the lesser susceptibility of SD as compared with LEH rats may be explained largely on the basis of differences in the production of the toxic metabolite rather than to differences in responsiveness of the liver cells to the toxic insult.

In agreement with earlier studies [1], the decreased susceptibility of diabetic LEH rats to acetaminophen hepatotoxicity as compared with normal LEH rats, was associated with enhancement of clearance of the drug by the nontoxic glucuronidation and sulfation pathways (Tables 1 and 3, Fig. 1). Thus, this diabetes-induced protection may be similarly explained in terms of a dramatic decrease in the contribution of the toxic pathway to the overall clearance of the drug (Table 8).

In contrast to the situation in LEH rats, diabetes did not confer protection on SD rats (Table 1, Fig. 1).

Table 8. Comparison of the effects of strain and the diabetic state on acetaminophen reactive metabolite fraction and hepatotoxicity

Animals*	$\frac{K'_{MA}}{\beta}$†	Mean score for hepatotoxicity‡
LEH		
Normal	0.076 ± 0.015§	1.1 ± 0.2§
Diabetic	0.033 ± 0.002‖	0.4 ± 0.1‖
SD		
Normal	0.047 ± 0.006‖	0 ± 0¶
Diabetic	0.036 ± 0.009‖	0 ± 0¶

* Rats received acetaminophen 6 days after STZ or vehicle.

† The reactive metabolite fraction was calculated by dividing the apparent rate constant for acetaminophen mercapturate formation (K'_{MA}) by the overall elimination rate constant (β) after a dose of 600 mg/kg [^{3}H]acetaminophen. Values are means ± S.E., N = 4; representative of three separate experiments.

‡ Mean score for liver necrosis 48 hr after acetaminophen (800 mg/kg) was calculated by multiplying the severity of lesion (0, 1+, 2+, etc.) by the number of animals showing that lesion and dividing the sum of scores for each group of rats by the total number of animals. See Table 1. Values are means ± S.E.

§–¶ Values with different superscrips are significantly different ($P < 0.05$).

Comparison of the metabolic data (Table 3) indicated that induction of the diabetic state in these SD animals was associated with modest enhancement of clearance of acetaminophen by sulfation and renal elimination with no change in the rate of elimination via the toxic pathway (as measured by urinary mercapturate). Of importance, clearance by glucuronidation was not enhanced. Further, since sulfation and renal elimination of acetaminophen are, relatively speaking, only minor pathways of clearance of the drug at hepatotoxic dose levels, their enhancement resulted in only a minor increase in β, and a correspondingly small and statistically nonsignificant reduction in the toxic metabolite fraction (K'_{MA}/β, Table 8). Thus, the lack of protection by the diabetic state in SD rats was matched by a lack of significant change in production of the toxic metabolite.

The biochemical mechanisms by which these differences in metabolic disposition of acetaminophen occur are not yet known. Studies of strain-dependent differences in drug metabolism that have been reported have focused on cytochrome P-450-dependent activities [18–26]. Less attention has been paid to strain-dependent differences in Phase II metabolism [27–29]. Rydell and colleagues [27, 28] considered the role of sulfotransferase in susceptibility to hepatocarcinogenicity of *N*-hydroxy-2-acetylaminofluorene and noted that SD rats showed significantly less activity toward this substrate than did Fischer 344 rats. In considering the sulfotransferase activity toward 3-methoxy-4-hydroxyphenylglycol in eight strains of rats, Maus *et al.* [29] reported generally similar hepatic activities.

In the present studies, the higher apparent rate constant for glucuronidation in normal SD rats as compared to normal LEH rats appeared not to be due to higher basal levels of UDPGA or acetaminophen glucuronyltransferase. In agreement with earlier studies [13], calculation from urinary metabolite data (Table 2) of the amount of UDPGA required for the synthesis of acetaminophen glucuronide (*ca.* 2 mmoles/kg) indicated that, at the high dose levels, the amount of UDPGA needed was very much greater than the basal UDPGA content of the liver at the time of acetaminophen administration (Table 5). Thus, acetaminophen glucuronide synthesis was dependent in both strains on the rate of UDPGA production during the time period of metabolism of the drug. Of importance, since the half-life of acetaminophen was different in the two strains of rats, and in normal and diabetic LEH rats, the rates of formation of UDPGA must have been different. As shown in Table 6, the calculated average rate of formation of hepatic UDPGA was significantly greater in SD than in LEH rats, and in diabetic as compared with normal LEH rats. In contrast, UDPGA production did not appear to be enhanced by diabetes in the SD animals.

The mechanism by which the sulfation capacity is greater in normal SD rats than that in normal LEH rats is also unknown. However, since the apparent rate constant for sulfation at a low dose of acetaminophen (Table 4) is similar in the two strains, it is likely that they have similar acetaminophen sulfotransferase enzyme levels analogous to the acetaminophen glucuronyl transferase situation as discussed above. Since the capacity-limitation of sulfation seen at higher doses of acetaminophen [3, 30] is believed to be due to rate of supply of inorganic sulfate for phosphoadenosine phosphosulfate (PAPS), synthesis [31], the observed higher sulfation capacity of SD rats suggests that they have a greater capacity to supply inorganic sulfate.

The enhancement of diabetic LEH rats of glucuronidation and sulfation of acetaminophen appears to be similarly due to enhancement of production of the cosubstrates, UDPGA and PAPS, rather than to increase in the levels of the enzymes. In SD rats in contrast, only PAPS formation showed evidence of enhancement by diabetes; UDPGA formation was apparently unchanged. The rate-limiting step in UDPGA synthesis *in vivo* is currently unknown. Previous *in vitro* studies have suggested that UDP-glucose dehydrogenase or levels/ratios of NADH/NAD^+ may regulate UDPGA formation [14, 15]. These hypotheses, however, were not supported by our experimental observations; that is, changes in the basal levels of UDP-glucose (Table 7), UDPGA (Table 5), UDP-glucose dehydrogenase (Table 7), and the ratio of NADH/NAD^+ were either minimal or did not reflect change in UDPGA production. Collectively, the data suggest that the differences in the rate of UDPGA synthesis during metabolism of large doses of acetaminophen arise from differences in the intermediary metabolism of glucose. Additional studies to explore the strain differences in glucuronidation may elucidate the control mechanisms of UDPGA synthesis.

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