Biochemical Pharmacology, Vol. 29, pp. 1167–1175.

0006-2952/80/0415-1167 $02.00/0

DRUG INTERACTIONS WITH ACETAMINOPHEN

EFFECTS OF PHENOBARBITAL, PREDNISONE AND 5-FLUOROURACIL IN NORMAL AND TUMOR-BEARING RATS

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(*Received* 23 *January* 1979; *accepted* 25 *October* 1979)

Abstract—Acetaminophen (APAP) was determined by a simplified gas chromatographic method, without derivatization, on a column packed with 3% OV17 on Gas Chrom Q. APAP, released enzymatically from its conjugates, was also so determined. Changes in urinary excretion patterns of APAP, its glucuronide (APAPGA) and its sulfate (APAPS) were studied as indicators of interference with metabolism of APAP. Effects of the presence of some tumors on APAP metabolism of hosts were examined, as were the effects of the following agents: phenobarbital, as a prototype of a hepatic enzyme inducer, prednisone and prednisolone, as alternate substrate inhibitors, and 5-fluorouracil (5-FU) and galactosamine, as possible modifiers of cofactors. Normal rats, Reuber H-35 hepatoma hosts and colon carcinoma hosts excreted 87.8 ± 8 per cent of a 300 mg/kg dose of APAP, in the form of APAP plus APAPGA and APAPS. The presence of colon carcinoma did not alter significantly the quantities of APAP metabolites appearing in the urine of the hosts. Hepatoma hosts excreted significantly higher amounts of APAPGA, and early urine samples also contained elevated APAPS. These effects of the tumors were related to the presence of APAP conjugating enzymes in the hepatoma, and their virtual absence in the colon carcinoma tissue. The above effects on conjugations contrast with known depressive effects of tumors on mixed function oxidation pathways of hosts. Phenobarbital pretreatment (80 mg/kg, i.p., daily for 7–9 days) of Sprague–Dawley, or ACI, rats increased (3- to 4-fold) the amount of urinary APAPGA excreted in the first 3 hr after injection of APAP, and decreased (2-fold) the amount of unchanged APAP excreted. Coadministration of prednisone, or prednisolone (14 mg/kg, i.p.), with APAP caused a small but significant decrease in urinary APAPGA, which is consistent with the previously reported inhibitory effects of these steroids on APAP glucuronidation *in vitro*. Treatment of rats with 5-FU (120 mg/kg, i.p.) 3 hr prior to administration of APAP produced slight decreases in urinary APAPGA and APAPS. Contrary to expectations, pretreatment with galactosamine, on a schedule known to markedly reduce UDP-sugar pools, did not decrease urinary APAPGA. The results suggest that drugs which can induce hepatic glucuronyltransferase, or which can compete with APAP for the enzyme, rather than those that can alter UDP-sugar pools, are likely to have marked effects on APAP metabolism.

Acetaminophen is extensively metabolized, chiefly to glucuronide and sulfate conjugates. Both glucuronidation and sulfation detoxify the drug; however, they are limited capacity pathways [1, 2]. Animal studies show that, when these pathways are saturated, a toxifying biotransformation route, leading to hepatotoxic metabolites, gains prominence [3]. A similar mechanism appears to operate in humans, since patients who developed liver damage after overdoses of the drug exhibited a plateau in the excretion of acetaminophen glucuronide, concurrently with an increased excretion of cysteine and mercapturic acid conjugates [4], the latter being the conjugates of hepatotoxic oxygenated metabolites of the drug [5–7]. Although severe hepatic necrosis and hepatic failure are known to occur only when excessive doses of acetaminophen are taken, there is evidence that therapeutic doses of acetaminophen can saturate sulfation and also may briefly overtax glucuronidation. This is seen as a prolonged plateau in the rate of sulfate excretion, and a flat maximum in glucuronide excretion which is decreased if a drug competing for glucuronidation is coadministered [1, 2, 4]. It was, therefore, of interest to probe the sensitivity of the two detoxifying pathways for acetaminophen to interference by various classes of drugs that may be co-administered to different patients, including cancer patients.

Many drugs which possess a hydroxy group can be envisaged as potential inhibitors of acetaminophen metabolism. We found several, including prednisone and prednisolone, capable of inhibiting acetaminophen glucuronidation *in vitro* [8]; we are now reporting on the effects of the latter *in vivo* in normal and in tumor-bearing rats. Drugs of another group, the antimetabolites of pyrimidines and purines, have the potential to alter the availability of cofactors for conjugation. For instance, 5-fluorouracil, known to be metabolized extensively to 5-fluorouridine triphosphate [9] and, to a lesser extent, to 5-fluorouridine diphosphoglucose [10] may readily interfere with UDPGA pools. We examined, therefore, its effect on acetaminophen metabolism, with special attention to glucuronidation. We compared its effect with that of galactosamine which is known to produce marked changes in UDP-sugar pools [11]. We also re-examined the effect of phenobarbital, which has

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been reported to increase acetaminophen glucuronidation by about 30 per cent [6, 12].

The studies were performed in rats, a species in which acetaminophen is metabolized chiefly via glucuronide and sulfate conjugations, and only 3–4 per cent by the mercapturic acid pathway [6], thus permitting us to focus on the two former pathways. The dose of acetaminophen was 300 mg/kg, that is 1935 mg/m^2, which is within the therapeutic range for an acetanilide type of analgesic in the rat [13], and falls within the human therapeutic range of 500–2000 mg/m^2.

Preliminary reports of parts of these studies have been given [14, 15].

MATERIALS AND METHODS

Chemicals. [^{3}H-(G)]*N*-Acetyl-*p*-aminophenol (APAP; acetaminophen) (sp. act. 370 mCi/mmole) was obtained from the New England Nuclear Corp., Boston, MA. Unlabeled acetaminophen, uridine 5′-diphosphoglucuronic acid (UDPGA, ammonium salt), UDP-*N*-acetylglucosamine (UDPAG), prednisone, prednisolone and D-galactosamine were purchased from the Sigma Chemical Co., St. Louis, MO. Phenobarbital (PB) and 5-fluorouracil (5-FU) were pharmaceutical preparations from the Roswell Park Memorial Institute Pharmacy. All other chemicals were the best available commercial grade.

Animals and treatments. Male or female Sprague–Dawley rats, weighing 200–300 g, were purchased from Charles River, Wilmington, MA. Female Sprague–Dawley rats were used as hosts for a transplantable colon carcinoma (initially described by Ward *et al.* [16], which was obtained from Dr. Y. Rustum of this Institute). Male ACI rats, weighing 170–250 g, were obtained from the Laboratory Supply Co., Indianapolis, IN, and were used as hosts for the Reuber H-35 hepatoma, as described earlier [17].

Acetaminophen (300 mg/kg), in saline solution, was injected i.p. in a volume equal to 3% of the body weight. Phenobarbital pretreatment consisted of i.p. injections of the sodium salt of the drug in saline in volume equal to 1% of body weight; 80 mg/kg were injected daily for 7–9 days as indicated, the last injection being 24 hr before administration of acetaminophen. Prednisone and prednisolone were injected i.p. in doses of 14 mg/kg, as suspensions in saline, in volumes equal to 0.3% of body weight. The injections were administered just before, or half an hour prior to acetaminophen injection. 5-Fluorouracil, at a dose of 120 mg/kg in saline solution, was injected 1–3 hr prior to acetaminophen, as indicated. Galactosamine solution in saline was administered i.p. at dose levels of 200 or 400 mg/kg, as indicated. Both 5-fluorouracil and galactosamine were injected in volumes equivalent to 0.3% of body weight.

Acetaminophen-injected animals were placed in glass metabolic cages with access to water *ad lib.* Periodic urine collections were made as indicated. Three to seven animals were used for each treatment group.

Determination of acetaminophen and its conjugates by a simplified gas chromatographic method. Urinary acetaminophen glucuronide and sulfate were estimated after their hydrolysis to acetaminophen, by incubating urine samples with 2000 units of β-glucuronidase or a mixture of β-glucuronidase with 70 units of sulfatase, per ml of incubate, containing 0.1 M acetate buffer at pH 5. The incubations were carried out for 18 hr at 37°. Urine (0.5 ml), or hydrolysate (0.5 ml), was saturated with sodium chloride, extracted with 10 ml of diethyl ether by shaking for 10 min, and centrifuged at 1600 *g* for 5 min. The ether extracts were evaporated to dryness and the residue was dissolved in small amounts of ethyl acetate (100–800 μl). The ethyl acetate solutions were injected onto the gas chromatographic column packed with 3% OV17 on Gas Chrom Q. The column temperature was 220°, as were the inlet and detector temperatures. Nitrogen carrier gas was passed through at the rate of 40 ml/min, hydrogen at 20 ml/min and air at 400 ml/min. Dimensions of the column were 2 m, 2 mm i.d. Good peaks of acetaminophen were obtained without derivatization when the range of the compound injected onto the column was between 0.2 and 3 μg. The retention time was approximately 3 min. Recoveries of acetaminophen determined by this method, when added to urine or plasma samples, were between 85 and 100 per cent, when acetaminophen was present in a concentration range of 5–2500 μg/ml.

Acetaminophen glucuronlytransferase assay. The microsomal glucuronyltransferase assay was carried out as described by Bolanowska and Gessner [8].

Acetaminophen sulfotransferase assay. Soluble fraction (100,000 *g* supernatant fraction) was incubated with tritiated acetaminophen (0.2–5 mCi/ml) at concentrations of 0.1–2 mM, plus 0.25 mM sodium sulfate, 5 mM magnesium chloride, 30 mM nicotinamide, and 5 mM ATP, in 0.08 M phosphate buffer at pH 7.4; 30 min-incubations were carried out at 37°. The reaction was stopped by freezing at −70°; samples were stored at −20° until analysis. The acetaminophen sulfate which formed was determined as a water soluble metabolite (ATP-dependent), left in the aqueous layer after ether extraction of unreacted acetaminophen, in a manner analogous to that described for acetaminophen glucuronide [8].

RESULTS

Gas chromatography of acetaminophen. In order to carry out these studies, we developed a simplified gas chromatographic method which we found more convenient than the commonly used colorimetric methods [18, 19]. We have used ether extractions of acetaminophen, as described by Brodie and Axelrod [18], and carried out analyses by a specific gas chromatographic method, which is a modification of that described by Prescott [20, 21]. We found that, without derivatization, good peaks of acetaminophen were obtained on columns packed with 3% OV17 on Gas Chrom Q. The limit of detection in the presence of interfering substances from biological fluids was 0.2 μg. Recoveries from biological fluids were between 85 and 100 per cent for urine or plasma samples containing actaminophen concentrations ranging from 2.5 to 2500 μg/ml.

Effects of tumors on acetaminophen metabolism in vivo. We examined the effects of a transplantable

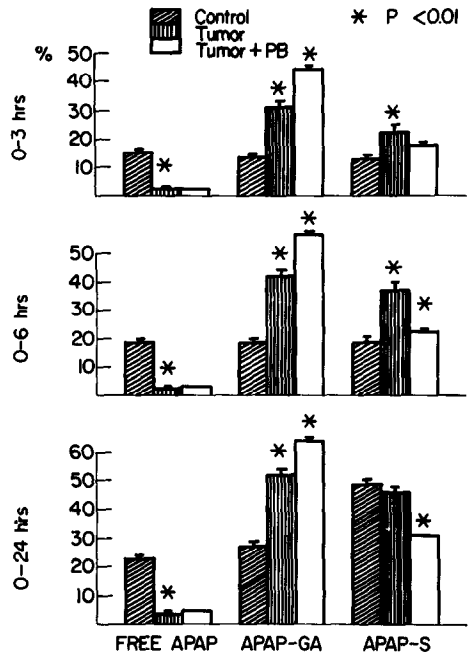

Fig. 1. Excretion of acetaminophen (APAP), acetaminophen glucuronide (APAPGA) and acetaminophen sulfate (APAPS) by ACI control rats and hepatoma Reuber H-35 bearing rats, untreated and phenobarbital (PB)-pretreated (7 days). Values are means ± S.E. P values were computed by Student's *t*-test. One hundred per cent = total 24 hr urinary excretion which accounted for 84.2 ± 7, 88.4 ± 3.4 and 79.8 ± 1.4 per cent of dose in control, tumor and tumor + PB groups, respectively.

hepatoma, Reuber H-35, on acetaminophen metabolism by comparing urinary excretion of unchanged acetaminophen and its metabolites, glucuronic acid and sulfate conjugates, by tumor hosts and control rats. The results are comparable to those we obtained previously from experiments using the model compound, *p*-nitrophenol [17]. In each case the hepatoma hosts excreted significantly higher amounts of the glucuronide conjugate of the phenolic compound. Additionally, in the case of acetaminophen, significant increases in the sulfate conjugate were also noted in the early urines (Fig. 1).

By contrast, no significant effect on acetaminophen metabolism was observed in rats bearing a transplantable colon carcinoma. Hosts with tumors ranging from 6 to 18 g (larger tumors tended to kill the rats) excreted acetaminophen metabolites in amounts comparable to control rats, although a trend to a decreased excretion of unchanged acetaminophen could be seen. Thus, the free drug accounted for 24.5 ± 5.5 and 15.5 ± 5.3 per cent of urinary acetaminophen excreted by control rats and tumor hosts respectively.

Effects of tumors on acetaminophen metabolism in vitro. In order to test for possible causes of increased metabolism of acetaminophen in hepatoma hosts, we examined hepatoma microsomal and cytosol preparations for their abilities to, respectively, glucuronidate and sulfate acetaminophen. It can be seen from Fig. 2 that hepatoma and liver microsomes have comparable glucuronyltransferase activities. Sulfotransferase activity of the hepatoma cytosol is also very similar to that of liver soluble fraction, except that the hepatoma enzyme appears to have a somewhat lower affinity for acetaminophen (Fig. 3). Thus, the results indicate that increased excretion of acetaminophen conjugates by the tumor

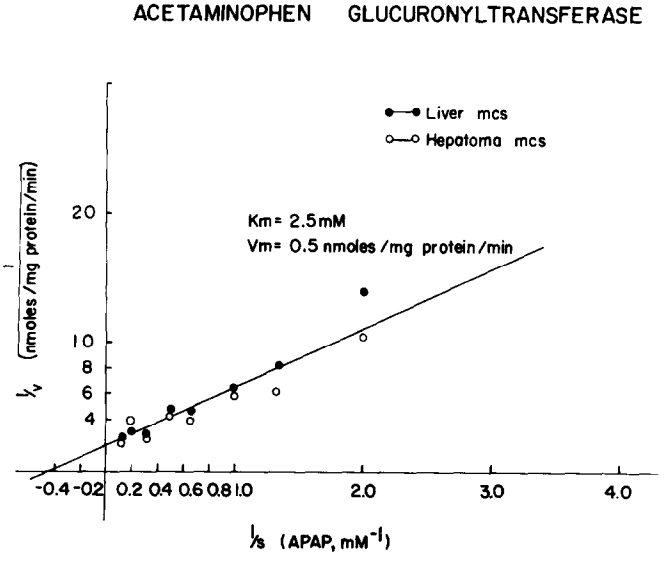

Fig. 2. Double reciprocal plot of *V* (in nmoles of acetaminophen glucuronide formed per mg of microsomal protein) against *S*, mM concentration of acetaminophen (APAP). Key: (●——●) liver microsomes and (○——○) hepatoma microsomes. Details are given under Materials and Methods.

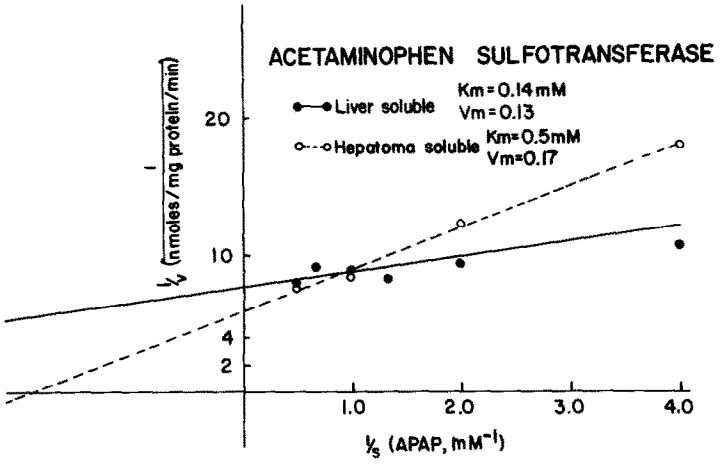

Fig. 3. Double reciprocal plot of V (in nmoles of acetaminophen sulfate formed per min per mg of soluble fraction protein) against S, mM concentration of acetaminophen (APAP). Key: (●——●) control liver and (○——○) hepatoma, soluble fractions. Details are given under Materials and Methods.

hosts is probably due to the contribution of the hepatoma to the metabolism of this drug. It is noteworthy that the amount of acetaminophen glucuronide excreted in 24 hr correlated with the weight of hepatoma, giving a correlation coefficient of 0.7 for tumor weights ranging from 4 to 30 g (Fig. 4).

The ability of colon carcinoma subcellular preparations to conjugate acetaminophen was tested similarly. Only trace amounts of glucuronidating and sulfating activity were found, thus explaining the lack of significant increases in urinary metabolites of acetaminophen in those hosts. Additionally, the results indicate that the presence of tumors, such as colon carcinoma, does not impair acetaminophen metabolism in the hosts. In this respect, the results are similar to the lack of effect of Walker 256 carcinoma on glucuronidation and sulfation of the model compound *p*-nitrophenol [17], and contrast with the effects of such tumors on mixed function oxidation pathways of hosts [22–26].

Effect of phenobarbital pretreatment. It has been reported previously that pretreatment of rats with 80 mg/kg of phenobarbital daily for 3 days produced only small (about 30–60 per cent) increases in excretion of acetaminophen glucuronide [6, 12]. By contrast, in our experiments, a 7- to 9-day phenobarbital pretreatment schedule resulted in a marked (3- to 4-fold) increase in the production of acetaminophen glucuronide, as demonstrated in two strains of rats (Table 1). This was accompanied by marked decreases in the excretion of acetaminophen sulfate and of free acetaminophen. The data also show that after the first 3 hr very little free acetaminophen was excreted.

Significant effects of phenobarbital pretreatment on metabolism of acetaminophen were also discernible in Reuber hepatoma hosts (Fig. 1). However, the changes, though statistically significant, were smaller in magnitude than those observed in control rats. This can be explained by the fact that increased metabolism due to hepatoma, and hence, fast depletion of the circulating substrate, left little scope for marked additional increases due to phenobarbital treatment.

Effects of prednisone and prednisolone. We found earlier [8] that both prednisone and prednisolone are inhibitors of acetaminophen glucuronidation in microsomal assays, and that the latter is somewhat more potent than the former. It was of interest to test *in vivo* effects of coadministration of these compounds with acetaminophen. The doses of the glucocorticoids chosen for our experiments represent approximately ten times the human doses used in cancer chemotherapeutic regimens. This is appropriate, since the drugs are subject to fast and extensive metabolism in the body [26–29], and rat basal metabolic rate is about ten times higher than that of humans.

Prednisone coadministered with acetaminophen produced a trend toward decreased glucuronidation, especially discernible in late urines; thus, the percentage excreted in the 0–24 hr urine was 24.7 ± 1.7

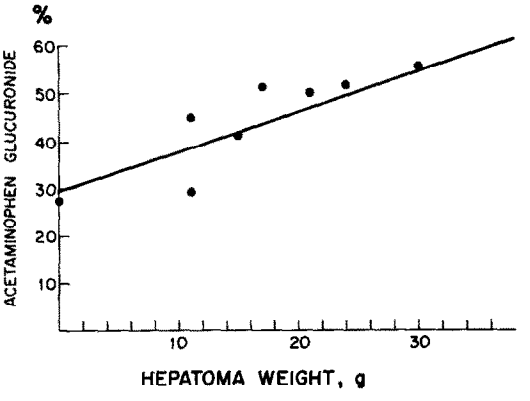

Fig. 4. Correlation between excretion of acetaminophen glucuronide (24 hr urine collection) and weight of hepatoma Reuber H-35; $r = 0.7$.

Table 1. Effects of phenobarbital pretreatement of male Sprague–Dawley and ACI rats on urinary metabolites of acetaminophen

Compound excreted	Rat group*	% Total excreted in urine†		
		0–3 hr	0–6 hr	0–24 hr
Acetaminophen	SD control	16.9 ± 2.1	19.5 ± 1.5	21.5 ± 1.5
	SD phenobarbital	7.0 ± 3.6	7.9 ± 3.6	8.8 ± 3.5
		P < 0.001‡	P < 0.001	P < 0.001
	ACI control	15.6 ± 2.1	19.3 ± 1.7	23.3 ± 1.7
	ACI phenobarbital	6.4 ± 0.7	6.8 ± 0.5	7.4 ± 0.2
		P < 0.001	P < 0.001	P < 0.001
Acetaminophen glucuronide	SD control	14.5 ± 1.9	19.9 ± 2.3	26.0 ± 2.8
	SD phenobarbital	45.9 ± 9.7	54.6 ± 5.1	60.4 ± 5.3
		P < 0.001	P < 0.001	P < 0.001
	ACI control	14.0 ± 2.4	19.0 ± 3.2	27.5 ± 3.2
	ACI phenobarbital	57.9 ± 6.7	63.8 ± 3.7	68.9 ± 1.4
		P < 0.001	P < 0.001	P < 0.001
Acetaminophen sulfate	SD control	18.0 ± 2.9	28.2 ± 3.6	52.5 ± 4.1
	SD phenobarbital	16.8 ± 4.8	22.5 ± 4.8	30.8 ± 4.7
		NS	P < 0.05	P < 0.001
	ACI control	13.0 ± 3.9	19.8 ± 4.9	49.1 ± 4.2
	ACI phenobarbital	13.0 ± 0.4	17.3 ± 0.9	23.7 ± 1.6
		NS	NS	P < 0.001

* Phenobarbital pretreatment was 9 days for SD rats and 7 days for ACI rats.

† Values are means ± S.D. One hundred per cent = total 24 hr urinary excretion which accounted for 87.0 ± 1.99, 83.2 ± 5.5, 84.2 ± 7.5 and 85.0 ± 5.1 per cent of dose in SD control, SD phenobarbital, ACI control and ACI phenobarbital groups, respectively.

‡ P values were computed by Student's *t*-test. Control and experimental groups were not significantly different from each other (NS) when P values were > 0.05.

instead of 33.1 ± 5.9 (P < 0.5). This was accompanied by a small, but statistically significant (P < 0.001), compensatory elevation of acetaminophen sulfate excretion (55.0 ± 1.7 vs 42.8 ± 1.4 per cent). The effects of prednisolone could be discerned only in the early urine; thus in the 0–3 hr urine, acetaminophen glucuronide decreased from 14.0 ± 2.4 to 9.2 ± 2.1 per cent (P < 0.02) (see Fig. 5).

We considered the possibility that the effects of these inhibitors might be magnified under conditions of more extensive glucuronidation (as would be the case in humans [30, 31]), and, therefore, examined their effects in hepatoma hosts. Under such conditions, prednisone, as well as prednisolone, appeared to produce significant decreases in acetaminophen glucuronidation, with concomitant significant increases in excretion of free acetaminophen (Fig. 5).

Effects of 5-fluorouracil and galactosamine. Effects of 5-fluorouracil were tested using a dose about ten times higher than a human chemotherapeutic dose, and one which has been used in animal studies [32, 33]. We looked for a 5-FU effect on acetaminophen metabolism, considering its possible modulation of uridine diphosphoglucuronic acid pools and hence of glucuronidation. One-hr pretreatment with 120 mg/kg of 5-fluorouracil produced a small but significant increase in the excretion of unchanged acetaminophen, but without significant decreases in glucuronidation or sulfation. Similarly, in phenobarbital-pretreated rats, 5-fluorouracil given 1 hr before acetaminophen produced no significant changes in urinary metabolites of acetaminophen (data not shown). A 3-hr pretreatment with 5-

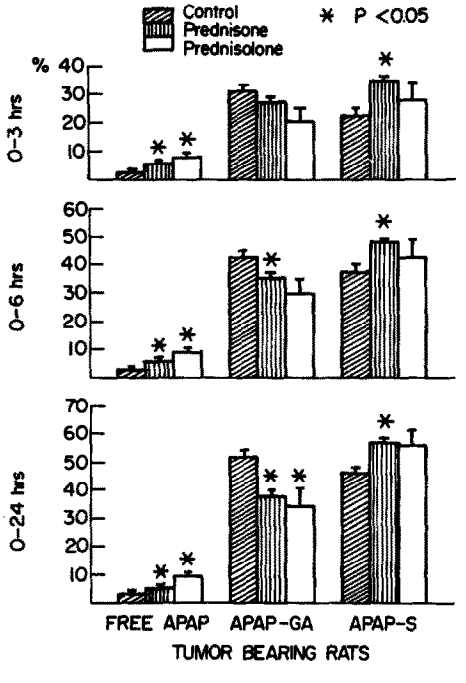

Fig. 5. Effects of prednisone and prednisolone on excretion of acetaminophen (APAP) and its glucuronide (APAPGA) and sulfate (APAPS) in hepatoma bearing rats. Values are means ± S.E. P values were computed by Student's *t*-test. One hundred per cent = total 24 hr urinary excretion which accounted for 88.4 ± 3.4, 103.7 ± 1.2 and 79.8 ± 1.4 per cent of dose in control, prednisone and prednisolone groups respectively.

Table 2. Effects of 5-fluorouracil and galactosamine treatments on urinary metabolites of acetaminophen

Compound excreted	Rat group*	% Total excreted in urine†		
		0–3 hr	0–6 hr	0–24 hr
Acetaminophen	Control	15.6 ± 2.1	19.3 ± 1.7	23.3 ± 1.7
	5-FU	18.7 ± 0.8	24.9 ± 1.9	30.5 ± 2.1
		$P < 0.01$‡	$P < 0.002$	$P < 0.001$
	200 Gal N	13.6 ± 2.4	17.4 ± 2.9	21.5 ± 3.7
	400 Gal N	13.9 ± 2.5	20.5 ± 2.5	26.0 ± 2.2
		NS	NS	NS
Acetaminophen glucuronide	Control	14.0 ± 2.4	19.0 ± 3.2	27.5 ± 3.2
	5-FU	11.6 ± 0.6	18.2 ± 1.1	28.0 ± 2.4
		$P < 0.05$	NS	NS
	200 Gal N	16.4 ± 2.1	23.0 ± 1.3	32.6 ± 0.8
	400 Gal N	13.0 ± 1.3	20.6 ± 1.3	26.4 ± 2.6
		NS	NS	NS
Acetaminophen sulfate	Control	13.0 ± 3.9	19.8 ± 4.9	49.1 ± 4.2
	5-FU	10.0 ± 1.3	15.2 ± 2.7	41.6 ± 1.4
		NS	MS	$P < 0.001$
	200 Gal N	15.0 ± 3.3	19.5 ± 2.7	45.9 ± 4.3
	400 Gal N	15.6 ± 0.9	22.6 ± 3.3	47.1 ± 2.8
		NS	NS	NS

* 5-FU was give 3 hr before acetaminophen; galactosamine was given concurrently with (200 mg/kg), or 0.5 hr before (400 mg/kg), acetaminophen.

† Values are means ± S.D. One hundred per cent = total 24 hr urinary excretion which accounted for 84.2 ± 7.5, 80.9 ± 4.6, 86.3 ± 7.5 and 79.9 ± 5.5 per cent of dose in control, 5-FU, 200 Gal N and 400 Gal N groups, respectively.

‡ P values were computed by Student's *t*-test. Control and experimental groups were not significantly different from each other (NS) when P values were > 0.05.

fluorouracil produced small decrease in acetaminophen glucuronide in the early urine, and a somewhat larger decrease in acetaminophen sulfate in the late urine (Table 2). The latter effect can be best understood if 5-fluorouracil alters availability of the sulfate donor, 3′-phosphoadenosine-5′-phosphosulfate (PAPS) (see Discussion).

A 200 mg/kg dose of galactosamine has been reported to affect acetaminophen metabolism in hamsters [34], but we saw no effects of such treatment in rats (Table 2). Galactosamine, which at a dose of 400 mg/kg is known to produce in rats marked effects on UDP-sugar pools within 0.5 hr of its administration [23], was administered 0.5 hr before acetaminophen. (This was considered to be appropriate timing since acetaminophen half-life is about 60 min in rats [34].) The treatment produced no decrease in acetaminophen glucuronide excreted in the urines (Table 2), thus indicating that availability of UDPGA, though undoubtedly markedly decreased, was not limiting to acetaminophen glucuronidation.

DISCUSSION

We have attempted to evaluate the potential for drug interactions with acetaminophen via various modes of interference with its metabolism. Effects of altered enzyme levels in the body were considered by examining the influence of two types of tumors and of phenobarbital pretreatment. The effects of inhibitors, such as prednisone and prednisolone, were examined, as well as possible effects of agents capable of altering cofactor availability, e.g. galactosamine and 5-FU. To study the interactions, we examined urinary excretion patterns, as they have been interpreted by others, both in human and animal studies, to reflect important changes in metabolism of acetaminophen [1–4, 6, 12, 25–38]. Thus, in humans it is known that subhepatotoxic doses are excreted chiefly as the glucuronide (about 60 per cent of the dose appears as urinary acetaminophen glucuronide, and 25 per cent as acetaminophen sulfate [1, 2, 31, 38]). Moreover, with increasing doses of acetaminophen [3, 37], there is a progressive increase followed by saturation, first of the sulfation pathway, then of glucuronidation, concurrently with increasing prominence of the ordinarily minor pathway of mercapturate conjugation [3, 37]. Such effects have been observed also in laboratory animals [6, 35], and the amount of mercapturate excreted have been correlated with the degree of hepatic necrosis, so that a graded response was observed [37] at seemingly subhepatotoxic doses. Thus, in experimental animals, increasing amounts of the urinary mercapturate of acetaminophen signify increased flux of the drug through the toxifying, mixed function oxidation pathway, and progressively greater amounts of hepatic necrosis. It can be surmised that in humans the same phenomenon can occur at overtly sub-hepatotoxic doses, which are in the upper therapeutic dose ranges; therefore, there should be concern about conditions that can impair the detoxifying glucuronidation and sulfation pathways.

We have used for our studies a 300 mg/kg dose of acetaminophen. This is a subsaturating dose for glucuronic acid conjugation in rat, and a subhepatotoxic dose in that animal [39]. Under these conditions, mercapturate conjugation accounts for only about

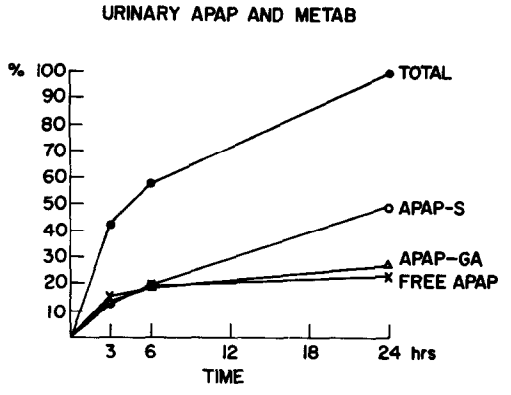

Fig. 6. Cumulative excretion of acetaminophen (APAP) and its glucuronide (APAPGA) and sulfate (APAPS) in ACI rats. One hundred per cent = total 24 hr urinary excretion which accounted for 84.2 ± 2.7 per cent of dose.

3 per cent of the dose [6]. We concentrated our efforts, therefore, on examining the effects of various pretreatments on the patterns of glucuronic acid and sulfate conjugates and on unmetabolized acetaminophen. In all the studies reported here, the amount of free acetaminophen plus its glucuronide and sulfate conjugates which was excreted in the 24 hr urine accounted for 87.8 ± 4.8 per cent of the dose, irrespective of the treatment group.

The results reported here for acetaminophen, together with those reported earlier for the model compound *p*-nitrophenol [17], show that glucuronidation and sulfation pathways are not impaired in tumor hosts. This contrasts with reported impairment of mixed function oxidase pathways [22–26]. Some tumors, such as hepatomas, can increase overall host metabolism. We attribute this to the ability of the tumor to metabolize the drug.

Phenobarbital pretreatment is known to induce drug-metabolizing enzymes, including glucuronyltransferase. Our results show that about a 3-fold increase can occur as a result of phenobarbital pretreatment. Jollow *et al.* [6] observed only about a 30 per cent increase in urinary glucuronide excretions of acetaminophen, administered to rats in doses ranging from 50 to 400 mg/kg. However, Jollow *et al.* [6] treated the rats with phenobarbital (75 mg/kg daily) for just 3 days, whereas we continued phenobarbital treatment for 7–9 days. Although a 3-day phenobarbital pretreatment is sufficient for induction of mixed function oxidases, a longer treatment is needed to optimally induce *p*-nitrophenol glucuronyltransferase [40]. Our results indicate that the latter also applies to induction of acetaminophen glucuronidation. A short-term, 3-day pretreatment would favor, preferentially, induction of the toxifying pathway of acetaminophen metabolism, which was observed by Mitchell *et al.* [35]; however, a longer pretreatment should offset this to some extent by a concomitant induction of glucuronidation, an important detoxifying pathway.

Phenobarbital pretreatment increased acetaminophen glucuronide conjugation and decreased sulfate conjugation. Normally, acetaminophen sulfate continues to increase at a steady rate for up to 24 hr after acetaminophen administration, after glucuronidation has virtually subsided (Fig. 6). However, when phenobarbital pretreatment is involved, acetaminophen is quickly used up by glucuronidation and very little of it is left unmetabolized (as is indicated by virtually no additional excretion of unchanged acetaminophen after the first 3 hr); there is, thus, very little acetaminophen for subsequent sulfation, ultimately resulting in much lower sulfate excretion. The bases for these events appear to be the relative affinities of acetaminophen glucuronyltransferase and sulfotransferase (see Figs. 2 and 3). It appears that the glucuronyltransferase has a much higher apparent K_m value for acetaminophen than does the sulfotransferase. Hence, glucuronide conjugation predominates when a high circulating level of acetaminophen is present and subsides when it falls, but sulfate becomes predominant when a low level of circularing acetaminophen is present and continues to be synthesized under such conditions. By contrast, the v_{max} for sulfation is lower than that for glucuronidation. Thus, sulfation behaves as a high affinity, low capacity pathway, and glucuronidation behaves as a low affinity, high capacity pathway. (A similar observation was made by Moldeus [41] who studied acetaminophen metabolism in isolated hepatocytes.) This may explain why sulfation is readily saturated but continues to function at a steady rate when acetaminophen-circulating levels fall, whereas glucuronidation is not readily saturated, and is most prominent when high levels of acetaminophen are present in the circulation. The latter occurs during the first hours after acetaminophen

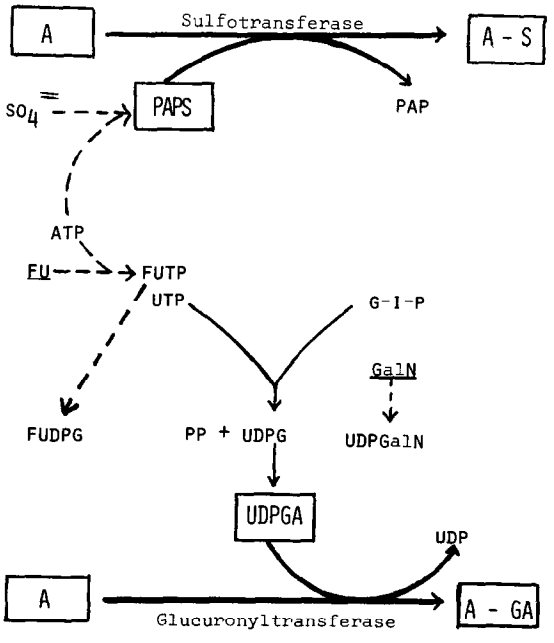

Fig. 7. Possible interactions of 5-fluorouracil and galactosamine with chief biotransformation pathways of acetaminophen. Key: A, acetaminophen; AS, acetaminophen sulfate; AGA acetaminophen glucuronic acid conjugate; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; 5-FU, 5-fluorouracil; and GalN, galactosamine.

dosage, and when high doses of acetaminophen are administered. A pattern of predominance of the glucuronide conjugate can be seen when high doses of acetaminophen are used, and of the sulfate conjugate at lower doses, by comparing the reports of other investigators [6, 42, 43].

With respect to inhibitors, we reported earlier that, *in vitro*, prednisolone is a better inhibitor than prednisone. The *in vivo* studies reported here corroborate this. In hepatoma hosts, however, such effects were somewhat masked by a greater variability in the response of these rats to prednisolone. It is noteworthy that no decrease in sulfation was observed in our studies, which suggests that prednisolone does not inhibit sulfation, though it is inhibitory to glucuronidation. It is interesting that Banerjee *et al.* [44] observed an increased hepatotoxity of acetaminophen in conjunction with prednisolone treatment.

Another group of drugs, antimetabolites of pyrimidines and purines, were envisaged as potential modifiers of uridine diphosphoglucuronic acid and 3′-phosphoadenosine-5′-phosphosulfate pools, the donor molecules for glucuronidation and sulfation respectively. The effects of 5-fluorouracil were examined in this context. Interference with these pathways can occur as depicted in Fig. 7. It was anticipated that its effects would be mainly on the uridine diphosphoglucuronic acid, since 5-FU is known to be extensively metabolized to 5-FUTP [9] which, in turn, can be metabolized to 5-FUDPG and 5-FUDPGA [10]. It was hypothesized that, if the availability of UDPGA were rate-limiting, 5-FU would reduce the glucuronidation of acetaminophen. Our results, in fact, show only a marginal effect on glucuronidation. This does not mean, however, that substantial changes in UDPGA pools did not take place. We found that galactosamine, at doses which markedly decrease UDP-sugar pools and UTP [11], had no significant effect on acetaminophen glucuronidation in our experiments. This contrasts with decreased glucuronidation of acetaminophen in hamsters treated with galactosamine [34] and may indicate a species difference in sensitivity of this pathway to modulation of the UDPGA pools. 5-FU actually had a much greater effect on sulfation than on glucuronidation in our experiments. This suggests that 5-FU, because of its extensive conversion to 5-FUTP, may be depleting ATP to an extent which causes a decreased pool of the active sulfate PAPS. Availability of sulfate as a limiting factor in the sulfation of drugs has been observed [45, 46], and recently it has been found that an increase in dietary sulfate can augment acetaminophen sulfate production and decrease toxicity of this drug [46, 47].

Acknowledgements—Thanks are due to Jo Ellen Budnick for her technical assistance. This work was supported by US Public Health Science Grant CA-13038.

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