Effect of Aspirin on a Subtoxic Dose of ^{14}C-Acetaminophen in Mice

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Abstract □ The interaction of ^{14}C-acetaminophen, 150 mg/kg (20 μCi/kg), and aspirin, 200 mg/kg po, was studied in male mice. The radiolabel was rapidly absorbed from the GI tract, achieving maximum blood levels 0.25 hr after oral dosing. Radioactivity in the blood equilibrated rapidly with the tissues and was concentrated in the liver and kidney. At 14 hr, most of the dose was eliminated in urine as the glucuronide, cysteine, sulfate, free drug, and mercapturate. Pretreatment with aspirin tended to reduce the rate and extent of acetaminophen absorption and altered the percentage of the dose excreted in the urine as sulfate, mercapturate, glucuronide, and cysteine. Interpretation of these data toxicologically as an indication of the potentiation of either toxicity or protection was not possible.

Keyphrases □ Aspirin—effect on absorption, excretion, and metabolism of acetaminophen, radiochemical analysis, mice □ Acetaminophen—absorption, excretion, and metabolism, effect of aspirin, radiochemical analysis, mice □ Absorption—acetaminophen, effect of aspirin, radiochemical analysis, mice □ Excretion—acetaminophen, effect of aspirin, radiochemical analysis, mice □ Metabolism—acetaminophen, effect of aspirin, radiochemical analysis, mice □ Radiochemistry—analysis, effect of aspirin on absorption, excretion, and metabolism of acetaminophen, mice □ Analgesics—effect of aspirin on absorption, excretion, and metabolism of acetaminophen, radiochemical analysis, mice

Acetaminophen, a widely used nonnarcotic analgesic, is remarkably safe in therapeutic doses. Recently, it has been used in combination with aspirin in analgesic formulations. In view of a previous report of an aspirin–phenacetin metabolic interaction (1) and the fact that acetaminophen is the principal metabolite of phenacetin, a survey of some animal species was initiated to determine if aspirin affected the fate of ^{14}C-acetaminophen.

In the rat, absorption and metabolism were both markedly altered by aspirin (2); in the guinea pig, aspirin appeared to have its greatest inhibitory effect on biliary and urinary excretion (3). As a continuation of this survey, the fate of a subtoxic dose of ^{14}C-acetaminophen alone and in the presence of aspirin was examined in the mouse, a species markedly more susceptible to acetaminophen-induced hepatotoxicity than rats or guinea pigs (4).

EXPERIMENTAL

Materials—Carboxyl-labeled ^{14}C-aspirin[1], specific activity 122 μCi/mg, and uniformly ring-labeled ^{14}C-acetaminophen[2], specific activity 44.64 μCi/mg, were synthesized. Gum tragacanth[3] and unlabeled aspirin[4] and acetaminophen[5] were obtained commercially. Swiss Webster male mice, 28 ± 3 g, were purchased locally[6].

Methods—Animals were acclimatized for at least 1 week before each experiment; both food and water were supplied *ad libitum*. Sixteen hours prior to each experiment, food, but not water, was withdrawn.

Establishing Subtoxic Dose—In view of the much higher toxicity of acetaminophen in the mouse compared to the rat or guinea pig (4), it was essential to establish that 150 mg/kg was a subtoxic level of acetaminophen. As an index of toxicity, plasma levels of glutamic–oxalacetic transaminase (EC 2.6.1.1) and glutamic–pyruvic transaminase (EC 2.6.1.2) were monitored. Mice were pretreated with vehicle or aspirin (200 mg/kg po) and then treated with acetaminophen (150 or 250 mg/kg po) 30 min later as previously described (3).

Sixteen hours following acetaminophen administration, the mice were decapitated and blood was collected in heparinized Natelson blood-collecting tubes. Blood samples were centrifuged in a clinical centrifuge. The plasma was assayed for transaminases at 37° by the UV method of Henry *et al.* (5), using standard assay reagents[7] and a biochromatic analyzer[8]. Control transaminase levels were determined using plasma from mice not receiving treatment.

Blood Profiles—Radiolabeled aspirin, 200 mg/kg po, was administered as a 0.25% gum tragacanth suspension at 25 μCi/kg (10 ml/kg) to determine the profile of ^{14}C-aspirin-derived radioactivity in the blood. All subsequent experiments were performed with cold aspirin, which was administered at 200 mg/kg po 30 min prior to dosing with ^{14}C-acetaminophen. Radiolabeled acetaminophen was administered at 150 mg/kg po (20 μCi/kg) as described previously (3). Mice dosed intravenously (tail vein) received a solution of acetaminophen (150 mg/kg) at 7 ml/kg containing 10% ethanol. Duplicate blood samples were collected at various times and processed as reported previously (6).

Tissue Distribution and Urinary Excretion—The distribution of orally and intravenously administered ^{14}C-acetaminophen in blood (10 μl) and tissues (25–100 mg) from vehicle- and aspirin- (200 mg/kg) pretreated mice, collected at 0.25, 1, and 4 hr following ^{14}C-acetaminophen (150 mg/kg), was examined by reported methods (3, 6). Urine was collected from groups of eight orally dosed mice housed in metabolism cages. The volume of urine excreted per animal, the urinary concentration of total carbon-14 expressed as micrograms per milliliter, and the cumulative total carbon-14 excreted were determined by liquid scintillation counting (6).

Urinary Metabolites—Acetaminophen and its metabolites were separated using the alkaline paper chromatography system (System C) described by Shahidi (7) and modified by Thomas *et al.* (2). Some urine samples were also chromatographed on Sephadex gel G-10 as previously described (2). In addition to the chromatographic separation of neat (unadulterated) urine, aliquots of β-glucuronidase[9]-treated urine were chromatographed on paper (2).

The cysteine conjugate was identified in mouse urine, which had been desalted by passing it through an ion-exchange column[10]. Following the elution of salts and other highly water-soluble impurities with distilled water, radioactivity was eluted with methanol. The eluent was dried under a nitrogen stream, taken up in water, and separated by Sephadex gel G-10 column chromatography (2). The fractions collected were monitored for both a positive ninhydrin test and radioactivity.

The fractions giving a positive ninhydrin test and containing high concentrations of radioactivity were lyophilized and chromatographed in the alkaline system (2) and the 1-butanol–acetic acid–water (4:1:1) system (acidic system) reported previously (8). In addition, a mass spectrum of the lyophilized sample was obtained by the direct insert probe method using a low-resolution mass spectrometer[11] at an ionization energy of 80 ev and a source temperature of 210°.

Data Analysis—With the exception of the paper and Sephadex gel G-10 chromatography data, isotopic assays were performed in duplicate. Transaminase activities were also determined on duplicate plasma samples. Means and standard errors were computed from averaged data; statistical significance was determined by the unpaired Student t test, with $p < 0.05$ being considered significant. In cases where percentages were compared, arc–sin transformed data were used (9).

[1] Amersham/Searle Corp., Arlington Heights, IL 60005.
[2] Mallinckrodt, St. Louis, MO 63160.
[3] Fisher Scientific Co. Ltd., Toronto, Canada.
[4] B.D.H. Canada Ltd., Toronto, Canada.
[5] Eastman Kodak Co., Rochester, N.Y.
[6] Biobreeding Labs of Canada Ltd., Ottawa, Canada.
[7] Abbott Laboratories, Montreal, Canada, and Boehringer Mannheim Corp., New York, NY 10017.
[8] ABA-100, Abbott Scientific Products Division, South Pasadena, CA 91030.
[9] Ketodase, Warner-Chilcott, Morris Plains, NJ 07950.
[10] Amberlite XAD-2, Polystyrene Polymers, B.D.H. Canada Ltd., Toronto, Canada.
[11] Hitachi Perkin-Elmer RMS-4.

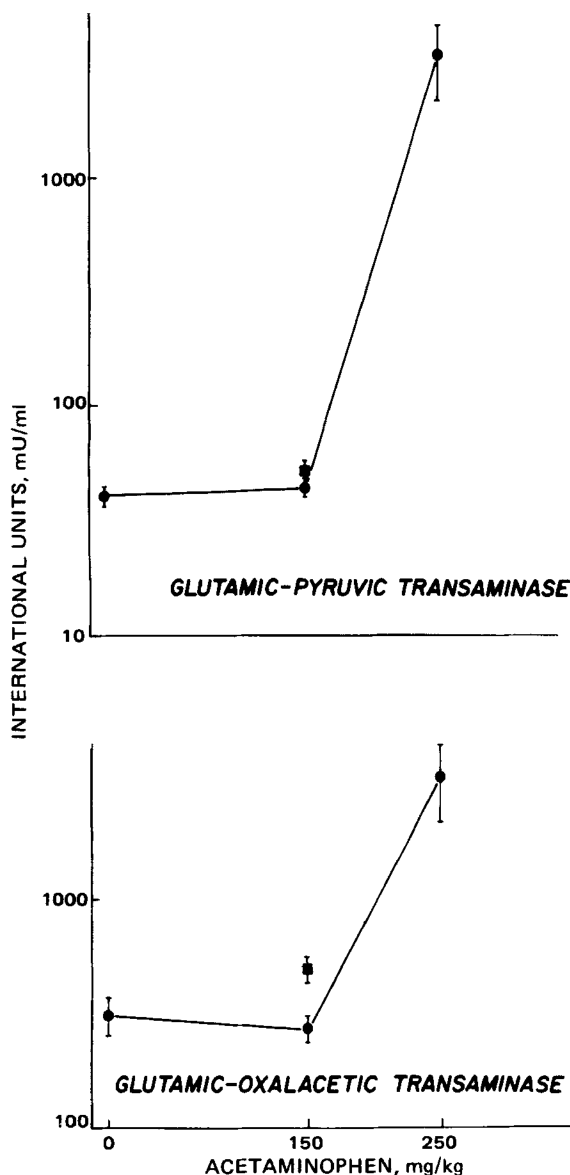

Figure 1—*Plasma transaminase dose–response curves in mice following administration of acetaminophen, 150 mg/kg po. Plasma glutamic–pyruvic transaminase and glutamic–oxalacetic transaminase levels were determined 16 hr following acetaminophen administration to mice orally pretreated with vehicle (●) or aspirin, 200 mg/kg (■), 0.5 hr prior to dosing with acetaminophen. Values are means ±SE from at least seven animals.*

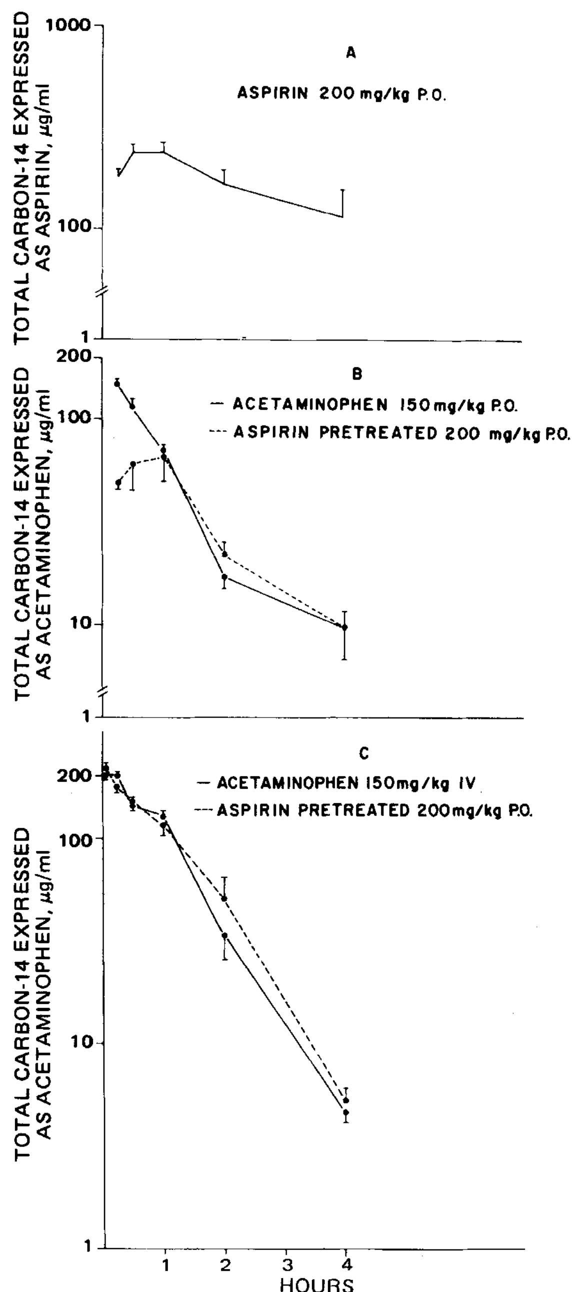

Figure 2—*Blood profiles of radioactivity following drug administration obtained from mice receiving: A, ^{14}C-aspirin, 200 mg/kg po; B, ^{14}C-acetaminophen, 150 mg/kg po; or C, ^{14}C-acetaminophen, 150 mg/kg iv. In both B and C, mice were orally pretreated with either vehicle (—) or cold aspirin, 200 mg/kg (- - -), 0.5 hr prior to receiving ^{14}C-acetaminophen. Values are means ±SE from three to five animals. In B, differences are significant at 0.25 and 0.5 hr at* $p < 0.001$ *and* $p < 0.05$, *respectively.*

RESULTS

As a criterion for establishing a subtoxic dose of acetaminophen, plasma transaminase activities were monitored and the dose–response was determined (Fig. 1). Vehicle-pretreated mice were dosed with different concentrations of acetaminophen to determine a "no effect level." At 150 mg/kg, vehicle-pretreated mice possessed plasma levels not significantly different from control animals; 250 mg/kg statistically elevated plasma transaminase levels, suggesting that this dose was hepatotoxic.

To ensure that 150 mg/kg remained a "no effect level" in the presence of aspirin, plasma transaminases were likewise monitored in aspirin-pretreated mice. Plasma glutamic-oxalacetic and glutamic–pyruvic transaminases were not significantly altered from control values (Fig. 1), establishing that acetaminophen, 150 mg/kg, alone or in combination with aspirin, 200 mg/kg, was a subtoxic dose.

Radioactivity in the blood of ^{14}C-aspirin-treated mice peaked 0.5–1 hr following administration (Fig. 2A). Consequently, all subsequent pretreatments with cold aspirin were administered 0.5 hr prior to ^{14}C-acetaminophen. Pretreatment with aspirin significantly reduced the blood levels of radioactivity at 0.25 ($p < 0.001$) and 0.5 ($p < 0.05$) hr following oral administration of ^{14}C-acetaminophen (Fig. 2B), whereas comparable blood levels were observed following intravenous administration (Fig. 2C).

The half-life and area under the blood profile curves following intravenous dosing ($t_{1/2} = 0.688 \pm 0.023$ hr; area = 238.8 ± 11.0 µg/ml × hr) were not altered by aspirin pretreatment ($t_{1/2} = 0.718 \pm 0.072$ hr; area = 240.6 ± 17.2 µg/ml × hr). This lack of statistical significance in the area under the blood profile curves between vehicle- and aspirin-pretreated mice was also seen in the oral study (vehicle pretreated = 182.5 ± 13.5 µg/ml × hr *versus* aspirin pretreated = 139.9 ± 11.0 µg/ml × hr), suggesting that aspirin pretreatment was not statistically altering the extent of absorption from the GI tract.

The distribution of radioactivity, expressed as micrograms of acetaminophen per milliliter or gram, in the blood, heart, liver, kidneys, and GI tract and contents at 0.25, 1.0, and 4.0 hr following oral or intravenous administration of 150 mg of ^{14}C-acetaminophen/kg is presented in Table

Table I—Effect of Aspirin on the Tissue Distribution of Radioactivity following ^{14}C-Acetaminophen[a]

Tissue	Hours	Oral		Intravenous	
		Vehicle Pretreated	Aspirin Pretreated	Vehicle Pretreated	Aspirin Pretreated
Blood	0.25	149.9 ± 7.3 (1.0)	49.4 ± 2.4 (1.0)**	202.2 ± 6.0 (1.0)	177.6 ± 5.0 (1.0)*
	1.0	70.2 ± 4.9 (1.0)	66.5 ± 16.5 (1.0)	128.4 ± 6.0 (1.0)	117.3 ± 15.8 (1.0)
	4.0	9.6 ± 2.9 (1.0)	9.6 ± 9.8 (1.0)	4.6 ± 0.5 (1.0)	5.3 ± 0.7 (1.0)
Heart	0.25	110.0 ± 6.9 (0.7)	39.2 ± 2.3 (0.8)**	148.3 ± 5.6 (0.7)	131.4 ± 3.8 (0.7)*
	1.0	40.2 ± 4.4 (0.5)	47.4 ± 12.0 (0.7)	82.3 ± 5.4 (0.6)	93.9 ± 10.2 (0.8)
	4.0	4.0 ± 1.0 (0.4)	6.8 ± 0.9 (0.7)*	3.6 ± 0.8 (0.8)	2.8 ± 0.2 (0.5)
Liver	0.25	516.2 ± 39.2 (3.4)	242.4 ± 23.4 (4.9)**	229.9 ± 9.5 (1.1)	207.1 ± 2.0 (1.2)
	1.0	310.6 ± 49.4 (4.4)	349.7 ± 72.2 (5.3)	165.1 ± 8.2 (1.3)	300.4 ± 127.5 (2.5)
	4.0	92.3 ± 39.1 (9.6)	97.9 ± 25.2 (10.2)	18.3 ± 2.2 (4.0)	20.0 ± 3.2 (3.8)
Kidney	0.25	310.3 ± 23.3 (2.1)	188.4 ± 40.4 (3.8)*	256.7 ± 3.4 (1.3)	237.1 ± 8.4 (1.3)
	1.0	233.5 ± 19.0 (3.3)	238.60 ± 55.8 (3.6)	229.5 ± 7.1 (1.8)	179.2 ± 33.9 (1.5)
	4.0	52.7 ± 10.0 (5.5)	79.6 ± 20.0 (8.3)	35.2 ± 3.5 (7.7)	27.7 ± 1.7 (5.2)
GI tract and contents	0.25	—	—	475.2 ± 20.5	450.2 ± 28.5
	1.0	—	—	557.6 ± 35.3	595.8 ± 163.2
	4.0	—	—	504.4 ± 61.4	588.8 ± 49.6

[a] Mice were orally pretreated with vehicle or aspirin (200 mg/kg) 0.5 hr prior to oral or intravenous administration of ^{14}C-acetaminophen (150 mg/kg). Radioactivity is expressed as grams per milliliter or grams of tissue. Statistical differences are indicated as $p < 0.05$ (*) and $p < 0.001$ (**). Values are means from three to five animals ± *SE*. Ratios of tissue to blood carbon-14 concentration are in parentheses.

Table II—Effect of Aspirin Pretreatment on Urinary Excretion of ^{14}C-Acetaminophen[a]

Hours	Pretreatment	Cumulative Volume of Urine per Animal, ml	Concentration, μg/ml	Cumulative Percentage of Dose Excreted
4	Vehicle	0.52 ± 0.12	2198.3 ± 328.8	25.3 ± 1.9
	Aspirin	0.62 ± 0.09	1950.5 ± 196.2	27.2 ± 2.4
12	Vehicle	1.31 ± 0.02	2008.7 ± 75.2	61.5 ± 3.3
	Aspirin	1.44 ± 0.17	1599.7 ± 207.0	57.8 ± 8.7
24	Vehicle	2.77 ± 0.05	532.0 ± 78.5	79.4 ± 2.2
	Aspirin	2.64 ± 0.21	737.7 ± 42.5	75.9 ± 6.5
48	Vehicle	5.19 ± 0.58	77.0 ± 27.1	83.3 ± 2.2
	Aspirin	4.67 ± 0.92	163.4 ± 35.2	83.8 ± 5.0

[a] Mice were divided into six groups of eight animals each and orally pretreated with vehicle or aspirin (200 mg/kg) 0.5 hr prior to receiving ^{14}C-acetaminophen (150 mg/kg po). Each value is the mean ± *SE* from three pools of eight mice.

I. In the oral study at 0.25 hr, blood, heart, liver, and kidney levels of carbon-14 from vehicle-pretreated mice were approximately two- to threefold higher than levels in corresponding tissues from aspirin-pretreated animals. At later time periods, the trend had reversed: tissues from aspirin-pretreated animals tended to possess higher levels of carbon-14 than tissues from control mice, although statistical significance could only be demonstrated in cardiac tissue at 4.0 hr.

With the exception of 0.25-hr blood and heart samples, vehicle- and aspirin-pretreated mice dosed intravenously with ^{14}C-acetaminophen gave comparable tissue carbon-14 levels. Blood and cardiac tissue collected at 0.25 hr from aspirin-pretreated mice possessed lower levels of radioactivity than the corresponding tissues from vehicle-pretreated mice.

Pretreatment with aspirin did not alter any of the urinary parameters examined (Table II) in mice dosed orally with ^{14}C-acetaminophen. Descending alkaline paper chromatography[12] of neat urine separated radioactivity into five distinct peaks (Fig. 3A), whereas urine samples treated with β-glucuronidase gave six peaks (Fig. 3B). Peaks 2, 3, 5, and 6 were previously identified in neat urine as the glucuronide, mercapturate, sulfate, and free acetaminophen, respectively (2). Peak 6 (Fig. 3B) increased following β-glucuronidase treatment and was attributed to acetaminophen derived from the hydrolysis of the acetaminophen–glucuronide conjugate. Peaks 1 and 4 were minor peaks and were not identified.

Preliminary TLC and GLC–mass spectrometry data suggested that peak 4 contained more than one ^{14}C-metabolite, at least one of which contained sulfur. Peak 2 (R_f 0.07–0.09) was tentatively identified as the acetaminophen–cysteine conjugate. Examination of the qualitative properties of the fraction isolated as the cysteine conjugate by Sephadex gel G-10 (Table III) confirmed the presence of the acetaminophen–cysteine conjugate in mouse urine, and a quantitative comparison of peak 2 and the acetaminophen–cysteine conjugate isolated by column chromatography gave comparable results.

The cumulative urinary excretion of free acetaminophen at 4, 8, and 14 hr following oral administration of ^{14}C-acetaminophen was not significantly altered by aspirin pretreatment (Table IV). During the first 8 hr, sulfate excretion was significantly reduced whereas mercapturate elimination was increased by aspirin pretreatment. By 14 hr, the effect of aspirin on cumulative urinary excretion of sulfate and mercapturate had disappeared and significant differences in the excretion of glucuronide and cysteine appeared. Glucuronide excretion was inhibited and the 14-hr cumulative excretion of the cysteine conjugate was increased by aspirin pretreatment.

[12] Whatman No. 1 filter paper.

DISCUSSION

The combination of orally administered aspirin and acetaminophen appeared to reduce the acetaminophen absorption rate during the absorptive phase (Fig. 2B), an observation previously reported for the rat (2) and guinea pig (3). Since urinary excretion (Table II) and the intravenous $t_{1/2}$ were comparable between vehicle- and aspirin-pretreated animals, elimination differences did not appear to be responsible for the altered blood profiles (Fig. 2B). In view of the fact that blood carbon-14 concentrations and areas under the blood concentration–time curves were comparable following intravenous administration to vehicle- and aspirin-pretreated animals, the reduced blood level of carbon-14 in aspirin-pretreated mice (Fig. 2B) was attributed to an inhibition of absorption.

The basis for this inhibitory effect of aspirin on absorption is not immediately apparent. Smith and Irving (10) reported that salicylate (100 mg) administered to rats reduced the gastric emptying rate. More recent reports indicated that acetaminophen absorption was dependent upon gastric emptying (11, 12). In view of these reports, retardation in absorption of radioactivity in aspirin-pretreated mice (Fig. 2B) was attributed to a reduction in the gastric emptying rate.

Tissue distribution of acetaminophen appeared to be dependent upon the route of administration. In the oral study, carbon-14 levels in the liver and kidneys during the 1st hr were 2.1–5.3-fold higher than blood concentrations (Table I); in the intravenous study, liver and kidney levels of radioactivity were only 1.1–2.5-fold higher than blood carbon-14 levels. Pretreatment with aspirin apparently had little effect on the distribution of intravenously administered acetaminophen. However, in the oral study, tissue-to-blood ratios of carbon-14 (Table I) tended to be higher in aspirin-pretreated mice than in vehicle-pretreated animals. These results suggested that ^{14}C-acetaminophen-derived radioactivity was more readily taken up following oral administration by tissues of aspirin-pretreated mice than by tissues from controls. This possibility prompted an examination of acetaminophen metabolism.

Table III—Properties of the Acetaminophen–Cysteine Conjugate[a]

Column chromatography	Cysteine conjugate/acetaminophen elution volume (ml), 38/85
Descending paper chromatography	R_f 0.28 in acidic system, R_f 0.07 in alkaline system
Color test	Ninhydrin, purple
Mass spectrometry	m/e ions 226, 209, 197, 183, 141, and 108

[a] The cysteine conjugate was isolated from Amberlite XAD-2 desalted mouse urine by Sephadex gel G-10 column chromatography. The acidic and alkaline paper chromatography systems were 1-butanol–acetic acid–water (4:1:1) and 2-propanol–water–ammonium hydroxide (20:2:1), respectively.

The major urinary metabolites of acetaminophen are glucuronide, mercapturate, sulfate, and free drug (2, 3, 13–16). In this study, chromatography of neat and β-glucuronidase-treated urine following 150 mg of ^{14}C-acetaminophen/kg separated radioactivity into six distinct peaks (Fig. 3). Four of these peaks were previously identified (2). Other peaks, with the exception of peak 2 (Fig. 3B), were minor and were not identified.

On the basis of migration in the descending alkaline paper chromatography system (Fig. 3B and Table III), peak 2 was tentatively identified as the acetaminophen–cysteine conjugate, previously reported (7, 17) to have R_f 0.10. To confirm the occurrence of the acetaminophen–cysteine conjugate in mouse urine, properties of the cysteine fraction were examined (Table III). It gave a positive ninhydrin test, which indicated a primary amine. Radioactivity in this fraction migrated in descending acidic and alkaline paper chromatography systems as single peaks with R_f values comparable to those previously reported (17). Mass spectrometry of the freeze-dried cysteine fraction (Table III) gave a fragmentation pattern identical to that reported for the acetaminophen–cysteine conjugate (16), unequivocally establishing the presence of the cysteine conjugate in mouse urine.

Glucuronide (27%) was the major urinary metabolite in 14-hr urine samples (Table IV), followed by cysteine (13.6%), sulfate (9.5%), free drug (3.9%), and mercapturate (1.9%). Jollow *et al.* (15) examined the 24-hr urine of mice dosed with ^{3}H-acetaminophen (100 mg/kg ip) and found radioactivity distributed as glucuronide (47.9%), sulfate (25.2%), free drug (13.4%), and mercapturate (12.4%) but failed to demonstrate the presence of the acetaminophen–cysteine conjugate. In view of their high glucuronide percentage and considering the fact that they eluted and then were treated with β-glucuronidase, the presence of the cysteine conjugate cannot be excluded as a possible contaminant of their glucuronide peak. On the other hand, a different strain of mouse (GP mice) from that used in this study (Swiss Webster mice) was utilized, which could account for the differences. A recent report (18) attested to mouse strain differences in inducibility of acetaminophen metabolism to produce hepatotoxicity, although acetaminophen metabolites were not examined *per se*.

The most significant metabolic effects of aspirin in this study were the reduction in sulfate and the increase in mercapturate at 4 and 8 hr (Table IV), effects previously reported in the rat (2). The biochemical basis for these effects was not established; however, Boström *et al.* (19) showed that salicylate inhibited the biosynthesis of mucopolysaccharide sulfates. These investigators suggested that the oxidative uncoupling action of salicylate reduced active sulfate (3′-phospho-5′-adenosine phosphosulfate) levels, thereby inhibiting sulfation. Since cysteine is a precursor for the synthesis of both the active sulfate and glutathione (15), it seems reasonable that reducing the active sulfate level would increase the pool of cysteine or precursors of cysteine (glutathione) available for conjugation with radioactivity derived from ^{14}C-acetaminophen. This result, in fact, was observed; aspirin-pretreated mice tended to excrete a larger percentage of the dose as cysteine and mercapturate than vehicle-pretreated animals (Table IV), but statistically significant differences could only be demonstrated in mercapturate excretion at 4 and 8 hr and cysteine excretion at 14 hr.

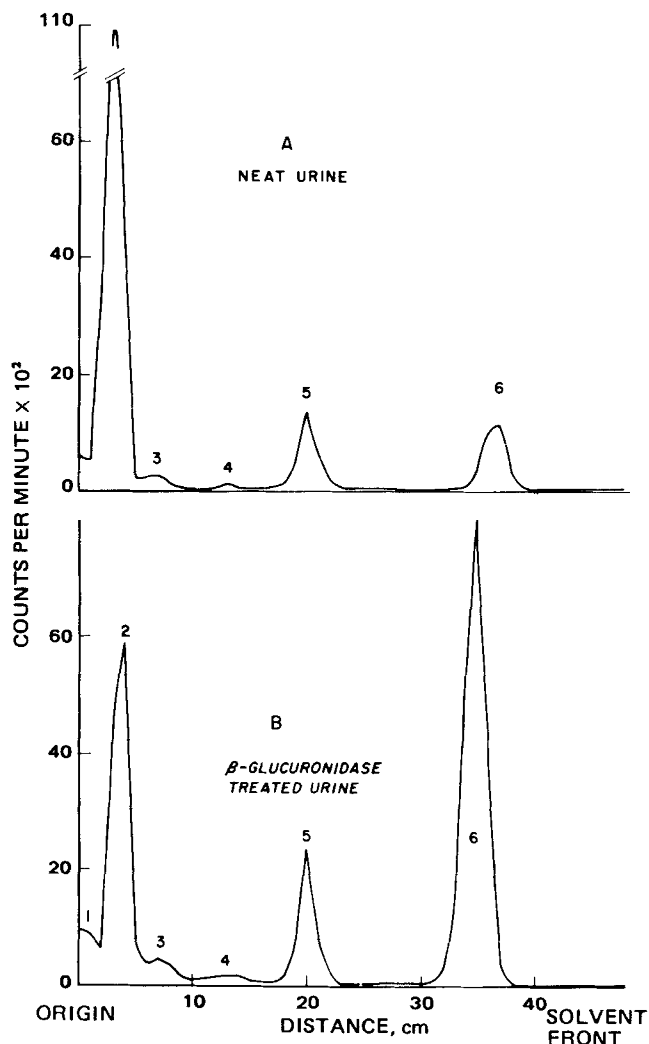

Figure 3—*Typical paper chromatographic separation of urinary metabolites of ^{14}C-acetaminophen in the mouse. Neat (A) and 16-hr β-glucuronidase-treated (B) urine, 10 and 20 µl, respectively, were applied as bands to filter paper and developed descendingly in 2-propanol–water–ammonium hydroxide (20:2:1). The paper was serially sectioned into 1-cm strips and assayed for carbon-14 by liquid scintillation counting. The separated peaks were: 1 and 4, unknown; 2, cysteine; 3, mercapturate; 5, sulfate; and 6, free acetaminophen. The increase in peak 6 following β-glucuronidase treatment was attributed to the glucuronide.*

Measurement of the acetaminophen–glutathione conjugate excreted in the urine as degradation products (mercapturate and cysteine conjugates) was suggested (20) as a suitable method for estimating the toxic pathway and the animal's susceptibility to acetaminophen. Since the susceptibility to acetaminophen-induced hepatotoxicity follows the order

Table IV—Effect of Aspirin on the Urinary Metabolites of ^{14}C-Acetaminophen in the Mouse[a]

Hours	Pretreatment	R_f 0.01–0.04, Metabolite 1	R_f 0.05–0.07, Glucuronide	R_f 0.07–0.09, Cysteine	R_f 0.14–0.18, Mercapturate	R_f 0.26–0.30, Metabolite 4	R_f 0.41–0.47, Sulfate	R_f 0.72–0.78, Acetaminophen
4.0	Vehicle	0.68 ± 0.15	11.18 ± 1.97	3.60 ± 0.67	0.40 ± 0.12	0.50 ± 0.06	3.16 ± 0.44	3.36 ± 0.90
	Aspirin	0.81 ± 0.06	8.25 ± 1.44	4.04 ± 0.22	1.41 ± 0.04*	0.43 ± 0.05	0.63 ± 0.02*	2.48 ± 0.24
8.0	Vehicle	0.95 ± 0.29	14.98 ± 1.47	5.89 ± 0.62	0.71 ± 0.11	0.73 ± 0.03	4.47 ± 0.16	3.70 ± 0.77
	Aspirin	1.23 ± 0.03	11.90 ± 1.03	6.44 ± 0.28	1.88 ± 0.10*	0.67 ± 0.10	0.99 ± 0.05**	2.78 ± 0.24
14.0	Vehicle	1.97 ± 0.59	27.00 ± 0.37	13.59 ± 0.10	1.87 ± 0.38	1.75 ± 0.18	9.51 ± 0.75	3.91 ± 0.72
	Aspirin	2.54 ± 0.30	21.16 ± 0.01*	17.63 ± 0.15*	3.88 ± 0.98	2.14 ± 0.54	7.33 ± 0.30	5.65 ± 0.55

[a] Mice were divided into six groups of eight animals each and orally pretreated with vehicle or aspirin (200 mg/kg) 0.5 hr prior to receiving ^{14}C-acetaminophen (150 mg/kg po). Metabolites were separated using a 2-propanol–water–ammonium hydroxide (20:2:1) descending paper chromatography system. Values are mean percentages of the dose ± *SE*. Statistical significance is indicated as $p < 0.001$ (**) and $p < 0.05$ (*) when compared to corresponding control group.

of mouse > rat > guinea pig (4), one would expect the excretion of glutathione degradation products in these three species to be mouse > rat > guinea pig. This pattern was observed; the mouse excreted 15.5% of the dose in 14 hr as glutathione degradation products (Table IV) compared to a 12-hr excretion of 5.1% in the rat (2) and 1% in the guinea pig (3). Excretion of glutathione degradation products in the mouse increased following aspirin pretreatment (Table IV). On the basis of the catabolite hypothesis (20), this result suggests that aspirin-pretreated mice are more susceptible to acetaminophen-induced hepatotoxicity than are vehicle-pretreated mice.

However, if aspirin increased the pool of cysteine or precursors of cysteine by reducing active sulfate levels, as already suggested, then the increased urinary excretion of glutathione degradation products could indicate an increase in the conjugative detoxication of the toxic metabolite or protection from acetaminophen-induced hepatotoxicity. In support of the latter hypothesis is a study where aspirin showed a protective effect against toxic doses of acetaminophen (21).

CONCLUSION

The metabolite profile of acetaminophen in the mouse was not exactly the same as that observed in humans (22, 23). However, the mouse biotransformed acetaminophen more like the human than either the rat (2) or the guinea pig (3). Since the mouse is more susceptible to acetaminophen hepatotoxicity than either the rat or the guinea pig (4) and biotransformation of acetaminophen in the mouse resembles that occurring in humans, the mouse is a better animal model than either the rat or guinea pig for toxicological studies involving acetaminophen.

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Colorimetric Determination of Aliphatic Acids

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Abstract □ A colorimetric method for the determination of carboxylic acids based on the dicyclohexylcarbodiimide-coupled reaction of 2-nitrophenylhydrazine and carboxylic acids is described. The product of the reaction is extracted into aqueous sodium hydroxide to produce a blue color. This method is suitable for the analysis of aliphatic acids, but aromatic acids do not react under these conditions.

Keyphrases □ Aliphatic carboxylic acids—colorimetric analysis in solutions □ Carboxylic acids, aliphatic—colorimetric analysis in solutions □ Colorimetry—analysis, aliphatic carboxylic acids in solutions

The recent application of the coupling agent dicyclohexylcarbodiimide in the analysis of carboxylic acids *via* hydroxamic acid formation (1) prompted investigation of this reagent to couple carboxylic acids with 2-nitrophenylhydrazine. An earlier study (2) showed that the hydrazide resulting from the reaction of 2-nitrophenylhydrazine with activated carboxylic acid derivatives such as acid anhydrides and acid chlorides gives an intense blue color in aqueous hydroxide solutions. This color formation was useful for the colorimetric analysis of these substances.

The objective of this study was to adapt the dicyclohexylcarbodiimide-coupled reaction of carboxylic acids and 2-nitrophenylhydrazine to produce a colorimetric method for the determination of carboxylic acids.

EXPERIMENTAL

Materials—Unless otherwise stated, analytical reagent grade chemicals were used. Acetonitrile was distilled from phosphorus pentoxide, with the fraction boiling at 81.5° being collected. Dichloromethane was distilled directly, with the fraction boiling at 39.5° being collected. 2-Nitrophenylhydrazine was recrystallized from water–methanol (85:15), yielding yellow-orange needles, mp 92–92.5°.