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Effect of Diethyl Ether on the Biliary Excretion of Acetaminophen[1] (41928)

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Abstract. The biliary and renal excretion of acetaminophen and its metabolites over 8 hr was determined in rats exposed to diethyl ether by inhalation for 1 hr. Additional rats were anesthetized with urethane (1 g/kg ip) while control animals were conscious throughout the experiment (surgery was performed under hexobarbital narcosis: 150 mg/kg ip; 30-min duration). The concentration of UDP-glucuronic acid was decreased 80% in livers from ether-anesthetized rats but was not reduced in urethane-treated animals when compared to that in control rats. The concentration of reduced glutathione was not affected by either urethane or diethyl ether. Basal bile flow was not altered by the anesthetic agents. Bile flow rate after acetaminophen injection (100 mg/kg iv) was increased slightly over basal levels for 2 hr in hexobarbital-treated control rats, was unaltered in urethane-anesthetized animals, and was decreased throughout the 8-hr experiment in rats exposed to diethyi ether for 1 hr. In control and urethane-anesthetized animals, approximately 30–35% of the total acetaminophen dose (100 mg/kg iv) was excreted into bile in 8 hr, while only 16% was excreted in rats anesthetized with diethyl ether. Urinary elimination (60–70% of the dose) was not altered by exposure to ether. Separation of metabolites by reverse-phase high-pressure liquid chromatography showed that ether decreased the biliary elimination of unchanged acetaminophen and its glucuronide, sulfate, and glutathione conjugates by 47, 40, 49, and 73%, respectively, as compared to control rats. Excretion of unchanged acetaminophen and the glutathione conjugate into bile was depressed in urethane-anesthetized animals by 45 and 66%, respectively, whereas elimination of the glucuronide and sulfate conjugates was increased by 27 and 50%, respectively. These results indicate that biliary excretion is influenced by the anesthetic agent and that diethyl ether depresses conjugation with sulfate and glutathione as well as glucuronic acid. © 1984 Society for Experimental Biology and Medicine.

Many endogenous and exogenous compounds are excreted into bile as the unchanged chemical, or more typically, as a conjugated metabolite. The most common synthetic reactions produce glucuronide, sulfate, or glutathione derivatives. For example, bilirubin, hexachlorophene, iopanoic acid, phenolphthalein, thyroxine, valproic acid, and vitamin D_3 are excreted into bile mainly as glucuronides while sulfobromophthalein and ethacrynic acid are secreted predominantly as glutathione conjugates (1, 2). Numerous factors such as species, dose, nutritional state, and exposure to pharmacological agents can affect biotransformation and biliary excretion (1, 2).

The phenolic drug, acetaminophen, is excreted into urine predominantly as the sulfate and into bile mostly as the glucuronide (3–8). However, unmetabolized acetaminophen and the glutathione conjugate are also found in urine and bile. Decreased metabolic conversion to the sulfate in some disease states or after sulfate depletion results in enhanced formation of other metabolites (3–5). Because metabolites of acetaminophen in both bile and urine are readily separated by reverse-phase high-pressure liquid chromatography and quantified (9), one can determine how exposure to various xenobiotics affects hepatic biotransformation.

The availability of cofactors and substrates can markedly alter the rates of conjugation reactions and can influence drug elimination.

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Hepatic UDP-glucuronic acid (UDPGA)[5] concentrations can be increased by pretreatment with microsomal enzyme inducers and decreased by exposure to gaseous anesthetics (10). Livers from rats under diethyl ether narcosis contain 80% less UDPGA than control rats (11, 12). This reduction in UDPGA by diethyl ether is sufficient to decrease the biliary excretion of the glucuronide conjugates of phenolphthalein, iopanoic acid, bilirubin, diethylstilbestrol, and valproic acid (13, 14). However, the elimination of phenolphthalein–glucuronide, which is not biotransformed before excretion, was not changed indicating transport function was not altered by exposure to diethyl ether (13). Whether exposure to diethyl ether affects other conjugative pathways besides glucuronidation is unknown. Thus, this study has evaluated the effect of diethyl ether on the biliary excretion of acetaminophen and its glucuronide, sulfate, and glutathione conjugates.

Materials and Methods. Acetaminophen (AA), AA-glucuronide, AA-sulfate and AA-mercapturate were obtained from Sterling-Winthrop, Sussex, United Kingdom. AA-glutathione was a gift from Dr. B. H. Lauterberg, Baylor College of Medicine, Houston, Texas. Reduced glutathione, 1-chloro-2,4-dinitrobenzene, 5,5′-dithiobis(2-nitrobenzoate), UDPGA, and urethane were purchased from Sigma Chemical Company, St. Louis, Missouri. Anhydrous diethyl ether, ethyl acetate, and HPLC grade methanol came from Fisher Scientific, St. Louis, Missouri.

Young adult male Sprague–Dawley rats, 260–320 g (Sasco, Omaha, Nebr.), were allowed tap water and laboratory rodent chow (Purina, St. Louis, Mo.) *ad libitum* and were maintained in quarters at 22–28°C with a 12-hr light/dark cycle before the experiments. Immediately prior to anesthesia, all animals were administered 20 ml saline/kg po to promote diuresis. Control rats were administered hexobarbital (150 mg/kg ip; 10 ml/kg in saline) to induce narcosis for surgery. Additional rats were anesthetized with either urethane (1.0 g/kg ip; 2 ml/kg in water) or diethyl ether (by inhalation with humidified oxygen). After onset of narcosis, the bile duct was isolated and cannulated with PE-10 tubing (Clay–Adams, Parsippany, N.J.). Acetaminophen (100 mg/kg; 2 ml/kg in 50% (v/v) propylene glycol/saline) was injected into a saphenous vein 30 min after the onset of narcosis. This dose of acetaminophen is not hepatotoxic in the rat (15). Rats were placed in restraining cages, and body temperatures were maintained at 37°C with a heat lamp to prevent hypothermic alteration of biliary excretion (16). The duration of hexobarbital anesthesia was 15–30 min after acetaminophen administration, and the rats were conscious throughout the rest of the experiment. Diethyl ether narcosis was maintained for 1 hr after acetaminophen injection, while urethane-treated rats were anesthetized for 8 hr. Bile was collected in tared test tubes at hourly intervals for 8 hr. Bile flow rate was determined gravimetrically assuming a density of 1.0. Urine was collected in a 10-ml beaker with a funnel placed under the caudal end of the rat.

Acetaminophen, AA-glucuronide, AA-sulfate, AA-glutathione, and AA-mercapturate were separated by reverse-phase HPLC on a C_{18} μBondapak column (Waters Associates, Milford, Mass.) as described by Howie *et al.* (9), with a Waters Model 6000A pump and Model 440 detector. Metabolites were identified by comparison to the retention times of known standards. The solvent was 1% acetic acid/methanol/ethyl acetate (90/15/0.1) and was filtered before use (Solvent Clarification Kit, Waters Associates). There were no interfering peaks in rat bile or urine. Concentrations of metabolites were calculated from an acetaminophen standard curve because the molar extinction coefficients are essentially the same for unchanged acetaminophen and its conjugates (9, 17). Urine and bile samples were diluted with the solvent mixture and centrifuged at 3500*g* before injection onto the column.

Hepatic concentrations of UDPGA were determined by the method of Watkins and Klaassen (12) and that of reduced glutathione by the procedure of Ellman (18). Activity of GSH-transferase toward 1-chloro-2,4-dinitro-

[5] Abbreviations used: UDPGA, UDP-glucuronic acid; AA-glucuronide, acetaminophen glucuronide; AA-sulfate, acetaminophen sulfate; AA-glutathione, acetaminophen-glutathione; AA-mercapturate, acetaminophen mercapturic acid; GSH-transferase, glutathione *S*-transferase.

benzene was measured by the spectrophotometric method of Habig *et al.* (19). These parameters were determined in livers excised from rats 1 hr after the onset of narcosis for each anesthetic agent.

Data were analyzed by a one-way analysis of variance followed by Duncan's new multiple range test to compare the means. Urine data were compared with Students *t* test. $P < 0.05$ was considered significant. Differences are routinely discussed as compared to hexobarbital-treated control rats who were usually conscious 15–30 min after administration of acetaminophen.

Results. Effects of the anesthetics and acetaminophen on bile flow are illustrated in Fig. 1. In vehicle-treated (50% propylene glycol/saline) control rats, bile flow was not affected by the various anesthetics. Bile flow rate decreased from 4.5 to 2.7 ml/hr/kg over 8 hr. There was no major change in bile flow immediately after emergence from either hexobarbital- (at 0–0.5 hr) or diethyl ether- (at 1.5–2.5 hr) induced narcosis. Injection of 100 mg acetaminophen/kg iv produced a pronounced choleresis to a maximal rate of 6.8 ml/hr/kg in hexobarbital control rats from 0 to 3 hr and a smaller increase to 5.2 ml/hr/kg in ether-anesthetized animals from 1–2 hr. No acetaminophen-induced choloresis was observed in urethane-treated rats and, thus, bile flow was significantly lower than in hexobarbital controls throughout the 8-hr experiment after acetaminophen administration.

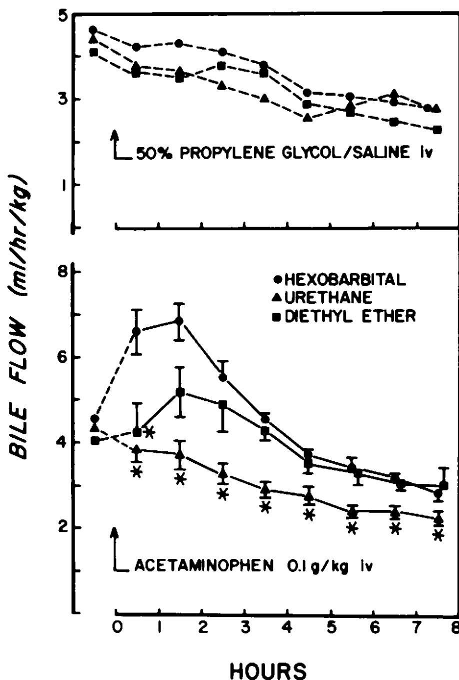

FIG. 1. Effect of anesthetic agents on bile flow rate in control and acetaminophen-treated rats. Control rats ($n = 2$) received 2 ml/kg of 50% propylene glycol/saline and average values are reported. Additional rats received 100 mg acetaminophen/kg iv and the values are means ± SE of six animals. Asterisks indicate results are significantly different from those of hexobarbital-treated rats at $P < 0.05$.

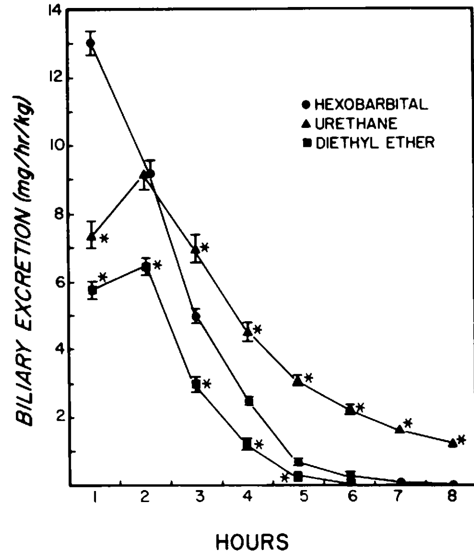

FIG. 2. Biliary excretion of total acetaminophen including its metabolites. Each point (mg equivalents of acetaminophen/hr/kg) represents means ± SE of five (diethyl ether), six (hexobarbital), or seven (urethane) rats. Acetaminophen (100 mg/kg) was injected iv at 0 hr. Asterisks indicate values are significantly different from hexobarbital-treated animals at $P < 0.05$.

Effects of the anesthetics on the biliary excretion of total acetaminophen including its metabolites are depicted in Fig. 2 as equivalents of acetaminophen. When compared to hexobarbital control, excretion was markedly reduced by 1 hr of exposure to diethyl ether, and secretion remained depressed throughout the entire experiment. Excretion in urethane-anesthetized rats was decreased in the first collection period but was higher than in hexobarbital-treated con-

trol rats from 3 to 8 hr. Maximal biliary excretion occurred in the first hour in hexobarbital rats and the second hour for animals anesthetized with urethane and diethyl ether. Total biliary excretion over 8 hr in both hexobarbital- and urethane-anesthetized animals was similar and was greater than 30% of the dose while having been only 16% in rats exposed to ether (Table I).

The time course for the biliary excretion of the parent drug and each conjugate is demonstrated in Fig. 3. The primary metabolite in bile was the glucuronide, with AA-sulfate second. Excretion of parent compound and metabolites in diethyl ether- and urethane-anesthetized rats was lower than that in controls during the first hour. By the fourth hour, elimination of AA-glucuronide and AA-sulfate was higher in animals anesthetized with urethane than that in hexobarbital control rats. Significantly lower quantities of AA-glutathione and acetaminophen were excreted by urethane- and ether-treated rats during the first 2 hr. Moreover, excretion of the glucuronide, sulfate, and glutathione conjugates and parent drug was depressed for 5, 5, 4, and 3 hr, respectively, in animals exposed to diethyl ether.

Excretion rates were cumulated for 8 hr and are listed in Table I. The predominant metabolite in rat bile is AA-glucuronide, with AA-sulfate, AA-glutathione and parent drug in decreasing order. When compared to excretion in hexobarbital-anesthetized control rats, cumulative excretion rates in urethane-treated animals for total acetaminophen, acetaminophen, AA-glucuronide, AA-sulfate, and AA-glutathione were 115, 55, 127, 151, and 34%, respectively, and in ether-anesthetized rats were 52, 53, 60, 50, and 27%. AA-mercapturate was not observed in bile from ether-treated animals, but was present in bile from hexobarbital- and urethane-anesthetized rats. Determination of metabolites in urine indicates a significant increase in AA-glucuronide elimination in rats anesthetized with diethyl ether while total excretion was not affected. Urinary elimination was not measured in urethane-treated rats because these animals did not micturate during the experiment.

Table II indicates that exposure to diethyl ether caused an 80% decrease in hepatic UDPGA concentration. Reduced glutathione content was not affected by the anesthetic agents, however, GSH-transferase activity toward 1-chloro-2,4-dinitrobenzene was decreased 15% in urethane- and ether-treated rats.

Discussion. Diethyl ether is widely used to induce anesthesia in laboratory animals and has been assumed to have little effect on xenobiotic biotransformation or drug elimination. However, recent evidence indicates both short-term and continuous exposure to ether demonstrably influences drug metabolism. For example, administration of diethyl ether throughout drug infusion and blood sampling reduced the total clearance and elimination rate for antipyrine and acetamin-

TABLE I. BILIARY AND RENAL EXCRETION OF ACETAMINOPHEN AND ITS METABOLITES IN RATS[a]

Group		Acetaminophen	AA-glucuronide	AA-sulfate	AA-glutathione	AA-mercapturate	Total
Hexobarbital[b]							
(control)	Bile	1.19 ± 0.14	16.8 ± 1.8	8.2 ± 0.9	4.66 ± 0.53	0.32 ± 0.13	31.1 ± 3.0
(n = 6)	Urine	5.07 ± 0.63	14.3 ± 1.9	40.3 ± 5.0	ND[c]	0.62 ± 0.11	60.2 ± 7.2
Diethyl ether[d]	Bile	0.63 ± 0.07*	10.1 ± 0.9*	4.2 ± 1.0*,**	1.27 ± 0.14*	ND[c]	16.2 ± 2.0*,**
(n = 5)	Urine	3.74 ± 0.43	21.3 ± 1.2*	43.3 ± 3.4	ND[c]	0.91 ± 0.12	69.3 ± 3.3
Urethane[e]	Bile	0.65 ± 0.15*	21.4 ± 4.4*	12.4 ± 1.8*	1.59 ± 0.31*	0.18 ± 0.04	35.8 ± 3.2
(n = 7)							

[a] Values represent means ± SE and are percentages of administered dose (100 mg/kg iv).
[b] 150 mg/kg ip (0.5–1 hr narcosis).
[c] Not detectable.
[d] 1 hr exposure (1 hr narcosis).
[e] 1.0 g/kg ip (8 hr narcosis).
* $P < 0.05$ as compared to hexobarbital.
** $P < 0.05$ as compared to urethane.

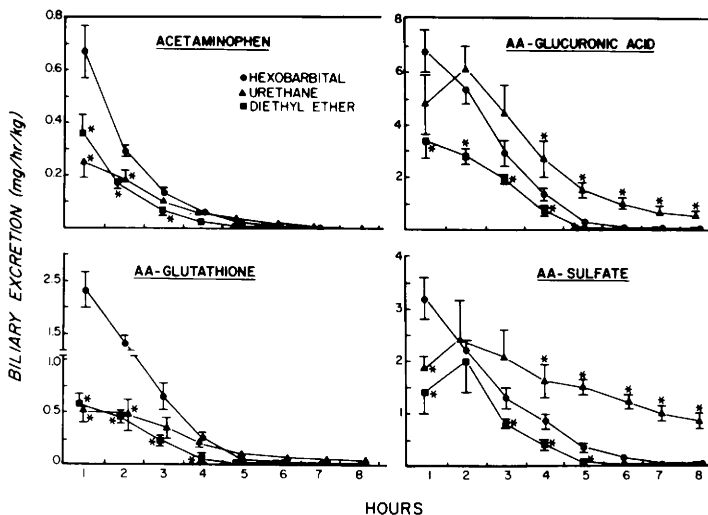

FIG. 3. Biliary excretion rates of acetaminophen, AA-glutathione, AA-glucuronide, and AA-sulfate following an iv dose of 100 mg acetaminophen/kg. Values represent the means ± SE of five (diethyl ether), six (hexobarbital), or seven (urethane) rats. Asterisks indicate significant differences from hexobarbital-treated animals at $P < 0.05$.

ophen (20), and iopanoic acid-glucuronide (21). Metabolism of these two compounds plus sulfanilamide was also decreased in isolated hepatocyte suspensions when ether was added to the medium (22). Moreover, diethyl ether inhibits the microsomal oxidation of barbiturates (23, 24), diphenylhydantoin (25) and antipyrine (26), the microsomal glucuronidation and cytosolic sulfation of acetaminophen (27), mitochondrial respiration (28), and protein synthesis (29). These studies emphatically demonstrate that diethyl ether is not innocuous, but is capable of producing dramatic effects on several intracellular processes that have different localizations.

Recent studies have indicated that profound reductions in the hepatic concentration of UDPGA occur within minutes of exposure to diethyl ether (11, 12). The 80% reduction in UDPGA levels (Table II) is similar to that observed in other studies (10, 13) and is sufficient to depress the biliary excretion of glucuronidated cholephils (13, 14). Elimination of the glucuronide conjugates of bilirubin, diethylstilbestrol, iopanoic acid, and valproic acid was decreased 41, 29, 76, and 28%, respectively (13). Plasma clearance of the parent compounds, and appearance of conjugates in bile and plasma were also reduced. The present study indicates that

TABLE II. EFFECT OF ANESTHETIC AGENTS ON THE HEPATIC CONCENTRATIONS OF UDP-GLUCURONIC ACID AND REDUCED GLUTATHIONE AND GSH-TRANSFERASE ACTIVITY[a]

Group	UDP-Glucuronic acid (nmole/g liver)	Glutathione (mM)	GSH-Transferase activity[b] (μmole/min/g liver)
Hexobarbital[c]	448 ± 30	7.17 ± 0.38	200 ± 2.7
Diethyl ether[d]	80 ± 11*,**	6.97 ± 0.16	174 ± 4.8*
Urethane[e]	370 ± 39	7.63 ± 0.82	168 ± 5.2*

[a] Values represent means ± SE of four rats.
[b] Activity measured toward 1-chloro-2,4-dinitrobenzene.
[c] 150 mg/kg ip (0.5–1 hr narcosis).
[d] By inhalation (1 hr narcosis).
[e] 1.0 g/kg ip (8 hr narcosis).
* $P < 0.05$ as compared to hexobarbital group.
** $P < 0.05$ as compared to urethane group.

total acetaminophen excretion into bile is reduced 48% (Table I, Figs. 2, 3) in rats exposed to ether. Moreover, depression of the biliary excretion of the parent drug, AA-sulfate, and AA-glutathione was also observed (Table II, Fig. 3). In contrast, urinary excretion of total acetaminophen is not affected although 33% more AA-glucuronide was in the urine of ether-anesthetized rats (Table I). This increase in urinary elimination may occur because metabolism of acetaminophen to glucuronide and sulfate conjugates as well as to a reactive compound that is converted to the mercapturic acid derivative is known to occur in the kidney (30). In addition, intestinal conversion of AA-glutathione to the mercapturate and/or cysteine conjugates (4) may explain the absence of AA-glutathione in urine (Table I).

The mechanism for these effects is not completely clear. The reduction in UDPGA levels is apparently due to increased degradation, because an increase was observed in the urinary concentration of two breakdown products, D-glucaric acid and L-ascorbic acid (31). Depression of AA-glutathione production may result from the decreased GSH-transferase activity (Table II). However, a number of fluorinated ethers are suicide inhibitors of the cytochrome *P*-450-dependent monooxygenases (32). It may be that diethyl ether can also inhibit the activation of acetaminophen to the reactive intermediate that is normally conjugated with glutathione. Sulfation of acetaminophen is substrate (or capacity) limited (3, 33–35), and activation of inorganic sulfate to 3′-phosphoadenosine-5′-phosphosulfate may be influenced by alterations in intracellular nucleotide levels (36). Inhibition of mitochondial respiration by diethyl ether (28) may reduce sulfate activation and hence compromise acetaminophen sulfation. Alternatively, since sulfation is more sensitive to changes in liver blood flow than glucuronidation or glutathione conjugation (37), the reduced AA-sulfate excretion may be the result of ether-induced hemodynamic changes (38, 39). Though the mechanism for these effects is complex and not completely understood, it is obvious that exposure to diethyl ether may produce wide ranging effects on many intracellular functions.

Comparison of the biliary excretion of acetaminophen and its metabolites in rats anesthetized with urethane to those under initial hexobarbital narcosis reveals several interesting observations. The greater choleresis in hexobarbital-treated rats (Fig. 1) after administration of acetaminophen may result from the higher excretion rates of AA-sulfate, AA-glutathione, and parent drug (Figs. 2, 3). In later collection periods (4–8 hr), elimination of the sulfate and glucuronide conjugates decreases below that in urethane-treated rats (Fig. 3) and thus, total excretion over the 8-hr experiment is not different (Fig. 2, Table I). However, it is possible that the increased bile flow rate also results from some secretion of the barbiturate into bile because both pentobarbital and phenobarbital are rapidly excreted into bile (28 and 18% of dose, respectively, in 6 hr) while bile flow rate was not altered (40). With its shorter biological half-life, hexobarbital excretion into bile, if occurring at all, would be expected to occur more rapidly than pentobarbital or phenobarbital and have only a transient effect on acetaminophen secretion. Other studies indicated that acute treatment with barbiturates or diethyl ether does not significantly alter bile flow rate but could depress secretion of anions into bile (21, 41). Our previous work indicating that exposure to diethyl ether did not affect the biliary transport of phenophthalein glucuronide (13) suggests that further studies are needed to completely delineate the effects of anesthesia on bile flow and biliary transport.

Quantification of acetaminophen and its metabolites in urine was not made in urethane-treated animals because the rats did not urinate. This is not unexpected since urethane, when administered ip, causes fluid accumulation in the peritoneum, impaired renal function, and decreased urine production (41).

In summary, this study further demonstrates that exposure to diethyl ether depletes the rat liver of UDPGA, depresses glucuronidation and reduces the excretion of AA-glucuronide into bile. Evidence is also presented that the biliary elimination of AA-sulfate, AA-glutathione, and unmetabolized acetaminophen are also reduced, which in-

dicates that acute exposure to diethyl ether affects several pathways of drug and xenobiotic conjugation.

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