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BILIARY EXCRETION AND ENTEROHEPATIC CIRCULATION OF 1-NITROPYRENE METABOLITES IN FISCHER-344 RATS

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Abstract—1-Nitropyrene (1-NP), present in diesel engine emissions, is a potent mutagen to bacteria, such as those found in mammalian intestinal tract, which contain nitroreductase enzymes. The purposes of this study were to determine the importance of bile as a route of excretion of 1-NP metabolites and to determine if reabsorption of biliary metabolites required the presence of intestinal bacteria. The bile ducts of male Fischer-344 rats were cannulated, 0.3 or 1.2 μmoles [^{3}H]1-NP was given i.v., and bile, urine, and feces were collected for 24 hr. Biliary excretion accounted for 70 (80%) or 170 (60%) nmoles of [^{3}H]1-NP after the low and high dose, respectively, with half-times for excretion of 1.7 hr ± 0.3 (± S.E.M.) and 3.4 hr ± 1.6 (± S.E.M.). Excretion of [^{3}H]1-NP equivalents in the urine was linearly related to dose, with 6 or 16 nmoles (8%) excreted in 24 hr. At the low dose, more radioactivity appeared in the urine in control rats compared to bile-duct cannulated rats, suggesting that reabsorption of 1-NP metabolites occurred. Pretreatment of rats with orally administered antibiotics prior to i.v. injection of 0.3 μmole [^{3}H]1-NP decreased radioactivity excreted in urine compared to untreated controls, suggesting that intestinal microorganisms may alter the biliary metabolites of 1-NP to facilitate reabsorption. Pretreatment of rats with buthionine sulfoximine, a glutathione depletor, decreased the excretion of certain biliary metabolites, suggesting that they were mercapturic acids of 1-NP metabolites. In summary, the results of these studies indicate that bile was an important route of excretion of nitropyrene metabolites. A portion of the excreted metabolites was reabsorbed from the gut, and this reabsorption required the presence of gut microorganisms.

A significant fraction of the mutagenic activity associated with diesel engine emissions is attributed to 1-nitropyrene (1-NP) [1]. Exposure to diesel exhaust emissions is widespread, and it is projected that during the next decade an increased number of light-duty vehicles will be powered by diesel engines, with a resultant increase in emissions.† Other sources for atmospheric 1-NP include industrial combustion processes [2, 3].

Studies of the fate of inhaled 1-NP aerosols by Fischer-344 rats indicate that radioactivity is rapidly absorbed from the lungs into the blood [4]. Much of this radioactivity later appears in the feces, indicating that 1-NP or metabolites of 1-NP are excreted in the bile. Bond *et al.* [5] demonstrated that in isolated perfused livers, 12% of the administered 1-NP is excreted in bile in 90 min. 1-NP is reductively metabolized by microorganisms typical of those in the intestines of mammals [6]. Other studies have shown that, *in vitro*, intestinal microflora are able to cleave and reductively metabolize the biliary metabolites of other nitro-aromatic compounds [7, 8]. These metabolites can be reabsorbed from the gut.

The purpose of our study was to determine if significant amounts of 1-NP were excreted *in vivo* in bile of rats given i.v. injections of 1-NP and if reabsorption of nitropyrene metabolites required the presence of gut microflora.

MATERIALS AND METHODS

Chemicals. The syntheses of [^{3}H]1-NP and 1-NP have been described previously [4, 9]. Briefly, [^{3}H]-pyrene was prepared by ICN (Irvine, CA) by ^3H_2O exchange with unlabeled pyrene obtained from the Eastman Kodak Co. (Rochester, NY). Approximately 12 mCi of [^{3}H]pyrene (2.3 Ci/mmole) was added to 12 mg of unlabeled pyrene and nitrated by reacting with dilute nitric acid according to the method of Bavin and Dewar [10]. Purification of the synthesized [^{3}H]NP was done by preparative thin-layer chromatography [4]. [4,5,9,10-^{14}C]1-Nitropyrene ([^{14}C]1-NP) (43 mCi/mmole; 98% pure) was custom synthesized by Amersham Searle, Inc. (Arlington Heights, IL). Aminopyrene (AP) was purchased from the Aldrich Chemical Co. (Milwaukee, WI). Acetylaminopyrene (AAP) was synthesized by treating AP (dissolved in methylene chloride) with acetic anhydride and pyridine. Arylsulfatase, β-glucuronidase, D-saccharic acid-1,4-lactone, and γ-glutamyl transpeptidase were obtained from the Sigma Chemical Co. (St. Louis, MO). DL-Buthionine-*S,R*-sulfoximine (BSO) was purchased

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† M. N. Ingalls and R. L. Bradow, *Seventy-fourth Annual Meeting of the Air Pollution Control Association* (Abstract), Vol. 81 (1981).

from the Chemical Dynamics Corp. (South Plainfield, NJ). Phosphatidyl choline was obtained from the Sigma Chemical Co., tetracycline hydrochloride from Pfipharmeces, Pfitzer, Inc. (New York, NY), neomycin sulfate from Eli Lilly & Co. (Indianapolis, IN), and ACS Certified dimethyl sulfoxide (DMSO) from the Fischer Scientific Co. (Fair Plains, NJ).

Animal maintenance and care. Male Fischer-344 rats, 17- to 20-weeks-old, were used in these studies. Rats (specific pathogen free) were born and raised in the barrier-maintained colony of the Inhalation Toxicology Research Institute. Rats were housed two per cage in polycarbonate cages with hardwood chip bedding and filter tops. Rooms were maintained at 68–72°F, with a relative humidity of 20–50% and a 12-hr light:dark cycle, with light starting at 6:00 a.m. Food (Lab Blox, Allied Mills, Chicago, IL) and water from bottles with sipper tubes were provided *ad lib.*

Surgical procedure. To investigate the biliary excretion and enterohepatic circulation of 1-NP metabolites, a glass vessel for bile collection was surgically implanted into the peritoneal cavity of rats [11]. During surgery, rats were maintained under anesthesia with 1.5% halothane in O_2. The common bile duct was cannulated with a 40-cm length of Intramedic PE 10 polyethylene tubing (Clay Adams, Parsipanny, NJ), the cannula was inserted into the collection vessel, and the sampling port was exteriorized. Control rats also underwent surgery and had glass vessels implanted with sampling ports exteriorized, but the bile duct was not cannulated.

Administration of 1-NP and collection of excreta. One hour after surgery, rats were given tail vein injections of 1-NP as a suspension consisting of 1-NP dissolved in 0.2 ml of DMSO, with 20 mg of phosphatidylcholine and 0.8 ml of 0.9% NaCl. Three control or bile-duct cannulated rats received 1.2 or 0.3 μmoles 1-NP/kg body wt. The specific activity of the dosing solution was adjusted so that each animal received 20 μCi of ^3H irrespective of the amount of 1-NP administered.

After injection, rats were placed in stainless steel metabolism cages. Food and water were supplied *ad lib.* Urine was collected on ice at 4, 7, 10, 16, and 24 hr after injection. Bile was removed at 1, 2, 3, 4, 5, 7, 12, 20, and 24 hr. The bile collection vessel was rinsed with 1.0 ml of saline, and the total volume withdrawn was recorded and samples stored at −80° until analysis.

Antibiotic pretreatment. To determine if the presence of microflora in the intestines was necessary for the reabsorption of 1-NP metabolites, additional rats were depleted of their intestinal microflora by p.o. administration of antibiotics [12]. Six rats were dosed daily for 5 days with tetracycline hydrochloride (50 mg/kg body wt) and neomycin sulfate (100 mg/kg body wt) suspended in 2% gelatin in 0.9% NaCl. Six control rats received solutions of 2% gelatin in 0.9% NaCl. On day 5, all rats were given tail vein injections of suspensions of either 1.2 or 0.3 μmoles 1-NP/kg body wt as described above. Immediately after injection they were placed in stainless steel metabolism cages, and urine and feces were collected at 6, 12, 18, 24, 36, and 48 hr. The collection period was extended in this study to allow more time for the excretion of radioactivity in the feces.

Quantitation of radioactivity. At the end of 24 and 48 hr (biliary excretion or gut-microflora studies, respectively), rats were killed and blood samples were taken by heart puncture. Liver, kidney, lung, and fat samples were removed, weighed, frozen, and stored for subsequent analysis. Samples of bile and urine were added to vials containing liquid scintillation fluid (Ready-Solv, Beckman, Fullerton, CA) and analyzed for total radioactivity in a Packard model 460C liquid scintillation spectrometer. Feces, caecum, large intestine, and small intestine (last three with contents) were homogenized in 2 vol. of deionized water, and samples were oxidized to ^3H_2O in a Packard model B306 Tissue Oxidizer. Samples of other tissues were oxidized directly without homogenization. The remaining carcass was homogenized in 2 vol. of water, and samples of the homogenate were also oxidized for determination of radioactivity.

Previous studies had indicated that, after metabolism of 1-NP, the tritium label became unstable, with the resultant formation of tritiated water [4]. To quantitate the formation of ^3H_2O, additional samples of urine and bile were dried under a stream of N_2 to remove any ^3H_2O before oxidation or radioanalysis. Weighed samples of feces, caecum, large intestine, small intestine and carcass homogenates were dried at 60° for 12 hr to remove any ^3H_2O before oxidation and liquid scintillation counting. Samples of other tissues were also dried at 60° to remove any ^3H_2O. Preliminary tests using homogenates of liver and feces spiked with ^3H_2O or [^3H] 1-NP indicated that ^3H_2O would evaporate under these conditions with no loss of 1-NP. Radioanalysis of samples of liver that were dried with or without prior homogenization gave similar results. Comparison of the radioactivity in these samples and samples that had been analyzed without prior drying indicated the amount of radioactivity that was due to tritiated water. The total amount of tritiated water in excreta, tissues, and carcass was calculated and accounted for 27 ± 5% of the dose for all studies. Total recovery of ^3H was 97%. The change in specific activity of the 1-NP caused by loss of tritium, as tritiated water, was taken into account in the analysis of the data. All data were expressed as 1-NP equivalents.

Half-times for excretion of 1-NP equivalents in bile were determined by least-squares linear regression analysis of the log of the fraction of radioactivity remaining to be excreted as a function of time. $T_{\frac{1}{2}} = -0.693/\lambda$, where λ equaled the slope of the regression line. Statistical analyses of data were performed by comparing means using a one-tailed Student's *t*-test. The level of significance was set at $P < 0.05$.

Biliary metabolites. To determine the nature of the biliary metabolites, six rats were anesthetized with halothane and the common bile ducts were cannulated as described above. Immediately after surgery, three rats were given i.p. injections of 1.8 g BSO/kg body wt in 0.9% NaCl. BSO is a potent inhibitor of γ-glutamylcysteine synthetase, the first enzyme in the glutathione biosynthesis pathway [13].

Control rats were given injections of 0.9% NaCl (1 ml/kg). After 2 hr, all six rats were given i.p. injections of 0.2 μmole [^{14}C]1-NP/kg body wt (8 μCi) in dimethyl sulfoxide. [^{14}C]1-NP was used in these studies to compare metabolite profiles using a stable radiolabel to profiles obtained using [^{3}H]1-NP. Bile was sampled from the collection vessels hourly for 4 hr, after which time the animals were killed.

Livers were removed from the rats, weighed and homogenized in 2 vol. of 1.15% KCl (4°). Portions (500 μl) of liver homogenate were mixed with 5 ml of trichloroacetic acid (10%, w/v), and the pellet obtained by centrifugation was exhaustively extracted [14] to determine the amount of ^{14}C covalently bound to hepatic macromolecules.

Analysis of 1-NP metabolites in bile. Biliary metabolites collected after injection of either [^{3}H]1-NP or [^{14}C]1-NP were separated at ambient temperature on a 250 nm × 4.6 mm column (RP-18, 10 μm, Alltech Assoc., Deerfield, IL) and attached to a Spectra Physics SP8100 high-performance liquid chromatograph (Spectra Physics Inc., San Jose, CA). Two different chromatography systems were used. The first employed a constant flow rate of 2.0 ml/min and a 35-min linear gradient from 40% methanol in Tris phosphate buffer (0.005 M; pH 2.75) to 100% methanol. In the second system, a constant flow rate of 2.0 ml/min and a 40-min linear gradient from 30% methanol in Tris phosphate buffer (0.005 M; pH 2.75) to 100% methanol was used. A 10-min hold at 45% methanol in buffer and a 5-min hold at 100% methanol was employed. For both chromatography systems, fractions (30 sec; 1 ml) of eluate were collected (LKB Instruments, Gaithersburg, MD) directly into mini-liquid scintillation-counting vials. Scintillation fluors (Ready Solv, Beckman Instruments) were added, and the samples were counted in a Packard model 460C liquid scintillation counter. Quench correction was performed by the automatic external standard method with counting efficiencies of >88%. Sufficient counts were accumulated for individual fractions associated with each peak to provide for <10% error with 95% confidence intervals. Retention times of the radioactive metabolites were compared with the standards 1-NP, AAP and AP.

Enzyme hydrolysis. Biliary metabolites were purified by HPLC as described above, and portions of the metabolites were incubated at 37° for 16 hr with β-glucuronidase and aryl-sulfatase as previously described [15]. Additional portions of the metabolites incubated at 37° without addition of enzyme served as controls. After incubation, samples were subjected to high-performance liquid chromatography (HPLC) and change in retention time of radioactivity, compared to controls, was examined. Saccharolactone (15 M) was included in some samples as an inhibitor of β-glucuronidase.

Additional portions of the biliary metabolites purified by HPLC were also incubated with γ-glutamyl transpeptidase in Tris glycine buffer (pH 8.5) at 25° for various times up to 1 hr. Control incubations were performed in the absence of enzyme. After incubation the samples were injected directly onto the HPLC column and change in retention time, compared to controls, was examined.

RESULTS

Biliary excretion. The appearance of 1-NP equivalents in bile of male rats after i.v. administration of 1-NP indicated that bile was the major route of excretion. With the concentrations used in this study, biliary excretion of 1-NP equivalents was dependent on the dose, with 70 (80%) or 170 (60%) nmoles of 1-NP-equivalents appearing in bile in 24 hr (Fig. 1). The half-times for excretion of [^{3}H]1-NP equivalents in the bile were not significantly different, 1.7 ± 0.3 hr (± S.E.M.) for the 0.3 μmole/kg dose and 3.4 ± 1.6 hr (± S.E.M.) for the 1.2 μmoles/kg dose.

In rats from which bile was collected, urinary excretion of [^{3}H]1-NP equivalents was linearly related to dose, with approximately 8% of the dose (6 or 16 nmoles) excreted in 24 hr by this route (Fig. 2). At the high dose, urinary excretion of 1-NP equivalents in control rats was equal to that found in rats with bile-duct cannulations. However, at the low dose, significantly more radioactivity ($P < 0.05$) was excreted in the urine of control rats, compared to those from which bile was collected.

In control rats, most of the radioactivity was found in the caecum, which accounted for 52–63% of the dose (Fig. 2). In contrast, less than 1% of the dose was found in the caecum of rats from which bile was collected. Control rats excreted some radioactivity in the feces (Fig. 2) compared to less than 1% of the dose found in the feces of animals from which bile was collected.

On a per organ basis, livers contained the highest percentage of the dose of the tissues examined for all rats, with from 5.5 to 1.7% (range) at the end of 24 hr. The highest concentrations of [^{3}H]1-NP equivalents were in the kidney, liver, and blood (Table 1). At the low dose, there was no significant difference in concentrations of [^{3}H]1-NP equivalents in tissues from control rats or rats from which bile was taken. At the high dose, liver, lung, and kidney of cannulated rats contained significantly higher concentrations of [^{3}H]1-NP equivalents.

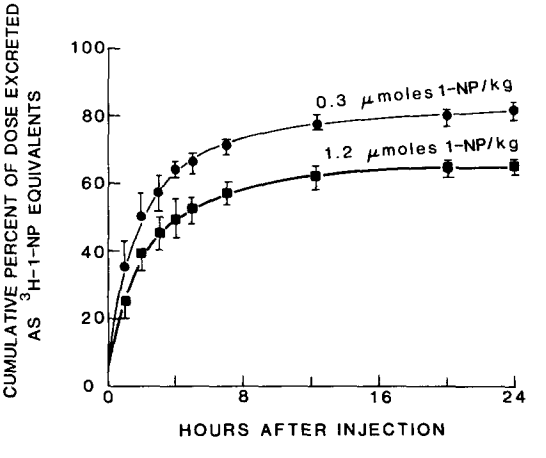

Fig. 1. Cumulative excretion of [^{3}H]1-NP equivalents in bile, with time after i.v. administration of either 0.3 or 1.2 μmoles 1-NP/kg body wt. Data are expressed as means ± S.E.M.

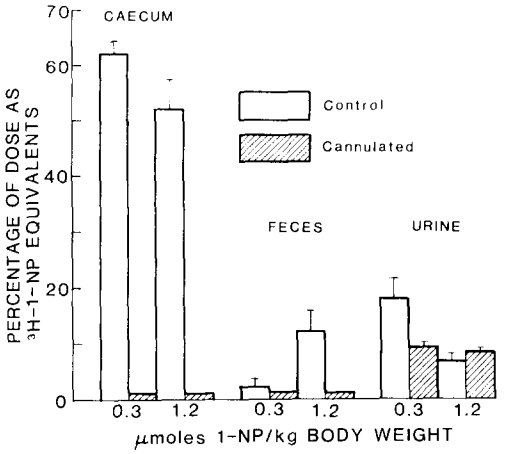

Fig. 2. Presence of [^{3}H]1-NP equivalents in caecum, feces or urine at 24 hr after i.v. administration of 0.3 or 1.2 μmoles 1-NP/kg body wt. Hatch bars represent means of three rats with bile-duct cannulations. Open bars represent means from three control rats that underwent surgery but did not have bile cannula implanted. Error bars represent means ± S.E.M.

Antibiotic pretreatment. In both control rats and rats pretreated with antibiotics, from 34 to 58% of the dose appeared in the feces by 48 hr after injection (Table 2). After injection of the low dose there was significantly more radioactivity ($P < 0.05$) excreted in the urine of control animals compared to that of rats pretreated with antibiotics. In rats injected with the high dose, there was no difference in the amount of radioactivity excreted in urine over the 48-hr period.

At the low dose, 24 hr after injection, 8.2 and 18.1% of the dose were excreted in the urine of rats pretreated with antibiotics and control rats respectively. This was similar to results seen at the end of 24 hr for rats with bile-duct cannulations and sham-operated controls (Fig. 2), suggesting that the presence of gut microorganisms was necessary for reabsorption of 1-NP biliary metabolites.

Biliary metabolites of 1-NP. Comparison of the high performance liquid chromatograms of biliary metabolites collected after i.v. injection of [^{3}H]1-NP or i.p. injection of [^{14}C]1-NP (Fig. 3) indicated that the profiles of metabolites produced were similar. None of the peaks co-chromatographed with the 1-NP or AP standards. In this system, AAP chromatographed with a retention time of 18 min (36 ml).

In rats pretreated with BSO and then given i.p. injections of [^{14}C]1-NP, the profile of biliary metabolites was different from that found in bile from controls (Fig. 4). One major peak, E, and two minor peaks, B and C, were significantly depressed compared to controls. Metabolite F co-chromatographed with authentic AAP. The retention times of metabolites A through E did not change when chromatographed following incubation with aryl-sulfatase or β-glucuronidase.

Treatment of purified metabolites (A through E) with γ-glutamyl transpeptidase caused a change in

Table 1. Tissue distribution of [^{3}H]1-NP equivalents in rats at 24 hr after injection of 1-NP

	Percentage of [^{3}H]1-NP administered per g of tissue*			
	Amount injected (μmoles/kg)			
	0.3		1.2	
Tissue	Bile-duct cannumeted	Control	Bile-duct cannulated	Control
Liver	0.30 ± 0.06	0.31 ± 0.08	0.71 ± 0.17†	0.22 ± 0.04‡
Lung	0.09 ± 0.02	0.09 ± 0.03	0.17 ± 0.02†	0.05 ± 0.004‡
Blood	0.23 ± 0.02	0.32 ± 0.10	0.32 ± 0.18	0.29 ± 0.03
Fat	0.11 ± 0.03	0.08 ± 0.03	0.13 ± 0.07	0.06 ± 0.01
Kidney	0.49 ± 0.06‡	0.48 ± 0.08‡	1.10 ± 0.18†	0.32 ± 0.02‡

* Data are expressed as means ± S.E.M. with N = 3.
†,‡ Values with different symbols indicate a significant difference in percentage of [^{3}H]1-NP equivalents remaining in tissues.

Table 2. Excretion of [^{3}H]1-NP equivalents 48 hr after injection of 1-NP

	Percentage of dose* as [^{3}H]1-NP equivalents in:					
	Urine		Feces		Caecum	
Dose (μmoles/kg)	Control	Antibiotic treated	Control	Antibiotic treated	Control	Antibiotic treated
0.3	24 ± 3†	14 ± 1‡	34 ± 13	39 ± 6	33 ± 11	33 ± 3
1.2	23 ± 3	18 ± 3	58 ± 14	42 ± 4	18 ± 13	31 ± 1

* Data are expressed as mean ± S.E.M. with N = 3.
†,‡ Values with different symbols indicate a significant difference in [^{3}H]1-NP equivalents measured in samples.

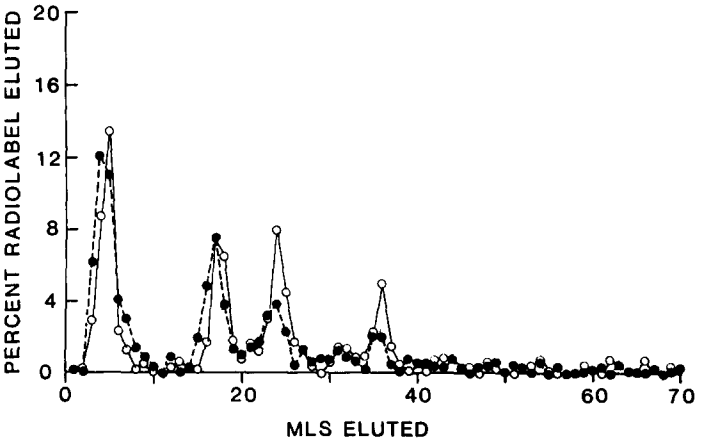

Fig. 3. Representative high-performance liquid chromatograms of [^{14}C]1-NP (○) or [^{3}H]1-NP metabolites (●) excreted in bile of rats injected i.p. or i.v. with [^{14}C]1-NP or [^{3}H]1-NP respectively. The chromatography system employed a constant flow of 2 ml/min and a 35-min linear gradient from 40% methanol in Tris phosphate buffer (0.005 M; pH 2.75) to 100% methanol. Approximately 20% variation in peak areas was seen in chromatography profiles for different animals receiving either form of 1-NP.

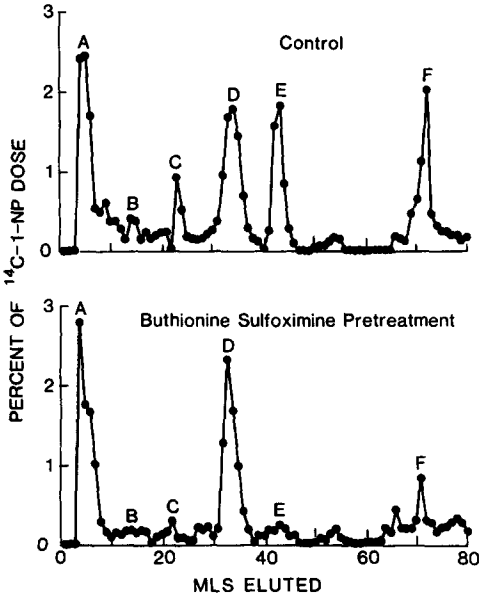

Fig. 4. High-performance liquid chromatogram of [^{14}C]1-NP metabolites excreted in bile from a control (A) and buthionine sulfoximine pretreated (B) rat. Metabolites were separated using a 40-min linear gradient from 30% methanol in Tris phosphate buffer (0.005 M; pH 2.75) to 100% methanol with 10- and 5-min holds at 45% and 100% methanol respectively.

retention time of only two of the metabolites, B and E. After treatment, peak B chromatographed with a retention time identical to C and peak E chromatographed with a retention time similar to D. The retention time of peaks A, C, and D were not affected by treatment with γ-glutamyl transpeptidase.

Quantities of total ^{14}C in the liver and ^{14}C covalently bound to liver macromolecules are shown in Table 3. In livers from control and BSO-pretreated rats, approximately 2% and 3%, respectively, of the total i.p. administered dose were detected. The differences between control and BSO-pretreated rats were not significant. Considerably smaller quantities of ^{14}C covalently bound to liver macromolecules were measured in both control and BSO-pretreated rats, and these amounts were not significantly different.

DISCUSSION

The results of this study indicate that bile was an important route of excretion of 1-NP metabolites. Comparison of the urinary excretion of [^{3}H]1-NP equivalents in rats with bile-duct cannulations to control rats indicated that urinary excretion of [^{3}H]1-NP equivalents was increased significantly in the control animals only at the low dose. This was in contrast to studies with other nitro compounds where enterohepatic circulation of metabolites plays

Table 3. Amount of ^{14}C and macromolecular covalent binding of ^{14}C in livers from rats with cannulated bile ducts treated i.p. with 1-NP*

	Total amount of ^{14}C in liver	Amount of ^{14}C covalently bound to macromolecules
Livers from control rats	0.4 ± 0.3	0.01 ± 0.003
Livers from BSO-pretreated rats†	0.5 ± 0.3	0.04 ± 0.02

* Values represent nanomole equivalents/g liver (mean ± S.E., N = 3).

† Rats were given by i.p. injections of 1.8 g buthionine sulfoximine (BSO)/kg body wt 2 hr prior to dosing with 0.2 μmole [^{14}C]1-NP/kg body wt.

a large role in disposition. Studies on the enterohepatic circulation of [^{14}C]2,4-dinitrotoluene (DNT), for example, indicate that, even with high doses of [^{14}C]2,4-DNT (190 μmoles/kg), 50% (5 μmoles [^{14}C]2,4-DNT equivalents) of the radioactivity excreted in the bile is reabsorbed [15]. In the studies reported here, only 13% (0.01 μmole [^{3}H]1-NP equivalents) of the [^{3}H]1-NP equivalents excreted in the bile was reabsorbed after injection of 0.3 μmole/kg.

Profiles of the biliary metabolites of both [^{14}C]1-NP and [^{3}H]1-NP indicated that no unmetabolized 1-NP was found in the bile of rats *in vivo*, although some AAP was excreted in bile. AAP is excreted in bile when [^{14}C]1-NP is added to the perfusate of isolated perfused liver preparations [5]. Unmetabolized 1-NP is not found in bile of these isolated perfused livers. In the perfused liver studies, less of the very polar metabolite A is excreted in bile compared to our study with intact animals. In contrast, a metabolite identified as a glucuronide conjugate is excreted in bile from perfused livers, but was only observed in very low amounts in our study. Similar to our studies, a large proportion of the biliary metabolites in the perfused liver studies appear to be glutathione derivatives. In studies reported here, pretreatment with BSO caused a decrease in some of the metabolites excreted in bile (peaks B, C and E). Since BSO is an inhibitor of γ-glutamylcysteine synthetase, the first enzyme in the glutathione biosynthesis pathway [13], the decrease in these three metabolites in the bile suggested that these compounds might be glutathione derivatives. Enzyme treatment (γ-glutamyl transpeptidase) caused a change in retention time of peaks B and E. This data suggested that these metabolites might be part of the mercapturic acid pathway. The conjugation of pyrene with glutathione and the appearance of pyrenol-mercapturic acids in the bile is well documented [16]. The observation that [^{3}H]1-NP metabolites were not extensively absorbed from the gut was consistent with the hypothesis that these may be glutathione-conjugated derivatives since these conjugates are less readily cleaved by gut flora than are glucuronide and/or sulfate conjugates [16, 17]. Although definitive identification of biliary metabolites has not been made in this study, other investigators also suggest that the 1-NP biliary metabolites may be glutathione-conjugated derivatives [18].

Pretreatment with BSO did not alter the amount of ^{14}C covalently bound to liver macromolecules compared to control rats. In addition, concentrations of covalently bound ^{14}C were similar (on a percent bound basis) in these rats, from which bile was collected, to concentrations found in both livers and lungs of rats at 4 hr after oral administration of 0.04 mmole [^{14}C]1-NP/kg (Paul Ayres, personal communication). This suggested that delivery of bile into the intestines was not a prerequisite for formation 1-NP metabolites that could covalently bind to macromolecules.

Intestinal microflora are capable of deconjugating and reductively metabolizing many biliary metabolites [7]. Once deconjugated, these metabolites are often more easily absorbed from the intestines. The results of the studies described here indicated that depletion of gut flora decreased urinary excretion of [^{3}H]1-NP equivalents at the low dose, suggesting that presence of gut flora was necessary for reabsorption of 1-NP biliary metabolites.

Other investigators [6, 19] have shown that metabolism of nitropyrene by microorganisms isolated from rat intestines increases the formation of DNA adducts associated with 1-NP. Intestinal microflora assume significance in the metabolism of a compound only in situations that allow interaction of the compound and microflora [16]. The results from the study described here indicated that bile was an important route of excretion of 1-NP metabolites. However, we have also shown these metabolites were not extensively reabsorbed from the gut. Whether the metabolites that were not reabsorbed could react with intestinal microorganisms and/or intestinal tissue before excretion remains to be determined.

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