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INCREASED BILIARY EXCRETION OF GLUTATHIONE IS GENERATED BY THE GLUTATHIONE-DEPENDENT HEPATOBILIARY TRANSPORT OF ANTIMONY AND BISMUTH

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Abstract—We have recently demonstrated that the hepatobiliary transport of arsenic is glutathione-dependent and is associated with a profound increase in biliary excretion of glutathione (GSH), hepatic GSH depletion and diminished GSH conjugation (Gyurasics Á, Varga F and Gregus Z, *Biochem Pharmacol* **41**: 937–944 and Gyurasics Á, Varga F and Gregus Z, *Biochem Pharmacol* **42**: 465–468, 1991). The present studies in rats aimed to determine whether antimony and bismuth, other metalloids in group Va of the periodic table, also possess similar properties. Antimony potassium tartrate (25–100 μmol/kg, i.v.) and bismuth ammonium citrate (50–200 μmol/kg, i.v.) increased up to 50- and 4-fold, respectively, the biliary excretion of non-protein thiols (NPSH). This resulted mainly from increased hepatobiliary transport of GSH as suggested by a close parallelism in the biliary excretion of NPSH and GSH after antimony or bismuth administration. Within 2 hr, rats excreted into bile 55 and 3% of the dose of antimony (50 μmol/kg, i.v.) and bismuth (150 μmol/kg, i.v.), respectively. The time courses of the biliary excretion of these metalloids and NPSH or GSH were strikingly similar suggesting co-ordinate hepatobiliary transport of the metalloids and GSH. However, at the peak of their excretion, each molecule of antimony or bismuth resulted in a co-transport of approximately three molecules of GSH. Diethyl maleate, indocyanine green and sulfobromophthalein (BSP), which decreased biliary excretion of GSH, significantly diminished excretion of antimony and bismuth into bile indicating that hepatobiliary transport of these metalloids is GSH-dependent. Administration of antimony, but not bismuth, decreased hepatic GSH level by 30% and reduced the GSH conjugation and biliary excretion of BSP. These studies demonstrate that the hepatobiliary transport of trivalent antimony and bismuth is GSH-dependent similarly to the hepatobiliary transport of trivalent arsenic. Proportionally to their biliary excretion rates, these metalloids generate increased biliary excretion of GSH probably because they are transported from liver to bile as unstable GSH complexes. The significant loss of hepatic GSH into bile as induced by arsenic or antimony may compromise conjugation of xenobiotics with GSH.

Glutathione (GSH§; γ-glutamylcysteinylglycine) is synthetized predominantly in the liver from where it is transported into both the circulation and bile [1]. Hepatobiliary transport of GSH is thought to control the biliary excretion of several endogenous (e.g. copper and zinc) and exogenous (e.g. cadmium, methylmercury, lead) metals [2–8]. Recently, biliary excretion of arsenic has also been shown to be GSH-dependent. Decreased biliary excretion of GSH produced either by depletion of hepatic GSH or by inhibition of bile canalicular transport of GSH resulted in diminished biliary excretion of arsenic in rats injected with sodium arsenite or arsenate [9]. Interestingly, the GSH-dependent hepatobiliary transport of arsenic is associated with a large increase in the biliary excretion of endogenous GSH [10, 11]. These observations prompted the suggestion that arsenic is transported into bile as an unstable GSH complex.

Antimony and bismuth are chemically related to arsenic and found also in group Va of the periodic table. Both antimony and bismuth are known to be excreted into bile in humans and animals [12–15] and to react with thiols *in vivo* [16, 17]. Therefore, it was of interest to investigate whether the biliary excretion of antimony and bismuth is also associated with the hepatobiliary transport of GSH. More specifically, our aim was three-fold: (1) to determine the effect of antimony and bismuth on the biliary excretion of endogenous GSH; (2) to determine whether the biliary excretion of antimony and bismuth is dependent on the hepatobiliary transport of endogenous GSH; and (3) to determine whether antimony and bismuth interfere with the formation and biliary excretion of xenobiotic GSH conjugates.

For studying the effect of antimony and bismuth on the hepatobiliary transport of endogenous thiols, intravenously injectable water soluble compounds, i.e. antimony potassium tartrate and bismuth ammonium citrate, were selected. The biliary excretion of both non-protein thiols (NPSH) and total (reduced + oxidized) glutathione (i.e. GSH + 2 GSSG) was measured in response to these metal

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§ Abbreviations: BSP, sulfobromophthalein; DEM, diethyl maleate; GSH, glutathione; GSSG, oxidized glutathione; ICG, indocyanine green; NPSH, non-protein thiols.

compounds. Biliary NPSH is composed of GSH and its thiol-group containing breakdown products (i.e. cysteinylglycine and cysteine) which are formed by intrabiliary hydrolysis initiated by γ-glutamyltransferase [18–20]. Therefore, the biliary excretion rate of NPSH is a better estimate for the rate of hepatobiliary transport of GSH than the biliary excretion rate of the unchanged GSH.

In order to study the GSH dependence of the biliary excretion of antimony and bismuth, the effect of diethyl maleate (DEM), which depletes hepatic GSH, and of sulfobromophthalein (BSP) and indocyanine green (ICG), which inhibit hepatobiliary transport of GSH, on the biliary excretion of antimony and bismuth was examined. In order to assess the possible interference of antimony and bismuth with GSH conjugation of xenobiotics, effect of these metals on the biliary excretion of BSP was studied. BSP undergoes conjugation with GSH in the liver and is excreted into bile both in unchanged and conjugated forms [21].

MATERIALS AND METHODS

Chemicals. Antimony potassium tartrate, bismuth ammonium citrate, reduced glutathione, NADPH and sulfosalicylic acid were purchased from Reanal (Budapest, Hungary). Ellman's reagent [5,5'-dithiobis(2-nitrobenzoic acid)] and glutathione reductase (type III) were from the Sigma Chemical Co. (St Louis, MO, U.S.A.) and metaphosphoric acid from Alfa Products (Danvers, MA, U.S.A.). BSP was obtained from Fluka (Buchs, Switzerland), ICG from Hynson, Wescott and Dunning (Baltimore, MD, U.S.A.) and DEM from Koch Light Labs. (Colnbrook, U.K.).

Animal experiments. Female, 12–16-week-old Wistar rats (Lati, Gödöllő, Hungary), housed at 23–26°, 55–65% relative air humidity and on a 12 hr light/dark cycle were used. Tap water and Altromin lab. chow (Lati) were provided *ad lib.* Experiments were started between 8 and 9 a.m. in order to exclude the effects of diurnal variations in hepatic GSH levels. Animals were anesthetized with urethane (1.2 g/kg, i.p.), and their body temperature was kept at 37° by means of heating lamps. After median laparotomy, the common bile duct was cannulated with a 23-gauge needle attached to a polyethylene tubing (PE-50). For maintaining patent airways, a tracheotomy was performed on each animal. With the exception of DEM, which was dissolved in sunflower oil and injected i.p., the test compounds (i.e. antimony potassium tartrate, bismuth ammonium citrate, BSP and ICG) were dissolved in distilled water and administered i.v. The dosages of the compounds and the timing of their administration are indicated in the figure legends. Bile was collected into pre-weighed microcentrifuge tubes in 20 min periods for 2 hr immediately after injection of the antimony or bismuth compounds. For determination of biliary NPSH and GSH excretion, bile was collected into microcentrifuge tubes embedded in ice and containing 400 μL 5% (w/v) metaphosphoric acid in order to prevent oxidation of thiols. The volume of bile was determined gravimetrically assuming unity for specific gravity. Biliary excretion rates of endogenous thiols, antimony, bismuth and BSP were calculated as the product of bile flow rate and their biliary concentration.

When examining the effect of antimony and bismuth on hepatic and renal NPSH levels, the liver and the left kidney of the urethane-anesthetized, bile duct-cannulated rats were removed 60 min after i.v. administration of antimony or bismuth. The tissue samples were immediately homogenized in 5 vol. of 4% ice-cold sulfosalicylic acid.

Analytical methods. The concentration of NPSH in deproteinized bile or tissue homogenates was measured with Ellman's reagent [22]. Biliary concentration of total GSH was determined according to the method of Tietze [23] using glutathione reductase.

Antimony and bismuth in bile were quantitated with an energy dispersive X-ray emission spectrometer (Atomki, Debrecen, Hungary). Sources of irradiation were ^{241}Am for measuring antimony and ^{109}Cd for bismuth, with excitation energies 60 keV (γ-ray) and 22.1 keV (X-ray), respectively. The intensity of the 26.3 keV energy X-ray emitted by antimony and the 10.8 keV energy X-ray emitted by bismuth in the samples and standards was measured for 20 min. Fifty microlitres of the bile samples or standard solutions containing known amounts of antimony potassium tartrate or bismuth ammonium citrate was absorbed into carrier tablets of 13 mm diameter. Constituents of the carrier tablets, being elements with low atomic numbers, did not interfere with the measurement of antimony and bismuth.

Biliary concentration of total (i.e. unconjugated and conjugated) BSP was measured spectrophotometrically at 580 nm after diluting the bile with 0.1 M sodium hydroxide. The proportion of conjugated and unconjugated BSP in bile was determined after separation by ascending paper chromatography using Whatman No. 1 paper and a mixture of *n*-butanol, acetic acid and water (4:1:2, v/v/v). Spots corresponding to the BSP compounds were visualized over ammonia vapor, cut out and eluted with water. The proportion of unconjugated and conjugated BSP in bile was calculated after measurement of absorbance of alkalinized eluates at 580 nm.

Statistical analysis. Data were compared by one-way analysis of variance followed by Duncan's test with $P < 0.05$ as level of significance.

RESULTS

Effect of antimony and bismuth on the biliary excretion of endogenous NPSH and GSH

The effects of intravenously injected antimony potassium tartrate and bismuth ammonium citrate on the rates of biliary NPSH excretion and bile flow are shown in Figs 1 and 2, respectively. In saline-injected control rats, the NPSH excretion and bile flow were nearly constant with only a slight decline during the second hour of the experiment. Sodium potassium tartrate (100 μmol/kg, i.v.) or ammonium citrate (200 μmol/kg, i.v.) did not influence biliary NPSH output (data not shown).

Antimony potassium tartrate (25–100 μmol/kg,

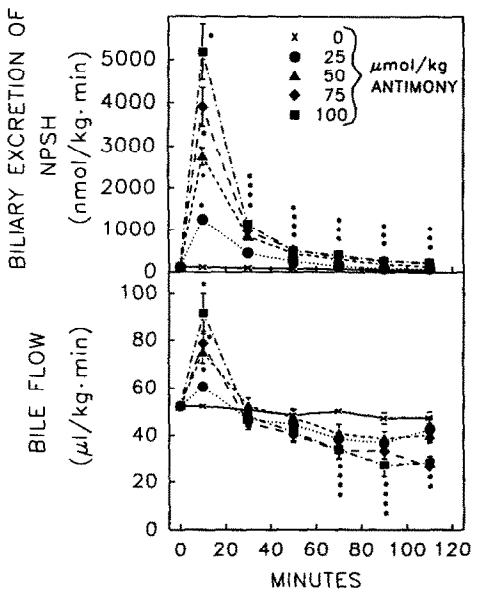

Fig. 1. Effect of antimony potassium tartrate on bile flow and biliary excretion of NPSH. Antimony potassium tartrate was injected i.v. at 0 min and bile was collected in 20 min periods for 2 hr thereafter. Symbols represent means ± SE of 6–8 rats. Asterisks indicate values significantly different ($P < 0.05$) from respective controls.

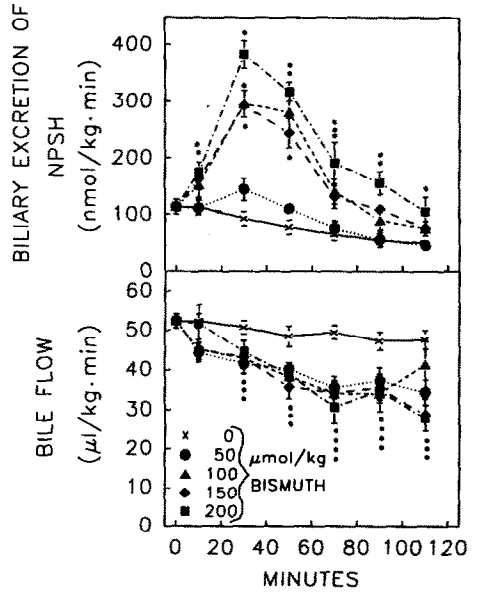

Fig. 2. Effect of bismuth ammonium citrate on bile flow and biliary excretion of NPSH. Bismuth ammonium citrate was injected at 0 min and bile was collected in 20 min periods for 2 hr thereafter. Symbols represent means ± SE of 7–9 rats. Asterisks indicate values significantly different ($P < 0.05$) from respective controls.

i.v.) produced an immediate and dramatic 10–50-fold increase in the biliary excretion of NPSH (Fig. 1, upper panel). Thereafter, thiol output rapidly decreased, and 2 hr after injection of antimony it was only 1.5–4-fold higher as compared to the control. Bile flow also responded to antimony with a dose-dependent increase (Fig. 1, lower panel). During the peak of NPSH excretion, bile production exceeded the control rate by 44–76% in all dosage groups except the 25 μmol/kg group. This transient acceleration of bile flow was followed by a dose-dependent decline below the control flow rate during the second hour of the experiment.

Bismuth ammonium citrate (50–200 μmol/kg, i.v.) also enhanced biliary NPSH excretion in a dose-related manner (Fig. 2, upper panel). However, both the extent and the time course of bismuth-induced enhancement in thiol excretion differed from that observed after antimony administration. Bismuth produced only a 4-fold maximal increase in NPSH excretion. In addition, the enhanced thiol excretion developed less rapidly, exhibiting a peak 20–40 min after bismuth administration, and receded gradually thereafter. Bismuth did not affect bile flow immediately after its injection (Fig. 2, lower panel). Later, however, the bile flow of bismuth-injected rats declined gradually until the end of the experiment when it became 30–42% lower than in control animals.

In order to determine whether the antimony- and bismuth-induced increases in biliary NPSH excretion resulted from enhanced excretion of GSH, the effects of antimony and bismuth on biliary NPSH and GSH outputs were simultaneously examined. As shown in Fig. 3, after injection of antimony potassium tartrate (50 μmol/kg, i.v.) or bismuth ammonium citrate (150 μmol/kg, i.v.) the time courses of biliary excretion of NPSH and GSH were similar. In addition, the magnitudes of the increases in NPSH and GSH excretion were also comparable following antimony or bismuth administration.

Effect of antimony and bismuth on hepatic and renal NPSH

The effects of antimony potassium tartrate (50 μmol/kg, i.v.) and bismuth ammonium citrate (150 μmol/kg, i.v.) on hepatic and renal NPSH concentrations were examined in anesthetized, bile duct-cannulated animals 1 hr after their administration (Fig. 4). Antimony significantly decreased (30%), however, bismuth did not influence the hepatic NPSH concentration. The renal NPSH concentration was reduced by 22% in antimony-injected rats, but was unaffected in bismuth-treated animals (Fig. 4).

Relationship between the biliary excretion of antimony or bismuth and NPSH

Biliary excretion of NPSH as well as of antimony or bismuth were measured over 2 hr after injection of antimony potassium tartrate (50 μmol/kg, i.v.) or bismuth ammonium citrate (150 μmol/kg, i.v.) in control rats and in rats injected with ICG, BSP or DEM (Figs 5 and 6). In control saline-injected rats, the biliary excretion of antimony followed a time course almost identical to the time course of NPSH

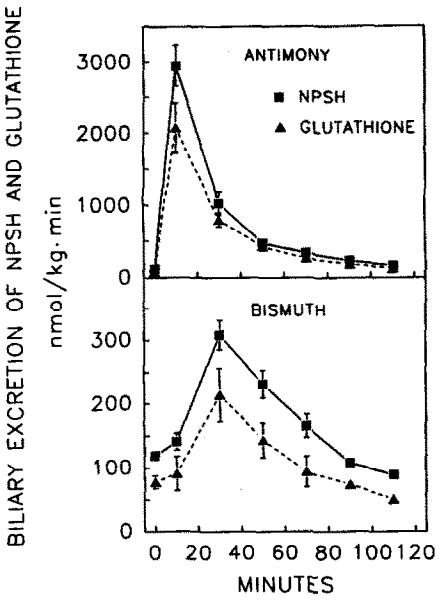

Fig. 3. Effect of antimony potassium tartrate and bismuth ammonium citrate on biliary excretion of NPSH and glutathione. Rats were injected i.v. with 50 μmol/kg antimony potassium tartrate or 150 μmol/kg bismuth ammonium citrate at 0 min and bile was collected in 20 min periods for 2 hr thereafter. GSH represents the sum of reduced and oxidized glutathione (GSH + 2 GSSG). Symbols represent means ± SE of 6 animals.

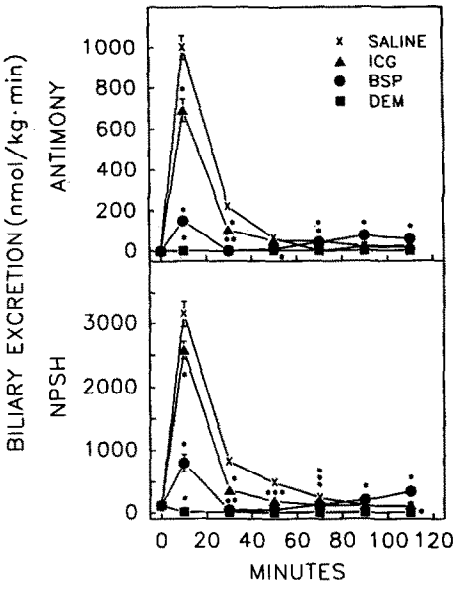

Fig. 5. Effect of ICG, BSP and DEM on the biliary excretion of antimony (upper panel) and NPSH (lower panel). Antimony potassium tartrate (50 μmol/kg, i.v.) was given 1 min after injection of ICG (25 μmol/kg, i.v.) or BSP (50 μmol/kg, i.v.) and 45 min after injection of DEM (4 mmol/kg, i.p.). Bile was collected in six consecutive 20 min periods immediately after the injection of antimony potassium tartrate. Symbols represent means ± SE of 6 animals. Asterisks indicate values significantly different ($P < 0.05$) from controls.

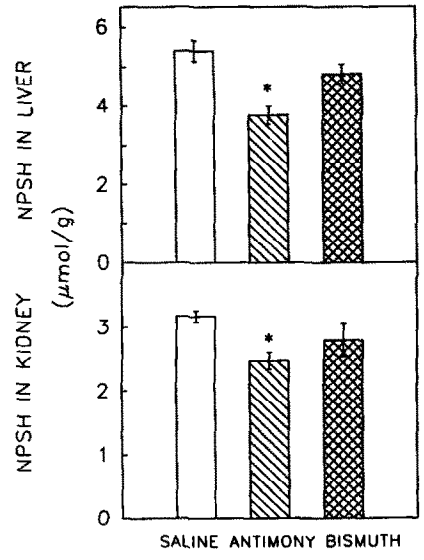

Fig. 4. Effect of antimony potassium tartrate and bismuth ammonium citrate on the hepatic (upper panel) and renal (lower panel) NPSH concentration. Antimony potassium tartrate (50 μmol/kg) or bismuth ammonium citrate (150 μmol/kg) were injected i.v. to bile duct-cannulated rats. Hepatic and renal NPSH concentrations were determined 1 hr after injection of the test compounds or saline. Bars represent means ± SE of 6 animals. Asterisks indicate values significantly different ($P < 0.05$) from control.

excretion with a large but transient increase immediately after administration of antimony (Fig. 5). Similarly, the time courses of the biliary excretion of bismuth and NPSH were also largely parallel in control rats (Fig. 6). Interestingly, the peak excretion rates of NPSH in rats injected with antimony or bismuth exceeded the peak excretion rates of antimony and bismuth 3- and 5-fold, respectively (Figs 5 and 6).

In rats injected with ICG, BSP or DEM, the time courses of the biliary excretion of antimony and NPSH were also comparable. These agents decreased the biliary excretion of antimony and NPSH to similar degrees. For example, the peak excretion rates of antimony and NPSH were reduced by 31 and 19%, respectively, in ICG-injected rats, by 85 and 75% in BSP-injected rats and by almost 100% in DEM-treated rats as compared to the control saline-injected rats (Fig. 5).

ICG, BSP and DEM also decreased the biliary excretion of bismuth and NPSH (Fig. 6). DEM reduced the excretion of both bismuth and antimony to essentially zero. BSP diminished the biliary output of bismuth only initially. Later (60–120 min), NPSH excretion returned to the control level whereas the rate of bismuth excretion even exceeded the then already declining bismuth excretion in control rats (Fig. 6). ICG, which permanently decreased the NPSH excretion by 60–80% below the pretreatment

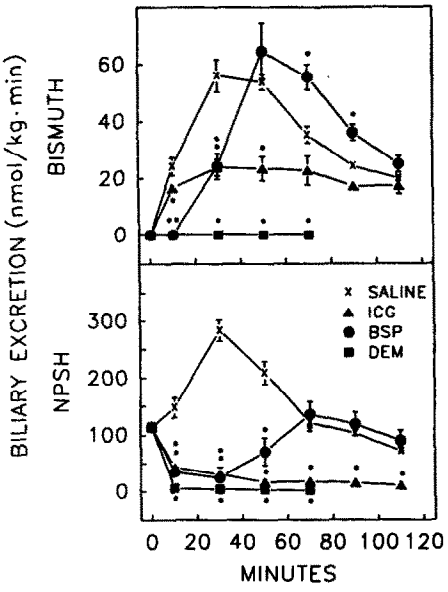

Fig. 6. Effect of ICG, BSP and DEM on the biliary excretion of bismuth (upper panel) and NPSH (lower panel). Bismuth ammonium citrate (150 μmol/kg, i.v.) was given 1 min after injection of ICG (25 μmol/kg, i.v.) or BSP (50 μmol/kg, i.v.) as well as 45 min after injection of DEM (4 mmol/kg, i.p.). Bile was collected in six consecutive 20 min periods immediately after the injection of bismuth ammonium citrate. Symbols represent means ± SE of 6 animals. Asterisks indicate values significantly different ($P < 0.05$) from controls.

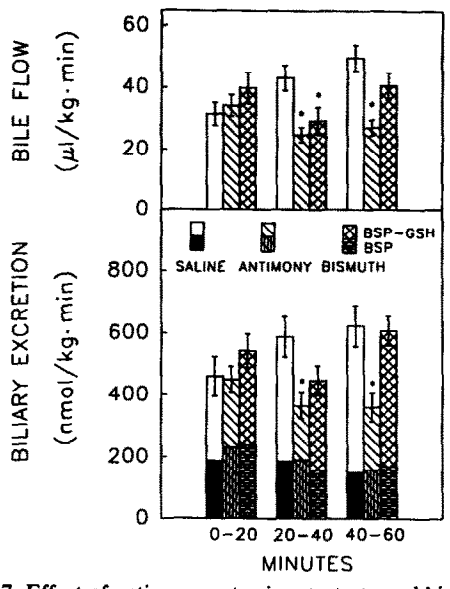

Fig. 7. Effect of antimony potassium tartrate and bismuth ammonium citrate on bile flow and the biliary excretion of BSP and its glutathione conjugate (BSP–GSH). Antimony potassium tartrate (50 μmol/kg) or bismuth ammonium citrate (150 μmol/kg) were injected i.v. 1 min after BSP (120 μmol/kg, i.v.) at zero time. Bile was collected in 20 min periods for 1 hr. Bars represent means ± SE of 6 animals. Asterisks indicate values significantly different ($P < 0.05$) from saline-treated (3 mL/kg, i.v.) controls.

rate, produced a moderate but prolonged depression of biliary bismuth excretion.

Antimony and bismuth were toxic to the DEM-pretreated animals. While in the administered dosages neither the metalloids nor DEM alone produced lethality, 80% of the rats receiving DEM and antimony potassium tartrate (50 μmol/kg, i.v.) died by the end of experiment, and no animal treated with DEM and bismuth ammonium citrate (150 μmol/kg, i.v.) survived the fourth bile collection period. Increased toxicity in DEM-treated and antimony- or bismuth-injected rats was also manifested as a more pronounced decrease in bile formation than in control rats receiving these metalloids alone (data not shown).

Effect of antimony and bismuth on biliary excretion of BSP

BSP is excreted into the bile partly in unchanged form and partly as a glutathione conjugate (BSP–GSH). Simultaneous administration of antimony potassium tartrate (50 μmol/kg, i.v.) significantly lowered the rate of total BSP excretion 20–60 min after injection (Fig. 7, lower panel). This decrease resulted solely from reduced excretion of conjugated BSP as the output of unconjugated BSP remained unchanged. Co-administration of bismuth ammonium citrate (150 μmol/kg, i.v.) did not influence significantly the biliary excretion of BSP or its GSH conjugate (Fig. 7, lower panel). Bile flow rate was significantly lower in antimony-injected animals than in controls in the second and third bile collection periods, and also in bismuth-injected animals in the second 20-min bile collection period.

DISCUSSION

It has been reported recently that arsenic administered as sodium arsenite or arsenate induces a profound increase in the biliary excretion of NPSH and GSH in rats [9–11]. This study demonstrates that trivalent antimony and bismuth administered as antimony potassium tartrate and bismuth ammonium citrate, respectively, also exert a similar effect (Figs 1–3). Antimony, which produced up to a 50-fold increase in the biliary excretion of NPSH, was 2–3 times more effective than the arsenicals, whereas bismuth, which increased NPSH excretion only up to 3-fold, was considerably less effective than arsenite or arsenate. Such a dramatic increase in biliary excretion of GSH or NPSH as observed after administration of antimony potassium tartrate has not yet been reported. As was observed with arsenicals, the biliary excretion rates of NPSH following administration of antimony or bismuth also closely correlated, both in time and magnitude, with the excretion rates of total GSH (Fig. 3). This observation indicates that enhanced excretion of NPSH results mainly from increased output of GSH.

Both antimony [12, 15] and bismuth [13, 14] are excreted into bile in humans or experimental animals to a significant extent. In fact, the extent of biliary

Table 1. Relationship between the biliary excretion rates of arsenic, antimony and bismuth and their effects on biliary excretion of NPSH and bile flow

Injected element	Dose (μmol/kg i.v.)	Biliary excretion (nmol/kg·min) Element	NPSH	Bile flow (μL/kg·min)
—	—	—	114 ± 13	52 ± 2
BiIII	150	59 ± 8	308 ± 23	44 ± 3
AsV	150	134 ± 16	1350 ± 140	61 ± 6
AsIII	50	286 ± 26	1640 ± 97	76 ± 5
SbIII	50	1000 ± 59	2950 ± 290	75 ± 5

Peak excretion rates and bile flow are presented after injection of bismuth ammonium citrate (BiIII), sodium arsenate (AsV), sodium arsenite (AsIII) and antimony potassium tartrate (SbIII) to urethane-anesthetized rats.

Data on the arsenicals are from Gyurasics *et al.* [9].

Values represent means ± SE of 6–10 rats.

excretion of antimony (i.e. over 50% of the dose in 2 hr) is larger than that reported for other metals [13]. Since there is a close correlation between the biliary excretion rate of arsenic and NPSH [9, 11], it was also of interest to determine if the antimony- and bismuth-induced increases in the biliary excretion of NPSH are related to excretion of antimony and bismuth into bile. Data presented in Figs 5 and 6 are indicative of such a relationship. There were striking similarities in the time courses of the biliary excretion of these metalloids and the NPSH control rate. Moreover, the biliary excretion of antimony and NPSH as well as of bismuth and NPSH were reduced, proportionally in general, by the GSH depletor DEM and the GSH transport inhibitors ICG and BSP (Figs 5 and 6). These observations support the conclusion that the hepatobiliary transport of antimony and bismuth, similarly to that of arsenic [9] and several heavy metals [4, 5], is dependent on the hepatobiliary transport of GSH. GSH-dependent biliary excretion of antimony most likely accounts for the observation that treatment of rats with buthionine sulfoximine, an inhibitor of GSH synthesis, diminished fecal but increased urinary excretion of antimony [15].

Our findings also suggest that transport of antimony and bismuth from the hepatocytes to the bile canaliculi generates hepatobiliary transport of GSH. Arsenical-induced increase of hepatobiliary transport of GSH has been hypothetically explained by assuming the formation of an unstable arsenic–GSH complex which is transported from the hepatocytes into the bile canaliculi but subsequently readily dissociates releasing the complexed GSH [11]. This hypothesis was based on the known affinity of trivalent arsenic to thiols and the dissociability of the monothiol-containing thioarsenites. Trivalent antimony [17] and bismuth [16] also react readily with sulfhydryl groups. Bailly *et al.* [15] found an antimony metabolite in the bile of rats injected with antimony trichloride which co-chromatographed on paper with the di-glutathione complex of antimony. Thus, based on the close chemical similarity of the trivalent antimony, bismuth and arsenic, we may hypothesize, until direct evidence is provided, that generation of biliary GSH excretion by antimony and bismuth is due to hepatic formation, biliary excretion and subsequent decomposition of unstable antimony–GSH and bismuth–GSH complexes.

Although enhancement of hepatobiliary transport of GSH appears to be a general feature of the investigated elements in group Va of the periodic table, their potency and efficacy is significantly different. As demonstrated in Table 1, the differences in the increments in biliary NPSH outputs following administration of these elements is related largely to the differences in the rate of the biliary excretion of these elements. For example, antimony was excreted into bile at a 17-fold higher rate than bismuth and produced a 15-fold larger net increment in biliary NPSH excretion rate than bismuth did (Table 1). Data in Table 1 also indicate that the net increments in NPSH excretion markedly exceed the excretion of the group Va elements. At the peak of NPSH and metalloid excretion, hepatobiliary transport of approximately three, nine, five and three thiol molecules was generated during the excretion of each metalloid molecule after administration of bismuth, arsenate, arsenite and antimony, respectively, at the indicated dosages. Thus, the excretion rates of the investigated group Va elements are different, as well as their potency to promote the hepatobiliary transport of GSH. The mechanism by which one molecule of these elements generates the transport of several molecules of GSH is unclear. Complexation of several GSH molecules by one metalloid ion or reabsorption of the metalloid ion from the biliary tree with subsequent re-excretion into bile with co-transport of additional GSH molecules, as suggested for arsenic [11], are possible alternatives.

The enormous increase in hepatobiliary transport of GSH after administration of antimony probably accounts for the decline in hepatic NPSH levels (Fig. 4). Bismuth, which produced a much smaller increase in biliary NPSH output, did not significantly diminish hepatic concentration of NPSH. Reduction of renal NPSH by antimony may be due to a direct effect of antimony on the kidney. Alternatively, it may also be secondary to depletion of GSH in the liver as hepatic GSH is considered to be the source of

substrate supply for GSH synthetis in extrahepatic organs including the kidney [24].

Antimony, but not bismuth, significantly diminished the biliary excretion of BSP. This effect is most likely due to antimony-induced inhibition of GSH conjugation of BSP rather than to inhibition of the hepatobiliary transport process. This conclusion is supported by the observation that antimony selectively reduced the excretion of conjugated BSP while not influencing the output of the unconjugated BSP (Fig. 7). Decreased conjugation of BSP may be due to decreased availability of hepatic GSH (Fig. 4) or interference with hepatic glutathione *S*-transferase activity. Arsenicals which influenced hepatobiliary disposition of BSP in an entirely similar fashion as antimony failed to change glutathione *S*-transferase activity in liver [9].

Antimonials, bismuth compounds and arsenicals are of toxicologic and/or therapeutic importance [15–17, 25, 26]. The present and previous reports [9, 11] demonstrate that these compounds (1) are excreted into bile in GSH-dependent fashion, (2) induce significant loss of hepatic GSH via the bile and (3) interfere with GSH conjugation in the liver. These features of the group Va elements may have important implications regarding their biologic fate and effects.

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