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EFFECT OF ARSENICALS ON BILIARY EXCRETION OF ENDOGENOUS GLUTATHIONE AND XENOBIOTICS WITH GLUTATHIONE-DEPENDENT HEPATOBILIARY TRANSPORT

ÁGNES GYURASICS, FERENC VARGA and ZOLTÁN GREGUS*
Department of Pharmacology, University Medical School of Pécs, H-7643 Pécs, Hungary

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Abstract—Sodium arsenite (25–100 μmol/kg, i.v.) and arsenate (75–300 μmol/kg, i.v.) injected into anaesthetized rats increased the biliary excretion of endogenous non-protein thiols (NPSH) in a dose-dependent fashion up to 24- and 31-fold, respectively. Simultaneously with NPSH, glutathione (GS) excretion was increased to a similar extent suggesting that the increment in biliary thiol output originated from enhanced hepatobiliary transport of GS. After administration of labelled arsenicals, biliary excretion of ^{74}As and NPSH followed similar time-courses. Biliary excretion of ^{74}As was more efficient after arsenite than arsenate administration corresponding to the greater potency of arsenite compared to arsenate to increase biliary output of NPSH. Coadministered sulfobromophthalein (BSP) inhibited the biliary excretion of ^{74}As and prevented the arsenical-induced increase in biliary NPSH. Thus, hepatobiliary transport of arsenic apparently proceeds coordinately with that of GS. However, excretion of each molecule of arsenic compound generates transport of several molecules of GS. Though mercuric, methylmercuric, cadmium and zinc ions are thought to be excreted into bile as complexes with GS, the marked arsenical-induced increase in GS excretion only doubled the biliary excretion of inorganic mercury and hardly influenced the transport of other metals into bile. This finding suggests that arsenicals markedly enhance biliary excretion of GS with a free thiol group but barely or not at all that of GS with a thiol group blocked by a firmly bound metal ion. Both arsenicals diminished the biliary excretion of BSP–glutathione conjugate after BSP administration presumably because they impaired conjugation of BSP with GSH due to decreased GS availability. It is assumed that arsenite, and arsenate after reduction to arsenite, forms an unstable complex with GS that is efficiently transported into bile resulting in increased biliary output of GS. It is demonstrated that arsenite-induced perturbation of hepatobiliary disposition of endogenous GS differentially affects biliary excretion of xenobiotics with GS-dependent hepatobiliary transport.

The liver plays a central role in glutathione (GS) homeostasis [1]. This tripeptide is synthesized predominantly in the liver and supplied to other organs via the blood and bile. After its hepatobiliary transport, GS undergoes partial oxidation and/or hydrolysis, the latter being initiated by γ-glutamyltransferase at the bile canalicular membrane [2–5]. Reduced GS and its thiol-containing hydrolysis products (i.e. cysteinylglycine and cysteine) can be quantitated by the thiol-reactive Ellman's reagent and are collectively called non-protein thiols (NPSH). GS is important in the elimination of several electrophilic xenobiotics. Some organic electrophiles, such as sulfobromophthalein (BSP), are conjugated with GS in a GS *S*-transferase-catalysed reaction with the resultant GS conjugates being excreted in the bile [6, 7]. Metal ions, such as cadmium [8], zinc [9], methylmercuric [10] and mercuric [11] ions, are thought to form complexes with reduced GS and, as such, are also eliminated via the bile. This process is apparently not influenced by GS *S*-transferases but rather by the hepatobiliary transport rate of GS-related thiols [12].

Transport of GS from liver to bile is affected by chemicals. GS depletors and cholephilic organic acids (e.g. BSP and indocyanine green) reduce, whereas certain enzyme inducers enhance excretion of NPSH in bile [12, 13]. The increase or decrease of biliary excretion of GS or NPSH results in respective alteration in transport from liver to bile of some exogenously given metals [12, 13].

Augmentation of biliary GS excretion by sodium arsenite in isolated perfused rat liver has recently been reported [14]. Arsenite appears to be far more effective in enhancing excretion of endogenous GS than any other agent. However, effect of arsenite *in vivo* on biliary output of GS has not been analysed in detail and its is unknown whether other arsenicals (e.g. arsenate, the form of inorganic arsenic prevalent in nature and dimethylcacodylic acid, the main urinary metabolite of inorganic arsenicals) exert a similar effect. Because rats excrete significant amounts of injected arsenic in bile [15, 16], it was also of interest to know whether the increment in biliary GS excretion following arsenical administration was related to hepatobiliary transport of arsenic. The present studies were designed to address these questions. In addition, these investigations also determined whether or not arsenical-induced enhancement of biliary GS excretion influences hepatobiliary transport of xenobiotics whose elimination via the biliary route is GS-dependent. For

* Correspondence should be addressed to: Zoltán Gregus, Department of Pharmacology, University Medical School of Pécs, Szigeti út 12, H-7643 Pécs, Hungary.

this purpose, effect of arsenicals on biliary excretion of BSP, cadmium, zinc, inorganic and methylmercury was also investigated. Preliminary data of these studies have been published [17].

MATERIALS AND METHODS

Chemicals. Ellman's reagent [5,5'-dithio-bis (2-nitrobenzoic acid)] and glutathione reductase (type III) were purchased from the Sigma Chemical, (St Louis, MO, U.S.A.; sodium arsenite ($NaAsO_2$) from Merck, Darmstadt, F.R.G.; metaphosphoric acid from Alfa Products, Danvers, MA; BSP from Fluka, Buchs, Switzerland; phenol-3,6-dibromphthalein disulfonate (DBSP) from Société d'Etudes et des Recherches Biologiques (SERB), Paris, France. Reduced glutathione, NADPH, mercuric chloride, zinc chloride, cadmium chloride, sodium arsenate ($Na_2AsHO_4 \cdot 7H_2O$) and sodium dimethylcacodylate were obtained from Reanal, Budapest, Hungary. [^{74}As]Arsenic acid (1.3 MBq/μg As), [^{203}Hg]mercuric chloride (132 MBq/mg Hg) and [^{203}Hg]methylmercuric chloride (0.545 MBq/mg Hg) were from Amersham International plc (Amersham, U.K.). [^{65}Zn]Zinc chloride (47 GBq/g Zn) was from Isotope Production and Reactor Centre, Otwock-Swierk, Poland and [^{109}Cd]cadmium chloride (86 GBq/mg Cd) from Tehsnabexport, Moscow, U.S.S.R. [^{74}As]Arsenite was produced from [^{74}As]arsenic acid by reduction with bisulfite and thiosulfate as described by Ray and Asher [18]. To ensure that all arsenic in the [^{74}As]arsenic acid solution is in the pentavalent form, [^{74}As]arsenic acid was kept under UV light for 4 hr before use [18].

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 lab chow (LATI, Gödöllő, Hungary) were provided *ad lib.* Experiments were started between 8 and 9 a.m. in order to eliminate influence of diurnal variations in hepatic GS levels. Rats were anesthetized with urethane (1.2 g/kg, i.p.) and their body temperature was maintained at 37° by means of heating lamps. In order to maintain patent airways, tracheotomy was performed on each animal. After median laparatomy, the bile duct was cannulated with a 23 gauge needle attached to a polyethylene tubing (PE-50). Arsenicals and the test compounds were dissolved in distilled water and injected into the saphenous vein in a volume of 3 mL/kg and in dosages indicated in the figures. In order to explore the whole capacity of arsenicals to increase biliary NPSH output, various dosages, up to the highest acutely tolerated dose, were selected. The radioactive dosages of arsenicals and the other metals were 0.2 and 1–2 MBq/kg, respectively. Coadministered chemicals were injected into the contralateral saphenous vein. Bile was collected into pre-weighed 1.5-mL microcentrifuge tubes in 20 min periods, immediately after injection of the arsenicals or test compounds. In experiments for determining excretion of NPSH or total GS, bile was collected into tubes embedded in ice and containing 0.4 mL 5% metaphosphoric acid in order to prevent oxidation of thiols [19, 20]. The volume of bile was determined gravimetrically, assuming unity for the specific gravity. Biliary excretion rates of endogenous thiols, arsenicals and test compounds (i.e. metals, BSP and DBSP) were calcualted as the product of bile flow and biliary concentration.

Analytical methods. Concentration of NPSH in deproteinized bile and liver homogenate was measured with Ellman's reagent according to Sedlak and Lindsay [21]. Biliary concentration of GS was quantitated according to the method of Tietze [22] using glutathione reductase.

The amounts of arsenicals and other metals in bile were determined by measuring radioactivity of the bile samples in a well type gamma scintillation counter. Standard solutions containing known amount of metal were also counted to calculate the dosimetry.

Biliary concentration of total BSP (i.e. unconjugated BSP plus its glutathione conjugate) and DBSP was determined spectrophotometrically at 580 nm after dilution of the bile samples with 0.1 M sodium hydroxide. In order to determine the proportion of unconjugated and conjugated BSP, bile samples were chromatographed on Whatman No. 1. paper in an ascending system using the mixture of *n*-butanol, acetic acid and water (4:1:2, v/v/v) as the mobile phase. Spots corresponding to the BSP compounds were visualized over ammonia vapor, cut out and eluted with water. Absorbance of the eluates measured at 580 nm after alkalinization were used to calculate proportion of unconjugated and conjugated BSP in bile.

Statistics. Comparison of data was performed by analysis of variance followed by Duncan's test with $P < 0.05$ as the level of significance.

RESULTS

Biliary excretion of endogenous thiols

Effect of sodium arsenite (25–100 μmol/kg, i.v.) and sodium arsenate (75–300 μmol/kg, i.v.) on biliary excretion of NPSH is demonstrated in Fig. 1. The largest dosages of these arsenicals were at the limit of acute tolerance, especially for arsenate which produced lethality 100–120 min after administration. Both arsenicals markedly enhanced biliary excretion of NPSH in a dose-dependent manner, however, their time courses of effect and potencies were different.

The trivalent arsenical produced a dramatic, 10–24-fold increase in the biliary excretion of NPSH immediately after administration. This effect of arsenite was short-lived with the rates of NPSH excretion rapidly declining with time. This decline was most rapid in rats injected with the largest arsenite dosage and excretion of NPSH returned to the control rates by two hours after injection of 100 μmol/kg arsenite.

In contrast to arsenite, administration of pentavalent arsenate resulted in a more sustained increase in NPSH excretion. For example, 75 μmol/kg arsenate elevated biliary output of NPSH steadily 6–10-fold from 20 to 120 min after injection. With increased dosage, arsenate induced larger

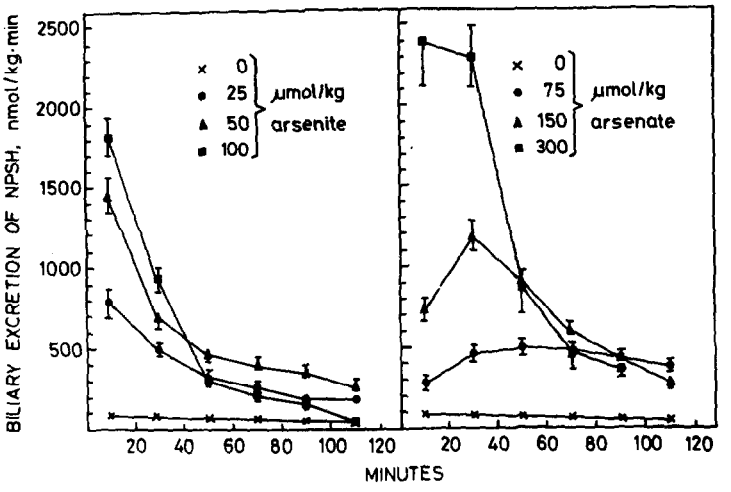

Fig. 1. Effect of arsenite and arsenate on biliary excretion of non-protein thiols (NPSH). Rats were injected with sodium arsenite or arsenate i.v. at 0 time and bile was collected in 20 min periods thereafter. Symbols represent means ± SE of 5–10 rats. With the exception of NPSH excretion at 100–120 min after administration of arsenite (100 μmol/kg), all values are significantly different ($P < 0.05$) from the respective control.

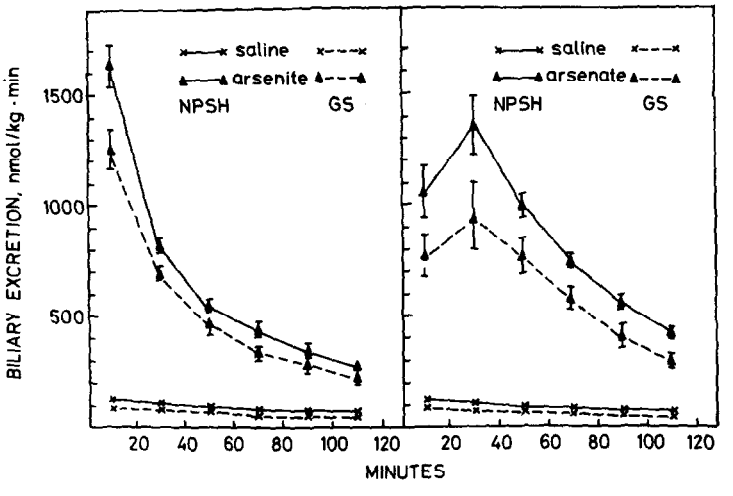

Fig. 2. Relationship between biliary excretion of non-protein thiols (NPSH) and total glutathione (GS) following arsenite or arsenate administration. Rats were injected with sodium arsenite (50 μmol/kg, i.v.) or sodium arsenate (150 μmol/kg, i.v.) at 0 time and bile was collected in 20 min periods thereafter. GS includes both reduced (GSH) and oxidized glutathione (2 GSSG). Symbols represent means ± SE of 7–8 rats. All values are significantly different ($P < 0.05$) from the respective controls.

enhancement in NPSH excretion (i.e. 17- and 31-fold after 150 and 300 μmol/kg, respectively), however, declines in NPSH excretion rate following the maximal arsenate-induced thiol excretion also became more pronounced.

In contrast to arsenite and arsenate, dimethylcacodylic acid, when injected intravenously in dosages of 100 and 500 μmol/kg, did not influence biliary excretion of NPSH (data not shown). In order to determine whether arsenical-induced enhancement in biliary excretion of NPSH resulted from increased excretion of GS, biliary excretion of both NPSH and GS was simultaneously measured in control, arsenite- and arsenate-injected rats. As shown in Fig. 2, arsenite (50 μmol/kg, i.v.) induced a 14-fold and 17-fold maximal increase in excretion of NPSH and GS, respectively. After arsenate injection (150 μmol/kg, i.v.), maximal enhancement of both NPSH and GS output was 13-fold. Furthermore, a close parallelism was observed in the time courses of biliary excretion of NPSH and GS not only in control animals, but also in rats injected with arsenite or arsenate. The rate of NPSH excretion exceeded the rate of GS excretion by 25–35% both in the control and arsenical-injected rats.

Influence of arsenicals on bile formation is demonstrated in Fig. 3. Initially, both arsenicals induced a choleresis with 40 and 45% maximal

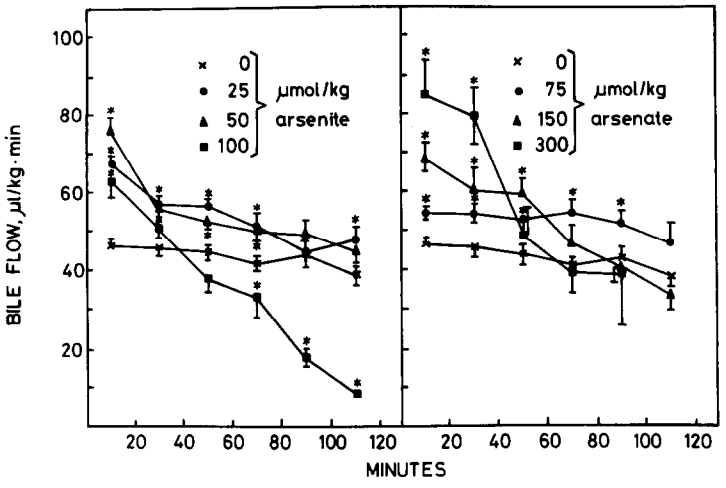

Fig. 3. Effect of arsenite and arsenate on bile production. Rats were injected with sodium arsenite or arsenate i.v. at 0 time and bile was collected in 20 min periods thereafter. Rats receiving the largest arsenate dosage died 100–120 min after arsenate administration. Symbols represent means ± SE of 5–10 rats. Asterisks indicate significant difference ($P < 0.05$) from control.

increases in bile flow following 50 µmol/kg arsenite and 300 µmol/kg arsenate, respectively. While the choleretic effect of arsenicals given in the lower dosages was apparent for 60–120 min after administration, choleresis produced by the largest dosages of arsenite or arsenate rapidly receeded and bile flow decreased below the control rate.

Relationship between biliary excretion of endogenous thiols and arsenicals

In order to determine whether or not arsenical-induced increase in biliary NPSH excretion is related to biliary excretion of arsenicals, ^{74}As-labelled arsenite or arsenate was injected to control rats and to rats that received BSP simultaneously. Biliary excretion of NPSH and ^{74}As in control and BSP-treated rats following administration of arsenite and arsenate are demonstrated in Figs 4 and 5.

Time courses of biliary excretion of NPSH and ^{74}As followed similar patterns in control rats. In rats injected with [^{74}As]arsenite, excretion rates of both arsenic and NPSH were highest immediately after arsenite administration followed by rapid declines in both arsenic and NPSH outputs (Fig. 4). In [^{74}As]arsenate injected rats, biliary excretion of ^{74}As and NPSH also changed in parallel reaching maximal rates by 20–40 min after administration and gradually declining thereafter (Fig. 5).

Hepatobiliary transport of both ^{74}As and NPSH responded in similar fashion to administration of BSP (Figs 4 and 5). Early after its administration, BSP markedly reduced outputs of [^{74}As]arsenite (−97%), [^{74}As]arsenate (−98%) as well as arsenite- and arsenate-induced increments in biliary thiol excretion (both −94%). Later after BSP administration, excretion of both ^{74}As and NPSH gradually increased and approached or even exceeded excretion rates of ^{74}As and NPSH in rats not injected with BSP (Figs 4 and 5).

Though biliary excretion of ^{74}As and the endogenous thiols responded similarly under various experimental conditions, the excretion rates (expressed as µmol/kg·min) of NPSH always exceeded the excretion rates of ^{74}As several fold. For example, during maximal arsenical excretion 5.6 and 9 times as much NPSH was excreted into bile than [^{74}As]arsenite and [^{74}As]arsenate, respectively.

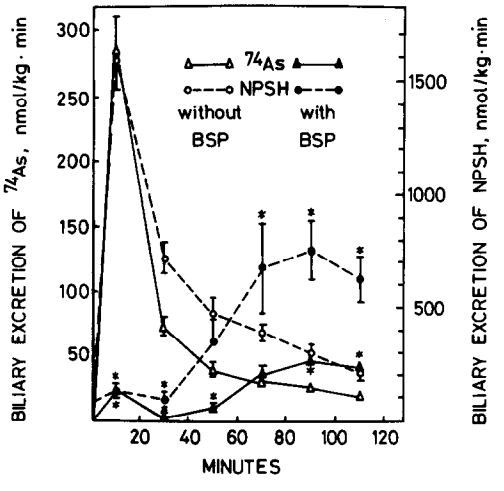

Fig. 4. Relationship between biliary excretion of ^{74}As and non-protein thiols (NPSH) after administration of arsenite with or without coadministration of sulfobromophthalein (BSP). Rats were injected with ^{74}As-labelled sodium arsenite (50 µmol/kg, i.v.) at 0 time with or without administration of BSP (50 µmol/kg, i.v.) one minute later. Symbols represent means ± SE of 5–10 rats. Asterisks indicate values of BSP-injected rats which are significantly different ($P < 0.05$) from the respective values of rats not receiving BSP.

Hepatic concentration of NPSH

Effect of arsenicals on hepatic NPSH levels was

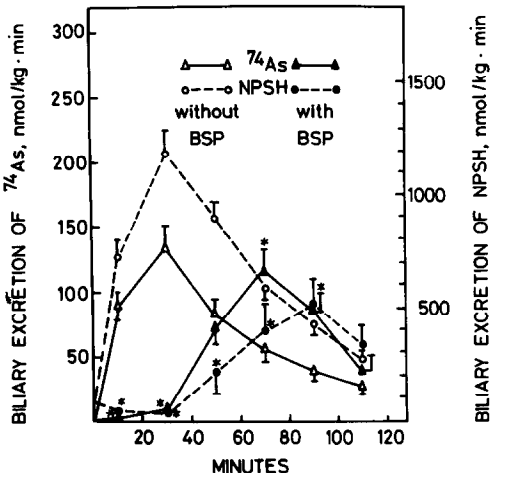

Fig. 5. Relationship between biliary excretion of ^{74}As and non-protein thiols (NPSH) after administration of arsenate with or without coadministration of sulfobromophthalein (BSP). Rats were injected with ^{74}As-labelled sodium arsenate (150 μmol/kg, i.v.) at 0 time with or without administration of BSP (50 μmol/kg, i.v.) one minute later. Symbols represent means ± SE of 5–10 rats. Asterisks indicate values of BSP-injected rats which are significantly different ($P < 0.05$) from the respective values of rats not receiving BSP.

examined after administration to anesthetized, bile duct-cannulated rats. NPSH levels were 6.63 ± 0.2, 5.02 ± 0.12, and 4.13 ± 0.09 μmol/g liver one hour after administration of saline, arsenite (50 μmol/kg, i.v.) or arsenate (150 μmol/kg i.v.), respectively. Thus, hepatic NPSH concentration was significantly ($P > 0.05$) diminished by both arsenite (25%) and arsenate (37%).

Biliary excretion of exogenous compounds

In order to investigate the influence of arsenical-induced increase in biliary thiol excretion on the hepatobiliary transport of xenobiotics whose excretion into bile is considered glutathione-dependent (i.e. metals and BSP), these xenobiotics were administered simultaneously with arsenite (50 μmol/kg, i.v.) or arsenate (150 μmol/kg. i.v.).

As shown in Fig. 6, both arsenicals significantly increased (up to 280% of control) the excretion rate of inorganic mercury. In contrast, biliary excretion of methylmercury remained unchanged and cadmium and zinc excretion was only minimally influenced by arsenicals.

Figure 7 depicts effect of arsenite and arsenate on biliary excretion of BSP, a cholephilic organic acid which appears in bile both in unchanged form and as the glutathione conjugate (BSP–GS). Neither arsenical influenced excretion of the parent compound. However, biliary excretion of BSP–GS was significantly decreased by both arsenite (up to −44%) and arsenate (up to −38%) 20–60 min after administration. Concomittantly, significant reduction in bile flow was also noted in rats injected with the arsenicals plus BSP as compared to rats receiving BSP alone (Fig. 7).

In contrast to BSP, the biliary excretion of DBSP (120 μmol/kg, i.v.), a non-metabolized analogue of BSP, was unaffected by coadministration of arsenite (50 μmol/kg, i.v.). Bile flow also remained unchanged in arsenite plus DBSP-injected rats as compared to rats receiving only DBSP (data not shown).

DISCUSSION

The present study demonstrates marked enhancement by arsenite of biliary thiol output in the anesthetized rat, confirming earlier findings obtained from isolated perfused rat liver [14]. In addition, arsenate, the form of inorganic arsenic most prevalent in the nature, but not dimethylcacodylic acid, the major metabolite of inorganic arsenicals, also enhances thiol excretion into bile.

The arsenical-induced increment in biliary thiol output is assumed to originate mainly or entirely from the increased hepatobiliary transport of GS. This assumption is supported by the similarities in the time courses of NPSH and GS excretion as well as by the similar magnitudes at which NPSH and GS outputs are enhanced by the arsenicals (Fig. 2).

The biliary excretion of ^{74}As follows a time course similar to that of the excretion of NPSH after administration of both arsenicals (Figs 4 and 5). Moreover, BSP influences the excretion of both ^{74}As and NPSH similarly (Figs 4 and 5). The close correlation in time, intensity and responsiveness to BSP between the simultaneous excretion of ^{74}As and thiols following arsenical administration suggests coupling of the hepatobiliary transports of the examined arsenicals and GS.

Complex formation between arsenic and reduced or oxidized GS has been hypothesized [14, 23]. Hepatobiliary transport of an arsenic-reduced GS complex may be the underlying cause of arsenical-induced biliary NPSH excretion. This complex, however, must be unstable, otherwise the Ellman's reagent could not react with the thiol group as it is impossible to detect any reaction between the purportedly very stable GS complex of inorganic mercury and Ellman's reagent (our observation). Drummond and Stern [24] also observed that reduced GS can be quantitated by the nitroprusside assay in the presence of equimolar amount of arsenite, though reduced GS could reverse, purportedly by complexation with arsenite, arsenite-induced inhibition of an acetoacetate-synthesizing enzyme *in vitro*. This finding was explained by an early observation [25] indicating that thioarsenites are almost completely dissociated.

It is interesting to note that the arsenical-induced increment in biliary thiol excretion exceeds the biliary excretion of ^{74}As several fold. For example, at the peak of arsenite output, the excretion of each molecule of arsenic compound induces excretion of 6–7 thiol molecules into bile (Fig. 4). The mechanism of this phenomenon is unknown. Although it is conceivable that more than one GS molecule complexes one arsenite molecule [23], it appears unlikely that the number of GS molecules in the complex would be as high as 6–7. If the arsenic–GS complex is indeed unstable, partial release of arsenic

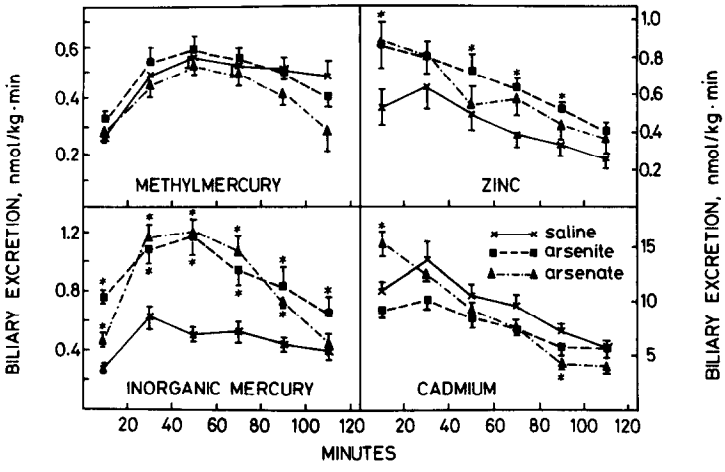

Fig. 6. Effect of arsenite and arsenate on biliary excretion of metals. Rats were injected i.v. with 10 μmol/kg mercuric chloride, methylmercuric chloride, zinc chloride or cadmium chloride followed by i.v. administration of saline (3 mL/kg), sodium arsenite (50 μmol/kg) or sodium arsenate (150 μmol/kg) one minute later. Bile was collected in 20 min periods after administration of the arsenicals. Symbols represent means ± SE of 5–8 rats. Asterisks indicate significant difference ($P < 0.05$) between arsenical- and saline-treated animals.

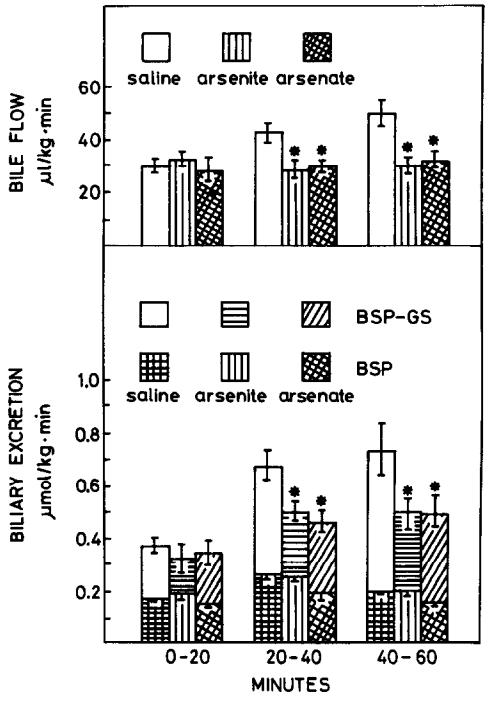

Fig. 7. Effect of arsenite and arsenate on the biliary excretion of sulfobromophthalein (BSP) and its glutathione conjugate (BSP–GS). Rats were injected with BSP (120 μmol/kg, i.v.) followed by i.v. administration of saline (3 ml/kg), sodium arsenite (50 μmol/kg) or sodium arsenate (150 μmol/kg) one minute later. Bile was collected in 20 min periods after administration of the arsenicals. Bars represent means ± SE of 8–10 rats. Asterisks indicate significant difference ($P < 0.05$) in bile flow or biliary excretion of BSP–GS between arsenical- and saline-treated animals. Biliary excretion of BSP did not differ significantly ($P < 0.05$) among the groups.

from the complex within the bile canaliculi could result in reabsorption of the liberated arsenic from the biliary tree and its subsequent reexcretion into bile as an arsenic–GS complex with the resultant hepatobiliary transport of an extra load of GS. Such intrahepatic cycling has been described for certain bile acids to explain their extreme choleretic effect [26]. It is known that biliary arsenic undergoes extensive reabsorption in the intestine [15], thus its reabsorption from the biliary tree is not unlikely.

Differences in chemical reactivity, disposition and biotransformation of inorganic arsenicals may explain the differences between arsenite and arsenate with respect to their biliary excretion (Figs 4 and 5) and their influence on biliary thiol output (Fig. 1). Arsenite is known to interact with thiol groups, whereas arsenate, which can substitute for phosphate in enzyme-catalysed reactions, is not thiol reactive [27–32]. Only after reduction to the trivalent oxidation state can pentavalent methylarsenicals form organosulfur derivatives with cysteine and reduced GS [27].

Arsenite is taken up by the liver to a considerably greater extent than arsenate [33, 34]. However, after arsenite administration, arsenic rapidly redistributes in rats, resulting in declining hepatic and increasing blood concentration [15]. The rapid hepatic uptake, and subsequent extensive hepatovascular transport may explain the initially high but rapidly subsiding biliary excretion rate of arsenite following its administration (Fig. 4), and that arsenite induces an immediate but transient increase in excretion of GS and NPSH in bile (Figs 1 and 2).

Arsenate is rapidly reduced to arsenite in experimental animals including rats [33, 35–38]. As early as 5 min after intravenous injection of sodium arsenate to rats, arsenite represents as much as 50% of total arsenic in blood [36]. Although no direct evidence is available, others' observations (e.g. poor hepatic uptake of arsenate compared to arsenite;

rapid conversion of arsenate to arsenite *in vivo*), and our findings (e.g. less efficient and more prolonged biliary excretion of ^{74}As after arsenate than arsenite; less potent but more prolonged stimulatory effect of arsenate than arsenite on biliary GS-excretion) are compatible with the following hypothesis. Arsenate in pentavalent form would not be excreted into bile to a significant extent and would not enhance biliary excretion of GS. Instead, arsenate would be gradually reduced in the body and transported into bile as arsenite with concomittant hepatobiliary transport of GS. Direct evidence for this hypothesis, however, remains to be presented.

Both arsenite and arsenate enhance bile flow, especially in the higher doses (Fig. 3). GS has recently been demonstrated as one of the osmotic driving forces in bile acid-independent bile formation [39]. Therefore, we assume, that arsenical-induced choleresis may be due to enhanced biliary GS output. The decreased bile flow after the initial choleresis, that is observed in rats injected with arsenicals at the largest dosages, is most probably a sign of arsenic toxicity.

Biliary excretion of exogenously administered mercuric, methylmercuric, cadmium and zinc ions has been shown to change in parallel with biliary excretion rates of endogenous GS and/or NPSH under various conditions [12, 13]. Therefore it appears paradoxical that despite the dramatic increase in hepatobiliary transport of GS and NPSH following administration of arsenite or arsenate, excretion of methylmercury, cadmium and zinc into bile is only slightly increased or even remained unchanged (Fig. 6). It was only inorganic mercury whose biliary excretion was clearly enhanced, though much less than that of endogenous thiols, by administration of 50 μmol/kg arsenite or 150 μmol/kg arsenate. The role of increased NPSH excretion in doubling the biliary excretion rate of inorganic mercury after arsenite administration is uncertain. The effect of arsenicals on hepatobiliary transport of metals may be complex which is also indicated by the observation that while 10 μmol/kg arsenite doubled biliary output of methylmercury [17], 50 μmol/kg arsenite failed to affect it (Fig. 6). In addition, if our hypothesis is correct that arsenite increases hepatobiliary transport of GS because it forms a rapidly excreted and unstable complex with this thiol, it appears logical that it cannot increase hepatobiliary transport of GS–metal complexes because the thiol group in these complexes is already blocked by firmly bound metal ions. Thus, limited responsiveness of metal excretion to arsenical-induced increase in biliary NPSH excretion further supports the above-described assumption regarding the mechanism of the enhanced biliary GS excretion after arsenical administration.

Both arsenite and arsenate significantly reduced excretion of BSP–GS following BSP administration (Fig. 7). This does not appear to be due to inhibition of hepatobiliary transport process by arsenicals because biliary excretion rates of both the parent compound (Fig. 7) and DBSP (data not shown), the unmetabolized analogue of BSP, remained unchanged following arsenical administration. Instead, diminished biliary excretion of BSP–GS may result from impaired conjugation of BSP with GS. Depressed conjugation of BSP, which would result in increased hepatic level of the cholestatic parent compound and diminished concentration of the choleretic BSP–GS [40], may also be responsible for the reduced bile flow during BSP excretion in arsenical-injected rats (Fig. 7). Decreased hepatic concentration of NPSH, which reflects reduced GS level in liver, can account, at least in part, for the impaired conjugation of BSP and, in turn, for diminished excretion of the conjugate into bile. GS *S*-transferase activities toward BSP in liver samples taken one hour after injection of arsenicals were comparable with those in untreated rats (our unpublished observation), thus inhibition of GS *S*-transferase, another likely mechanism of impaired conjugation, could not be confirmed.

In summary, this study demonstrates that both arsenite and arsenate markedly enhance excretion of endogenous NPSH in bile due to increased hepatobiliary transport of GS which is related to simultaneous hepatobiliary transport of arsenic. In spite of the greatly increased biliary GS output, biliary excretion of some toxic metals, which are transported into bile as complexes with GS, is little affected. However, arsenite and arsenate may reduce hepatic GS availability and impair conjugation of xenobiotics with GS.

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