Biochemical Pharmacology, Vol. 56, pp. 1381–1389, 1998.

ISSN 0006-2952/98/$19.00 + 0.00
PII S006-2952(98)00189-0

Role of Glutathione and Methylation in the Biliary Excretion of Selenium. The Paradoxical Effect of Sulfobromophthalein

Ágnes Gyurasics, Pál Perjési† and Zoltán Gregus*‡*

*Department of Pharmacology, University Medical School of Pécs, Pécs; and †Department of Medical Chemistry, University Medical School of Pécs, Pécs, Hungary

ABSTRACT. Biotransformation of selenite involves both reactions with GSH and methylations. Therefore, the role of GSH, methylation, and the hepatobiliary GSH transporter was investigated in the biliary excretion of selenium in rats injected with sodium [^{75}Se]selenite (1–10 μmol/kg, i.v.). Biliary output of selenium exhibited an apparent capacity limitation with an approximately 3 nmol/kg · min maximal rate and a dose-related decline in the fractional excretion. HPLC analysis of bile indicated absence of selenite and presence of selenodiglutathione (GS-Se-SG) and/or its hydrolysis products as the major biliary selenite metabolites. Depletion of hepatic glutathione by D,L-buthionine-[S,R]-sulfoximine or diethyl maleate decreased selenium excretion into bile by 60 and 80%, respectively. In contrast, inhibitors of methylation, i.e. periodate-oxidised adenosine or ethionine doubled the rate of biliary selenium excretion. While indocyanine green—an inhibitor of hepatobiliary GSH transport—failed to influence biliary selenium output, sulfobromophthalein (BSP)—another inhibitor of this sort—dramatically enhanced it. This effect was found to be a function of the dose of both selenite and BSP. The degree of BSP-induced enhancement of the selenium excretion rate gradually increased with elevation of the selenite dose, approaching 20-fold at 10 μmol/kg selenite. In contrast, the stimulatory effect of BSP on biliary selenium output was maximal at 50–100 μmol/kg and gradually lessened with elevation of the BSP dose above 100 μmol/kg. In summary, this study revealed that the biliary excretion of selenium depended on availability of hepatic GSH, probably for formation of GS-Se-SG, the putative cholephilic selenite metabolite. Methylation counteracted selenium excretion into bile and thus may contribute to the apparent capacity limitation of biliary selenium excretion. Finally, selenium output into bile was insensitive to inhibitors of the hepatobiliary GSH transporter, and was enhanced, paradoxically, by BSP several-fold. The mechanism of this unexpected effect is explored in the adjoining article. BIOCHEM PHARMACOL **56**;10:1381–1389, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. selenium; sulfobromophthalein; biliary excretion; methylation; glutathione; selenodiglutathione

Conjugation with GSH, the most abundant nonprotein thiol in cells, plays a key role in detoxification and elimination of electrophilic compounds. The GSH conjugates of organic electrophiles (e.g. BSP)§ and GSH complexes of several metal ions (e.g. mercury, methylmercury, cadmium, and zinc) are preferentially excreted into bile [1, 2]. GSH is also involved in the hepatobiliary disposition of the metalloids arsenic, antimony, and bismuth. We have recently provided circumstantial evidence for biliary excretion of these metalloids as unstable glutathione complexes [3–5]. It has also been demonstrated that the hepatobiliary transport of these metalloids is markedly diminished by chemicals that deplete hepatic GSH or inhibit the transport of GSH from liver to bile [3–5].

The biologic fate of another metalloid, the essential and anticarcinogenic selenium, is also intimately linked to GSH. Selenite is known to be biotransformed into GS-Se-SG and subsequently into glutathionyl selenol (GS-SeH) [6]. Thus, it is possible that selenium is excreted into bile in GSH-bound form and that biliary excretion may be significant in the disposition of selenium, because GSH conjugates are cholephilic compounds. Furthermore, excretion of selenium into bile, like that of arsenic, may depend on the availability of hepatic GSH and the activity of hepatobiliary GSH transporters. The present studies have been designed to test these hypotheses. For this purpose, we investigated the biliary excretion of selenium in rats injected with sodium [^{75}Se]selenite. Specifically, we tested the responsiveness of biliary selenium excretion to depletors of hepatic GSH, such as BSO and DEM [7, 8], and inhibitors of hepatobiliary transport of GSH, such as BSP and ICG [1, 4], and analysed the bile for GSH-containing

‡ Corresponding author: Zoltán Gregus, Department of Pharmacology, University Medical School of Pécs, Szigeti út 12, H-7643 Pécs, Hungary. Tel. 36-72-324-122; FAX 36-72-211-761; E-mail: gregus@apacs.pote.hu.

§ *Abbreviations*: BSO, D,L-buthionine-[S,R]-sulfoximine; BSP, sulfobromophthalein; CSH, cysteine; CGSH, cysteinylglycine; CS-Se-SC, selenodicysteine; CGS-Se-SCG, selenodicysteinylglycine; DEM, diethyl maleate; GSH, glutathione; GS-Se-SG, selenodiglutathione; GS-Se-SC, mixed selenotrisulfide of GSH and CSH; GS-Se-SCG, mixed selenotrisulfide of GSH and CGSH; ICG, indocyanine green; and PAD, periodate-oxidised adenosine.

Received 9 September 1997; accepted 9 April 1998.

selenium metabolites. In addition, because the above-mentioned GSH-dependent steps in the biotransformation of selenite are followed by successive methylations to produce methylselenol, dimethyl selenide, and trimethylselenonium ion, the role of methylation in the biliary elimination of selenium was also investigated. For this purpose, the effects of PAD, an indirect inhibitor of methyltransferases [9, 10], and ethionine, a depletor of *S*-adenosylmethionine [11], on the biliary excretion of selenium were also examined. Our studies with BSP unexpectedly revealed that this cholephilic organic acid dramatically increased the biliary excretion of selenium. Because of its potential significance, this striking observation was analysed further. Some of the descriptive characterisation of this finding is presented in this work, whereas an adjoining paper [12] deals with the mechanistic analysis of BSP-induced increase in biliary excretion of selenium.

MATERIALS AND METHODS

Chemicals

Sodium selenite ($Na_2SeO_3 \cdot 5H_2O$), selenium dioxide, D,L-ethionine, GSH, CSH, adenosine, sodium periodate, and urethane were purchased from Reanal. BSP was obtained from Fluka, ICG from Hynson, Wescott and Dunning, DEM from Koch-Light Laboratories and BSO as well as CGSH from Sigma Chemical Co. Sodium [^{75}Se]selenite was obtained from the Radioisotope Centre, Otwock-Swierk, Poland and Amersham International Plc. PAD was prepared by incubation of adenosine with sodium periodate according to the method of Hoffman [9]. Immediately before use, a saturated aqueous solution of PAD was prepared and its PAD concentration was determined spectrophotometrically as described by Tandon *et al.* [13].

[^{75}Se]GS-Se-SG and related selenotrisulfides were prepared by the method of Ganther [14]. In brief, 0.0167 M selenious acid solution was prepared by dissolution of crystalline SeO_2 in 0.1 M hydrochloric acid. To 75 μL of the ice-cold selenious acid solution (1.25 μmol H_2SeO_3) 1.5 μCi [^{75}Se]Na_2SeO_3 solution (1.1 μL) was added, and the obtained solution was mixed with 50 μL 0.1 M ice-cold GSH solution (5.0 μmol GSH) made up in deoxygenated water. The mixture was kept in ice for a few minutes then stored at −20°. An aliquot of this solution was diluted 200-fold before HPLC analysis. Similar incubations were performed using CSH, CGSH, as well as 1:1 molar mixtures of GSH plus CSH or CGSH instead of GSH to prepare the analogous selenotrisulfides that might be formed *in vivo* from GS-Se-SG.

Animal Experiments

Female 12- to 16-week-old Wistar rats (LATI, Gödöllö, Hungary) weighing 220–260 g were used. The animals were kept at 22–25° room temperature, at 55–65% relative air humidity, and on a 12-hr light/dark cycle and provided with tap water and lab chow (Altromin, LATI, Gödöllö, Hungary) *ad lib*.

Rats were anaesthetised with urethane (1.2 g/kg, i.p.) and their body temperature maintained at 37° by means of heating lamps. To maintain patent airways, tracheotomy was performed on each animal. After median laparotomy, the common bile duct was cannulated with the shaft of a 23-gauge needle attached to a PE-50 tubing (Clay-Adams). Sodium [^{75}Se]selenite dissolved in saline was injected into the left saphenous vein in a volume of 3 mL/kg and at doses indicated in the figures. The radioactivity injected was 7–15 μCi/kg when excretion of total [^{75}Se] was quantified, but was approximately 250 μCi/kg when bile was analysed for selenite metabolites. After administration of selenite, bile was collected into preweighed 1.5-mL microcentrifuge tubes for 2 hr in 20-min periods. For selenite metabolite analysis, bile was collected into tubes kept at subzero temperature in a cooling box.

To deplete hepatic GSH, rats were pretreated i.p. with DEM (4 mmol/kg in 3 mL/kg of corn oil) 1 hr before injection of selenite or with BSO (5 mmol/kg in 10 mL/kg of saline 4 hr before selenite). To inhibit hepatobiliary transport of GSH, rats were injected with BSP (50 μmol/kg in 3 mL/kg of water) or ICG (25 μmol/kg in 3 mL/kg of water) into the right saphenous vein 1 min after selenite administration. To inhibit methylation, rats were pretreated i.p. with D,L-ethionine (500 mg/kg in 20 mL/kg saline) 2 hr before injection of selenite or with PAD (50 μmol/kg, in 5–10 mL/kg of saline, depending on the actual concentration of PAD in the solution) 30 min before selenite.

Analysis

The amount of selenium excreted in bile was determined by measuring the radioactivity of the collected bile samples in a well-type γ scintillation counter. Standard solutions containing a known amount of [^{75}Se]selenite were also counted to calculate the dosimetry. Bile flow was measured gravimetrically taking 1.0 as the specific gravity.

Bile was analysed for metabolites of [^{75}Se]selenite by HPLC using the pumps, injector, reversed phase guard and analytical columns, and radioactivity detector specified in the adjoining paper [12]. For HPLC analysis, 50 μL of bile from [^{75}Se]selenite-injected rat was deproteinised by addition of 200 μL of methanol. The resultant precipitate was sedimented by centrifugation and resuspended in 250 μL of 80% methanol and recentrifuged. The combined supernatants were evaporated in a SpeedVac (Savant Instruments), and the residue was dissolved in 250 μL of water. Twenty microliters of this solution was injected into the HPLC. Gradient elution with a combined flow rate of 1 mL/min was performed using eluent A containing 45% methanol and 5 mM tetrapentylammonium bromide in water and eluent B containing 60% methanol, 5 mM tetrapentylammonium bromide, and 10 mM sodium sulfate in water. Elution was performed for 8 min with 100% A, which was

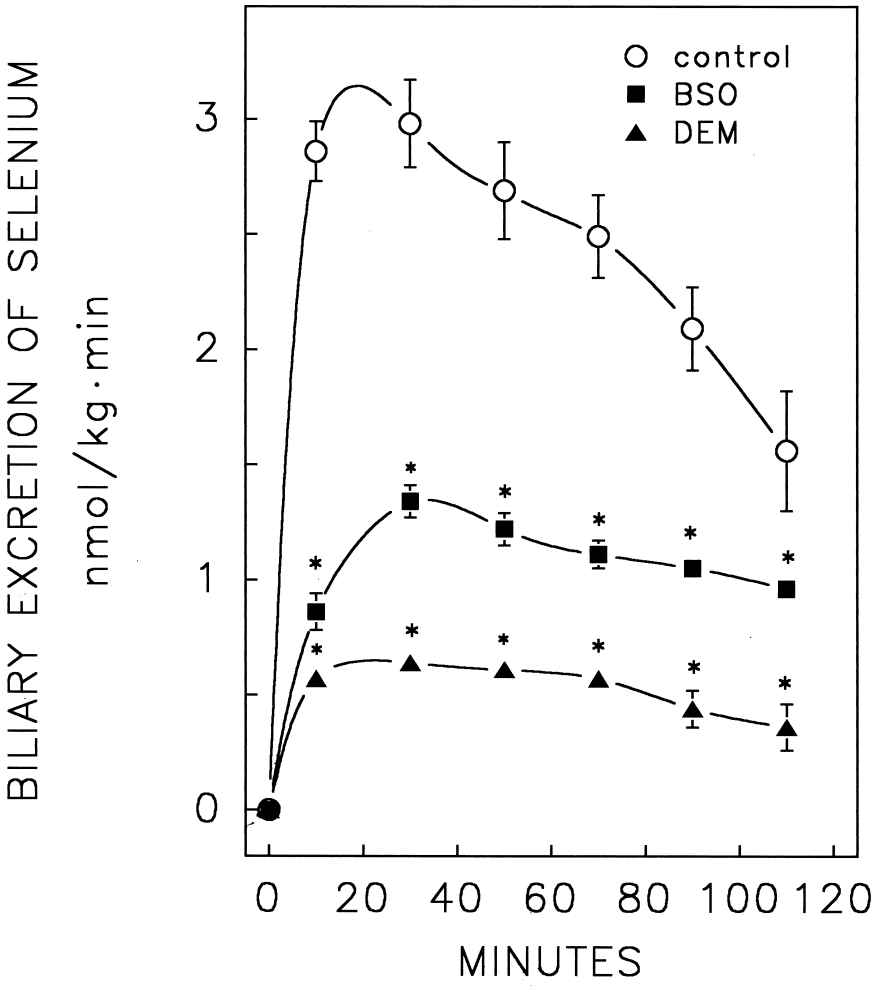

FIG. 1. Effect of glutathione depletors on the biliary excretion of selenium. Rats were pretreated with BSO (5 mmol/kg, i.p.) or DEM (4 mmol/kg, i.p.) 4 hr and 1 hr before injection of [^{75}Se]selenite, respectively. After administration of sodium [^{75}Se]selenite (10 μmol/kg, i.v.) at time 0, bile was collected in 20-min periods. Symbols represent means ± SEM of 4–6 rats. Asterisks indicate significant difference ($P < 0.05$) from control.

then changed linearly to 100% B by 10 min, after which this condition was maintained.

Comparison of data were performed by ANOVA followed by Duncan's test with $P < 0.05$ as the level of significance.

RESULTS

Role of GSH in the Biliary Excretion of Selenium

To assess the role of hepatic GSH availability in the biliary excretion of selenium, the effect of the GSH depletor DEM and BSO on the biliary excretion of selenium was studied in rats injected with sodium [^{75}Se]selenite (10 μmol/kg, i.v.). As demonstrated in Fig. 1, selenium appeared in the bile of control rats immediately and was excreted at a steady rate (2.5–3 nmol/kg · min) for 80 min before dropping below 2 nmol/kg · min by 2 hr. During this time period, approximately 3% of the selenium dose was eliminated via the bile. In rats pretreated with the GSH depletors, the biliary excretion rate of selenium was significantly lower than in control rats throughout the experiments. DEM, which depletes GSH by consuming it by conjugation, decreased the maximal biliary excretion rate of selenium by 80%, while pretreatment with BSO, which inhibits GSH synthesis, lowered it by 60%.

To study the possible mechanism for the observed dependence of biliary selenium excretion on hepatic GSH availability, the bile of a [^{75}Se]selenite-injected rat was analysed by HPLC linked to a radioactivity detector for [^{75}Se]selenite, [^{75}Se]GS-Se-SG and the possible hydrolysis products of this selenotrisulfide. Figure 2 demonstrates that the main [^{75}Se]-containing compound(s) in [^{75}Se]selenite-injected rat bile (top) coelute(s) with the synthetic [^{75}Se]GS-Se-SG (bottom) at 11.5 min. HPLC analysis did not reveal the presence of CS-Se-SC, selenite, or CGS-Se-SCG in the bile; these compounds eluted at 1.70, 3.50, and 7.08 min, respectively (not shown). Similar analyses of the incubates containing [^{75}Se]selenite plus GSH and CSH or CGSH indicated one prominent peak with the same retention time as that of GS-Se-SG (not shown). Therefore, it cannot be excluded that the peak in the chromatogram of the bile from selenite-injected rat (Fig. 2, upper panel) represents not only GS-Se-SG but also GS-Se-SC and GS-Se-SCG.

Role of Methylation in the Biliary Excretion of Selenium

To investigate the role of methylation in the biliary excretion of selenium, sodium [^{75}Se]selenite (10 μmol/kg,

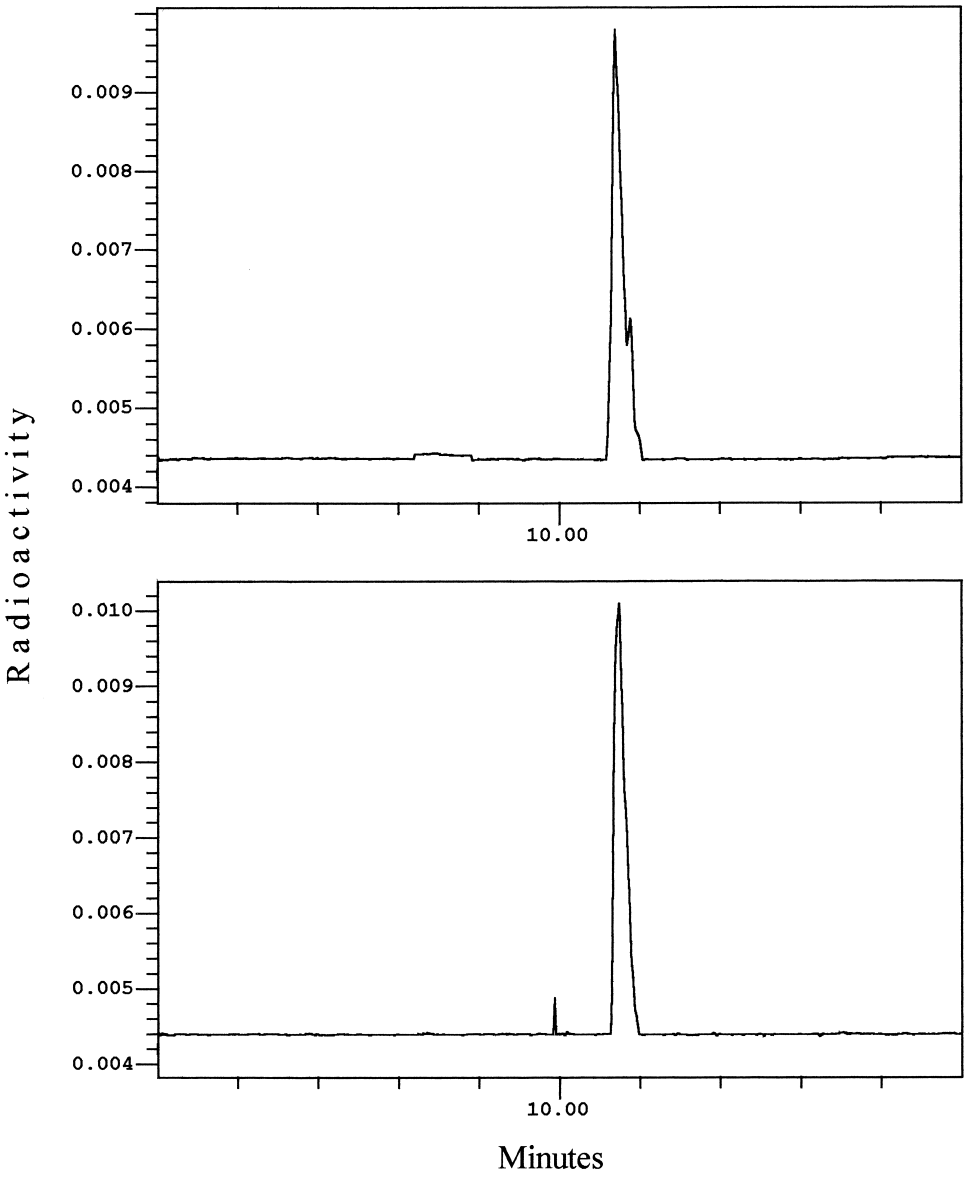

FIG. 2. Representative HPLC analysis of a bile sample collected from a [^{75}Se]selenite-injected rat (top) and of synthetic [^{75}Se]GS-Se-SG (bottom). The analysed bile sample was collected from a rat 20–40 min after injection of sodium [^{75}Se]selenite (10 μmol/kg, 250 μCi/kg, i.v.). Preparation of the bile sample for analysis and [^{75}Se]GS-Se-SG as well as the analytical conditions are described in Materials and Methods.

i.v.) was administered to rats pretreated with inhibitors of methylation. The effects of pretreatments with PAD–an indirect inhibitor of methyltransferases–and ethionine–a depletor of S-adenosylmethionine–are demonstrated in Fig. 3. Both PAD and ethionine pretreatment resulted in a significant, approximately two-fold elevation in biliary selenium output, starting from the second 20-min bile collection period.

Effect of Hepatobiliary GSH Transport Inhibitors on the Biliary Excretion of Selenium

Figure 4 shows the biliary excretion of selenium under the influence of ICG and BSP, cholephilic organic acids which are known to acutely inhibit the hepatobiliary transport of GSH. Injection of ICG (25 μmol/kg, i.v.) was without any significant effect on selenium excretion into bile. Unexpectedly, BSP (50 μmol/kg, i.v.) not only failed to inhibit the biliary excretion of selenium, but even substantially enhanced it. The stimulatory effect of BSP was apparent throughout the experiment, but was greatest 20–60 min after BSP administration. In this time period, selenium excretion rates were as much as 15–20 times higher than in controls. Because of its potential significance, the effect of BSP on the biliary excretion of selenium was analysed with respect to the dose of BSP, the dose of selenite, and the timing of BSP administration relative to injection of sodium selenite.

The effect of BSP on selenium excretion varied markedly with its dose (Fig. 5). These variations were seen in both the extent and the time course of the effect of BSP. The maximal (almost 20-fold) increase in selenium excretion observed within the time frame of the experiment was evoked by BSP injected at doses of 50 and 100 μmol/kg. Both the lower dose (25 μmol/kg) and, curiously, the higher doses (150 and 200 μmol/kg) of BSP were less effective. In addition, the onset of the maximal BSP-

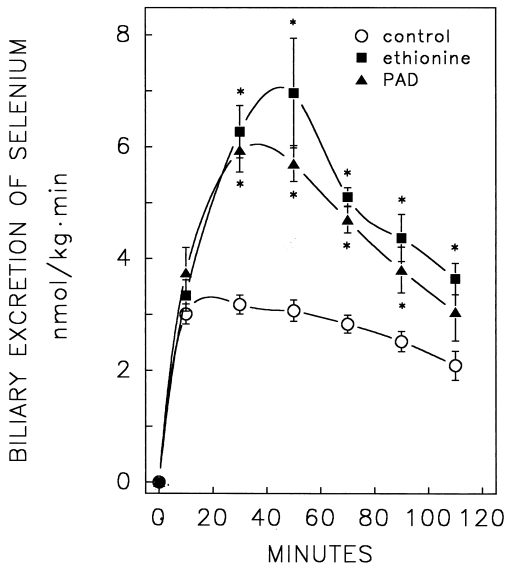

FIG. 3. Effect of inhibitors of methylation on the biliary excretion of selenium. Rats were pretreated with PAD (50 μmol/kg, i.p.) or with ethionine (500 mg/kg, i.p.) 30 min and 2 hr before administration of [^{75}Se]selenite, respectively. After injection of sodium [^{75}Se]selenite (10 μmol/kg, i.v.) at time 0, bile was collected in 20-min periods. Symbols represent means ± SEM of 4–6 rats. Asterisks indicate significant difference ($P < 0.05$) from control.

induced increase in the biliary excretion of selenium was clearly dependent on the dose of BSP. Injected at 25, 50, and 100 μmol/kg doses, BSP maximally augmented the biliary excretion of selenium at 20–40, 40–60, and 60–100

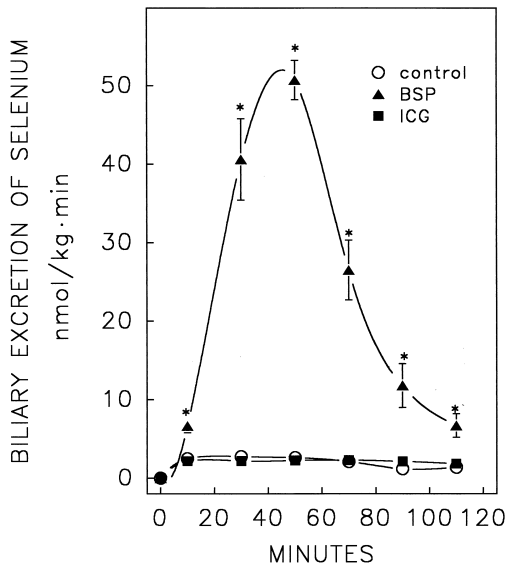

FIG. 4. Effect of inhibitors of hepatobiliary glutathione transport on the biliary excretion of selenium. Rats were injected with sodium [^{75}Se]selenite (10 μmol/kg) into the left saphenous vein and 1 min later (at time 0) with BSP (50 μmol/kg) or ICG (25 μmol/kg) into the right saphenous vein. Bile was collected in 20-min periods thereafter. Symbols represent means ± SEM of 4–6 rats. Asterisks indicate significant difference ($P < 0.05$) from control.

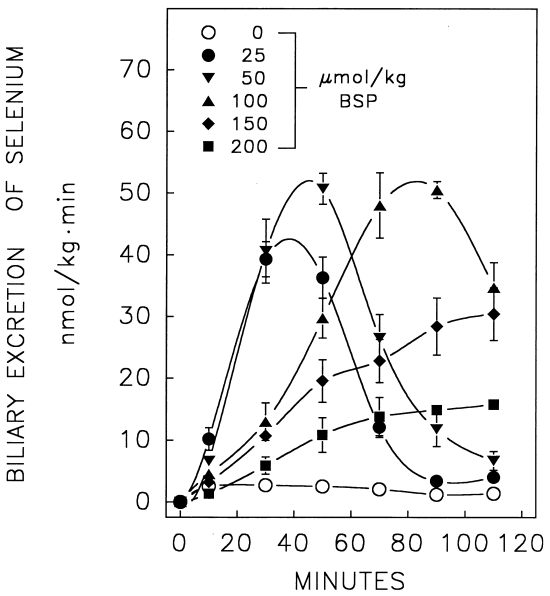

FIG. 5. Dose-dependent effect of BSP on the biliary excretion of selenium. Rats were injected with sodium [^{75}Se]selenite (10 μmol/kg) into the left saphenous vein and 1 min later (time 0) with BSP (25–200 μmol/kg) into the right saphenous vein. Bile was collected in 20-min periods thereafter. Symbols represent means ± SEM of 4–7 rats. Selenium excretion rates in BSP-injected rats are significantly different ($P < 0.05$) from those in rats not receiving BSP (dose = 0), with the exception of the rates at 0–20 min in rats injected with the three largest doses of BSP.

min after administration, respectively. With higher doses, maximal increases may have occurred at 2 hr after BSP administration or even later, because the excretion rate of selenium in rats injected with 150 and 200 μmol/kg of BSP rose steadily throughout the experiment.

The effect of BSP (50 μmol/kg) on biliary excretion of selenium in rats injected with sodium [^{75}Se]selenite in different doses (1–10 μmol/kg) is depicted in Fig. 6. When given alone, the excretion rate of selenium was little dependent on its dose, with the peak rates reaching approximately 1.5, 2.0, 3.0, and 3.0 nmol/kg · min following administration of selenite at doses of 1, 2.5, 5.0, and 10 μmol/kg, respectively. In this order, approximately 16, 8, 6, and 3% of the i.v. injected selenium doses appeared in bile within 2 hr. Coadministration of BSP with any of the selenite doses resulted in significant increases in biliary selenium output (Fig. 6). However, these increases became larger when the higher selenite doses were given. For example, while the maximal BSP-induced enhancement of selenium excretion rate was five-fold in rats receiving 1 μmol/kg of selenite, it was 8-, 12- and 18-fold, respectively, in the animals injected with 2.5, 5, and 10 μmol/kg of selenite.

In the above experiments, BSP was injected 1 min after administration of sodium selenite, and the bile collection was immediately started. We also investigated whether BSP also enhances the biliary excretion of selenium when injected at a later point after the selenite administration.

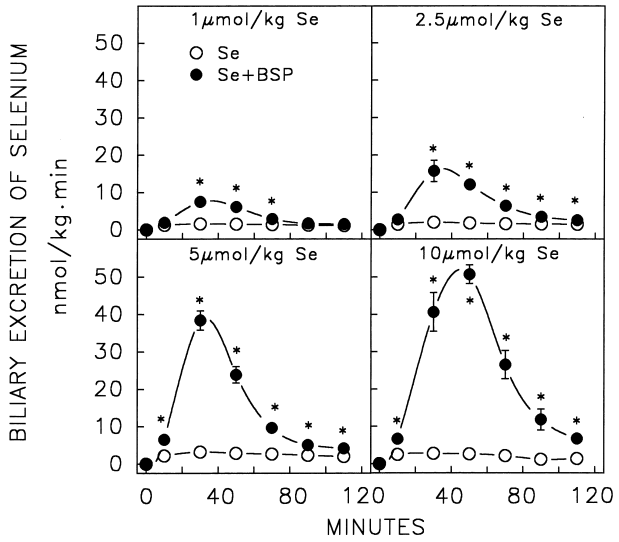

FIG. 6. Effect of BSP on the biliary excretion of selenium administered at different doses. Rats were injected with sodium [^{75}Se]selenite (Se; 1–10 μmol/kg) into the left saphenous vein and 1 min later (time 0) with BSP (50 μmol/kg) into the right saphenous vein. Bile was collected in 20-min periods thereafter. Symbols represent means ± SEM of 4–6 rats. Asterisks indicate selenium excretion rates in rats injected with selenite and BSP that are significantly different ($P < 0.05$) from those in rats receiving selenite alone.

The effect of BSP (50 μmol/kg, i.v.) given either 1 min or 2 hr after selenite (2.5 μmol/kg, i.v.) on the biliary excretion of selenium is shown in Fig. 7. It was found that selenium excretion into bile was almost equally stimulated by the immediate and the delayed BSP administration, with 8- and 6-fold increases, respectively, in the peak excretory rates.

DISCUSSION

We have recently demonstrated in rats that i.v. administered trivalent arsenic and antimony, neighbouring metalloids in group Va of the periodic table, are avidly transported into the bile with as much as 11% and 40% of the dose, respectively, appearing in bile within 20 min. Furthermore, the biliary excretion of these metalloids was markedly suppressed after depletion of hepatic GSH as well as by ICG and BSP, known inhibitors of hepatobiliary GSH transport [4, 5]. Selenium is located next to arsenic in group VIa of the periodic table. This chemical proximity and the similarities in metabolic fate of these two metalloids prompted us to analyse the role of GSH in the hepatobiliary transport of selenium as well. Although biliary excretion of selenium has been demonstrated in rats [15–17], factors governing selenium transport into bile have not been delineated.

This study has revealed both similarities and differences in the biliary excretion of selenium and arsenic. Similarly to arsenic, whose excretion into bile was almost abolished by DEM-induced GSH depletion in arsenite-injected rats [4], the hepatobiliary transport of selenium in selenite-injected rats was also diminished after chemical depletion of hepatic GSH (Fig. 1). This was demonstrated using either DEM or BSO, which deplete GSH through different mechanisms, i.e. by increasing its consumption and inhibiting its synthesis, respectively. The larger diminution in biliary output of selenium after DEM than after BSO is probably related to the fact that DEM is a more efficient depletor of hepatic GSH than BSO [18, 19]. Thus, while adequate availability of GSH in the liver is essential for biliary excretion of arsenic, GSH supply is also an important determinant in the hepatobiliary transport of selenium.

The mechanism underlying the GSH-dependent biliary excretion of arsenic and selenium is probably also similar. GSH forms complexes with arsenite *in vitro* and circumstantial evidence indicates that such complexes exist in the bile of arsenite-injected rats [3, 4], although the presence of GSH-bound arsenic in bile has not been demonstrated directly. Similarly to arsenite, selenite also reacts with GSH *in vitro* and forms GS-Se-SG [14]. This compound has been found in selenite-exposed HeLa cells [20] and in the urine of selenite-exposed young rats [21]. Here we present, apparently for the first time, chromatographic evidence for the presence of GSH-containing selenotrisulfides, but the absence of unchanged selenite, in the bile of a selenite-injected rat. The selenite metabolite found in the rat bile is most likely GS-Se-SG as it coelutes with synthetic GS-Se-SG (Fig. 2). Whereas we obtained no evidence that

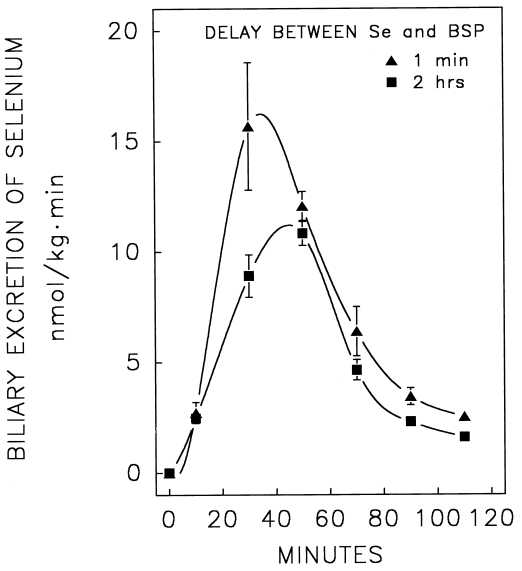

FIG. 7. Effect of immediate and delayed administration of BSP on the biliary excretion of selenium. Rats were injected with sodium [^{75}Se]selenite (Se; 2.5 μmol/kg) into the left saphenous vein and 1 or 120 min later with BSP (50 μmol/kg) into the right saphenous vein (time 0). Bile was collected in 20-min periods thereafter. Symbols represent means ± SEM of 4–6 rats. All selenium excretion rates beyond 20 min are significantly higher ($P < 0.05$) than those in rats receiving 2.5 μmol/kg of selenite alone that are presented in Fig. 6, upper right panel.

CS-Se-SC or CGS-Se-SCG are significant biliary metabolites of selenite, we could not exclude the possibility that the bile might also contain some GS-Se-SCG and GS-Se-SC. Presence of these latter mixed selenotrisulfides in bile would not be surprising, because they could be formed by γ-glutamyl transferase-mediated hydrolysis of GS-Se-SG in analogy to production of the mixed disulfide of GSH and CGSH (GS-SCG) from glutathione disulfide (GS-SG) [22]. Alternatively, these mixed selenotrisulfides may be formed in thiol exchange reactions [23] between GS-Se-SG and CGSH or CSH present in bile [24], but not within the hepatocytes, because γ-glutamyl transferase is not an intracellular enzyme and hepatic concentrations of CGSH and CSH are very low compared to GSH [25]. It is supposed that formation of GS-Se-SG in the liver depends on hepatic GSH concentration. Indeed, HeLa cells incubated with selenite produced a significant amount of GS-Se-SG, whereas HeLa cells depleted of GSH by BSO produced very little [20]. It is also likely that GS-Se-SG is a cholephilic compound because GSH and glutathione disulfide are also readily transported into bile [22, 24, 26, 27]. Thus, formation of GS-Se-SG from selenite in the liver and subsequent hepatobiliary transport of this selenotrisulfide would account for the dependence of biliary selenium output on hepatic GSH availability (Fig. 1), as this has been observed for other metals [1, 2, 28] and metalloids in group Va of the periodic table [4, 5] that are known or thought to be transported into bile as GSH complexes.

Differences in the biliary excretion of arsenic and selenium are multiple. The rate and dose-dependence of the biliary excretion of these metalloids are clearly dissimilar. While the maximum rate of biliary excretion of arsenic is as high as 300 nmol/kg · min [3], that of selenium is only 3 nmol/kg · min (Fig. 1). The fraction of dose excreted in 2 hr into bile is 24–37% for arsenic and dose-independent in a relatively wide dose-range [16]. In contrast, calculations from data presented in Fig. 6 indicate that the fraction of the selenite dose excreted into bile in 2 hr by control female rats gradually declines from 16 to 3% when the dose of selenite is elevated from 1 to 10 μmol/kg. A similar tendency has been observed in male rats [16]. Thus, the biliary excretion of selenium exhibits apparent capacity limitation in the dose-range studied.

The mechanisms that limit the biliary output of selenium in rats injected with large doses of selenite are unclear. Theoretically, depletion of hepatic GSH by selenite could account for such a finding; however, even the largest dose of selenite employed in this study does not influence the concentration of GSH in the liver [17]. Biotransformation of selenite into metabolites that are not excreted into bile may also be a factor. Selenite is known to be converted into dimethyl selenide, which is volatile and readily exhaled. Interestingly, the fraction that is exhaled as dimethyl selenide increases with elevation of the selenite dose [13, 29, 30]. Therefore, methylation may limit the biliary excretion of selenium at higher selenite doses. To test this hypothesis, we examined the effect of PAD and ethionine on the biliary excretion of selenium in rats given 10 μmol/kg selenite. PAD, an indirect inhibitor of methyltransferases [9], has been demonstrated to markedly diminish the pulmonary excretion of dimethyl selenide in rats [13]. Although no direct evidence has been provided that ethionine, a depletor of *S*-adenosylmethionine [11], also inhibits methylation of selenium, our observation presented in the accompanying paper [12] suggests that ethionine does exert such an effect. Pretreatment with either PAD or ethionine doubled the biliary excretion of selenium in selenite-injected rats (Fig. 3), indicating that methylation indeed counteracts the excretion of selenium into bile. In rats, methylation is a quantitatively less significant pathway in the biotransformation of arsenite, as rats excrete only 6% of the arsenite dose in 24 hr in methylated forms [31]. In comparison, as much as 12% of the dose is exhaled in 2 hr as dimethyl selenide by rats injected with selenite [12]. Thus, methylation is a prominent pathway in selenite biotransformation and may indeed be responsible, at least in part, for the dose-related decline in the fractional biliary output of this metalloid at high selenite doses. Mechanistically, however, it is unclear how methylation could counteract biliary excretion of selenium, because the accepted metabolic scheme of selenite (see [12]) indicates that it is hydrogen selenide formed from GS-Se-SG, and not GS-Se-SG, that is subject to methylation.

Selenium and arsenic also differ from each other in the responsiveness of their hepatobiliary transport to inhibitors of bile canalicular GSH transporter. Biliary excretion of arsenic, like that of antimony and bismuth, is diminished by cholephilic organic acids, such as ICG and BSP, that inhibit the hepatobiliary transport of GSH [4, 5]. In contrast, ICG failed to influence the output of selenium into bile (Fig. 4). As demonstrated in the adjoining article [12], the biliary excretion of selenium in selenite-injected rats was also unaffected by dibromosulfophthalein, another inhibitor of the bile canalicular GSH transporter [26]. Surprisingly, BSP dramatically enhanced the biliary excretion of selenium (Fig. 4). All these findings indicate that the hepatobiliary transport of exogenous selenium, unlike that of arsenic, is not sensitive to inhibitors of the bile canalicular GSH transporter. This may result from the lack of involvement of this transporter in the biliary excretion of selenium. Multidrug resistance protein (MRP2), thought to be identical to canalicular multispecific organic anion transporter (cMOAT) [32], could also translocate GS-Se-SG across the bile canalicular membrane because a subtype of this transporter accepts GS-SG as a substrate [33]. It is unclear, however, how the GSH transport inhibitors we used influence the activity of multidrug resistance protein (MRP2). Alternatively, another action of these organic acids may counterbalance or, in the case of BSP, even overcompensate for the inhibitory action of these cholephilic organic acids on the hepatobiliary transport of selenium. For example, dibromosulfophthalein inhibits not only the bile canalicular, but also the sinusoidal GSH transporter [26]. If the selenium species in the liver

were substrate for both of these transporters, inhibition of the sinusoidal transporter-mediated hepatovascular transport could, in theory, partially offset a decrease in hepatobiliary transport mediated by inhibition of the canalicular transporter. However, this is unlikely, because the GSH conjugate of BSP (BSP-SG), which is an inhibitor of the sinusoidal but not the canalicular GSH transporter [26], failed to increase the biliary excretion of selenium in selenite-injected rats [12]. Nevertheless, the coming paper [12] provides evidence for an *in vivo* chemical interaction between the injected BSP and selenium, which can very efficiently overcompensate for any inhibitory effect that BSP might exert on the hepatobiliary transport of selenium.

Because of the physiological importance as well as the antitoxic and anticarcinogenic properties of selenium, the stimulatory effect of BSP on the biliary excretion of this metalloid was subjected to detailed analysis. This has indicated that BSP produces this remarkable effect not only when given concurrently with selenite but also after delayed administration (Fig. 7). Very unusual dose-response relationships as a function of the dose of either BSP (Fig. 5) or selenite (Fig. 6) have also been revealed by these studies. The findings that the effectiveness of BSP in inducing an early increase in the rate of biliary excretion of selenium is directly related to the dose of selenite (Fig. 6) but is inversely related to the dose of BSP above 100 μmol/kg (Fig. 5) cannot be rationalised based on the studies described here. However, as tentative explanations for these phenomena have been provided by the mechanistic studies presented in the forthcoming publication, they will be given therein [12].

In summary, this work demonstrates that the biliary excretion of selenium depends on hepatic GSH availability, probably because selenite is biotransformed into GS-Se-SG in the liver and is transported into bile in this form. Furthermore, the biliary excretion of selenium is counteracted by its methylation, which may account, at least in part, for the apparent capacity limitation in the biliary selenium excretion at higher selenite doses. Finally, this study demonstrates that the hepatobiliary transport of selenium is insensitive to inhibitors of the bile canalicular GSH transporter and, moreover, that it is dramatically enhanced by BSP. The mechanism of this unexpected finding is dealt with in a subsequent paper.

This publication is based on a study supported by the Hungarian National Scientific Research Fund (OTKA T-016542), the Hungarian Ministry of Social Welfare (ETT 371/1996), and the Hungarian Academy of Sciences (AKP 97-69 3,2). The authors thank Angéla Schön for technical assistance and Katalin Gyulai and Ildikó Nagy for typing the manuscript.

References

1. Ballatori N, and Clarkson TW, Biliary excretion of glutathione and of glutathione-metal complexes. *Fundam Appl Toxicol* **5:** 398–403, 1985.
2. Gregus Z, and Klaassen CD, Excretion of metal ions: Mechanisms and influencing factors. In: *Handbook on Metal-Ligand Interactions in Biologic Fluids*. Ed. Berthon G, Vol. 1, pp. 445–460. Marcel Dekker, New York, 1995.
3. Gyurasics Á, Varga F, and Gregus Z, Effect of arsenicals on biliary excretion of glutathione and xenobiotics undergoing conjugation with glutathione. *Biochem Pharmacol* **41:** 937–944, 1991.
4. Gyurasics Á, Varga F, and Gregus Z, Glutathione-dependent biliary excretion of arsenic. *Biochem Pharmacol* **42:** 465–468, 1991.
5. Gyurasics Á, Koszorú L, Varga F, and Gregus Z, Increased biliary excretion of glutathione is generated by glutathione-dependent hepatobiliary transport of antimony and bismuth. *Biochem Pharmacol* **44:** 1275–1281, 1992.
6. Ganther HE, Pathways of selenium metabolism including respiratory excretory products. *J Am Coll Toxicol* **5:** 1–5, 1986.
7. Griffith OW, Depletion of glutathione by inhibition of biosynthesis. *Methods Enzymol* **77:** 59–63, 1981.
8. Plummer L, Smith BR, Sies H, and Bend JR, Chemical depletion of glutathione *in vivo*. *Methods Enzymol* **77:** 50–90, 1981.
9. Hoffman JL, The rate of transmethylation in mouse liver as measured by trapping *S*-adenosylhomocysteine. *Arch Biochem Biophys* **205:** 132–135, 1980.
10. Hoffman JL, and McConnell KP, Periodate-oxidized adenosine inhibits the formation of dimethylselenide and trimethylselenonium ion in mice treated with selenite. *Arch Biochem Biophys* **254:** 534–540, 1987.
11. Svardal AM, Ueland PM, Aarsaether N, Aarsland A, and Berge RK, Differential metabolic response of rat liver, kidney and spleen to ethionine exposure. *S*-Adenosylamino acids, homocysteine and GSH in tissues. *Carcinogenesis* **9:** 227–232, 1988.
12. Gregus Z, Perjési P, and Gyurasics Á, Enhancement of selenium excretion in bile by sulfobromophthalein: Elucidation of the mechanism. *Biochem Pharmacol*, **56:** 1391–1402, 1998.
13. Tandon SK, Magos L, and Webb M, The stimulation and inhibition of the exhalation of volatile selenium. *Biochem Pharmacol* **35:** 2763–2766, 1986.
14. Ganther HE, Reduction of the selenotrisulfide derivative of glutathione to a persulfide analog by glutathione reductase. *Biochemistry* **10:** 4089–4098, 1971.
15. Levander OA, and Baumann CA, Selenium metabolism VI. Effect on arsenic of the excretion of selenium in the bile. *Toxicol Appl Pharmacol* **9:** 106–115, 1966.
16. Gregus Z, and Klaassen CD, Disposition of metals in rats: A comparative study of fecal, urinary and biliary excretion and tissue distribution of eighteen metals. *Toxicol Appl Pharmacol* **85:** 24–38, 1986.
17. Gregus Z, Gyurasics Á, and Koszorús L, Interaction between selenium and group Va-metalloids (arsenic, antimony and bismuth) in the biliary excretion. *Environ Toxicol Pharmacol* **5:** 89–99, 1998.
18. Holeski CJ, and Eaton DL, Effects of glutathione depletion by buthionine sulfoximine (BSO) and diethyl maleate (DEM) on biliary excretion of aflatoxin B1 (AFB) conjugates and covalent AFB-DNA binding. *Toxicologist* **6:** 150, 1986.
19. Costa LG, and Murphy SD, Effect of diethylmaleate and other glutathione depletors on protein synthesis. *Biochem Pharmacol* **35:** 3383–3388, 1986.
20. Frenkel GD, Falvey D, and MacVicar C, Products of the reaction of selenite with intracellular sulfhydryl compounds. *Biol Tr Elem Res* **30:** 9–18, 1991.
21. Ostadalova I, Babicky A, and Kopoldova J, Selenium metabolism in rats after administration of toxic doses of selenite. *Physiol Bohemosl* **37:** 159–164, 1988.

22. Madhu C, Gregus Z, Cheng CC, and Klaassen CD, Identification of the mixed disulfide of glutathione and cysteinylglycine in bile: Dependence on γ-glutamyl transferase and responsiveness to oxidative stress. *J Pharmacol Exp Ther* **262:** 896–900, 1992.
23. Nakagawa T, Aoyama E, Kobayashi N, Tanaka H, Chikuma M, Sakurai H, and Nakayama M, Thiol exchange reactions involving selenotrisulfides. *Biochem Biophys Res Comm* **150:** 1149–1154, 1988.
24. Gregus Z, Stein AF, and Klaassen CD, Age-dependence of the biliary excretion of glutathione-related thiols in rats: Role of γ-glutamyltranspeptidase. *Am J Physiol* **253:** G86–92, 1987.
25. Gregus Z, Stein AF, and Klaassen CD, Effect of inhibition of γ-glutamyltranspeptidase on biliary and urinary excretion of glutathione-derived thiols and methylmercury. *J Pharmacol Exp Ther* **242:** 27–32, 1987.
26. Kaplowitz N, Fernández-Checa JC, Kannan R, Garcia-Ruiz C, Ookhtens M, and Yi J-R, GSH transporters: Molecular characterization and role in GSH homeostasis. *Biol Chem Hoppe-Seyler* **377:** 267–273, 1996.
27. Akerboom PM, and Sies H, Transport of glutathione, glutathione disulphide, and glutathione conjugates across the hepatocyte plasma membrane. *Methods Enzymol* **173:** 523–534, 1989.
28. Gregus Z, Stein AF, Varga F, and Klaassen CD, Effect of lipoic acid on biliary excretion of glutathione and metals. *Toxicol Appl Pharmacol* **114:** 88–96, 1992.
29. Hirooka T, and Galambos JT, Selenium metabolism. I. Respiratory excretion. *Biochim Biophys Acta* **130:** 313–320, 1966.
30. McConnell KP, and Roth DM, Respiratory excretion of selenium. *Proc Soc Exp Biol Med* **123:** 919–921, 1966.
31. Buchet JP, and Lauwerys R, Study of factors influencing the *in vivo* methylation of inorganic arsenic in rats. *Toxicol Appl Pharmacol* **91:** 65–74, 1987.
32. Müller M, and Jansen PLM, Molecular aspects of hepatobiliary transport. *Am J Physiol* **272:** G1285-G1303, 1997.
33. Leier I, Jedlitschky G, Buchholz U, Center M, Cole SPC, Deeley RG, and Keppler D, ATP-dependent glutathione disulphide transport mediated by the *MRP* gene-encoded conjugate export pump. *Biochem J* **314:** 433–437, 1996.