GSH release in bile as influenced by arsenite

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1. INTRODUCTION

Biliary release of glutathione disulfide (GSSG) and glutathione-*S*-conjugates by the isolated perfused liver under different metabolic conditions has been studied by several investigators [1–3]. Sies et al. [1,3] have shown that efflux of GSSG occurs during oxidation of drugs such as hexobarbital and ethylmorphine as well as during hydroperoxide metabolism. In the isolated liver there is also a transport of reduced glutathione (GSH) into the perfusate space [4]. However, it has also been reported that relatively high concentrations of GSH may be excreted in the bile produced in situ [5a,b].

The present study, performed mainly with isolated perfused rat liver, concerns the release of both reduced and oxidized glutathione in bile after arsenite exposure. It has previously been shown that arsenic is eliminated via this route [6] and compounds such as selenite are known to interact with arsenite in a way that increases their respective elimination in the bile [7,8]. Furthermore, the form of As, e.g., if complexed to small molecular weight compounds, or the mechanism(s) involved in its biliary excretion, have not been described. Similar studies have indicated an involvement of GSH in the excretion of zinc and other metals [9,10] in rat bile in situ.

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2. MATERIALS AND METHODS

Perfusions were performed with livers from male Sprague–Dawley rats (200–230 g). The animals had food and water ad libitum. Chemicals used were at least of reagent grade and purchased from local commercial sources.

A recirculating perfusion system was used [11] and the perfusate (100 ml) was Krebs-Henseleit buffer supplemented with 25 mM Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulphonic acid), pH 7.4, and saturated with an oxygen–carbon mixture (95:5). Perfusate flow was maintained at 3.5–5 ml/min and g liver and the pressure was 20 cm H_2O. After an equilibration period of 15–20 minutes, bile was collected at ten minute intervals for 1 h. Aliquots of bile were deproteinized with metaphosphoric acid immediately after collection and stored on ice until assayed at the end of the experiment. Arsenite was usually added to the perfusate at 30 min. [^{74}As]arsenite, prepared by reduction of [^{74}As]arsenic acid (Amersham, England) with sulphite [12], was added to the perfusate (about 0.5 μCi/100 ml). The bile flow was 6–10 μl/min. It was increased to about the double volume when arsenite was added, and then decreased towards control values at the end of the perfusion. The liver retained its normal macroscopic appearance, and the activity of lactate dehydrogenase in the perfusate was unchanged during a 60 min perfusion.

GSH and GSSG were assayed fluorimetrically [13] in the bile and in portions of the liver after de-

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proteination. A method based on high performance liquid chromatography was also used to assay GSH and GSSG in the bile [14]. Sulfobromophtalein (BSP) was measured at 578 nm after dilution of bile samples with 0.1 M sodium hydroxide [15]. Thin-layer chromatography of bile aliquots was performed on cellulose plates using a solvent system of isobutyric acid/NH_4OH/H_2O (66:33:1, by vol.). Spots containing radioactivity were identified with photographic film.

3. RESULTS AND DISCUSSION

In agreement with previous reports on hemoglobin free perfusions [1,3,4] only small amounts of GSH was excreted in bile ($\simeq$0.03 nmol · g liver^{-1} · min^{-1}) under control conditions. As shown in fig.1 the amount of GSH increased in bile after arsenite addition. The amount recovered after 30 min was related to the amount of arsenite added and with increasing concentrations of arsenite, bile flow rates also increased (table 1). The GSH concentration in bile was also dependent on the time elapsed after the addition of arsenite. In the samples taken during the last 10 min of perfusion less GSH was detected than in the samples taken during 10–20 min. When repeated doses of arsenite were administered at 10-min intervals the biliary GSH concentration remained high for a longer period, and the amount recovered after 30 min of perfusion with arsenite was more than doubled, as compared to the effect of a single dose (fig.2). It thus seems as arsenite stimulated GSH release in bile only during a certain phase of its metabolism. This interpretation is compatible with the results obtained by Klaassen [6], who showed that, after a single dose of arsenite, biliary excretion of As occurred mainly during the first 15 min.

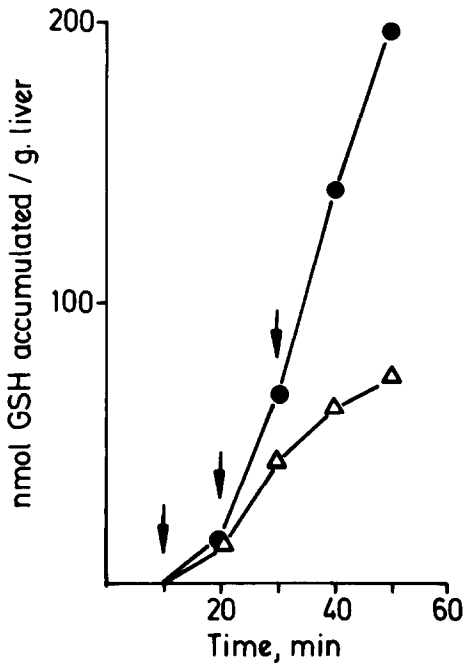

Fig.2. Accumulated amount of GSH released in bile after arsenite addition: (△) arsenite (1 μmol) added at 10 min; (●) three doses of arsenite (1 μmol) added at 10, 20 and 30 min. Values are mean of three experiments.

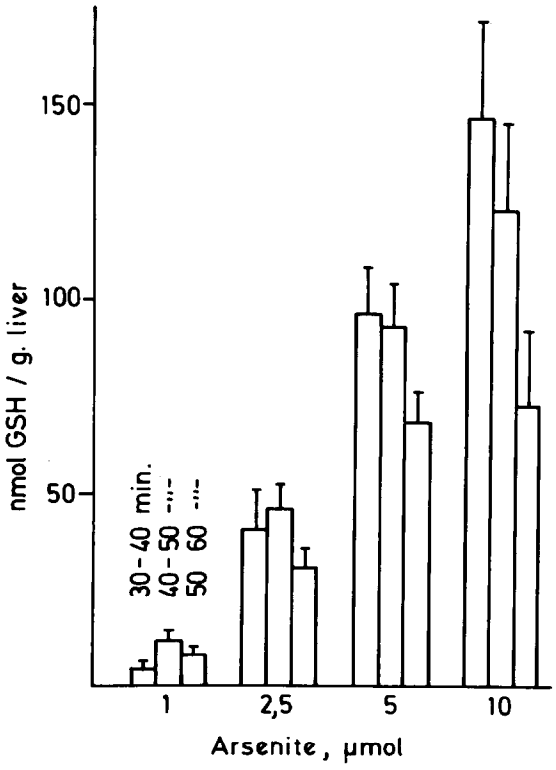

Fig.1. Content of GSH in bile from isolated rat liver perfused with different concentrations of arsenite. Arsenite was added at 30 min. Bars indicate mean ± SE of 4–6 separate experiments and show the amount of GSH excreted during three 10 min intervals.

Table 1

Amount of glutathione and arsenic excreted into the bile and hepatic glutathione content of perfused rat liver

Additions	nmol arsenic excreted in bile/30 min × g liver	GSH	GSSG	GSH + 2 GSSG	Bile flow (μl/min)	Hepatic GSH content[c] (μmol/g liver)
		(nmol excreted in bile/30 min × g liver)				
1 μmol arsenite	–[a]	25	46	117	7±0.7	5.7
2.5 μmol arsenite	20	118	72	262	9.2±0.4	5.5±1.0
5 μmol arsenite	52	257	90	438	11.4±1.3	4.7±1.2
10 μmol arsenite	–[a]	342	100	543	12.5±1.2	3.6±0.7
5 μmol selenite[b] + 5 μmol arsenite		241	122	485		

[a] Not determined

[b] 5 μmol selenite was added 10 min prior to the addition of arsenite

[c] GSH was assayed in the liver at the end of perfusion

Experiments were performed with radiolabelled arsenite as described in Materials and Methods. Values depicted are mean ± SE of 3–6 experiments.

The decreased biliary excretion was related to a redistribution and binding of hepatic As.

The study on in situ produced rat bile [5] indicates GSH efflux under physiological conditions. We repeated some of these experiments and the concentration of GSH was found to be approximately 1.2 mM. When arsenite was injected in the tail vein (10 μmol/kg rat) the concentration of GSH in bile was more than 4-fold increased during the first 20 min.

Other possible effects of arsenite on glutathione turnover were also studied. The amount of GSSG excreted in the bile under control conditions was 0.7–0.9 nmol/min g liver which is slightly less than previously reported [4]. During perfusion with arsenite the biliary GSSG content increased (table 1) while the amount in the perfusate was only slightly raised (not shown). The amount of GSH in the perfusate did not increase over control levels. There was no consistent stoichiometric relationship between the amounts of GSH and GSSG, but to what extent oxidation of GSH in the bile affected the values given in table 1 is not known. It has been claimed that this may occur rapidly in bile [5]. However, the total amount of glutathione, recovered in bile (table 1) was always less than the amount apparently lost in the liver indicating that part of it may have been retained in the liver in a not readily detectable form.

As could be expected from a previous study [8] about 10% of a given dose of arsenite (when given as ^{74}As) was recovered in the bile after 30 min (table 1). Thin-layer chromatography of the bile showed that the major part of ^{74}As was associated with a ninhydrin positive spot eluting together with GSSG. No radioactivity was associated with GSH. These results, as well as the fact that as much as 10 mol of glutathione was detected per mole of arsenite in the bile, indicate that at least part of GSH was excreted as such.

1 μmol arsenite did not markedly affect BSP excretion in bile, while 2.5 μmol and 10 μmol of arsenite decreased BSP excretion in a dose dependent fashion (fig.3). This may reflect a competition for a common excretion mechanism for biliary products (cf [16]).

If GSH is excreted in bile xenobiotic conjugates may form already in the intestinal lumen. Such an interaction may lead to inactivation and/or a delayed absorption of ingested compounds. In an effort to substantiate these thoughts selenite was also tested, as this compound is of nutritional importance and has been shown to readily react with GSH to form poorly absorbed metabolites [17,18].

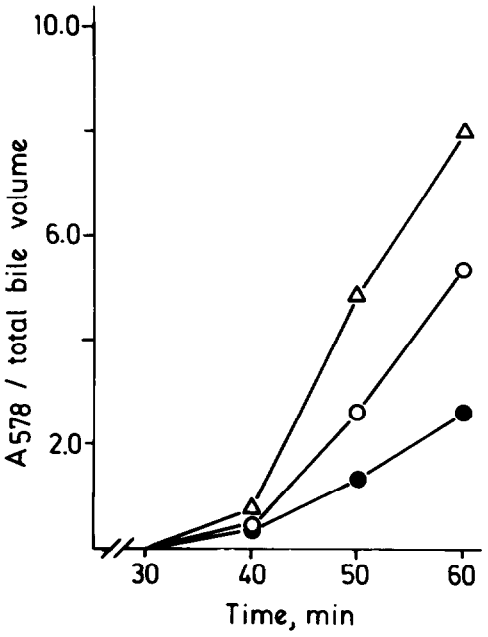

Fig.3. Effect of arsenite on the excretion of BSP into the bile from the perfused rat liver. Arsenite, (○) 2.5 and (●) 10 μmol, was added at 20 min and BSP (5 mg to 100 ml of perfusion fluid) at 30 min. Bile was collected at 10 min intervals. (△): Control experiment where BSP was added at 30 min.

It was found that selenite alone did not provoke GSH secretion under the same conditions as arsenite did (not shown). However, it was also noted that selenité did not markedly inhibit the effect of arsenite (table 1), so it seems possible that selenite absorption can be impaired by arsenite induced GSH secretion.

In conclusion, the results presented here show that arsenite in non-toxic amounts induced GSH secretion in bile from isolated liver and increased GSH secretion in bile produced in situ. The possibility that under certain conditions biliary GSH secretion may affect the bioavailability of ingested compounds is currently under study.

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