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Bile Secretion of Trace Elements in Rats with a Congenital Defect in Hepatobiliary Transport of Glutathione

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ABSTRACT. Bile secretion of trace elements, analyzed by proton-induced x-ray emission, was studied in rats with a congenital defect in hepatobiliary transport of organic anions [Groningen Yellow (GY) rats], in which the process of bile secretion resembles that of the neonatal period. Bile flow (−41%) and biliary glutathione secretion (−99%) were drastically impaired in GY rats compared with controls. Plasma concentrations of all detectable trace elements (Fe, Cu, Zn, Mo, Br, and Se), as well as that of simultaneously determined Ca, were similar in GY and age-matched control Wistar rats. Bile concentrations of Fe, Mo, Br, and Ca were also similar in both groups, resulting in a ~40% reduction of their secretion rates in GY rats. The concentrations of Zn (−62%) and Mn (−64%) were significantly lower in GY rats in contrast to that of Cu, which was 50% higher. Se could not be detected in bile of either group. Recovery in bile (% dose/3 h) after i.v. injection of $MnCl_2$, $CuSO_4$, or SeO_2 (1 mg metal/kg) was lower in GY rats than in controls: Mn, 26 and 35%; Cu, 2.6 and 5%; and Se, 1.5 and 5%, respectively. Injection of $ZnSO_4$ did not lead to increased Zn secretion in GY rats, and only 1.1% of the dose was recovered in controls. Thus, the hepatic handling of different endogenous and exogenously administered trace metals is affected to a variable extent in the GY rat. For a number of metals (*e.g.* Fe, Mo), this may be related to the reduced bile flow; for others (*e.g.* Zn, Mn, Cu), other regulatory factors appear to be responsible. (*Pediatr Res* 28: 339–343, 1990)

Abbreviations

GSH, glutathione
GY, Groningen Yellow
PIXE, proton-induced x-ray emission

The immaturity of hepatic excretory function in the neonatal period is well established. Hepatobiliary transport of several organic anions (1) and of bile acids (2) is severely impaired in neonatal animals when compared with that in adults. In addition, bile flow is markedly reduced (3, 4). Bile is the main route for elimination of certain trace elements (5, 6). The mechanism(s) involved in transport of metals from hepatocytes into bile is largely undefined. GSH may play an important role in this process; in a series of papers (7), Ballatori and Clarkson have provided evidence that the bile secretion of mercury and of GSH are coupled in the rat. A role for GSH has also been suggested in the secretion of copper (8), zinc (8, 9), silver (10), chromium (11), and cadmium (12). Bile secretion of GSH occurs via a carrier-mediated transport system (13, 14), which is immature in the suckling rat and develops only at weaning (4).

A mutant rat strain, which has recently been bred and characterized in our laboratory (15, 16), provides a useful tool to study GSH-dependency of biliary metal secretion. These rats (GY strain) express a congenital defect in hepatobiliary transport of a variety of organic anions, including bilirubin diglucuronide (16), bile acid sulfates (16), and bile acid glucuronides (17), whereas transport of amino acid conjugated bile acids is not affected (15). Available data suggest that the defect is similar to that in the rat strain previously described by Jansen *et al.* (18). GSH is virtually absent in bile of these animals (16, 19), despite elevated hepatic GSH concentrations (19). Bile flow is reduced in GY rats due to a 53% reduction of the so-called bile acid-independent fraction of bile flow (15); this may, at least partly, be caused by the absence of GSH in bile (20). Overall, the process of bile formation in GY rats therefore resembles that described in neonatal animals (2, 3). The aim of our study was to compare bile secretion of endogenous and i.v. administered trace metals in GY and control rats, to gain an insight into the GSH-dependency of these secretory processes.

MATERIALS AND METHODS

Animals. Male normal Wistar and GY Wistar rats weighing 280–300 g were used throughout the study. GY rats, bred at the Central Animal Laboratory of the University of Groningen, express a congenital defect in hepatobiliary transport of organic anions, resulting in conjugated hyperbilirubinemia. The rat strain has been described in detail elsewhere (15–17). The rats were kept on a 12 h light-12 h dark schedule and had free access to food and water before the experiments. Lab food (Hope Farms B.V., Woerden, The Netherlands) contained 195, 72, 63, and 22.4 mg/kg of iron, manganese, zinc, and copper, respectively. All experiments were performed according to the institutional guidelines for the care and use of laboratory animals in research.

Experimental procedures. Experiments were performed between 1100 and 1500 h to exclude effects of circadian variations in bile flow (21). The rats were anesthetized with sodium-pentobarbital (60 mg/kg body wt), and anesthesia was maintained by injection of small doses of the drug during the experiment. The rats were equipped with a silastic catheter (Silastic, Dow Corning, Midland, MI; inner diameter 0.5 mm, outer diameter 0.94 mm) in the jugular vein and in the common bile duct, as previously described (22). Body temperature was maintained at 37.5–38°C by means of a heating pad. Bile samples were collected in preweighed vials for a period of 4 h in 30-min fractions. After

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collection of two bile samples and a blood sample to assess baseline values, a solution of $MnCl_2$, $CuSo_4$, $ZnSO_4$, or SeO_2 in saline (1 mg metal/kg body wt, all obtained from E. Merck, Darmstadt, FRG) was injected via the jugular vein catheter and bile collection was continued for another 3 h. At 2 h after injection, a second blood sample was taken. Blood was centrifuged and bile and plasma samples were stored at −20°C until analysis.

Analytical procedures. All vials and test tubes were washed with concentrated HNO_3 and rinsed with bidistilled water before use. Bile production was determined gravimetrically, assuming a density of 1.0 g/mL for bile. The concentrations of trace elements and of calcium were determined in a single sample by PIXE. PIXE analysis allows accurate determination of nonvolatile elements with $Z > 19$ and an abundance exceeding 0.5 μg/mL in a single sample (23). Biliary concentrations of glutathione (GSH + 2GSSG) were determined according to Griffith (24).

Statistics. The statistical significance of differences between means was calculated using Wilcoxon's rank sum test; p values <0.05 were regarded as significant.

RESULTS

The plasma and bile concentrations of detectable trace elements are given in Table 1. Because the concentration of Ca is determined simultaneously by the PIXE procedure (23), values for this element are included for comparative purposes. As previously reported (15, 16), bile flow was significantly lower in GY rats than in controls. GSH concentrations were very low in bile of GY rats (0.005 *versus* 3.2 mM in controls), as already described by us (16) and by others (19). Plasma concentrations of all detectable trace elements (Fe, Cu, Zn, Mo, Br, and Se) as well as those of Ca were similar in control and GY Wistar rats (Table 1). The biliary concentrations of Fe, Mo, Br, and Ca were also similar in both strains of rats. As a consequence, bile secretion of these elements was reduced in GY rats to an extent similar to the reduction in bile flow (~40%). The concentrations of Mn and Zn were significantly lower in bile of GY rats than in controls, whereas that of Cu was 51% higher. The output rate of endogenous Cu was, therefore, not significantly reduced in GY rats. Mn and Se were below detection limits in plasma and bile, respectively, in both strains of rats. Bile to plasma concentration ratio was very low with Se, Zn, and Fe, but higher with Mn. The concentrations of Ca, Mo, and Br, however, were similar in plasma and bile. The bile to plasma concentration ratio for Zn was significantly lower in GY rats than in controls, whereas that for Cu tended to be higher. No significant differences were observed between the two strains of rats for the other elements.

Figure 1 shows the cumulative bile secretion of Cu, Mn, Se, and Zn after i.v. administration of these metals to control and GY rats. In all experiments, cumulative excretion in bile was lower in GY rats than in controls. Mn was secreted very rapidly into bile; at 3 h after injection, 35% of the dose had already been secreted in controls and 23% in GY rats. Corresponding values for Cu were 5 and 2.6%, and for Se, 4.5 and 1.5%, respectively. Recovery of Zn was only 1.1% in controls and a large variation between animals was observed, whereas GY rats did not secrete exogenously administered Zn into bile. Removal rate of all metals from plasma appeared to be similar in both strains of rats (Fig. 2). Plasma Cu concentrations were very similar before and at 2 h after injection of $CuSO_4$, and Mn was below detectable limits before and 2 h after its injection. In contrast, plasma concentrations of Se and Zn were still markedly elevated 2 h after administration of these metals (2.3-fold and 6-fold, respectively).

DISCUSSION

Biliary secretion is a principal pathway for elimination of a number of trace elements from the body, and this process may play a role in the homeostatic regulation of these elements. Cu represents the best known example of this class of elements; a blockade of hepatobiliary Cu transport, as in Wilson's disease (25), results in its hepatic accumulation and eventually leads to liver cell damage. Despite numerous investigations, the mechanism(s) of hepatobiliary metal transport is still poorly understood. Much research in the past two decades has been focused on the biliary elimination of i.v. administered mercury. It is now well established that under a variety of experimental conditions there is a close correlation between the secretion rates of GSH and those of inorganic mercury and methylmercury (7). The process is thought to proceed via intracellular formation of a metal-GSH complex, which is subsequently secreted into bile by the GSH-transporting system. This transport system is immature in the neonatal rat and develops only at weaning (4). As a consequence, suckling rats show a markedly reduced capacity to secrete mercury into bile (4). A role of GSH has also been proposed in bile secretion of a number of trace metals, including Cu, Zn, Ag, and Cr (7–12). However, available evidence is limited, and differences may exist between hepatic handling of endogenous and exogenously administered metals (26). In addition, there may be multiple pathways for a certain metal to reach the bile canaliculus (27).

In our study, we have systematically evaluated the role of GSH in bile secretion of trace metals in the rat by combining the analytical technique of PIXE with the availability of a rat strain with a congenital defect in biliary GSH secretion. The molecular background of the secretory defect in GY rats has not yet been fully characterized. Yet, the process of bile formation in these animals resembles that described for the neonatal rat (2, 3).

The observation that the plasma concentrations of all detectable trace elements were very similar in GY and control rats,

Table 1. *Plasma and bile concentrations, bile flow, and biliary output rates in GY Wistar rats relative to control Wistar rats**

	Plasma concentration		Bile concentration		Biliary secretion
	Control	GY	Control	GY	GY *vs* C (%)
Ca	3.3 ± 0.3	3.1 ± 0.3	3.0 ± 0.9	2.8 ± 0.6	−40†
Mn	nd	nd	5.0 ± 1.5	1.8 ± 1.6†	−78†
Fe	56.9 ± 18.9	47 ± 14.1	17.5 ± 6.6	19.7 ± 6.2	−31
Cu	25.8 ± 6.9	31.1 ± 8.4	26.4 ± 3.5	39.9 ± 4.2†	−7
Zn	30.0 ± 6.0	31.6 ± 12.7	6.8 ± 3.4	2.6 ± 0.8†	−80†
Mo	3.8 ± 0.9	3.4 ± 1.1	3.7 ± 1.2	4.0 ± 1.1	−39†
Br	136 ± 27.9	125 ± 31.9	131 ± 30	137 ± 42	−38†
Se	5.3 ± 0.6	5.6 ± 1.4	nd	nd	
Bile flow			1.38 ± 0.21	0.81 ± 0.19†	−41†

* Concentration of Ca (mM) and trace elements (μM) in plasma and bile; bile flow (mL/h). Values are mean ± SD. Controls (C), $n = 9$; GY rats (GY), $n = 8$. nd, not detectable.

† Significantly different from controls.

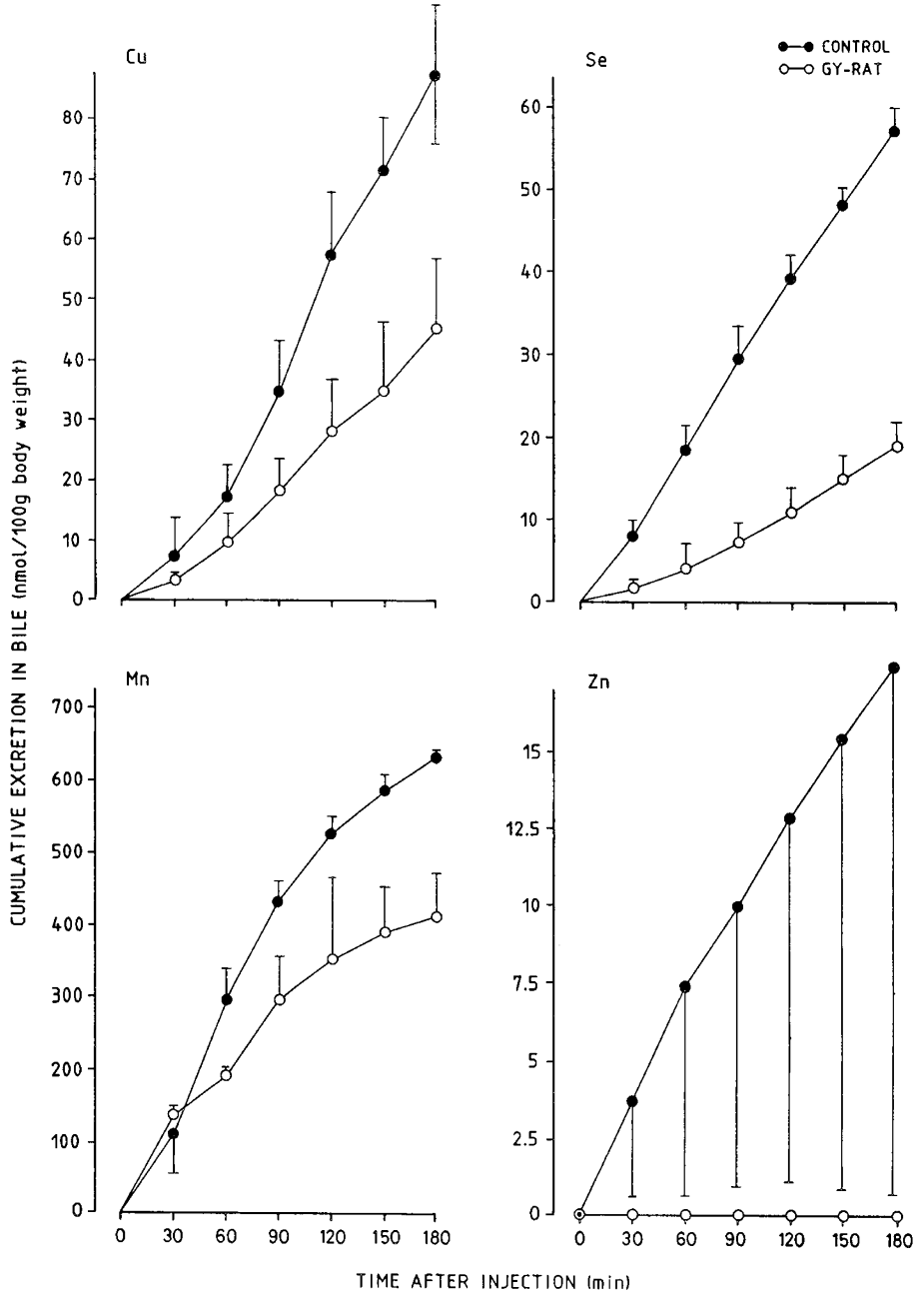

Fig. 1. Cumulative bile secretion of metals in GY (*open circles*) and control (*closed circles*) rats, after i.v. injection of 1 mg/kg of Cu, Se, Mn, or Zn. $n = 3$ in all experiments; *bars* indicate SD.

despite reduced bile secretion rates of most elements, indicates that the mutant animals are able to maintain their homeostasis. However, it should be kept in mind that plasma levels do not necessarily reflect the whole-body status of a metal. For a number of metals (Br, Mo, and Fe), as well as for Ca, it was found that the biliary concentration in GY rats and control rats was virtually identical. As a result of the lower bile flow in the former, bile secretion of these elements was proportionally reduced. These data are in accordance with recent studies from our laboratory, in which it was shown that secretion rates of the mentioned elements closely follow changes in bile flow caused by variations in bile acid output (Dijkstra M, Kuipers F, Havinga R, Smit EP, de Vries JJ, Vonk RJ, unpublished data). This and the observed bile to plasma ratio of approximately 1 for Br, Mo, and Ca suggest that their secretion is largely attributable to the convective flow of water and soluble elements from plasma to bile induced by the osmotic forces generated by canalicular secretion of ions, such as bile acids. The low bile to plasma ratio of Fe may be caused by the fact that Fe in plasma is tightly bound to proteins (29) and is therefore not freely diffusible. However, it has been shown in rats that Fe is released from hepatocytic lysosomes into bile by exocytosis under the condition of dietary iron overload (31). It is possible that there is more than one pathway for biliary Fe secretion, and that the relative importance of these pathways depends upon the Fe status of the animal.

Biliary secretion of endogenous Mn and Zn in GY rats was impaired to a larger extent than bile flow was reduced. In the case of Zn, this can probably be attributed to a strong GSH-dependency of its bile secretion (8, 9), which is also evident from the observation that biliary Zn secretion did not increase after i.v. administration of Zn in GY rats. In control rats, only a very small fraction of injected Zn was eliminated via bile, which is in accordance with the concept that the main pathway for its elimination is not via bile but across the intestinal wall (31). It is

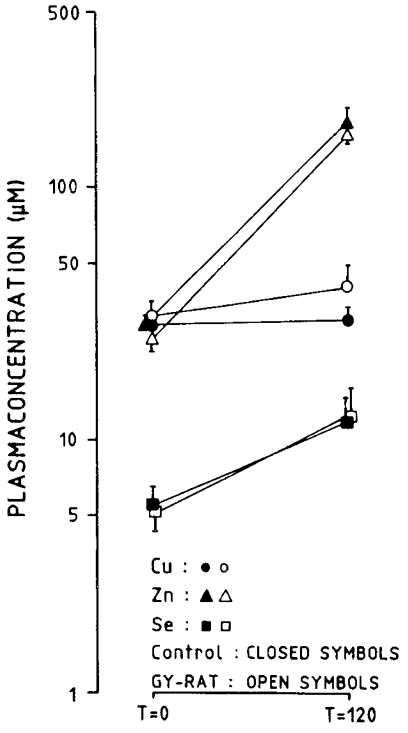

Fig. 2. Plasma concentrations of Cu (●, ○), Zn (▲, △), and Se (■, □) before ($T = 0$) and at 2 h ($T = 120$) after i.v. administration of 1 mg/kg body wt of either metal to control (*closed symbols*) or GY rats (*open symbols*). Mn was below detection limits both before and 2 h after administration of this metal. $n = 3$ in all experiments; *bars* indicate SD.

well established that Mn is very rapidly secreted into bile in adult animals (32). Available data suggest that active transport processes are involved in Mn secretion (33), although these processes have not yet been characterized. Association with bile pigments has been suggested to play a role in Mn secretion (34). It is therefore possible that impaired Mn secretion in GY rats is related to retention of conjugated bilirubin in the livers of these animals. The genetic defect in GY rats may thus indirectly inhibit Mn disposition into bile; the observation that secretion of endogenous and exogenous Mn is not affected to the same degree in GY rats suggests that different processes may operate in removal of Mn from the different sources. Plasma levels of Mn below detection limits in both strains of rats, even after i.v. Mn administration, probably result from the very efficient hepatic clearance of this element (6, 32).

Se could not be detected in bile under basal conditions, in agreement with experimental data that it is mainly eliminated via urine (6). However, a substantial amount of Se was secreted into bile after i.v. injection of Se in control rats, indicating that under conditions of Se excess the biliary pathway becomes of quantitative importance. From our data, we cannot conclude whether under these conditions impaired Se secretion in GY rats is due to reduced bile flow, absence of biliary GSH, or both.

The only element for which basal bile secretion was not significantly impaired in GY rats was Cu; its higher bile concentration compensated for the lower bile flow in these animals. This clearly indicates that secretion of endogenous Cu can proceed independently of GSH. Our data therefore do not support the conclusions of Alexander and Aaseth (8), who reported a decrease of endogenous Cu secretion after treatment of rats with diethylmaleate to deplete hepatic and biliary GSH. However, we found that secretion of exogenously administered Cu was markedly impaired in GY rats. This, together with our recent observation that a similar inhibition of exogenous Cu secretion occurs in control rats pretreated with diethylmaleate (26), suggests that GSH is required for efficient removal of excess Cu from the body. Apparently, multiple pathways exist for biliary output of Cu, which depend to a varying degree on GSH secretion. It is therefore conceivable that in situations with impaired GSH secretion—for instance, in the neonatal period—the liver is more susceptible to damaging effects of Cu overload.

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