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Biliary Excretion of Cadmium

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Biliary excretion of cadmium was studied in two groups of five rats which were given repeated injections of ^{109}Cd. The first group was given a total amount of 6 mg Cd/kg which was 6 times as much as the second group. The elimination of cadmium in bile, urine, and feces was measured 4 to 5 weeks after the last injection.

The relative biliary excretion of cadmium was the same in both groups, about 0.015% of body burden per 24 hr. This is higher than the urinary excretion, which was 0.001% but, less than the total fecal elimination of cadmium, which averaged 0.034% during the same time.

Introduction

Biliary excretion of metals is an important pathway contributing to gastrointestinal excretion of metals, and it has therefore been considered of interest to gain more knowledge in this field (*1*). A number of animal experiments have shown that various metals, such as arsenic, copper, lead, manganese, mercury, and zinc are excreted via bile. Depending on type of animal, dose, and metal administered, the initial 24 hr excretion in bile has been reported to be in the order of 2 to 31% of a given dose (*2–7*). Some of the mentioned metals are excreted into the bile against a large plasma to bile concentration gradient, and the biliary excretion has thus been considered to be the result of an active secretion (*8*).

At least three of these excreted metals, i.e., arsenic, manganese, and mercury, are reabsorbed again from the gut in an enterohepatic circulation (*6, 9–11*).

Biliary excretion of cadmium directly after a parenteral dose has been measured in rats by a number of authors (*8, 12–18*). Such studies have shown that initially the excretion of cadmium via bile is high. The excretion is dependent on dose in such a way that a higher dose gives a higher relative excretion. The biliary excretion during the first 24 hr has been reported to be of the order of 0.3 to 5.7% of the given dose. After the initial rapid excretion of cadmium in bile, it rapidly levels off; Nordberg et al. (*16*) reported that during day 2 and 3 the relative excretion drops to about 0.04% of the given dose.

Results on cadmium in bile and biliary excretion of cadmium in man are few and incomplete. To our knowledge, only four reports have been published which present data on cadmium in human bile. Two of them, both based on autopsy materials, have given averages of 0.3 μg Cd/g bile and 2.2 μg Cd/g bile, respectively (*19, 20*). Since these reported values appear to be very high and analyses were carried out by atomic absorption spectrophotometry (ASS) without background correction for interfering substances such as sodium chloride (*21*), the validity may be questionable.

The two other reports, one Japanese and one Swedish study, are based on bile samples collected by operations (*22, 23*). Cadmium analyses in these studies were also made with AAS but after extraction with ammonium pyrrolidine dithiocarbamate in methyl isobutyl ketone (APDC/MIBK). Cadmium concentrations in both studies were found to be log-normally distributed with geometric averages of 2.0 ng/g and 1.1 ng/g, respectively. The validity of these latter analytical results must however also, as pointed out by the Swedish authors (*23*), be regarded as uncertain until cross-checking with other analytical methods such as neutron activation analysis has been made.

If the latter analytical results are correct, they do indicate that biliary excretion could be of great importance with regard to elimination of cadmium in humans. A daily excretion of 500 ml of bile containing 1.1 ng Cd/g would thus result in elimination of 0.55 μg Cd/day. This estimate is low but still slightly higher than the average 24-hr urinary excre-

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tion of cadmium among middle-aged Swedish people (*24*).

The present study has been conducted in order to ascertain whether significant amounts of cadmium are excreted via bile in rats a long time after exposure, i.e., in a situation more similar to human exposure and excretion. Biliary excretion of cadmium was also related to total fecal and total urinary excretion.

Materials and Methods

Two groups of five male Sprague-Dawley rats (weight 190–210 g in the first group and 340–440 g in the second group) were pretreated with repeated subcutaneous injections of cadmium chloride. The first group was given 12 injections of 0.5 mg Cd/kg body weight. The injection solution, which has a cadmium concentration of 0.2 mg Cd/ml, was tagged with 250 μCi ^{109}Cd, giving a specific activity of 17 mCi/g Cd.

The second group of five rats received eight subcutaneous Cd injections of lower concentration, 0.125 mg/kg (0.05 mg Cd/ml), but higher specific activity, 360 mCi/g Cd. The dose was decreased in the latter group to enable a comparison of biliary excretion at different body burdens in rats. The higher specific activity was introduced in order to make it possible to measure quantitatively the cadmium in urine and serum samples.

Between 4 and 5 weeks after the last injection a cannula was introduced surgically into the common bile duct and bile was collected according to methods previously described (*16*). Bile was sampled as long as possible, i.e., until the animals died. Urethane, about 900 mg/kg given intraperitoneally, was used as anesthetic. One week before the operation the latter group of rats was placed in metabolism cages for two periods of 24 hr, and the total urine and feces was sampled. In addition one blood sample from each rat in this group was taken from the portal vein during the operation. The blood samples were allowed to stand for about 1 hr and were subsequently centrifuged to separate serum from blood cells.

Cadmium concentrations in tissues and fluids, calculated by two measurements of radioactivity, were compared with standards made from injection solutions according to the usual methods as described previously (*25*). The body burden of cadmium was measured by whole body counts.

Results

Individual cadmium concentration in liver and kidneys and body burdens of cadmium are given in Table 1, together with daily cadmium excretion in bile in percent of body burden. The average concentration of cadmium in bile was 0.014 μg ± 0.008 Cd/ml, n = 10, in the first group and 0.0025 μg ± 0.0007 Cd/ml, n = 10, in the second group. The ratio between these concentration figures, 5.6, agrees with the fact that the first group was given 6 times as much cadmium on a body weight basis. The relative 24 hr excretion in percent of body burden ranged from 0.005% to 0.021%, average 0.015%, in the first group and from 0.008 to 0.020%, average 0.013%, in the second group. The results show that cadmium excretion in bile is related to total given dose or to body burden of cadmium. Total body burden of cadmium in rats is at this exposure situation about twice the liver content of cadmium.

Table 2 and Figure 1 present the 24 hr excretion of cadmium in feces and urine as measured in two periods of 24 hr by the second group of rats. Urinary excretion of cadmium averaged 0.0010% of body burden, and fecal excretion averaged 0.038%. In rats at this exposure situation the results show

Table 1. Cadmium concentration in organs and cadmium excretion.

Rat no.	Cd concentration in liver, μg/g	Cd concentration in kidney, μg/g	Body burden of Cd, mg	Biliary excretion of Cd per 24 hr, of body burden (bb)[a] Day 1 % of bb	Day 1 ml	Day 2 % of bb	Day 2 ml	Day 3 % of bb	Day 3 ml
1:1	72.0	51.6	1.4	0.015	13.5	(0.004)	(2.4)		
1:2	102.8	66.4	1.4	0.016	14.9	0.021	12.5	0.019	10.5
1:3	70.2	55.5	1.5	0.007	10.2				
1:4	71.4	64.7	1.3	0.021	18.0	(0.008)	(13)		
1:5	39.8	54.3	1.1	0.005	6.3	0.001[b]	3.7	0.003[b]	5
2:1	8.7	7.4	0.35	0.020	18.7	(0.004)	(7.5)		
2:2	19.8	7.0	0.43	0.015	18.9	(0.003)	(7.6)		
2:3	11.0	6.2	0.37	0.015	18.8	0.012	18.2		
2:4	11.4	7.0	0.36	0.012	19.4	(0.006)	(13.2)		
2:5	9.4	5.9	0.35	0.012	14.8	0.008	11.1	(0.003)	(2.1)

[a] Values in parentheses do not cover the whole period of 24 hr.
[b] Values excluded from estimation of average and range.

Table 2. Urinary and fecal excretion of cadmium in 24 hr.

Rat no.	Period 1				Period 2			
	Urine		Fecal		Urine		Fecal	
	μg Cd/24 hr	%/24 hr	μg Cd/24 hr	%/24 hr	μg Cd/24 hr	%/24 hr	μg Cd/24 hr	%/24 hr
2:1	0.0052	0.0015	0.086	0.024	0.0063	0.0018	0.096	0.027
2:2	0.0024	0.0006	0.084	0.020	0.0039	0.0010	0.105	0.024
2:3	0.0030	0.0008	0.083	0.022	0.0024	0.0006	0.245	0.066
2:4	0.0013	0.0004	0.096	0.027	0.0050	0.0014	0.198	0.055
2:5	0.0029	0.0008	0.135	0.039	0.0045	0.0013	0.283	0.081

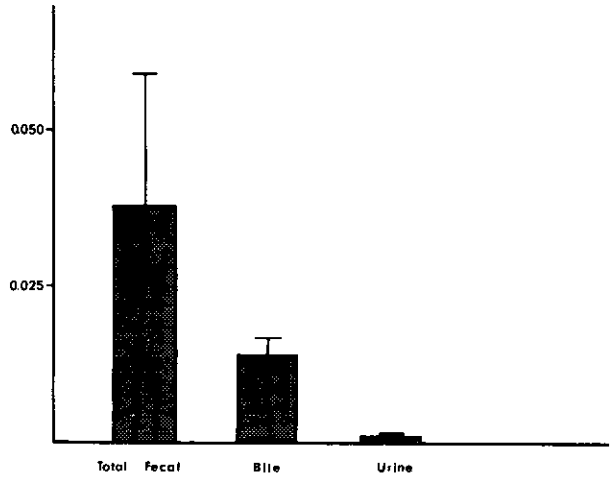

FIGURE 1. Average 24 hr excretion of cadmium in the rat as a percentage of body burden.

that biliary excretion is more important than urinary excretion, but also that biliary excretion of cadmium represents only about one third of the total gastrointestinal excretion.

The average cadmium concentrations in blood cells and serum taken from the portal vein of the second group of five rats were 0.073 ± 0.020 and 0.020 ± 0.013 μg Cd/ml, respectively. The ratio of average cadmium concentration in bile and in serum was 0.0025/0.020 = 0.12.

Discussion

Daily biliary excretion of cadmium in rats 4 to 5 weeks after parenteral administration of cadmium was found to be of the order of 0.015% of the body burden. The relative excretion of cadmium in bile in percent of body burden of cadmium, as measured by whole body counting, was the same in both groups of rats, independent of the total administered cadmium dose. This finding is not in accordance with acute exposure studies, where higher dose levels have been shown to give a higher relative excretion (*8, 13, 14*) but does agree with the metabolic model of cadmium metabolism in humans proposed by Kjellström and Nordberg (*26*), and with empirical data from humans where cadmium levels in bile on group basis are related to liver concentrations of cadmium (*23*).

Serum levels of cadmium in this study were higher than the bile concentration. In contrast to this report, Klaassen and Kotsonic reported (*8*) a bile to plasma gradient of cadmium shortly after cadmium administration which was higher than 1, which indicates that cadmium is actively secreted from serum to bile. One possible explanation for this discrepancy in results is that only a minor portion of the plasma-borne cadmium is available to excretion in bile. After parenteral administration of cadmium most of the plasma-borne cadmium is initially bound to high molecular proteins, but 4 days after exposure the major part of plasma bound cadmium is recovered in low molecular fractions, probably metallothionein, which firmly binds cadmium (*27*). It can thus be speculated that only free or loosely bound cadmium ions are available to active secretion into the bile, and that a long time after exposure only a very small portion of the plasma-borne cadmium is available to active secretion by the liver cells.

Of interest is the comparison between biliary excretion and urinary excretion. In rats, biliary excretion apparently is more important than urinary excretion: biliary excretion on an average was 0.014% of body burden whereas urinary excretion was found to be about 0.001%. In humans, biliary excretion of cadmium has not been sufficiently studied, but recent data suggest that biliary excretion of cadmium is pertinent also with regard to cadmium metabolism in humans (*23*). The possibility of an enterohepatic circulation of cadmium has been suggested (*12, 17*), but no data are available at present.

The total fecal excretion of cadmium presented in this report shows that cadmium is also excreted by other routes than via bile, since average bile excretion was in the order of 0.014% of the body burden, whereas the total fecal excretion, i.e., combined bile and gastrointestinal, averaged 0.038%. That the

fecal elimination of cadmium after parenteral administration is dominant in rodents has formerly been reported from several studies (*21*). During parenteral dosing of cadmium to mice, Nordberg (*28*) observed a ratio of daily fecal to daily urine excretion of cadmium of the order of 5 to 25. After exposure had ceased, the fecal excretion among mice dropped, whereas the urinary excretion remained about the same, supporting the idea that urinary excretion is more related to body burden of cadmium than fecal excretion (*28*). Even if the material in the present report is limited to five rats, the results do show that fecal elimination is dominating in rats 5 weeks after the last administration of cadmium.

Reports on gastrointestinal excretion of cadmium among humans have not been presented. The technical problems, and if radioactive cadmium is being used, ethical problems, with such studies are obvious, but there is a need for such studies in order to make it possible to make a complete metabolic model for cadmium in humans (*26*).

In conclusion, this paper shows that biliary and fecal excretion of cadmium is important even long time after exposure in rats. Further research is needed in order to conclude whether this has any significance for human metabolism of cadmium.

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