Biochemical Pharmacology, Vol. 31, No. 9, pp. 1761–1764, 1982.
Printed in Great Britain.

0006-2952/82/091761-04 $03.00/0

METABOLISM OF CADMIUM IN THE NEONATE: EFFECT OF HEPATIC ZINC, COPPER AND METALLOTHIONEIN CONCENTRATIONS ON THE UPTAKE OF CADMIUM IN THE RAT LIVER

RICHARD MASON

Toxicology Research Unit, Medical Research Council of New Zealand, University of Otago Medical School, P.O. Box 913, Dunedin, New Zealand

(*Received* 23 *September* 1981; *accepted* 18 *December* 1981)

Abstract—The accumulation and subcellular distribution of Cd^{2+} (1 mg/kg body wt, i.p.) in the liver of the neonatal rat is age-dependent. At 4 hr after treatment the liver Cd^{2+} contents in the 12-day-old, 20-day-old and adult rat are similar and greater than in the 2-day-old animal. The differences in hepatic Cd^{2+} concentration in the older age groups are consistent with the nonlinear weight gain of the liver in the developing animal. In the hepatic cytosol Cd^{2+} is incorporated into a high molecular weight and metallothionein fractions and transferred from the former to the latter. This process occurs less rapidly with increasing age. The uptake of Cd^{2+} by the whole liver and the hepatic metallothionein is not related to the total liver concentration of Zn^{2+} or copper and is not significantly influenced by the concentration of pre-existing metallothionein or the concentration of thionein-bound Zn^{2+} or copper. The results are discussed in relation to the possible effects of Cd^{2+} on the liver metabolism and tissue distribution of Zn^{2+} and copper in the developing animal.

The uptake of the toxic heavy metal Cd^{2+} by the liver is an important process since the liver is a significant site of storage and the metal is transferred slowly to the kidney, which is the critical organ in chronic Cd^{2+} exposure. Much of the Cd^{2+} in the liver and kidney is bound to metallothionein, a low molecular weight, cysteine-rich protein which also contains Zn^{2+} and copper [1].

The uptake of Cd^{2+} by the liver may be mediated by its binding to metallothionein. Pretreatment of adult rats with Zn^{2+}, for example, elevates the hepatic Zn-thionein content and results in isolated hepatocytes which show greater uptake of Cd^{2+} than controls [2]. In the liver of the newborn rat the concentration of thionein-bound Zn^{2+} is high and declines rapidly to the adult level soon after weaning [3]. High concentrations of the Zn^{2+}-rich metalloprotein in the livers of newborn Sprague–Dawley rats do not appear to significantly influence the binding of Cd^{2+} to metallothionein or the uptake of the metal by the liver [4, 5]. The hepatic metallothionein in the newborn rat also contains copper, the content of which increases between 10 and 14 days of age and then, like Zn^{2+}, declines rapidly. It is not known whether the change in metal composition of the metalloprotein which occurs during this period alters the biochemical response of the liver to the influx of Cd^{2+}.

In the present investigation the time course of the initial uptake of Cd^{2+} and its subcellular distribution in the liver of the 12-day-old Wistar rat was compared with that in the 2-day-old, 20-day-old and adult animal. Special attention was given to determining the relationship between the uptake of Cd^{2+}, the liver concentrations of copper and Zn^{2+}, and the metal composition and content of hepatic metallothionein.

MATERIALS AND METHODS

Animals. Random bred Wistar rats were used in the study. Newborn (2-day-old), suckling (12-day-old) and weanling (20-day-old) animals were obtained from first, second or third litters routinely culled to 10 at birth and weaned at 21 days of age. The dams were fed the standard I.C.I. (Dunedin, New Zealand) pelleted diet and tap water *ad lib.* throughout the pregnancy and nursing periods. Weanling and adult rats were also maintained on this diet.

Experimental. The 2-day-old and 12-day-old groups each contained 5 males and 5 females. The 20-day-old and adult groups contained only females and consisted of 5 and 3 animals respectively. The 2-day-old, 12-day-old and 20-day-old pups were separated from their mothers 3 hr before the experiment and all animals were prevented access to food and water during the experiment. Each animal received a single i.p. injection of Cd^{2+} (1 mg/kg body wt) as $CdCl_2$ in 0.1 ml (adult), 0.2 ml (20-day-old) or 0.5 ml (12-day-old and 2-day-old) isotonic saline/100 g body wt. Controls received similar treatment but without Cd^{2+}. Groups were killed by decapitation 30 min, 1 hr, 2 hr or 4 hr after Cd^{2+} injection. Livers were removed, weighed individually and portions of tissue digested and then analysed for Cd^{2+}, Zn^{2+} and copper by atomic absorption spectrometry [6].

The remaining tissue, or in the adult groups equal portions of tissue, from each animal in the group was pooled, homogenized in 3–4 vol. 10 mM Tris–

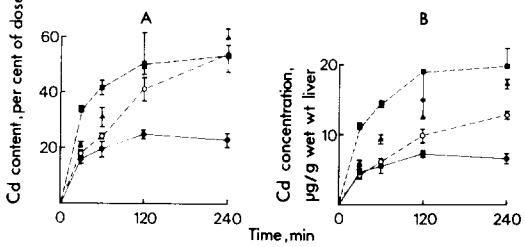

Fig. 1. Content (A) and concentration (B) of total hepatic Cd^{2+} as a function of time after treatment of adult and newborn rats of different ages with Cd^{2+} (1 mg/kg body wt, i.p.). Values are the means obtained from 3 separate experiments with the S.E.M. shown by the vertical bars. ●——●, 2-day-old; ■----■, 12-day-old; ▲·····▲, 20-day-old; ○—·—·—○, adult.

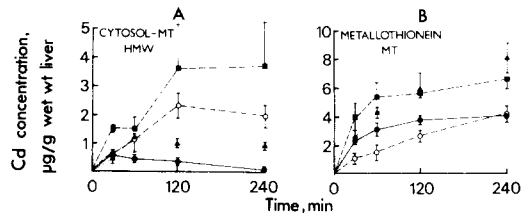

Fig. 3. Concentration of non-thionein-(A) and thionein-bound Cd^{2+} (B) in the hepatic cytosol as a function of time after treatment of adult and newborn rats of different ages with Cd^{2+} (1 mg/kg body wt, i.p.). Cytosolic fractions were prepared as described in Fig. 2 and separated by gel filtration chromatography into nonthionein (cytosol–MT, $V_e/V_o = 1$) and metallothionein (MT, $V_e/V_o = 2$) fractions as described in Materials and Methods. ●——●, 2-day-old; ■———■, 12-day-old; ▲·····▲, 20-day-old; ○·—·—·—○, adult.

HCl buffer, pH 8.0 and separated into particulate and cytosolic fractions by centrifugation at 27,000 *g* for 15 min and at 100,000 *g* for 1 hr. The metal content of the cytosol was estimated by analysing a portion of the post-microsomal supernatant solution for Cd^{2+}, Zn^{2+} and copper as described above.

The cytosol (equivalent to 1–2 g wet wt liver) was further fractionated on a column of Sephadex G-75 (85 × 1.5 cm) with 10 mM Tris–HCl buffer, pH 8.0 at a flow rate of 12–15 ml/hr as the eluant. Column eluate fractions were analysed for Cd^{2+}, Zn^{2+} and copper without digestion.

RESULTS AND DISCUSSION

The Cd^{2+} contents of the liver of the 12-day-old, 20-day-old and adult rats at 4 hr were similar whereas in the 2-day-old animal it was much lower (Fig. 1(A)). The liver of the 2-day-old (body wt, 6.99 ± 0.05 g), 12-day-old (body wt, 21.3 ± 0.5 g), 20-day-old (body wt, 34.7 ± 1.9 g) and adult (body wt, 96.4 ± 1.6 g) animals accounted for 3.52, 2.73, 3.48 and 4.17% of body weight respectively. Thus the differences in total liver Cd^{2+} concentration in the older animals, but not in the 2-day-old animal, can be accounted for by the nonlinear weight gain of the liver relative to body weight. Consequently, the total liver Cd^{2+} concentration was highest at all times in the 12-day-old rat. At 4 hr, total liver Cd^{2+} ranged from 6.7 μg/g wet wt tissue in the 2-day-old to 12.9 μg/g in the adult, 17.4 μg/g in the 20-day-old and 19.9 μg/g in the 12-day-old animal (Fig. 1(B)). The present results are in general agreement with those reported by Wong and Klaassen [4]. Figure 1(A) shows, however, that the liver Cd^{2+} content was much lower in the 2-day-old rat following the intraperitoneal injection of Cd^{2+} than in the older animals, whereas Wong and Klaassen reported that the Cd^{2+} content of the liver of the 4-day-old rat after intravenous injection of Cd^{2+} is similar to that in the 20-day-old animal [4].

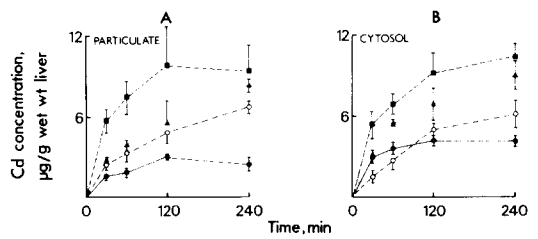

Fig. 2. Concentration of hepatic Cd^{2+} as a function of time after treatment of adult and newborn rats of different ages with Cd^{2+} (1 mg/kg body wt, i.p.). Particulate and cytosolic fractions were prepared by ultracentrifugation of pooled liver extracts from individual experiments. Values are the means (±S.E.M.) obtained from 3 separate experiments. (A) particulate fraction, (B) cytosolic fraction. ●——●, 2-day-old; ■———■, 12-day-old; ▲·····▲, 20-day-old; ○—·—·—○, adult.

The time course of the accumulation of Cd^{2+} in the liver was age-dependent. The maximum liver Cd^{2+} concentration probably occurred at 1 hr in the 2-day-old and at 2 hr in the 12-day-old animal. In the 20-day-old and adult animals Cd^{2+} continued to accumulate at least until 4 hr after its administration (Fig. 1(B)).

The time course of the concentration of Cd^{2+} in the particulate (Fig. (A)) and cytosolic fractions (Fig. 2(B)) of all age groups paralleled that in the whole tissue (Fig. 1(B)). In the hepatic cytosol of all age groups Cd^{2+} was incorporated into a high molecular weight fraction (>70,000) and a low mol. wt, metal-binding fraction which also contained Zn^{2+} and copper, had the same gel filtration elution characteristics (Sephadex G-75) and is subsequently referred to as metallothionein. The distribution of Cd^{2+} between these subfractions was age-dependent. Figure 3 confirms that the metal was transferred from the high mol. wt fraction to the metallothionein (see, e.g., Ref. 7). This process occurred most rapidly in the liver of the 2-day-old rat and appeared to take place more slowly with increasing age.

The concentration of pre-existing metallothionein (Σ ng ions Zn + Cu/g wet wt liver) was highest in the 2-day-old rat and decreased with increasing age (Table 1, Fig. 4). Since the liver Cd^{2+} content (Fig. 1(A)) was lower in the 2-day-old animal than in the older animals, it follows that high concentrations of the endogenous metallothionein did not significantly promote the uptake of the metal by the liver. It is

Table 1. Concentration of copper and Zn^{2+} in the livers of rats of different ages

		2-Day-old	12-Day-old	20-Day-old	Adult
			(μg metal/g wet wt liver)		
Zn					
	Total	99.35 ± 6.19	55.35 ± 9.02	40.54 ± 2.17	33.15 ± 0.57
	Thionein	37.73 ± 2.39	5.49 ± 1.18	4.45 ± 1.10	1.21 ± 0.01
Cu					
	Total	29.77 ± 3.64	59.51 ± 3.43	29.71 ± 6.18	6.21 ± 0.73
	Thionein	8.86 ± 0.98	9.90 ± 1.02	5.43 ± 1.45	0.68 ± 0.19

Liver cytosol was prepared by ultracentrifugation of pooled liver extracts. Metallothionein was isolated by gel filtration chromatography using a column (85 × 1.5 cm) of Sephadex G-75 with 10 mM Tris–HCl buffer, pH 8.0 as eluant. Values are the means ± S.E.M. of three groups at each age.

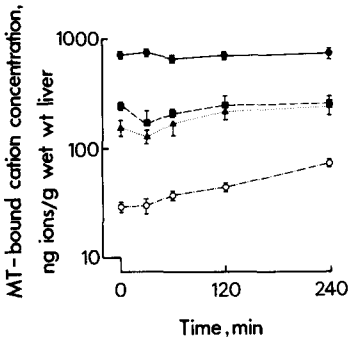

Fig. 4. Concentration of hepatic thionein-bound metals (ΣZn + Cu + Cd) as a function of time after treatment of adult and newborn rats of different ages with Cd^{2+} (1 mg/kg body wt, i.p.). Cytosolic fractions were prepared as described in Fig. 2 and metallothionein isolated by gel filtration chromatography. The values are the mean ± S.E.M. obtained from 3 separate experiments. ●——●, 2-day-old; ■———■, 12-day-old; ▲·····▲, 20-day-old; ○·—·—·—○, adult.

also apparent that the Cd^{2+} content of the liver (Fig. 1(A)) and the uptake of Cd^{2+} by metallothionein (Fig. 3(B)) was not related simply to the concentration of pre-existing metallothionein (Fig. 4) or the concentration of thionein-bound Zn^{2+} or copper (Table 1).

The effect of high concentrations of hepatic (Zn,Cu)-thionein in the newborn rat in promoting the uptake of Cd^{2+} may be nullified by the high concentrations of Zn^{2+} and copper in the liver. Stacey and Klaassen [2] for example reported that in isolated rat hepatocytes, the initial rapid phase of Cd^{2+} uptake is decreased when Zn^{2+} is added to the medium. In the present experiments the uptake of Cd^{2+} by the liver was least in the 2-day-old rat which had high concentrations of hepatic Zn^{2+} but Fig. 1(A) and Table 1 show that the Cd^{2+} content of the liver was not inversely proportional to the liver concentration of Zn^{2+} or copper. These results suggest that the increase in liver copper concentration between 10 and 14 days of age (Table 1 and see Ref. 3) probably does not significantly influence the uptake of Cd^{2+}.

The concentration of thionein-bound Cd^{2+} in the liver of the 2-day-old rat at 4 hr (38 ng ions/g wet wt) was low relative to that of thionein-bound Zn^{2+} (580 ng ions/g wet wt) and copper (138 ng ions/g wet wt) in the untreated animal (Table 2). Thus it was not possible to unequivocally discern the loss of these metals concomitant with the uptake of Cd^{2+}. Table 2 shows, however, that in the 12-day-old, 20-day-old and adult animals copper was lost from the hepatic metallothionein within 30 min of Cd^{2+} administration. The concentration of thionein-bound metals in the livers of the 2-day-old and 12-day-old animals remained constant, whereas in the 20-day-old and particularly in the adult animals it increased, therefore indicating increased synthesis of the metalloprotein (Fig. 4). The present results are consistent with those of Wong and Klaassen [4] who demonstrated that treatment of Sprague–Dawley rats with

Table 2. Effect of Cd^{2+} treatment on the binding of Zn^{2+} and copper to hepatic metallothionein

Time after Cd^{2+} injection (min)	2-Day-old		12-Day-old		20-Day-old		Adult	
	Zn	Cu	Zn	Cu	Zn	Cu	Zn	Cu
	(ng ions/g wet wt liver)							
0	580	138	85	155	69	85	19	11
30	600	125	64	68	76	28	15	6
60	536	93	85	73	99	30	15	8
120	525	139	48	147	104	57	13	7
240	591	125	45	148	121	54	24	9

Adult and newborn rats of different ages were treated with Cd^{2+} (1 mg/kg body wt, i.p.). Cytosolic fractions were prepared (for details, see Fig. 2 and Materials and Methods) and metallothionein isolated by gel filtration chromatography.

Values are the means obtained from 3 separate experiments.

Cd^{2+} (1 mg/kg body wt, i.v.) increases the concentration of hepatic metallothionein in the 21- and 70-day-old rat but not in the 4-day-old animal. Bell [5] showed that the 5-day-old rat can respond to high doses of Cd^{2+} (6 mg/kg body wt) with the induction of metallothionein synthesis. The present experiments show moreover that the increase in metallothionein synthesis occurred when the molar concentration of thionein-bound Cd^{2+} was similar to or exceeded that of copper. Thus from Fig. 3(B) and Table 2 it can be calculated that the molar concentrations of thionein-bound Cd^{2+} were approximately equal to that of copper in the 20-day-old animal and greater than that of copper in the adult animal. In the 2-day-old and 12-day-old animals, which did not synthesize additional metallothionein, the molar concentration of thionein-bound copper was always considerably greater than that of Cd^{2+}. These findings are consistent with the hypothesis that Cd^{2+} competes with copper for incorporation into the pre-existing protein and, in the 20-day-old and adult rat, results in the formation of a (Zn,Cd,Cu)- or (Cd,Zn,Cu)-thionein, with a lower rate of turnover than the pre-existing protein. This could lead to the induction of thionein synthesis by Cd^{2+} in response to the accumulation of the free metal in the cytosol.

The primary function of the metallothionein in the liver of the newborn rat may be to control potential changes in the nonthionein Zn^{2+} concentration of the liver cytosol and as a source of the metal when the need to sustain essential metabolic sites is of prime importance [3]. This mechanism would be facilitated by the rapid turnover of (Zn,Cu)-thionein which is a function of its metal composition [8, 9]. Bakka *et al.* [10] showed that when Cd^{2+} is administered to the pregnant rat, the concentration of thionein-bound Cd^{2+} in the newborn rat liver is low and does not affect the subsequent metabolism of Zn^{2+}. The present results show that at 4 hr after the administration of Cd^{2+} to the newborn rat, the hepatic metallothionein is also a Zn^{2+}-rich protein (Zn:Cu:Cd = 16.2:3.4:1.0). Therefore the presence of thionein-bound Cd^{2+} in the liver of the newborn animal is unlikely to influence hepatic Zn^{2+} metabolism. The uptake of Cd^{2+} in the liver of the 12-day-old rat, however, results in the formation, after 4 hr, of a copper-rich protein (Cu:Cd:Zn = 3.3:1.3:1.0) which also contains a significant proportion of Cd^{2+}. If the rate of turnover of this protein was reduced by the incorporation of Cd^{2+} it is possible that the loss of thionein-bound Zn^{2+} and copper which occurs during neonatal growth may be inhibited, thereby altering the liver metabolism and tissue distribution of these metals.

Acknowledgements—The author wishes to thank Miss Sarah McLaren for valuable technical assistance and Professor E. G. McQueen for helpful comments.

REFERENCES

1. M. Webb, *Biochem. Soc. Trans.* **3**, 632 (1975).
2. N. H. Stacey and C. D. Klaassen, *Tox. appl. Pharmac.* **55**, 448 (1980).
3. R. Mason, A. Bakka, G. P. Samarawickrama and M. Webb, *Br. J. Nutr.* **45**, 375 (1981).
4. K. L. Wong and C. D. Klaassen, *Tox. appl. Pharmac.* **53**, 343 (1980).
5. J. U. Bell, *Tox. appl. Pharmac.* **54**, 147 (1980).
6. R. Mason, *Biochem. Pharmac.* **30**, 2427 (1981).
7. M. Cempel and M. Webb, *Biochem. Pharmac.* **25**, 2067 (1976).
8. I. Bremner, W. G. Hoekstra, N. T. Davies and B. W. Young, *Biochem. J.* **174**, 883 (1978).
9. K. Cain and D. Holt, *Chem. Biol. Interact.* **28**, 91 (1979).
10. A. Bakka, G. P. Samarawickrama and M. Webb, *Chem. Biol. Interact.* **34**, 161 (1981).