Biochemical Pharmacology, Vol. 21, pp. 2767-2771. Pergamon Press, 1972. Printed in Great Britain.

PROTECTION BY ZINC AGAINST CADMIUM TOXICITY

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(*Received* 10 *February* 1972; *accepted* 8 *May* 1972)

Abstract—Pre-injection of male rats with Zn^{2+} (20 μmoles) 24 hr before the administration of Cd^{2+} (4 μmoles) partially protects against the changes in weight of the testes that are characteristic of the early stages of Cd^{2+}-injury. This pre-treatment with Zn^{2+} induces the synthesis in the livers of the rats of the Cd^{2+}-binding protein and, in consequence, Cd^{2+} ions accumulate and are thus immobilized more rapidly than in the livers of normal animals. It is probable that this accumulation does not occur simply by replacement of Zn^{2+} by Cd^{2+} in the pre-synthesized binding protein, but that the synthesis of this protein, when induced by the former cation, is stimulated rapidly and without lag on subsequent administration of the latter.

ALTHOUGH in the rat and certain other mammalian species, excess Zn^{2+} is known to protect against the selective injury to the testis produced by a single, subcutaneous injection of a sub-toxic dose of Cd^{2+}[1-5] the mechanism of this protection has not been established. It seems unlikely that this is due to the competitive inhibition of the displacement by Cd^{2+} of Zn^{2+} from metalloenzymes necessary for gametogenesis, as initially considered by Pařízek,[1,2] since the primary action of Cd^{2+} is known now to be on the vascular system.[6] Furthermore, only small amounts of Cd^{2+} accumulate in the damaged testis, whereas the Zn^{2+} content increases for about 30 weeks to a level 9–10 times greater than normal.[7]

In the preceding paper[8] it was shown that the subcutaneous injection of Cd^{2+} into the male rat was followed by the production of a specific binding protein in the liver. The synthesis of this protein, which was not present in the normal liver, was induced also by excess Zn^{2+}. This observation, coupled with the fact that Zn^{2+} is most effective as an antagonist of Cd^{2+} when administered at least 24 hr before the latter cation,[5] suggested the possibility the Cd^{2+} ions accumulate, and are thus immobilized more rapidly in the livers of animals that contain the pre-synthesized binding protein. An investigation of this hypothesis, which provides one mechanism that may contribute to the protective effect of Zn^{2+} against Cd^{2+} toxicity, is reported in this paper.

MATERIALS AND METHODS

Young male rats (220–230 g body wt) of the laboratory "hooded" strain were injected intraperitoneally with an aqueous solution of $(CH_3COO)_2Zn$ (20 μmoles) made isotonic with NaCl and, after a further 24–48 hr with $CdCl_2$ (4·0 μmoles), the animals being killed by cervical dislocation at suitable intervals thereafter.

Tissues were analysed for Cd^{2+} and Zn^{2+} as described previously.[7] The Cd^{2+}-binding protein of the liver cell sap was isolated by gel filtration on Sephadex G 75, and purified by column chromatography on DE 52 cellulose.[8] Protein was determined by the method of Lowry *et al.*[9]

RESULTS

As expected from the observations of Mason and Young[5] that a molar ratio of $Zn^{2+}:Cd^{2+}$ of between 45:1 and 30:1 was necessary for complete protection against testicular damage induced by Cd^{2+}, the amount of Zn^{2+} that was used in the present work significantly reduced, but did not entirely prevent the changes in weight of the

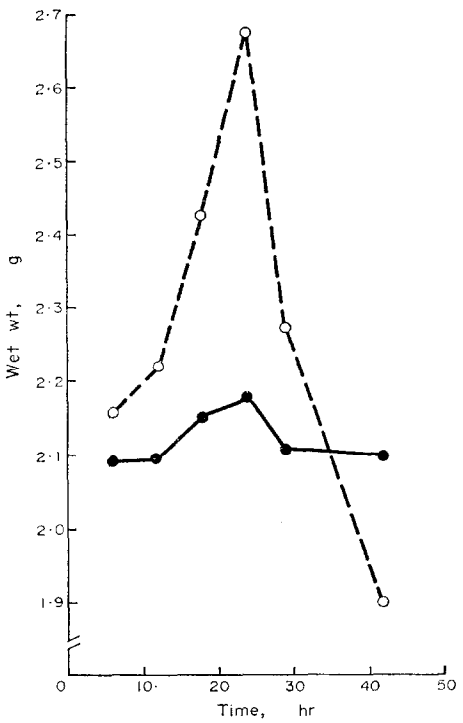

FIG. 1. Effect of pre-injection with Zn^{2+} on early Cd^{2+}-injury in the rat testes. Young adult male rats (220–230 g body wt) were injected intraperitoneally with $(CH_3COO)_2Zn^{2+}$ (20 μmoles). After 24 hr these animals, together with a group of normal males of the same age were injected subcutaneously with $CdCl_2$ (4·0 μmoles). Two or three animals of each series were killed at 6 hr intervals during the next 42 hr and the livers, kidneys and testes removed. The figure shows the change in the mean wet weight of the testes with time after the injection of Cd^{2+} in the normal (○– – –○) and Zn^{2+}-injected (●——●) rat.

testis that are characteristic of the early stages of Cd^{2+}-injury (Fig. 1, see also Ref. 7). Nevertheless, in the livers of these animals, in which the synthesis of the binding protein had been stimulated by Zn^{2+}, Cd^{2+} accumulated more rapidly and to higher levels than in the livers of control rats, injected with Cd^{2+} only (Table 1). In the kidney, however, preinjected Zn^{2+} had less effect, not only on the content of total Zn^{2+} (Table 1), but also on the uptake of Cd^{2+} (Table 1).

Fractionation of liver cell sap preparations showed that Cd^{2+} was accumulated solely by the specific binding protein which, as previously shown,[8] was identical with Zn^{2+}-protein IV, and that the more rapid accumulation of the cation in the livers of animals preinjected with Zn^{2+} (Fig. 2b, d, f) was correlated with the presence of this protein (Fig. 2a). An unexpected feature of these results (Fig. 2), which were

TABLE 1. EFFECT OF PREINJECTION WITH Zn^{2+} ON THE ACCUMULATION OF Cd^{2+} BY RAT LIVER AND KIDNEY

Time (hr) after Cd^{2+} injection	Contents of Zn^{2+} and Cd^{2+} (μg/g wet wt)							
	Liver				Kidney			
	Animals preinjected with Zn^{2+}		Normal rats		Animals preinjected with Zn^{2+}		Normal rats	
	Zn^{2+}	Cd^{2+}	Zn^{2+}	Cd^{2+}	Zn^{2+}	Cd^{2+}	Zn^{2+}	Cd^{2+}
6	46·0	21·6	28·2	16·6	20·4	2·35	19·7	3·04
12	47·5	25·9	34·2	20·0	20·8	5·49	18·9	4·00
18	47·5	31·0	38·2	21·5	22·4	7·62	21·9	4·05
24	52·5	35·0	47·5	22·4	21·3	5·18	18·7	3·88
29	52·5	38·0	49·2	24·0	19·3	7·02	21·2	6·54
42	55·0	42·0	52·2	30·0	22·7	8·13	18·4	9·95

Samples of liver and kidney from the experimental animals (see legend to Fig. 1 for details) were analysed as described in the Materials and Methods section.

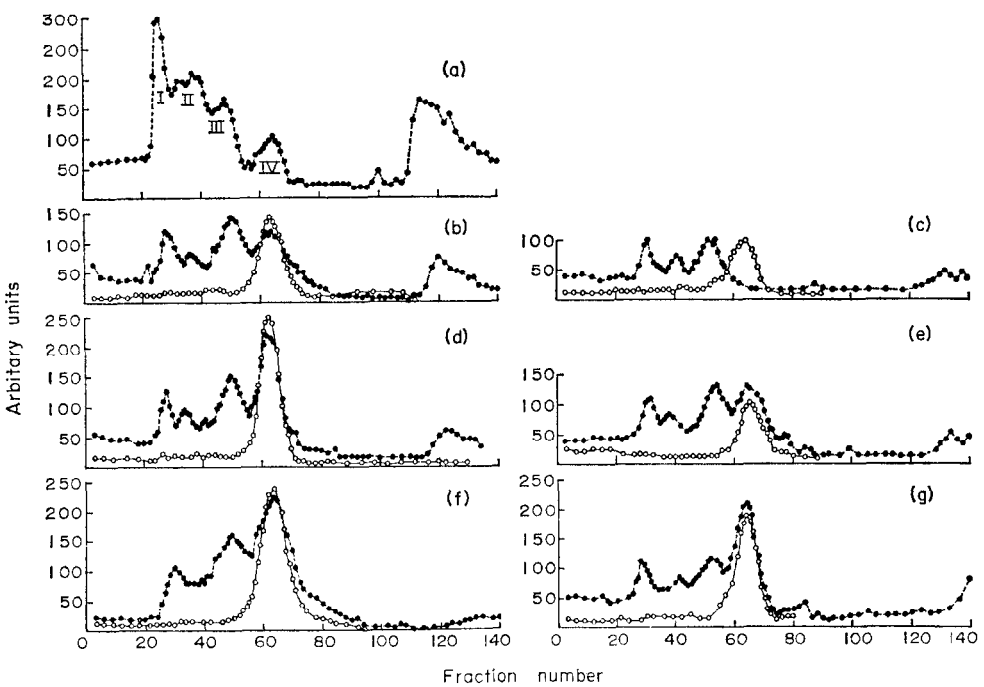

FIG. 2. Effect of pre-injection with Zn^{2+} on the accumulation of Cd^{2+} in the soluble fraction of rat liver. Cell sap preparations from the livers of male rats pre-injected with Zn^{2+} were made at (a) 0, (b) 6, (d) 12 and (f) 30 hr after the injection of Cd^{2+}, and from normal males at (c) 6, (e) 12 and (g) 30 hr after the injection of Cd^{2+} (see legend to Fig. 1 for experimental details). These were fractionated by gel filtration on columns (4 × 75 cm) of Sephadex G75, the eluates (5 ml fractions) being analysed for Zn^{2+} (●– – –●) and Cd^{2+} (○——○). The appropriate fractions from each run were combined, concentrated and analysed quantitatively for protein, Cd^{2+} and/or Zn^{2+} (see Materials and Methods).

obtained about 18 months after those reported in the preceding paper,[8] was the relatively low content in the livers of all animals of Zn^{2+}-protein II. The reason for this was not investigated, but seasonal and/or dietary variations seem possible contributory factors.

TABLE 2. EFFECT OF PREINJECTION WITH Zn^{2+} ON THE RECOVERY OF Cd^{2+} AND THE Cd^{2+}-CONTENT OF THE BINDING PROTEIN IN THE LIVERS OF MALE RATS

Time (hr) after Cd^{2+} injection	Recovery of Cd^{2+} in the binding protein (μg/g wet wt liver)		Cd^{2+} content (μg/mg protein) after fractionation on DE 52 cellulose	
	Animals preinjected with Zn^{2+}	Normal Male rats	Animals preinjected with Zn^{2+}	Normal Male rats
6	16·5 (76·5)	9·1 (54·9)	1·58	2·66
12	22·7 (87·6)	13·6 (68·0)	2·61	2·70
29	38·5 (100)	18·0 (75·0)	3·09	2·89

Figures in parentheses give the percentage of the total Cd^{2+} content of the liver (see Table 1) that was recovered in the binding protein.

From each of the separations shown in Fig. 2, the fractions that contained Cd^{2+} were combined and analysed to determine the recovery of Cd^{2+} in the Cd^{2+} binding protein. A portion of the remainder of each sample was concentrated and refractionated, first on Sephadex G 75, and then on DE 52 cellulose (see Materials and Methods). The fractions that contained the Cd^{2+}-binding protein were combined and analysed quantitatively for Cd^{2+} and protein as described in the Materials and Methods section.

Analyses of the pooled Cd^{2+} containing fractions from the gel filtration experiments of Fig. 2 are recorded in Table 2. These results show that for at least 30 hr after the injection of Cd^{2+} into the normal male rat, the concentration of Cd^{2+} (μg Cd^{2+}/mg protein) in the binding protein remained approximately constant, although the total amount of Cd^{2+} in this fraction increased with time. This implies that the binding protein becomes saturated with Cd^{2+} immediately it is synthesized, and that the content of this protein in the liver increases with the uptake of the cation. Not all of the total Cd^{2+} of the liver was recovered in the binding protein (Table 2) and it is probable that the remainder was associated with particulate cellular components, particularly the nuclei (see e.g. Heath and Webb[10]).

In the livers of animals preinjected with Zn^{2+} not only was a higher percentage of the tissue Cd^{2+} recovered in the binding protein, but also the total content of Cd^{2+} accumulated in this fraction was greater than in the liver of the normal male (Table 2). The latter, however, was not due simply to the substitution of Cd^{2+} for Zn^{2+} in the presynthesized binding protein (although this was possible during the early stages of Cd^{2+} accumulation), since the Zn^{2+} content of the binding protein fraction also increased; apparently by transfer of the cation from Zn^{2+}-proteins I and III (Fig. 2a, b, d and f).

DISCUSSION

The present results show that pre-injection of male rats with Zn^{2+} (20 μmoles) 24 hr before the administration of Cd^{2+} (4 μmoles) partially prevents the injurious

effects of the latter cation on the testes. This pre-treatment with Zn^{2+} induces in the livers of these animals the synthesis of a protein with high binding-affinity for Cd^{2+} (Ref. 8) and it seems that, in consequence, Cd^{2+} ions accumulate and thus are immobilized more rapidly than in the livers of normal rats. It is probable that this accumulation does not occur by replacement of Zn^{2+} in the pre-synthesized binding protein by Cd^{2+} but, after induction by Zn^{2+}, the synthesis of this protein is stimulated readily and without lag by the subsequent injection of Cd^{2+}. Since Zn^{2+} and Cd^{2+} induce the same protein it is likely that pre-treatment of rats with extremely small amounts of Cd^{2+} also would protect against the subsequent injection of a larger dose of the cation. During the induction of rhabdomyosarcomata by metallic cadmium,[11] for example, there is a slow feed of Cd^{2+} into the circulation as the implanted metal gradually dissolves. Under the latter conditions Cd^{2+} accumulates to high levels (e.g. 400 μg Cd^{2+}/g wet wt tissue) in the liver[10] as a complex with the binding protein[8] yet, in the male rat, the testes remain histologically and biochemically normal (J. C. Heath and M. Webb, unpublished results).

Acknowledgements—The technical assistance of Miss Diane Jackson and Mr. G. Payton in this work is gratefully acknowledged.

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