CADMIUM-INDUCED SELECTIVE LESIONS OF SENSORY GANGLIA*†

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Toxic doses of cadmium salts are known to cause morphologic lesions in various organs (kidney, lung, testis and ovary) of experimental animals (1–4). It was suggested that cadmium inhibits several enzyme systems, in particular those containing sulphydryl groups (1) and more recently the fact that pretreatment with zinc prevents intoxication by this metal has led to the hypothesis that cadmium possesses an antimetabolic activity for zinc (5, 6). Further interest in cadmium has arisen from findings which suggest that it plays a physiologic role in the renal cortex (7, 8), and even participates in the pathogenesis of arteriosclerosis (9).

While studying the calcifying activity of heavy metals, we observed that in the rat the parenteral administration of various cadmium salts provoked acute hemorrhagic lesions in the Gasserian and sensory spinal ganglia (10). Here we should like to report a more detailed investigation of this phenomenon.

MATERIALS AND METHODS

Five experiments were performed using groups of 10 guinea pigs, golden hamsters, and mice (their body weight is indicated in Table 1) and 150 Sprague-Dawley rats with a mean initial body weight of 205 g (range 193 to 211 g), which were divided into 15 equal groups. All animals were supplied by the Robidoux Farm (Montreal, Quebec, Canada).

The following compounds were given under conditions to be described later: Cadmium chloride (CdCl₂, Fisher Scientific Company, Fair Lawn, N.J., U.S.A.); Zinc acetate (Zn[C₂H₃O₂]·2H₂O, Fisher Scientific Company); Ferrie-dextran (Imferon®, Benger Laboratories, Holmes Chapel, England); Glutathione (GSH; Nutritional Biochemical Corp., Cleveland, Ohio, U.S.A.); Sodium pyrophosphate (Na₃P₂O₇·10H₂O, J. T. Baker Chemical Co., Phillipsburg, N.J., U.S.A.).

The surgical interventions employed were: total transection of the spinal cord (exposed through a small skin incision at the level of the first lumbar vertebra and then severed with small scissors after sectioning the intervertebral muscles and ligaments) and transectioning of the right sciatic and femoral nerves (exposed through a suprapubic midline incision of the abdominal wall).

The animals were maintained exclusively on Purina Laboratory Chow (Purina Co. of Canada), and tap water throughout the experiments which were terminated, unless

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498
otherwise stated, by killing the survivors with chloroform, 4 days after cadmium chloride injections. On autopsy, the intensity of the lesions was gauged in terms of an arbitrary scale of 0 = no lesion, 1 = just detectable, 2 = moderate, and 3 = most severe lesions. The mean intensities of these lesions together with the percentage of animals affected are listed in the tables. Specimens of the affected organs were fixed in alcohol-formol (4 parts of absolute alcohol and 1 part of 10 per cent neutral formalin) or in Susa solution for staining with hematoxylin and phloxine, the PAS technic or a trichrome stain (phosphotungstic acid, aldehyde fuchsin and alcian blue) which is particularly useful in illustrating thrombi and hemorrhagic suffusions (11, 12). It should be noted that in our experience keeping sensory ganglia for 4 to 24 hours in Susa solution provides a good preservation for the cells whereas alcohol-formol does not.

RESULTS

Comparative Action of CdCl₂ on Different Laboratory Animals: Table 1 shows that CdCl₂ (given subeutaneously in the interseapular region in a dose of 1 mg per 100 g of body weight always in 1 ml of water) has comparable toxic effects on sensory ganglia in all species examined.

Chronologic Development of the CdCl₂-Induced Lesions: For this experiment we used 5 groups of rats; one group served as untreated controls and the others were injected subeutaneously in the interseapular region with 2 mg of CdCl₂ in 1 ml of water and subsequently killed 1 hour, 5 hours, 24 hours and 4 days after the treatment.

No changes were observed in the animals killed 1 hour after the CdCl₂ injection.

In the group killed 5 hours after treatment, some animals had small hemorrhagic spots, located mainly in the Gasserian ganglion. Histologic examination revealed that the main changes consisted of small, irregular hemorrhages around the ganglionic cells, which showed a loss of Nissl substance, and in nerve fibers, particularly at the periphery of the ganglia appearance of PAS and hematoxylin positive deposits (fig. 1). The latter were most visible in the specimens fixed in alcohol-formol.

Twenty-four hours and 4 days after the treatment the animals showed similar changes which are therefore described together. On clinical examination, the rats appeared listless, although they retained strongly to a painful stimulus and showed an increase in irritability. No motor changes were observed. In rare instances, on autopsy, we noted foci of hepatic necrosis or

<table>
<thead>
<tr>
<th>Gr.</th>
<th>No. of Animals</th>
<th>Species</th>
<th>Body Weight (gm.)</th>
<th>Ganglionic Lesions</th>
<th>Mortality %</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Scale 0-3)</td>
<td>(%)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>Guinea pig</td>
<td>470</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>Rat</td>
<td>200</td>
<td>3</td>
<td>100</td>
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</tr>
<tr>
<td>4</td>
<td>10</td>
<td>Mouse</td>
<td>25</td>
<td>2</td>
<td>100</td>
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</table>

TABLE 1
Comparative Toxicity of Cadmium Chloride on Various Species
Fig. 1. Irregular distribution of several PAS positive masses in fibers at the periphery of the Gasserian ganglion, 3 hours after CdCl₂ administration, Alcohol-formol; PAS: × 460.

Fig. 2. Loupe magnification of the Gasserian ganglion "in situ" 24 hours after CdCl₂ administration. The lesion is sharply limited to the ganglionic tissue.
ovarian bleeding but observed no changes in the kidney, heart, or lungs. In addition, ganglionic lesions were present in all animals. Macroscopically, the affected organs appeared dark red because of massive hemorrhages sharply localized in the ganglionic tissue (fig. 2). Histologically, the most striking change was represented by hemorrhagic suffusions which were almost invariably located around the ganglionic cells (fig. 3); a small degree of leucocyte infiltration was also present. Moreover, we observed in several cells, karyorrhexis or nucleolar pyenosis with lysis of the cytoplasm (fig. 4); other cells (particularly those located at the periphery of the ganglia) appeared normal; in the fibers, PAS positive masses or granules were irregularly distributed throughout the ganglion. The sympathetic ganglia appeared normal.

**Influence of Different Nervous Lesions on Cadmium Intoxication:** Three groups of rats received the same dose of CdCl₂ as in the second experiment (table 2). The transection of the spinal cord, right sciatic and femoral nerves was performed under light ether anesthesia 10 days before CdCl₂ administration. As seen in Table 2 transection of the spinal cord did not influence the development of the lesions in the ganglia situated either above or below the first lumbar vertebra. On the other hand, the severity of hemorrhagic lesions induced by CdCl₂ in the ganglia corresponding to the transected nerves was considerably diminished in comparison to the contralateral ganglia and those situated at different levels.

![Fig. 3. Hemorrhagic suffusions around the nervous cells of the Gasserian ganglion 4 days after CdCl₂ administration. Alcohol-formol; aldehyde fuchsin, phosphotungstic acid and alcian blue; × 460.](image-url)
Fig. 4. A: Normal appearance of Gasserian ganglion cells of a control animal. B: Degenerative changes and necrosis of some cells in the ganglion in which there are hemorrhages, 4 days after CdCl₂ administration; PAS positive granules and a minor degree of leucocyte infiltration are also noticeable. Susa; PAS; × 400.
**TABLE 2**

*Influence of Neural Lesions on Cadmium Chloride Toxicity in the Rat*

<table>
<thead>
<tr>
<th>Gr.</th>
<th>No. of Animals</th>
<th>Treatment*</th>
<th>Gasserian Ganglion</th>
<th>Thoracic Ganglia</th>
<th>Lumbar Ganglia</th>
<th>Mortality %</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Scale 0-3)</td>
<td>%</td>
<td>(Scale 0-3)</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>None</td>
<td>3</td>
<td>100</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>Spinal cord transection</td>
<td>3</td>
<td>100</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>Right sciatic and femoral nerve transection</td>
<td>3</td>
<td>100</td>
<td>2</td>
<td>100</td>
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</tbody>
</table>

* In addition, all animals received CdCl₂ 2 mg in 1 ml of water s.e.

**Action of Zinc Acetate and Iron-Dextran on the CdCl₂-Induced Lesions:** It has been reported that zinc acetate prevents the testicular lesions induced by CdCl₂ (5) and that iron-dextran prevents the calcification of subcutaneous tissue induced by the same metal (18). Therefore, we investigated the effects of these two compounds in the inhibition of lesions of sensory ganglia induced by CdCl₂. We utilized the same amounts of zinc acetate and iron-dextran as were reported in the literature (5, 18). On the first day 3 groups of rats received CdCl₂ subcutaneously in the intercapular region using a dose of 1.32 mg (equal to 0.03 mM per kg) in 1 ml of water (3, 21). Zinc acetate was injected subcutaneously in a dose of 44 mg (the total amount being equal to 3 mM per kg) in 3 ml of water and ferric dextran in a dose equal to 30 mg of iron in 1 ml of water, intraperitoneally. Both compounds were given at 3 different times, 5 hours before, simultaneously with and 19 hours after cadmium administration. The pretreatment with zinc acetate prevented the development of ganglionic lesions, whereas iron-dextran proved ineffective in this respect (table 3).

**Influence of Glutathione and Sodium Pyrophosphate on CdCl₂ Intoxication:** In the following experiment we examined the possibility of inhibiting the CdCl₂-induced lesions of sensory ganglia by glutathione and sodium pyrophosphate. Glutathione has for a long time been known to counteract the action of heavy metals (20) in vitro and in vivo, and sodium pyrophosphate has been reported to prevent the soft-tissue calcification induced by heavy metals or rare earths (24, 25). We injected CdCl₂ and the potentially prophylactic agents intravenously because in preliminary experiments, not reported here, the most constant results were obtained by administration through this route. On the first day, 3 groups of rats were injected intravenously with 900 µg of CdCl₂ in 1 ml water. In our animals this amount was the highest tolerated dose of CdCl₂. Immediately preceding this injection, glutathione (150 mg)
TABLE 3
Action of Zinc Acetate and Ferrie-Dextran on Cadmium Chloride Intoxication in the Rat

<table>
<thead>
<tr>
<th>Gr.</th>
<th>No. of Animals</th>
<th>Treatment*</th>
<th>Ganglionic Lesions (Scale 0-3)</th>
<th>%</th>
<th>Mortality %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>None</td>
<td>2</td>
<td>100</td>
<td>0</td>
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<tr>
<td>2</td>
<td>10</td>
<td>Zinc acetate</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>Ferrie-dextran</td>
<td>2</td>
<td>100</td>
<td>20</td>
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</table>

* In addition, all animals received CdCl₂ 1.32 mg in 1 ml of water s.c.

TABLE 4
Action of Glutathione and Sodium Pyrophosphate on Cadmium Chloride Intoxication in the Rat

<table>
<thead>
<tr>
<th>Gr.</th>
<th>No. of Animals</th>
<th>Treatment*</th>
<th>Lesions</th>
<th>Mortality %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liver</td>
<td>Sensory Ganglia</td>
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<td></td>
<td></td>
<td></td>
<td>(Scale</td>
<td>%</td>
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<tr>
<td>1</td>
<td>10</td>
<td>None</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>Glutathione</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>Sodium pyrophosphate</td>
<td>3</td>
<td>100</td>
</tr>
</tbody>
</table>

* In addition, all animals received CdCl₂ 900 µg in 1 ml water i.v.

and sodium pyrophosphate (5 mg) each in 1 ml of water were administered in the same manner (table 4). The animals treated only with CdCl₂ exhibited the same neural lesions as those described above and seen on macroscopic and histologic examination; the change of route of administration did not alter the effect of CdCl₂ on sensory ganglia. In addition, however, extensive hepatic necroses were present which caused the death of several animals (table 4). Glutathione pretreatment suppressed the toxic action of CdCl₂ but sodium pyrophosphate did not.

DISCUSSION

The pharmacologic properties of cadmium were considered for many years to be similar to those of zinc, and it was only recently that new findings have stimulated interest in certain toxic and physiologic actions of this ion. Moreover, the increased industrial utilization of cadmium in the recent decades has revealed its occupational hazards (13, 14). The experiments described here point out hitherto unknown toxic effects of cadmium on the nervous system, although they give little information concerning the mechanism of action. It is generally accepted that this metal acts primarily on the central nervous system abolishing first the reflexes, secondly the sensation of pain, and finally causing death by asphyxia due to pulmonary edema (1). As long ago
as 1893, De Simone noted that subeutaneous injection of cadmium chloride abolishes the sense of pain in dogs (15); however, this observation has never been confirmed. In this connection it is noteworthy that we did not observe signs of analgesia in our animals after CdCl₂ treatment.

It is known that cadmium forms complexes with lipids especially with phospholipids and today it is used as a histologic fixative for these substances (16). This fact may explain the toxicity of the metal for neural tissue but not its selective localization in sensory ganglia. It is tempting to associate this localization with the special structure of ganglionic neurons, but at present we have no proof for this speculation.

The results of our third experiment show that transection of the afferent nerves with the consecutive atrophy in the corresponding ganglia diminishes the toxicity of cadmium for these organs. This fact is reminiscent of the previous observation that hypophysectomy decreases cadmium-induced lesions in the testis (5). The results of the fourth and fifth experiments are consonant with some of the recognized pharmacologic activities of glutathione and zinc (5, 20). On the other hand, iron-dextran and sodium pyrophosphate, both effective inhibitors of the calcifying properties of cadmium chloride (18, 19) do not alter the neural lesions induced by this salt.

In human pathology there is no counterpart of the nervous lesions which appear in experimental animals after cadmium administration. The PAS and hematoxylin positive masses found in the ganglionic fibers resemble those described in the Gasserian ganglion of patients with trigeminal neuralgia (17). They may also be compared to the large fragments of myelin (Markballen) which are observed in degenerative diseases of the nervous system (17).

Several authors suggest that cadmium damages the testis by an action on its vessels (5, 21, 22, 23). The early appearance of hemorrhagic suffusions in the sensory ganglia may also indicate that cadmium possesses specific vascular toxicity. However, the simultaneous appearance of PAS and hematoxylin positive masses in the ganglionic fibers suggests that this metal may also act directly on neural tissue. In any event, the ganglionic cells seem to be secondarily affected when compared to the fibers and vessels.

Although further studies will be needed to clarify the mechanism of cadmium action, we can conclude that this ion possesses a selective toxicity for sensory ganglia and that zinc acetate and glutathione are effective inhibitors of this action.

**SUMMARY**

In several species of animals the parenteral administration of cadmium chloride induces an acute hemorrhagic lesion of the Gasserian and sensory spinal ganglia. This lesion is characterized by hemorrhagic suffusions around ganglion cells, several of which exhibit karyorrhexis or nuclear pyknosis with lysis of the cytoplasm. In the nerve fibers there are distinctive PAS and hematoxylin positive masses. Pretreatment with zinc acetate or glutathione suppresses the toxicity of cadmium chloride.
REFERENCES