Regional Differences in Severity of Cadmium-induced Lesions in the Peripheral Nervous System in Mice

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Summary. Adult mice were injected s.c. with a single toxic dose of cadmium chloride. After intervals of 24 h to 7 days, various peripheral ganglia and the sciatic nerve were examined by light and electron microscopy for injurious effects of cadmium on blood vessels, nerve cells, axons and the connective tissue sheaths. In addition, horseradish peroxidase (HRP) was used to detect changes in the permeability of blood vessels, perineurium, and cellular membranes.

Endothelial cell damage with hemorrhages were constant findings in sensory ganglia. In the superior cervical ganglion and the sciatic nerve, hemorrhages and interstitial edema occurred in about 50% of the animals. No vascular lesions were present in the celiac ganglion.

Damage to nerve cells and axons occurred in sensory but not in sympathetic ganglia. Intravenously injected HRP entered the cytoplasm of several neurons in the sensory ganglia in a diffuse manner, indicating damage to their cell membranes with loss of selective permeability. The axoplasm of some myelinated axons was also stained by peroxidase.

Only minor ultrastructural changes were observed in the perineurial cells of the sciatic nerve or peripheral ganglia in cadmium-treated mice. The perineurial diffusion barrier to peroxidase in the sciatic nerve and the superior cervical ganglion was not affected by the cadmium injection.

Acute cadmium intoxication in adult mice therefore appears to leave the perineural structure and permeability to HRP unaffected, whereas the extent of vascular damage differs in different regions of the peripheral nervous system without any obvious relation to the normal permeability to macromolecules. This variation in the intensity of vascular lesions may reflect a functional difference between endothelial cells in different regions of the peripheral nervous system, but the precise nature of this hypothetical difference is unknown.

Key words: Cadmium — Mice — Nervous system — Light and electron microscopy — Horseradish peroxidase (HRP)

Exposure of human beings and laboratory animals to cadmium salts has been associated with a variety of pathological changes, such as nephropathy, arteriosclerosis, hypertension, liver damage, and pulmonary disorders (Prodan 1932a, 1932b; Wilson et al. 1941; Paterson 1947; Friberg 1950, 1952; Schroeder 1964; Carroll 1966). Acute cadmium intoxication in the rat is followed by profound damage to the testis, affected mainly by injury to the testicular blood vessels (Parizek 1956, 1957; Gunn et al. 1963). In 1966, Gabbiani showed that cadmium causes hemorrhagic and necrotic lesions in sensory ganglia. These lesions, like those of the testis, are thought to be primarily due to a vascular injury, and previous ultrastructural studies of cadmium-induced lesions in peripheral ganglia (Schlaepfer 1971; Gabbiani et al. 1974) have therefore been focused on alterations in ganglionic vessels. Morphological changes in endothelial cells of sensory ganglia were found to start as irregular dilatation of intercellular clefts with partial damage to the endothelial cell membrane (Gabbiani et al. 1974). Later, degenerative lesions developed, with large gaps in the endothelial continuity and extravasation of erythrocytes (Schlaepfer 1971; Gabbiani et al. 1974).

I have previously studied the intra- and extracellular distribution of exogenous protein tracers in peripheral ganglia and nerves of normal mice (Arvidson et al. 1973; Arvidson 1977, 1979a, 1979b). The present study concerns the effects of acute cadmium intoxication on the structure of various tissue components of peripheral sensory and autonomic ganglia and the sciatic nerve in mice. It was considered...
of special interest to compare the severity of the cadmium-induced lesions in various types of peripheral ganglia, because previously it was noted in one study that the sympathetic ganglia appeared normal after cadmium injection (Gabbiani et al. 1967) despite similarities in morphology and permeability of blood vessels in various types of ganglia (Jacobs et al. 1976; Jacobs 1977; Arvidson 1979a). Macromolecular tracers have been used in the present investigation to study the influence of a pathologic condition on their distribution within ganglia and nerves.

Materials and Methods

The experiments were performed on adult male albino mice of the NMRI strain (Anticimex, Stockholm, Sweden). Their weights ranged from 30–35 g. The animals had free access to a conventional laboratory chow diet (Astra-Ewos AB, Södermåla, Sweden) and tap water. The number of mice in different experimental groups (see below) and the survival times are given in Table 1.

In all cadmium-injected animals, a single dose of cadmium chloride (CdCl$_2$, analytical grade, Fisher Scientific Company, Fair Lawn, New York, USA) was given by s.c. injection. CdCl$_2$ was dissolved in sterile water at a concentration of 1 mg/ml. The dose of CdCl$_2$ in all groups of animals was 5 mg/kg body weight (b.w.). Similar doses have previously been used in experimental studies of acute cadmium intoxication (Gabbiani et al. 1974; Hoffman et al. 1975). The trigeminal ganglion, a lumbar dorsal root ganglion, the superior cervical ganglion and the celiac ganglion were excised for investigation. In addition, the sciatic nerve and a slice from the parietal cortex were removed.

The animals were divided into five groups depending on the experimental procedure.

In Group 1 the ganglia of cadmium-treated mice were investigated by light microscopy and the frequency of hemorrhages and nerve cell necrosis was determined. The animals were killed by exsanguination 24 h, 48 h, 72 h, and 7 days after the cadmium injection. Controls were injected s.c. with sterile water and killed 24 h later. The ganglia, the sciatic nerve and the brain were dissected out and immersed for 4 h in 0.16 M cacodylate buffer with 3% glutaraldehyde, pH 7.3. After washing in 0.16 M cacodylate buffer overnight, the tissues were dehydrated in graded ethanols and embedded in JB4 plastic. Sections 2 μm thick were stained with hematoxylin and cosin (H.E.), toluidine blue, and PAS and were mounted in Eukitt.

In Group 2 the same tissues were investigated by electron microscopy in animals killed at the same time intervals after the cadmium injection as those in Group 1 and in controls treated in the same way as those in the first group. The animals were fixed by whole-body vascular perfusion for 15 min, using the same fixative as above preceded by a short rinse with isotonic saline. After additional fixation by immersion for 4 h at 4°C, the tissue samples were washed overnight in 0.16 M cacodylate buffer with 5% sucrose. The tissue blocks were then fixed for 90 min in 2% OsO$_4$, stained "en bloc" with 2% maleate buffered uranyl acetate for 15 min (Reese and Karnovsky, 1967), dehydrated in alcohol, and embedded in Epon plastic. Thin sections were stained with lead citrate and uranyl acetate and examined in a Jeol 100 C electron microscope.

In Group 3 the animals were injected i.v. with colloidal carbon after pretreatment with cadmium. Intravenous injection of carbon is often used as a tool for detecting vascular injuries (Cotran et al. 1967: review). The mice in this group were injected with cadmium chloride and killed by vascular perfusion 24 h later. Thirty minutes before being killed they were injected with colloidal carbon (Pelikan Company, Hannover, Federal Republic of Germany, batch no. C11/1431a) in a dose of 0.1 ml/100 g b.w. (Cotran et al. 1967). Controls were injected with colloidal carbon in the same way without pretreatment with cadmium. The same tissues as in Group 1 were removed and prepared for light microscopy. The sciatic nerve, the trigeminal ganglion, and the superior cervical ganglion were also examined by electron microscopy.

In Group 4, the distribution of i.v. injected HRP was studied in cadmium-treated mice. Animals to be used for investigation by light microscopy were killed 1, 2, 4, 8, and 24 h after the cadmium injection. HRP (Type II, Sigma Chemical Co., St. Louis, Missouri, USA) was injected into the femoral vein 30 min before the animals were killed. Controls were injected with HRP without pretreatment with cadmium. The dose of the peroxidase was 2 mg/10 g b.w. (Arvidson 1977, 1979a). The animals were killed by exsanguination or vascular perfusion. The same tissues as in Group 1 were removed for light microscopy. Frozen sections 10 μm thick were cut and incubated as described by Graham and Karnovsky (1966). The sections were then dehydrated rapidly, mounted in Eukitt, and viewed unstained.

Animals to be used for electron microscopy were killed 4, 8, and 24 h after the cadmium injection. The sciatic nerve and the trigeminal and superior cervical ganglia were cut into thin slices with a razor blade, incubated for peroxidase as described by Graham and Karnovsky (1966), and prepared for electron microscopy as described above.

In Group 5 the perineurial diffusion barrier to peroxidase was investigated in superior cervical ganglia and sciatic nerves of cadmium-treated mice. The animals were injected with cadmium and 24 h later the superior cervical ganglion or the sciatic nerve was exposed under barbital anesthesia. About 10–15 μl of the tracer solution (HRP 20 mg/ml in isotonic saline, pH 7.4) was then applied locally. After 60 min, the animals were killed by an overdose of

Table 1. Experimental plan used for light and electron microscopic studies of cadmium-induced lesions in the peripheral nervous system

<table>
<thead>
<tr>
<th>Experimental group (see text)</th>
<th>Survival times</th>
<th>Number of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24, 48, 72 h, 7 days</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>24, 48, 72 h, 7 days</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>24 h</td>
<td>6</td>
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<tr>
<td>4</td>
<td>1, 2, 4, 8, 24 h</td>
<td>20</td>
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<tr>
<td>5</td>
<td>24 h</td>
<td>6</td>
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Fig. 1a–c. Plastic sections 2 μm thick stained with toluidine blue. a Trigeminal ganglion, control mouse. A group of nerve cells with large, light and small, dark neurons. The nuclei are located centrally and have prominent nucleoli. The extracellular space is not expanded. × 730. b Superior cervical ganglion, cadmium-treated mouse, survival time 24 h. In the center of the figure, extravasated erythrocytes have accumulated between the nerve cells. The neurons and nerve fibers are spread apart by interstitial edema. × 560. c Trigeminal ganglion, cadmium-treated mouse, survival time 24 h. In several neurons (arrows) a lightly staining, structureless material has accumulated at the periphery of the cytoplasm. A few nerve cells are shrunken, with vacuolization of the cytoplasm and nuclear pyknosis (arrow heads). × 578.