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Distribution of ^{109}Cd in the Nervous System of Rats After Intravenous Injection*

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Summary. The distribution of i.v. injected ^{109}Cd within the nervous system was studied in rats 24 h and 1 week after the injection. Measurements by gamma scintillation showed a high uptake of cadmium in peripheral sensory and autonomic ganglia, whereas the uptake was low in the brain, cerebellum, and spinal cord. The accumulation of cadmium in the sciatic nerve was significantly higher than in the brain and spinal nerve roots, but lower than in ganglia.

At autoradiography no labeling was seen in the major part of the brain parenchyma, but an accumulation of the metal was observed in structures outside of the blood-brain barrier (BBB), such as the hypophysis, meninges, choroid plexus and pineal gland. Within the peripheral nervous system (PNS), autoradiography showed accumulation of cadmium in the dorsal root ganglia.

The results show that the distribution of ^{109}Cd within the nervous system is correlated to regional variations in vascular permeability, blood vessels of different regions permitting penetration of different amounts of the protein-bound cadmium into the nervous tissues. The accumulation of cadmium in certain nervous structures may have relevance for some of the neurotoxicologic effects of this metal that have been demonstrated in animal experiments.

Key words: Cadmium – Nervous system – Blood-brain barrier – Peripheral ganglion

Introduction

Chronic exposure of humans to cadmium causes preferential damage to the kidneys, liver, and lungs (Friberg et al. 1974; Fassett 1975). Toxic effects on the nervous system have also been reported and include anosmia (Friberg 1950; Adams and Crabtree 1961), damage to the autonomic innervation of the gut (Baader 1952), and CNS symptoms (Vorobjeva 1957).

In animal studies, several neurotoxic effects of cadmium have been documented. In adult rats and mice, single toxic doses of cadmium chloride cause hemorrhagic and necrotic lesions in the sensory ganglia (Gabbiani 1966), and administration of cadmium to newborn animals, has been found to result in hemorrhagic encephalopathy (Gabbiani et al. 1967; Webster and Valois 1981; Wong and Klaassen 1982). Long-term low-level exposure to cadmium may cause peripheral neuropathy in adult rats (Sato et al. 1978). In immature rats, behavioral changes have been observed following cadmium exposure (Squibb and Squibb 1979; Rastogi et al. 1977). A survey of the neurotoxic effects of cadmium has been published recently (Arvidson 1983).

In previous studies on the distribution of radioactively labeled cadmium in animals, a low uptake has been observed in the brain (Walsh and Burch 1959; Nordberg and Nishiyama 1972; Buhler et al. 1981; Cahill et al. 1983). However, the part of the brain that has been investigated has not been specified in these reports. In two earlier reports on autoradiographic studies in mice, the distribution of cadmium in the brain is described only briefly (Berlin and Ullberg 1963; Nordberg and Nishiyama 1972). The cadmium distribution within the PNS has not been analyzed previously. The aim of the present investigation was to give a more complete picture of the distribution of i.v. injected ^{109}Cd in both the CNS and PNS of rats.

Material and Methods

The experiments were performed on 13 2-month-old female Wistar rats, ranging in weight from 190 to 200 g. The animals were kept individually in plastic cages at room temperature. They were fed a standard laboratory chow diet (EWOS AB, Södertälje, Sweden) and had free access to tap water. Cadmium-

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109 (specific activity 0.81 mCi/μg) was obtained as cadmium chloride in 0.1 M HCl from the Radiochemical Centre, Amersham (England). The solution was neutralized with 0.1 M NaOH before used.

The animals were injected with 10 μCi of the isotope in 0.15 ml of the neutralized solution, corresponding to a dose of approximately 0.045 μg of Cd/kg body weight. The solution was injected into the left saphenous vein under ether anesthesia, using a Leitz operating microscope.

After 24 h and 1 week, the animals were killed, and samples were taken from the CNS and PNS. Samples from the CNS consisted in tissue blocks taken from the parietal cortex, cerebellum, and thoracic spinal cord. Those from the PNS comprised about 2.5 cm of both sciatic nerves from the mid-thigh region, the trigeminal and superior cervical ganglia, the L_1 and L_2 dorsal root ganglia, and lumbar spinal nerve roots. Before counting, the sciatic nerves were cleared of as much epineurial fat as possible. Samples were also taken from the central part of the right liver lobe, from the kidney, and from blood for comparison with nervous tissues.

Care was taken to transfer the specimens from the animal to the balance immediately to minimize weight loss by evaporation. The specimens were then placed in glass tubes, and the radioactivity was counted in a Packard auto-gamma scintillation spectrometer. The counting efficiency was 38.6%, and the values were corrected for background activity. The accumulation of ^{109}Cd was expressed as dpm/mg wet weight. For statistical analysis, Student's *t*-test was used.

Autoradiography was performed for comparison with the results obtained by gamma scintillation. Since measurements by gamma scintillation showed similar values 1 day and 1 week after the injection, only one animal with a survival period of 1 week was investigated. In a previous autoradiographic study in mice weighing 26–31 g, a dose of 10 μCi of ^{109}Cd was used (Nordberg and Nishiyama 1972). For the rats of the present study weighing 190–200 g, a dose of 50 μCi was considered appropriate. The animal was killed by CO_2 asphyxiation, frozen in carbon dioxide-hexane, and embedded in carboxymethyl cellulose. From the frozen blocks 20 μm sagittal whole-body sections were prepared on tape, and autoradiography was performed by the method described by Ullberg (1954, 1977). The sections were freeze-dried at −20° C for 48 h, apposed to X-ray film (Agfa structurix D7), and exposed for 3 weeks. After exposure, the films and the sections were separated, and the films were developed in Kodak D19 developer for 5 min at 20° C, fixed, and rinsed. The sections were stained with hematoxylin-eosin (HE), dehydrated in a series of ethanol, and mounted on glass slides in Euparal (GBI Laboratory Ltd., Manchester, Great Britain).

Results

The accumulation of ^{109}Cd in various structures of the nervous system 24 h and 1 week after the injection is shown in Fig. 1. In Fig. 2, the uptake of cadmium in dorsal root ganglia and extraneural tissues (liver, kidney, and peripheral blood) is compared. Values recorded after 1 week were approximately the same as those obtained 24 h after injection. The highest accumulation of cadmium was seen in peripheral ganglia (dorsal root, trigeminal, and superior cervical ganglia), whereas very low values were obtained for samples from the CNS (parietal cortex, spinal cord, and cerebellum). Accumulation of ^{109}Cd in the sciatic

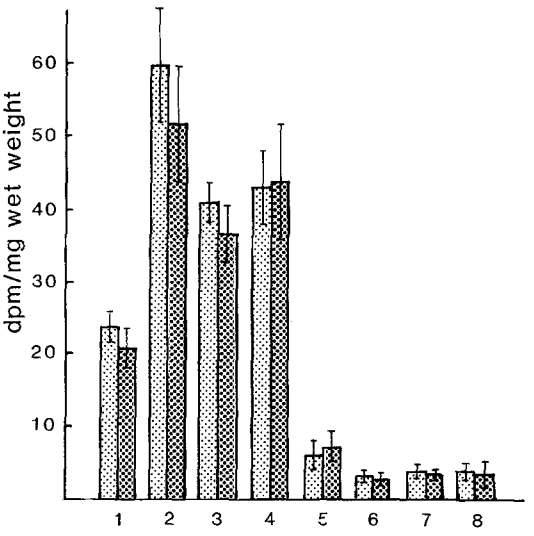

Fig. 1. Accumulation of ^{109}Cd in samples from the nervous system of rats. *Stippled bars* to the *left* represent animals killed 24 h after the injection and *stippled bars* to the *right* animals killed after 1 week. The results are expressed as mean ± SD (*verticals bars*) of six animals. *1* sciatic nerve, *2* dorsal root ganglion, *3* trigeminal ganglion, *4* superior cervical ganglion, *5* nerve roots, *6* parietal cortex, *7* spinal cord, *8* cerebellum

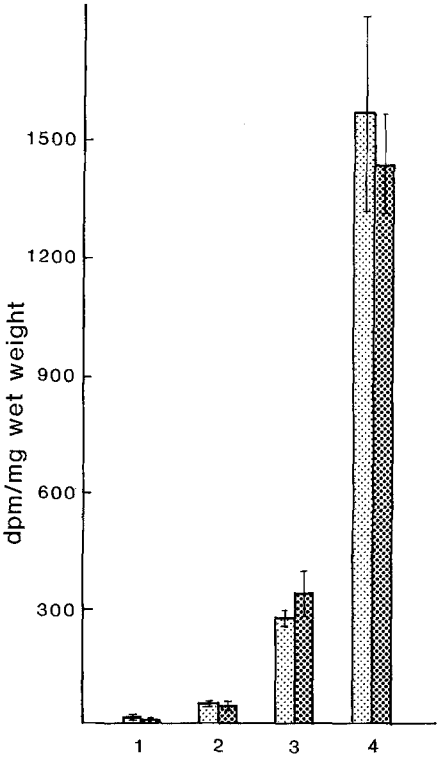

Fig. 2. Comparison between the accumulation of ^{109}Cd in dorsal root ganglia and extraneural tissues. *Stippled bars* to the *left* represent animals killed 24 h after the injection and those to the *right* animals killed after 1 week. The results are expressed as mean ± SD (*vertical bars*) of six animals. *1* blood, *2* dorsal root ganglion, *3* kidney, *4* liver

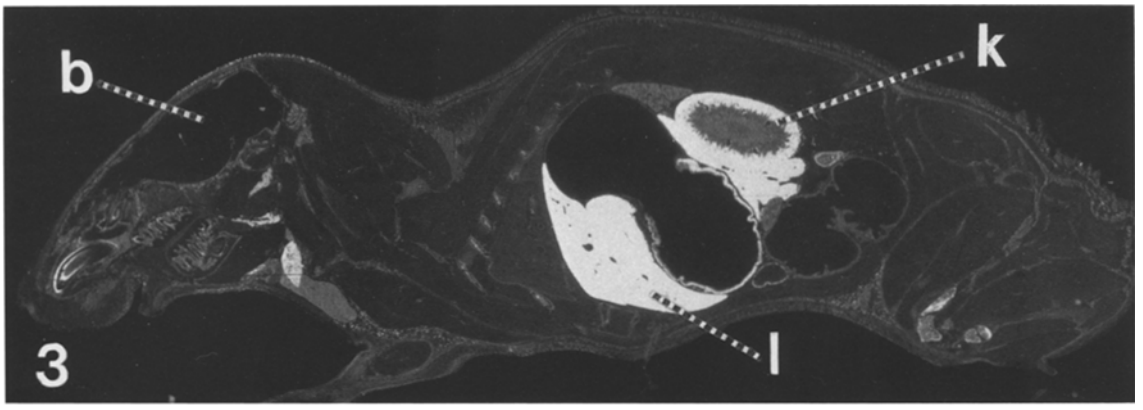

Fig. 3. Whole-body autoradiogram of a rat killed 1 week after i.v. injection of 50 μCi of ^{109}CdCl2. The metal has accumulated preferentially in the liver (*l*) and renal cortex (*k*). There is no detectable uptake in the brain parenchyma (*b*)

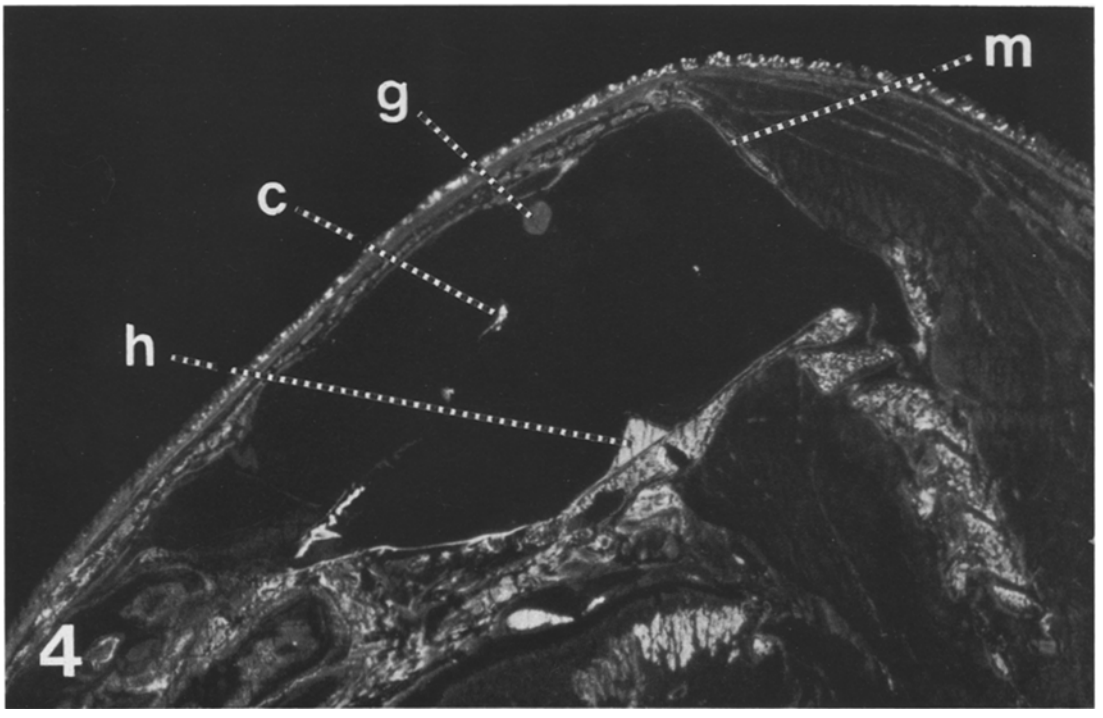

Fig. 4. Autoradiogram of a sagittal section of the brain. There is no uptake of cadmium in the brain parenchyma, but cadmium has accumulated in the hypophysis (*h*), choroid plexus (*c*), pineal gland (*g*), and meninges (*m*)

nerve was roughly intermediate between that in ganglia and in the CNS.

The accumulation of ^{109}Cd in the sensory and autonomic ganglia was significantly greater ($P < 0.01$) than in the sciatic nerve. The activity in spinal nerve roots was much lower than in the sciatic nerves, but significantly higher ($P < 0.01$) than in samples from the CNS. The uptake of cadmium in the dorsal root ganglia was relatively low as compared with that in the kidney and liver (Fig. 2).

Whole-body autoradiography showed preferential labeling of the liver and renal cortex (Fig. 3). Within the CNS, there was no detectable uptake in the parenchyma of the brain or in the spinal cord (Figs. 4, 5), but the hypophysis, meninges, choroid plexus, and pineal gland were labeled (Fig. 4). Paramedian sections passing through the dorsal root ganglia showed labeling of the ganglia (Fig. 5a, b). The ganglia were identified by comparing the autoradiogram with the corresponding section stained with hematoxylin-eosin (HE). The findings were the same for ganglia in the cervical, thoracic, and lumbar parts of the spinal cord. The parenchyma of the adjacent spinal cord was not labeled (Fig. 5a, b).

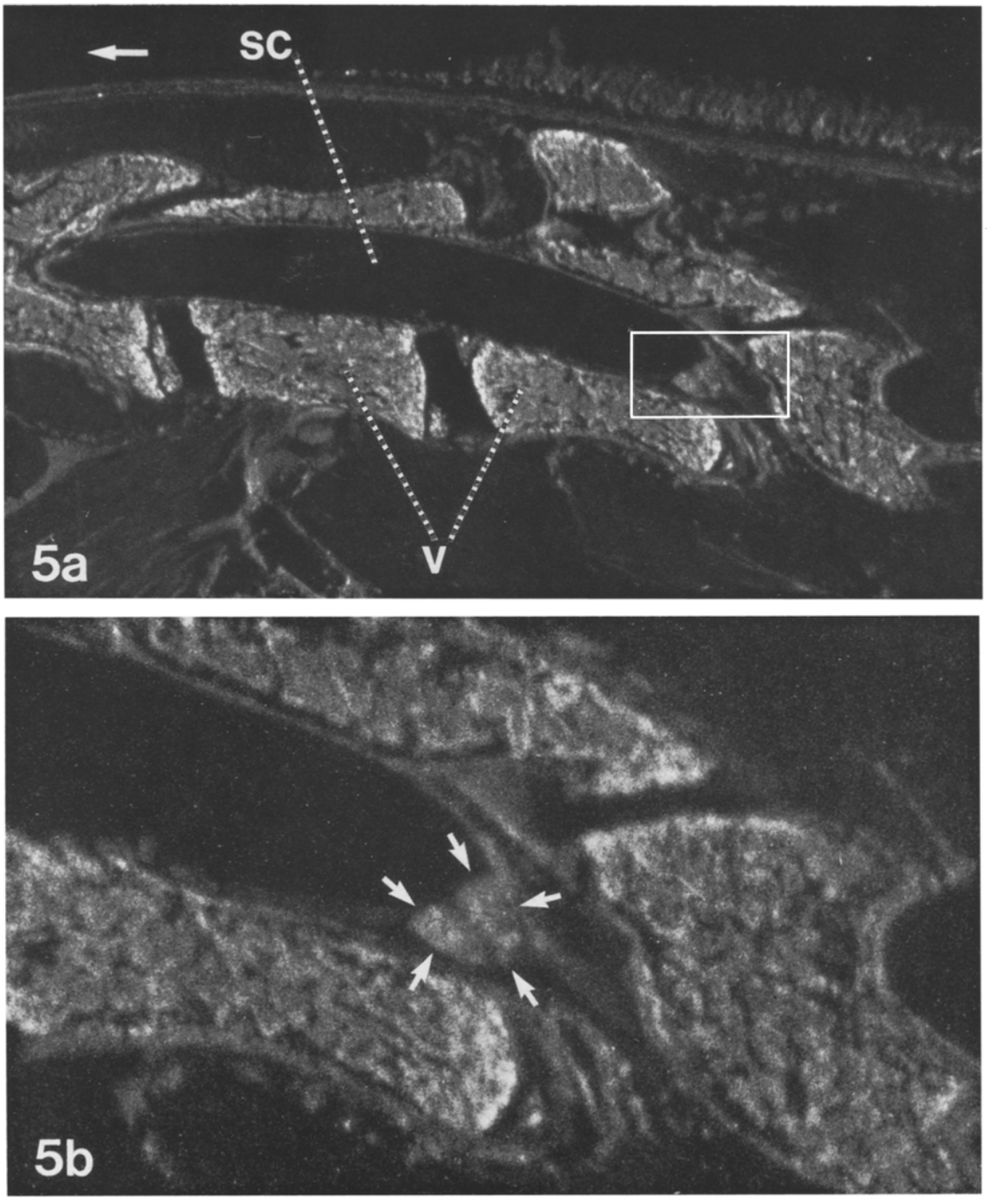

Fig. 5. a Part of whole-body autoradiogram showing a paramedian section passing through the thoracic spinal cord (*sc*) and vertebrae (*v*). The *arrow* points into the cranial direction. Within the area enclosed by a rectangle, the section goes through a dorsal root ganglion. **b** Detail of **a**, showing the dorsal root ganglion (*arrows*). Note that cadmium has accumulated in the ganglion, whereas there is no detectable uptake of the metal in the parenchyma of the adjacent spinal cord

Discussion

After parenteral administration in rodents, cadmium will bind to different proteins of plasma and erythrocytes. From investigations in mice, Nordberg (1972) concluded that during the first hour after injection, the cadmium in both plasma and erythrocytes was bound to proteins with molecular weights corresponding to albumin or larger. Two to 4 days after injection, the cadmium was predominantly localized in the erythrocytes, with only low concentrations in the plasma. At that time, cadmium in erythrocytes

was distributed about equally between relatively large proteins and a small protein of the size of metallothionein. Chen et al. (1975) studied the binding of ^{109}Cd to proteins of the rat plasma and reported that 1 h after s.c. injection, 59% of the cadmium in the blood was found in the plasma, where most of it was bound to a protein with a MW of about 48,000. In another study, the distribution of ^{109}Cd in rat plasma was investigated by gel permeation chromatography. Five hours after s.c. injection, cadmium was associated with a plasma fraction of approximately 77,000 daltons (Gasiewitz and Smith 1976).

The present study has shown preferential accumulation of cadmium in peripheral ganglia and a low uptake of the metal in the brain parenchyma. These findings can be explained by regional differences in vascular permeability, vessels of different regions permitting penetration of different amounts of the protein-bound cadmium into the tissues. Thus, in rats and mice, the blood vessels of peripheral autonomic and sensory ganglia are fenestrated and highly permeable to proteins, such as albumin, horseradish peroxidase, and ferritin (Olsson 1971; Arvidson et al. 1973; Jacobs et al. 1976; Arvidson 1979). In contrast, blood vessels of the CNS are highly impermeable and efficiently restrict the passage of proteins across the vessel wall (Reese and Karnovsky 1967; Rapoport 1976).

The deposition of cadmium in ganglia implies that the ganglionic neurons will be exposed to higher concentrations of cadmium than nerve cells in regions protected by the BBB. To what extent this may lead to damage to the neurons in ganglia is not known at present. However, in tissue culture studies of rat dorsal root ganglia, low concentrations of cadmium in the medium caused degenerative changes in the neurons (Tischner and Schröder 1972). Sato et al. (1978) exposed rats to low concentrations of cadmium in the drinking water for 18–31 months and reported a slight reduction in nerve cells of dorsal root ganglia, with a concomitant increase in satellite cells.

The accumulation of cadmium in the sciatic nerve was significantly greater than that in the CNS or nerve roots. Part of this difference is probably due to uptake of cadmium by the connective tissue sheaths surrounding the nerve. The fact that in rodents the endoneurial blood vessels are more permeable than those of the brain parenchyma (Bradbury and Crowder 1976; Arvidson 1977, 1984; Malmgren and Olsson 1980) may also have contributed to the high uptake of cadmium in the nerve as compared to the CNS.

Although the uptake of cadmium by the parietal cortex was very low, autoradiography showed strong labeling of areas outside the BBB, such as the choroid plexus, pineal gland, and hypophysis. Uptake of cadmium by the hypophysis and choroid plexus has been observed in previous autoradiographic studies in mice (Berlin and Ullberg 1963; Nordberg and Nishiyama 1972), whereas the observation of cadmium accumulation in the pineal gland appears to be new. Studies are in progress in our laboratory to find out whether cadmium also accumulates in other regions of the brain which lack a BBB and to what extent such a concentration of cadmium in certain regions might cause damage to the nervous tissue.

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