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Demonstration of mercury in the human brain and other organs 17 years after metallic mercury exposure

H. Opitz[1], F. Schweinsberg[2], T. Grossmann[3], M.F. Wendt-Gallitelli[4] and R. Meyermann[1]

[1]*Department of Neuropathology,* [2]*Hygiene-Institute,* [3]*Institute of Pathology, and* [4]*Institute of Physiology, University of Tübingen, Germany*

Abstract. A male subject became exposed to metallic mercury vapor at work in 1973. He excreted 1,850 mg Hg/l urine initially. Controls of urine mercury excretion after D-penicillamin administration led to the assumption of a total body clearance of mercury latest since 1976. Subsequently he developed an organic psychosyndrome without detectable signs of classical mercurialism. He never returned to work again and died of lung cancer in 1990. In different organs (brain, kidney, and lung) which were sampled at autopsy elevated levels of mercury were documented by atomic absorption analysis. Histological examination of the tissue by the Danscher and Schroder method, which is specific for mercury, showed a highly positive staining in the majority of nerve cells and cells of other organs. Ultrastructurally mercury could be demonstrated by elemental x-ray analysis within lipofuscin deposits. The lipofuscin content was increased in the mercury positive nerve cells as demonstrated by a strong positive autofluorescence.

Key words: metallic mercury-intoxication – lipofuscin – lysosomal storage – D penicillamin

Introduction

Inorganic mercury poisoning is usually due to occupational exposure. Among all possible mechanisms of exposure, inhalation of Hg vapor is probably the most common and frequent one. Significant occupational exposure to elementary mercury vapor occurs in a variety of industrial branches, for example in the chloralkali industry [Lindstedt et al. 1979], in the manufacture of fluorescent tubes, batteries, instruments, and to a lower extent in dental practice [Akesson et al. 1991]. On inhalation exposure to elemental mercury vapor about 80% of the element is absorbed into the blood [Clarkson et al. 1988]. Most of the absorbed mercury is excreted via urine and feces. Within the different organs kidney and brain are the main targets of mercury deposits.

Estimations of exposure to mercury are often based on determination of the concentrations in blood and urine, which mainly reflect recent exposure. Mercury deposits in the kidneys and the brain, however, were shown to have a very slow turnover [Takahata et al. 1970] and, thus, accumulate during a long period after exposure. Consequently the levels in blood and urine may reflect neither the body burden nor the concentrations within the organs. Some lines of evidence indicate that complexing agents can mobilize only mercury which was stored after recent exposure but do not affect slow body pools or mercury after long-term exposure [Molin et al. 1991].

Here we present a patient who clearly was exposed to mercury vapor for a short time. He never suffered from typical clinical signs of mercury intoxication. Treatment with complexing agents failed to increase renal excretion mercury serum levels, which was interpreted as restitutio ad integrum. Post mortem, however, it could be demonstrated that mercury was still stored within the brain in high amounts which might correspond to the unspecific psychosyndrome and painful sensations of which the patient had suffered since intoxication.

Case report

A male subject, aged 57 at death, had worked for 13 years in the recycling of mercury from amalgams with a mercury content of 1 – 2%. He suffered from an acute exposition of mercury vapor at the age 41. Immediately after this intoxication he excreted 1,850 mg Hg/l urine. After therapy with D penicillamin the excretion declined to 154 mg Hg/l urine 4 weeks later. Initially and in the following years he suffered from tiredness, dizziness, and burning abdominal pain. None of the symptoms, however, could be related to an acute or chronic mercury intoxication. Additionally, a latent diabetes mellitus and an organic psychosyndrome

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Correspondence to Prof. Dr. R. Meyermann, Institut für Himforschung, Calwerstraße 3, 72076 Tübingen, Germany.

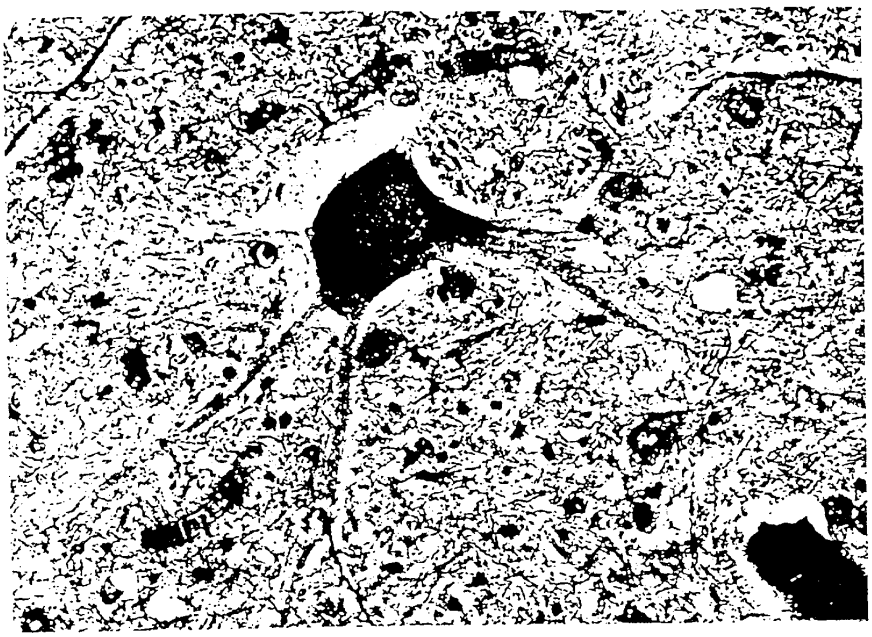

Fig. 1 Anterior horn cell with dense granular deposits in the cytoplasm representing silver-coated mercury deposits. Danscher and Schroder's method counter-stained with hematoxylin. Original magnification × 630.

are described. Several medical certificates, up to 1986, stated that the patient's organs should have been cleared of mercury latest since 1976. Since that time no raise in mercury excretion could be provoked by D penicillamin. He died of lung cancer in 1990 without having returned to work since 1975.

Pathological examination of the brain

The examination of the brain showed a metastase of the known lung cancer in the left parietal lobe. The tumor contained large necrotic areas and was superinfected with fungi. The upper part of the vermis was atrophied. All other cerebellar regions did not reveal any loss of cerebellar granular cells. No loss of neurons in the visual cortex or other changes indicated any previous mercury poisoning when the brain was studied by conventional neuropathological stains.

Histochemical identification of mercury

Paraffin sections (10 nm) were cut from various regions of the brain and other organs including the cerebral cortex, hippocampus, basal ganglia, cerebellar cortex, spinal cord, peripheral nerves, lung, kidney, thyroid gland, cardiac muscle, and liver. After deparaffinization the sections were stained by the silver precipitation method of Danscher and Schroder [1979]. Sections from nervous tissue of corresponding regions of an age-matched male subject without known exposure to mercury were stained as a control.

Quantitative determination of mercury in tissue samples

Samples of formalin-fixed brain, kidney, lung, thyroid gland, heart, liver, and of the fixation medium were treated with HNO_3/H_2SO_4 under pressure at 180° C for 6 hours and analyzed for mercury by flameless atomic absorption spectrometry.

X-ray elemental analysis

Tissue specimens of selected regions (cerebellum and visual cortex) were glutaraldehyde-fixed and processed for electron microscopy by routine methods. Ultrathin sections were cut at approximately 400 nm and mounted on carbon-coated copper grids. Electron-dense deposits and adjacent cytoplasmic areas of these preparations were studied by energy-dispersive x-ray spectral analysis at an accelerating voltage of 100 KV for 1,100 sec with a Link equipment attached to a Philips Electronmicroscope.

Results

Using conventional neuropathological stains no loss of neurons in the visual cortex or in the cerebellum was found.

The Danscher and Schroder method which labels specifically mercury within tissue sections revealed an intense staining of neurons as well as cells of other organs. The staining resulted in black granules, sometimes clustered in black masses in a perinuclear location which is typical for lysosomes and lipofuscin granules. Those neurons which were labeled by silver granules displayed also a strong positive autofluorescence and an increase in lipofuscin content (Figure 6).

The staining intensity varied from one neuron to the other. It was most prominent in neurons of the basal ganglia and the motor neurons of the spinal anterior horn (Figure 1). The cerebral cortex neurons of the occipital lobe displayed a preferential staining when compared to those of the frontal cortex. In the hippocampus the small granule cells of the fascia dentata were not stained whereas the

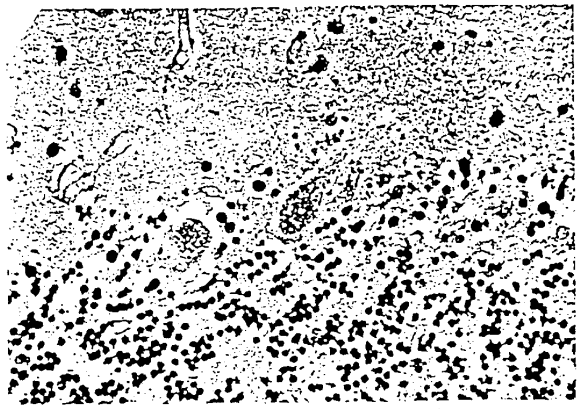

Fig. 2 Cerebellar cortex. Fine granular deposits of silver coated mercury are seen in the cytoplasm of Purkinje cells, granular cells and Bergmannglia-cells. Method see Figure 1. Original magnification × 250.

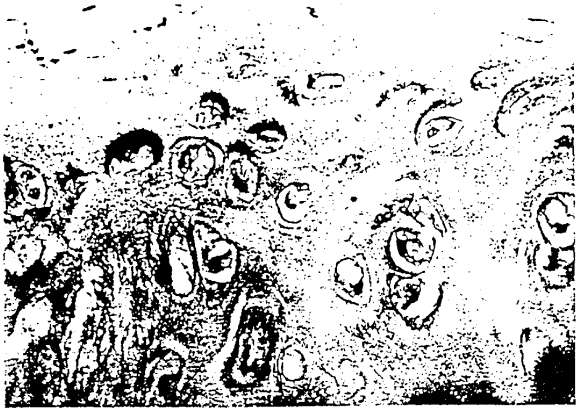

Fig. 3 Hyaline cartilage of the bronchiolarsystem. Perinuclear staining in chondrocytes and in the extracellular matrix. Method see Figure 1. Original magnification × 250.

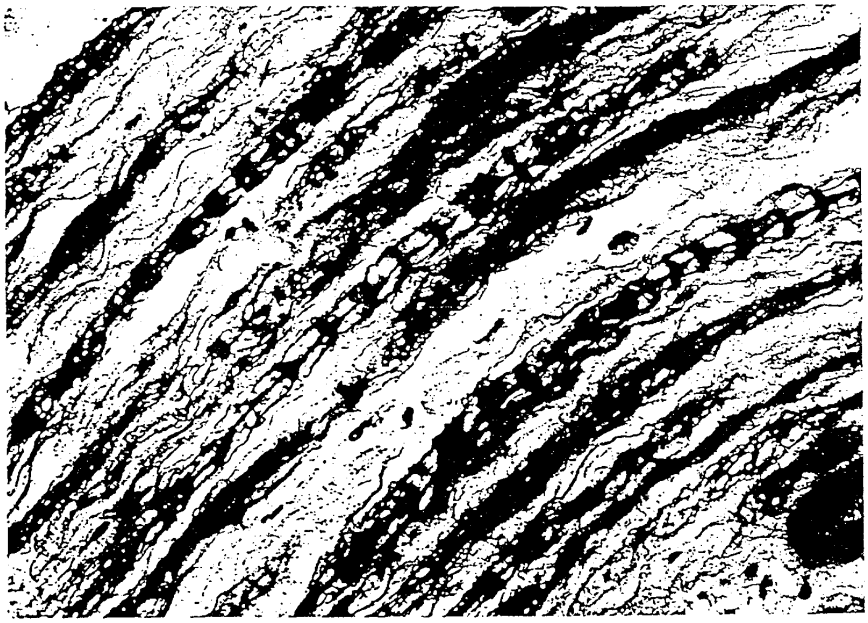

Fig. 4 Peripheral nerve. Dark precipitates in the Schwann cell cytoplasm and intra-axonal. Danscher and Schroder's method × 250.

pyramidal cells were weakly positive. Within the Purkinje cells of the cerebellum fine granules were stained positively for mercury. The distribution of those granules extended sometimes to the dendrites (Figure 2). The granule cells and the Bergmann glia contained positively stained material within their scanty cytoplasm. Darkly stained deposits were also found in neurons of the brain stem.

Within spinal nerve roots the reaction product could be located within the cytoplasm of Schwann cells but was also found intraaxonal (Figure 4).

In tissue sections from unexposed control subjects nothing was stained which was also true for lipofuscin deposits.

Cells which were labeled in other organs are summarized in Table 1.

Electron microscopy with x-ray elemental analysis confirmed the metallic mercury within lipofuscin deposits (Figure 6). Adjacent cytoplasmic areas being free of lysosomes served as negative controls.

Chemical analysis

Table 2 lists the mercury concentrations in different brain areas and other examined organs.

Table 1 Cells which were labeled in other organs

Kidney	tubular cells
Liver	von Kupffer cells (Figure 5)
Lung	perivascular connective tissue, some bronchiolar chondrocytes (Figure 3)
Thyroid gland	epithelial cells
Heart	no staining

In comparison to mercury concentrations in different organs of the general population without known mercury exposure (Table 3) our figures show a marked increase in brain especially cerebellum, kidney, and lung.

Discussion

Three remarkable facts have to be stressed in the demonstrated case report and will be discussed in detail:

- Although the patient was exposed to high concentration of metallic mercury vapor which resulted in an excretion of 1,850 mg Hg/l urine he never developed so-called typical clinical signs of mercurialism.
- The Danscher and Schroder method is a very sensitive qualitative method to detect Hg deposits in the tissue of patients who were challenged with metallic mercury even a long time ago (17 years in the presented case). The method also detects bound deposits.
- Stimulation of the Hg excretion by complexing agents seems not a sufficient approach to evaluate whether or not an individuum has been restituted from a Hg intoxication.

Comment

The present case contrasts to the case reported by Hargreaves et al. [1988] because of the absence of typical mercurialism symptoms after the acute exposure to metallic mercury vapor, although the mercury contents in the organs of both cases are similar. The presented patient suffered from tiredness, dizziness, and burning abdominal pain, and showed signs of an organic psychosyndrome and a latent diabetes mellitus. Elevated glucose tolerance curves or mild glucosuria are known to occur in the sequence of metallic mercury poisoning [Kark 1979]. There is evidence that the onset of symptoms of inorganic mercury poisoning may be delayed up to 30 years [Chang 1980] which depends on the frequency and the dose of exposure. Thus, the present case is surely within the range of variable symptoms described as a consequence of inorganic mercury intoxication and the missing of typical mercurialism may be due to the short time of exposure to high mercury concentrations. Spinal root ganglia have been shown to store preferentially inorganic mercury and other heavy metals [Schionning et al. 1991, 1993].

Unfortunately we could not check spinal root ganglia for mercury storage. The alterations of sensory function like the described painful abdominal sensations, however,

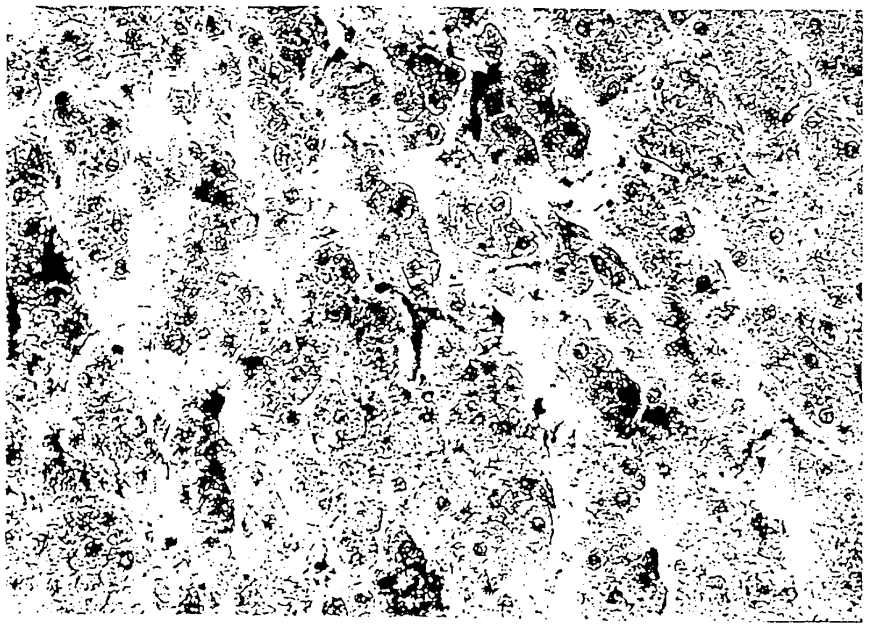

Fig. 5 Liver. Dark granular deposits in the cytoplasm of a Kupffercell. Danscher and Schroder's method × 630.

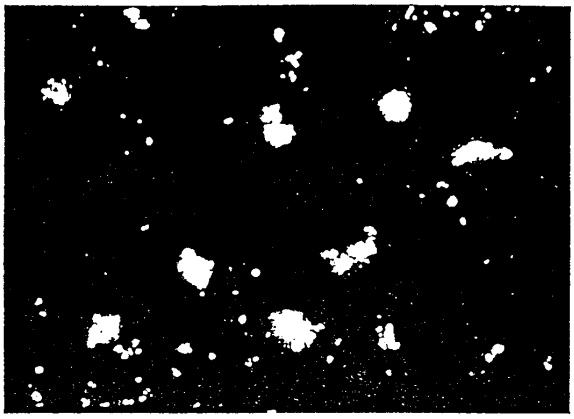

Fig. 6 Cortical nerve cells showing a strong autofluorescence with UV-light. Original magnification × 250.

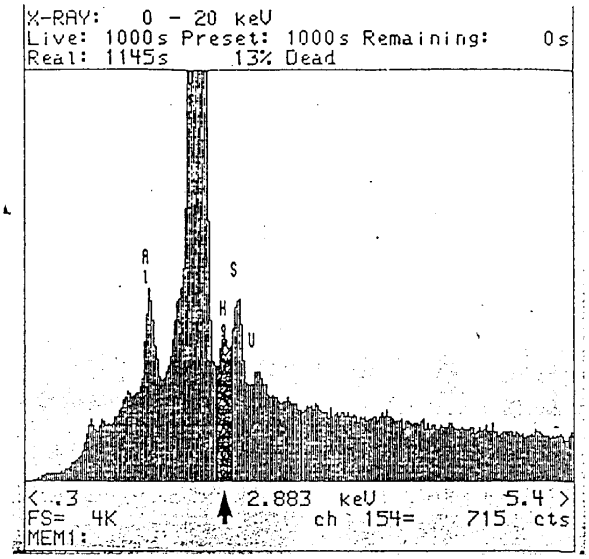

Fig. 7 Elemental x-ray analysis of a lipofuscin deposit showing a peak for mercury (Hg). The large peak for osmium is due to the osmification of the tissue in the routine electronmicroscopic procedure. In an adjacent area of cytoplasm free of lipofuscin deposits no mercury could be detected (not shown).

Table 2 Mercury concentrations (µg/kg w.w.) in different brain tissues and in other organs

Organ	Mercury concentration (µg/kg weight)
Brain	
cerebellum	2,190
occipital	1,090
thalamus	1,010
putamen	250
caudate nucleus	110
Kidney	1,650
Lung (tumor-free)	600
Lung (tumor)	< 20
Liver	70
Thyroid gland	250
Formalin (µg/l)	0.6

may account for the presence of mercury. Alterations of sensory function after chronic exposure to elemental mercury have been confirmed by others (e.g. [Albers et al. 1988]).

The sensitive and specific silver precipitation method [Danscher and Schroder 1979], which is also called autometallography [Danscher 1984], is known to demonstrate mercury bound to both sulphur and selenium in mammalian tissue [Danscher and Moller-Madsen 1985]. By this method combined with electron microscopy it is shown that mercury deposits are exclusively located in lysosomes of neurons, astrocytes, endothelial cells, and ependymal cells [Schionning and Moller-Madsen 1991]. The lysosomal storage of mercury by CNS cells fits with a general pattern for heavy metal accumulation. Experimentally, lysosomal uptake has been demonstrated for copper, mercury, lead, iron, aluminum, and tellurium in brain and other tissues (reviewed by [Braheny and Lampert 1980]). If the intralysosomally accumulated mercury alters the function of this organelle or if it is merely an inert endproduct of a detoxification process is still debated. The present case raises the question about the pathomechanism of lipofuscin accumulation in nerve cells without concomitant loss of neurons which is a prominent feature in neuronal ceroid-lipofuscinosis. On the one side intralysosomally accumulated mercury might be able to induce an increase of lipofuscin content possibly by inactivating lysosomal enzymes which is also known for tellurium [Duckett and White 1974]. On the other side the number of lysosomes might increase as a consequence of decreased lysosomal function as described by Yip and Chang [1981] after methyl-mercury poisoning. We stained samples of juvenile ceroid-lipofuscinosis with the Danscher-Schroder method without any positive labeling of the lipofuscin containing cells. This control demonstrated that an increased lipofuscin storage by other reasons is not linked with the storage of mercury.

80% of inhalated elemental mercury vapor enters the circulating blood. Within the blood the initial distribution

Table 3 Mercury concentrations (µg/kg w.w.) in different organs of the general population as given in the recent literature

Organ	Nylander 1991	Bauer 1989 (n = 48)	Schiele 1981 (n = 51)	own observations (n = 34)
Cerebellum			8 (< 5 – 229)	
Cerebrum	11 (2 – 23) (n = 17)	5 (0.5 – 16)	6 (< 5 – 17)	
Kidney	280 (22 – 808) (n = 12)	250 (14 – 1,300)	78 (< 2.5 – 453)	290 (30 – 2,600)
Lung				40 (20 – 100)
Liver			78 (8 – 458)	110 (50 – 400)

of mercury is about equally distributed in both plasma and erythrocytes. A large fraction of the absorbed mercury is excreted in the urine and feces. The rest is mainly deposited in kidney and brain. It is believed that the initial blood level after mercury intoxication reflects the dose to which the organism was exposed. Mercury in both the kidney and the brain, however, is very slowly cleared from the tissue [Takahata et al. 1970]. Thus, the storage of mercury can accumulate during chronic exposure. Consequently, the levels in blood and urine can reflect neither the body burden nor the concentrations within the critical organs. Molin et al. [1991] have demonstrated that mercury which is mobilized by a single dose of complexing agent mainly reflects the release of mercury from readily accessible body pools. The output from pools with a slow turnover rate is yet concealed. The same findings are confirmed by the present case. The treatment with the complexing agent D penicillamin has appearently not cleared the affected organism from the poison as demonstrated by high mercury concentrations in brain, kidney, and lung. Therefore, medical certificates cannot be based on the assumption that the absence of a detectable increase in urine mercury excretion after treatment with complexing agents signals a completed elimination of accumulated body mercury.

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