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PERSISTENT MERCURY IN NERVE CELLS 16 YEARS AFTER METALLIC MERCURY POISONING

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Persistent mercury in nerve cells 16 years after metallic mercury poisoning

A male subject, after exposure to mercury metal at work in 1968, developed classical signs of mercurialism from which he made a slow clinical recovery. He subsequently developed psychoneurotic symptoms and became an alcoholic; he never returned to work and died in 1984. No histological changes relevant to mercury intoxication were found in the brain, but staining by Danscher & Schroeder's method for mercury showed many positively staining lysosomal dense bodies in a large proportion of nerve cells, and the presence of mercury was confirmed by elemental X-ray analysis. The mercury content of the brain was increased, much of it being present in colloidal form.

Introduction

Poisoning by inorganic mercury in the majority of cases is considered to be reversible on removal from the source of intoxication (Hunter, 1959) but in severely affected cases the symptoms and signs of mercurialism may persist (Teleky, 1955; Takahata *et al.*, 1970). In the few reported cases studied post-mortem, no significant structural changes have been noted (Brigatti, 1949) with the exception of the unique cases of chronic calomel poisoning reported by Davis *et al.* (1974). It is of some interest, therefore, to record a case of metallic mercury poisoning in whom excess mercury was found in the tissues at post-mortem study 16 years later. Although there was no histological evidence of mercury toxicity, staining by Danscher & Schroeder's (1979)

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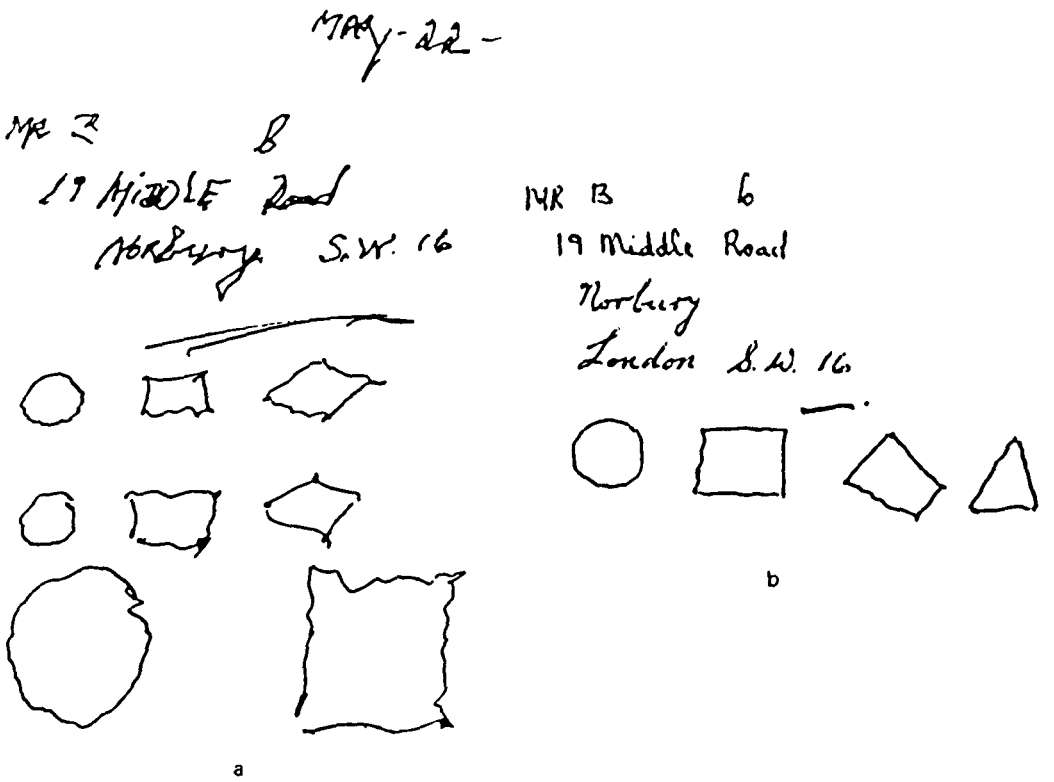


Figure 1. Examples of the handwriting and drawing of the patient B.C.. **a**, shortly after admission to the National Hospital, Queen Square and **b**, 6 months later. Note the substantial improvement of the tremor.

method revealed positive material in many nerve cells, confirmed by X-ray analysis to be mercury.

Case report

B.C., a male aged 50 at death, had worked for about 18 months filling mercury thermometers before he was seen at the National Hospital, Queen Square, by Dr K. Zilkha in April 1968. He presented complaining of tremor in both hands, drowsiness, constipation and a foul taste in the mouth. His tremor had insidiously become worse over the previous 3 months having begun in his hands and then spread to involve his legs and later his whole body. The tremor was worse in the company of others and when he was excited, his speech was tremulous and writing became increasingly difficult (Figure 1). On examination, the protruded tongue was tremulous. There was marked tremor of the outstretched hands, dysarthria, and titubation, but no sensory disturbances or motor weakness. There was a blue discoloration of the inside of the lower lip and of the lower gum.

The urine contained 1015 μg of mercury in a 24 h specimen (1400 ml) on the day of admission and 406 μg in a 24 h specimen (1120 ml) on the second day. The blood mercury content was 48·1 μg/100 ml. Electroencephalogram examination showed mild diffuse non-specific abnormal activity.

Despite treatment with *N*-acetyl penicillamine, he was slow to recover, and by August 1968 he still showed tremor, poor concentration and an intellectual deficit, but his handwriting had improved considerably (Figure 1a & 1b). He was discharged in November 1968, though he continued to complain of forgetfulness and of having odd ideas but when seen in May 1969 his tremor had greatly improved. He was never subsequently employed, became increasingly alcoholic and he stated that he suffered from epileptic fits for which he was given treatment. In August 1984, he was brought in dead to King's College Hospital, Denmark Hill, having collapsed at home.

POST-MORTEM EXAMINATION (DR S. CORDNER, GUY'S HOSPITAL)

The lungs contained inhaled gastric contents. Superficial scars of old contusions were present in the temporal lobes, and on cutting the brain slight ventricular dilatation was seen. Histological examination of the brain using conventional neuropathological stains did not reveal any loss of cerebellar granular cells or other changes to suggest previous mercury poisoning.

HISTOCHEMICAL IDENTIFICATION OF MERCURY

Paraffin sections (10 μm thick) were cut from various regions of the brain, including the cerebral cortex, hippocampus, cerebellar cortex, spinal cord and spinal ganglia. After dewaxing, the sections were stained by the silver precipitation method of Danscher & Schroeder (1979) and control sections from nervous tissue of an age-matched male with no known exposure to mercury were stained in parallel.

X-RAY ELEMENTAL ANALYSIS

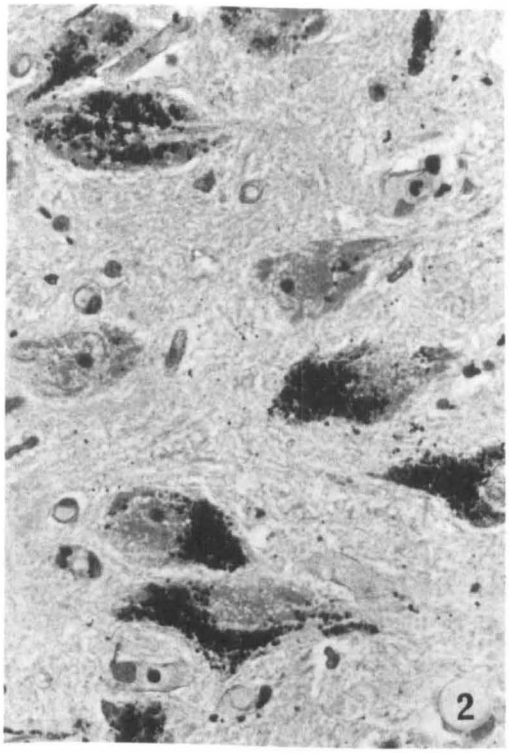
Attempts to detect mercury by this method in unstained paraffin sections were not successful. Paraffin sections stained by the Danscher & Schroeder method were therefore used. They were processed conventionally for electron microscopy and ultrathin sections were then cut at approximately 400 nm and mounted on copper grids supported by a carbon film. These thin sections were analysed over positive and negative staining areas using a JEOL 100 CX instrument fitted with KEVEX 700-Q EDAX at an accelerating voltage of 100 kV for 100 s and employing a beam of less than 10 nm.

CHEMICAL ANALYSIS

Portions of fresh kidney, liver and brain taken at post-mortem examination were initially analysed for mercury by atomic absorption spectrometry at the Poisons Unit, Guy's Hospital, and subsequently the total and colloidal state of the mercury was determined at the Toxicology Unit, Carshalton by the method of Magos *et al.* (1984).

Results

In the various brain sections stained by the Danscher & Schroeder method, many nerve cells in all regions examined contained abundant black granules about the size of lysosomal dense bodies, often occurring where lysosomes and lipofuscin granules are normally found (Figures 2–5). The amount in each cell varied considerably, depending partly upon the plane of the section, but they seemed to be most abundant in neurons of the substantia nigra. In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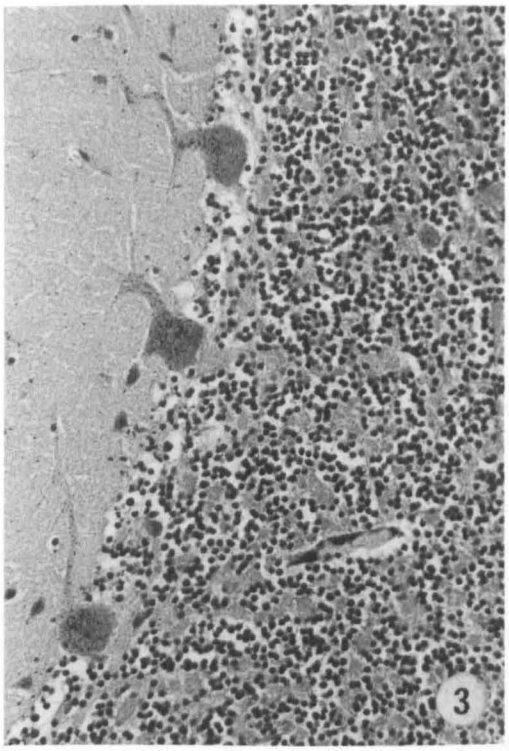
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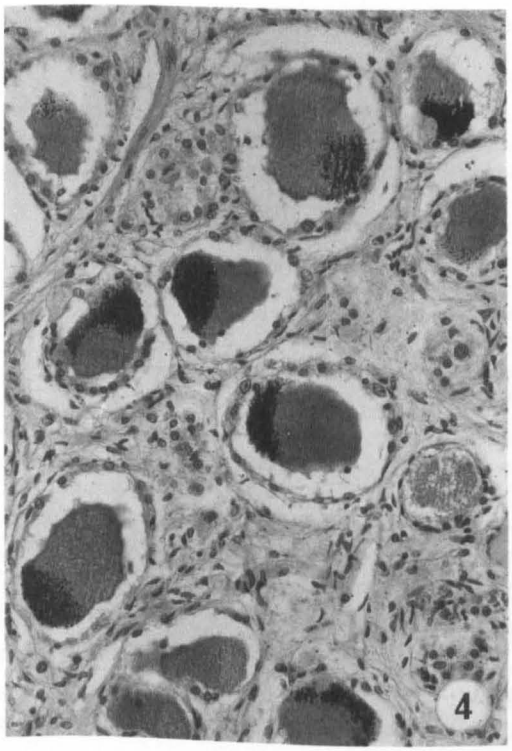
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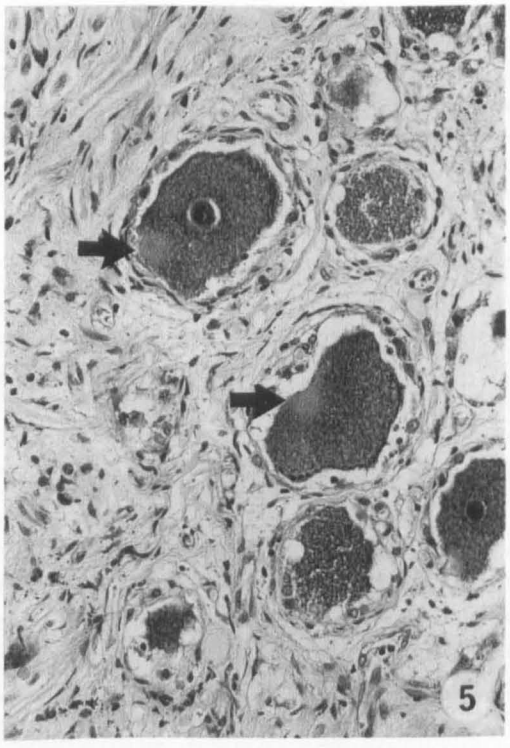
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Table 1. Mercury concentrations in fresh organ samples taken at post-mortem examination

	Mercury concentrations (ppm)		
	Laboratory 1	*Laboratory 2*	
Organ	*Total Hg*	*Total Hg*	*Colloidal Hg*
Kidney	2·6	1·73	0·87
Liver	0·04	0·06	<0·01
Brain			
Occipital	4·3	2·23	1·00
Temporal	1·09	0·97	0·35
Cerebellum	1·16	0·93	0·90

the cerebral cortex, some large pyramidal neurons contained considerable quantities while in others it was scanty or absent. In small neurons, there were frequently a few granules. The small cells of the dentate fascia appeared to be free of stained material, as were the hippocampal pyramidal cells, except for a few heavily stained large cells in the H_5 region. In the cerebellum, the cytoplasm of most Purkynë cells had a fine sprinkling of black granules that often extended into the dendrites. Granule cells generally showed small numbers of black grains in their cytoplasm. Large neurons in the brain stem and in the anterior horns of the spinal cord either showed dense clusters of black granules or a fine sprinkling of granules throughout the cytoplasm; very few had none at all. Neurons of spinal ganglia usually had large clusters of black granules. Satellite cells were unstained, but small black granules were constantly seen in the perinuclear cytoplasm of peripheral nerve Schwann cells. Small numbers of black granules were constantly present in many astroglial cells and in cells associated with vessel walls. No deposits of silver were seen in sections from the unexposed control subject, even though lipofuscin granules and lysosomal dense bodies were readily visible (Figure 5).

Figure 2. Neurons of the substantia nigra. Note the dense precipitate in the majority of cells. Small black granules are also visible in glial cell cytoplasm in the neuropil. Danscher & Schroeder's method counterstained with haematoxylin. ×475.

Figure 3. Cerebellar cortex. There is a fine black precipitate in the perikarya and dendrites of the Purkynë cells. Method as Figure 2. ×275.

Figure 4. Dorsal root ganglion cells showing clumps of dense black particles lying to one side of each cell. Virtually no precipitate is present in the intervening connective tissue. Method as Figure 2. ×475.

Figure 5. Dorsal root ganglion from a control subject not known to have been exposed to mercury. No precipitate present. Arrows point to clusters of lipofuscin granules. Danscher & Schroeder's method. ×475.

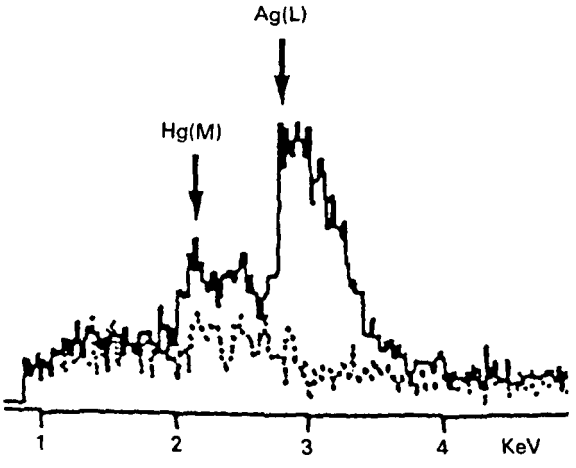

Figure 6. X-ray microanalysis trace (solid) from a region of a nerve cell containing precipitate showing a peak for mercury (Hg (M)) and a larger peak for silver (Ag (L)): background levels are shown by the dotted trace from an adjacent unstained area of cytoplasm.

Electron microscopy confirmed that all the electron dense silver deposits of stain lay within lysosomal dense bodies, and X-ray elemental analysis showed peaks typical for silver over positive areas; in addition, a small peak at 2·2 KeV was also present consistent with the M peak of mercury (Figure 6).

CHEMICAL FINDINGS

Table 1 lists the mercury concentrations in kidney, liver and in different brain areas. Differences in the findings in the two laboratories are negligible for liver, cerebellum and temporal cortex, but the second laboratory found 33% less mercury in the kidney and 48% less in the occipital cortex than had been found in the first. Since tissue pieces and not homogenates had been received by each for analysis, uneven tissue distribution may have been responsible for these discrepancies. Table 1 also shows that most of the deposited mercury was in colloidal form, most likely as HgSe or HgS.

Discussion

The present case stands in marked contrast to the two examples of chronic calomel poisoning reported by Davis *et al.* (1974). Our present subject had been exposed to elemental mercury vapour for 18 months. He suffered from mercurialism from which he slowly recovered after exposure had ceased and he died 16 years later. The two reported subjects of Davis and colleagues (1974) had taken calomel for constipation daily for 6 and 25 years respectively from which they developed mild ataxia and dementia, in addition to erethism and intention tremor. The concentrations of accumulated mercury in their kidneys (42 and 25 ppm) and in liver (one patient 25 ppm) were considerably higher, and in the brain (3·3 to 6·0 ppm in different cortical brain areas) only slightly higher than the figures for the present case. A common feature between our case and the first calomel victim was that a substantial

Table 2. Reported 'normal' tissue concentrations of mercury in relevant organs from research groups working in North America

	*Mercury concentrations (ppm)**			
Organ	*Group 1*	*Group 2*	*Group 3*	*Group 4*
Kidney	0·76 ± 1·20 0·006–6·40 (95)			0·76 0·075–3·36 (20)
Liver	0·25 ± 0·29 (0·008–1·43) (95)			0·17 0·026–0·545 (20)
Brain				
Cerebrum	0·081 ± 0·105 0·008–0·47 (61)			
Occipital lobe		0·30 ± 0·45 0·08–1·69 (12)	0·054 ± 0·062 <0·008–0·220 (11)	
Temporal lobe		0·24 ± 0·48 0·04–1·72 (12)	0·039 ± 0·028 0·007–1·20 (11)	
Cerebellum	0·13 ± 0·194 0·006–0·96 (60)	0·44 ± 0·53 0·08–1·85 (12)	0·10 0·038,0·160 (2)	

Location of groups: Group 1, Seattle (Wa) (Mottet & Body, 1974); Group 2, Buffalo (NY) (Olzewski *et al.*, 1974); Group 3, Northern Ontario, Canada (Smith & Bewcastle, 1977); Group 4, Denver and Los Alamos (Stein & Moss, 1974).

*Values are means ± SD and the range. Figures in parentheses are the number of samples.

proportion of the deposited mercury was found to be in colloidal form (Magos, 1980).

At post-mortem examination of the calomel-poisoned cases, diffuse loss of granule cells from the cerebellum was found. Timm's stain for mercury was positive in neurons of the inferior olives and dentate nuclei, but less so in Purkynë cells, substantia nigra and anterior horn cells. While this stain is not specific for mercury, chemical analysis noted above also suggested retention and storage of mercury in neurons of many regions, although the intoxication was of an unusually chronic kind.

As far as the concentrations of mercury are concerned, the first question is how far the values for our case shown in Table 1 relate to normal values for mercury in the tissues. Unfortunately, there are no established normal values for UK residents and therefore we must refer to tissue concentrations reported from other regions for comparison. Selection of data has been based upon comparability of blood mercury levels. For example, since mercury concentrations in the blood of people in Japan with no recorded

occupational exposure to mercury are about six times higher (Suzuki *et al.*, 1971; Fujita & Takabatake, 1977) than those of UK residents (Haxton *et al.*, 1979), tissue mercury concentrations published from Japan (Yukawa *et al.*, 1980) are not suitable for comparison. Furthermore, since the mean blood mercury concentrations reported from the USA (Magos & Clarkson, 1972; Baglan *et al.*, 1974; Pitkin *et al.*, 1976) and Canada (Dennis & Fehr 1975) range from 1·5 to 8·7 ng/ml and are comparable with the 3·5 ng/ml found in residents along the English Channel (Haxton *et al.*, 1979), North American tissue mercury levels offer the best comparison, with the following two provisos. The first is that post-mortem material is usually derived from hospital populations and may not, therefore, be representative of the general population, and secondly no attempt is usually made to trace an occupational history of mercury exposure from the subjects, though most studies have excluded the possibility of the subjects receiving mercury therapeutically. Comparison of the data in Tables 1 and 2 shows that our patient had higher mercury concentrations in his kidneys and brain, and lower liver levels, than the mean 'normal' values. As individual values are available from the first three groups in Table 2, it is possible to derive frequency distribution curves for liver and kidneys from group 1 data and an integrated frequency distribution curve for the CNS based on data from all three sets of data. When mercury had been estimated in more than one region of the CNS the mean was used.

The derived frequency distribution curve for liver indicates that 0·04 to 0·06 ppm is at the lower end of the population values since only 15% of the individuals fall below 0·05 ppm, while 58% lie above 0·10 ppm. In contrast to liver, the 1·7 or 2·6 ppm renal mercury concentration in our subject is at the higher end of the curve since only 21% of the population values are above 1·0 ppm, 9% above 2·0 ppm and 6% above 3·0 ppm. Concentrations in the brain reached or exceeded the upper limit of the 'normal' population values as only 2% of normal individuals exceeded 1·0 ppm while none was greater than 2·0 ppm.

Thus the mercury content of our subject's brain was undoubtedly high by comparison with figures from North America. This, taken with the histochemical evidence, confirmed by X-ray analysis, suggests that most of the mercury present in the brain had reached the organ in the lipid soluble and diffusible elemental form and had become oxidized there. This apparently resulted in extensive subsequent sequestration and storage in lysosomal dense bodies, and perhaps elsewhere within neurons. Our case is thus similar in many ways to the two cases reported by Takahata *et al.* (1970). These were mercury miners, exposed for 5 and 10 years respectively, with clinical signs of mercurialism that continued until they died more than 10 years after exposure ceased. The Japanese authors also found the mercury content of nervous tissue was increased in amounts comparable to our case; the greatest concentrations were in the occipital lobe and the substantia nigra.

Localization in the occipital lobe is of some interest because of the extremely high density noted above of small neurons in the occipital region by comparison with the so-called 'agranular' cortex, such as frontal and parietal regions. However, this propensity for small neurons to accumulate mercury did not appear to hold true for the cerebellar cortex. We also found histochemically that substantia nigra neurons appeared to show the greatest quantities of stainable material by Danscher & Schroeder's method. The reason for this is unknown.

From the present findings and those of Takahata *et al.* (1970), it seems clear that residual mercury, as detected by the sensitive and specific silver precipitation technique (Danscher & Schroeder, 1979), is mostly, if not entirely, confined within lysosomal dense bodies and presumably has remained sequestered there from shortly after the time of its first entry into the tissue. It is possible that there has been some slow expulsion of mercury from neurons to glial cells, and indeed many cells appeared not to contain any deposits at all. But, in view of the amounts still remaining in other cells 16 years after exposure, any removal of this sequestered material must have been very slow, and it must be assumed to be securely bound and unlikely to affect neuronal function adversely. Whether the subsequent mental state of this individual was in any way related to the mercury in his nerve cells is impossible to decide, since his mental and psychological state before exposure was unknown. Only further studies might clarify this important point.

Acknowledgements

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