

AUTOMETALLOGRAPHIC TRACING OF MERCURY IN PILOT WHALE TISSUES IN THE FAROE ISLANDS

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ABSTRACT

Objectives. Autometallography (AMG) was applied for tracing mercury in long-finned pilot whales (*Globicephala melas*) harvested in the Faroe Islands.

Results. Ample mercury accumulation was found in kidney tubules, in contrast to the largely unstained glomeruli. Hepatocytes, as well as liver macrophages, exhibited high mercury uptake. The muscle tissue accumulated only scant amounts of mercury, primarily around the nuclei of the striated muscle cells. At the ultrastructural level, mercury was found to accumulate intracellularly in lysosomes, and extracellularly in the basement membranes of vessels. The results were verified by proton induced X-ray emission (PIXE) analysis, and it was established that the tissue contained no other AMG traceable metals.

Conclusion. The use of AMG analysis on autopsies and biopsies is suggested as a tool for evaluating mercury pollution.

Keywords: Heavy metals, Silver enhancement, Pilot whales

INTRODUCTION

In the Faroe Islands, the long-finned pilot whale (*Globicephala melas*) has been harvested for centuries in a drive-fishery which still provides an important supply of meat and blubber for human consumption (1). The pilot-whaling is a drive fishery (2) where entire schools sighted, from land or from boat, in the vicinity of the islands are driven ashore by small fishing boats under the guidance of fore-

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men, and dispatched on the beach by a group of men waiting there. Therefore, pilot-whaling has always been a well organized and social event. Today, the hunt is still communal and non-commercial in character, and meat and blubber are shared freely amongst the local inhabitants; there is no factory processing. The pilot-whaling catches whole schools, offering scientists one of the very few possibilities in wild game biology to study both sexes, all ages and sexual stages from the same date and the same school. A detailed description of this whale hunt, known as a *grind* in Faroese, is given in (2). This type of whaling has no special whaling boat, or whaler, and the share is free. The hunt is sustainable and done in a humane way as agreed by the North Atlantic Marine Mammal Commission (NAMMCO) and the International Whaling Commission (IWC).

It has been suggested that the high uptake of mercury in long-finned pilot whales might cause adverse effects in the whales, i.e. be directly damaging to their capability of surviving (3-6). Furthermore, the consumption of foodstuffs containing high levels of mercury may be hazardous to humans, as methyl mercury is a well-established neurotoxicant that can have serious adverse effects on the development and function of the human central nervous system, especially when the exposure occurs prenatally (7, 8). A Faroese birth cohort of children prenatally exposed to methyl mercury from maternal consumption of pilot whale products was established in 1986-87. The observed median umbilical cord blood-mercury level was 24.2 µg/l (8, 9), whereas the estimated umbilical cord mercury level for diseased Minamata children is about 216 µg/l (10). In seven-year-old children from the Faroese birth cohort, mercury dose-related deficits in attention, language, and memory have been observed (11), leading to a decrease in the recommended exposure limits for mercury, e.g. only 6 gram of pilot whale meat per day for a woman weighing 60 kg.

The autometallographic (AMG) technique for tracing mercury is based on the capability of small clusters of mercury sulphide/selenide molecules to catalyse an AMG silver amplification. It has been demonstrated that potassium cyanide (KCN) cannot dissolve mercury sulphide/selenide clusters in tissue sections while all other AMG traceable metals will be dissolved (12-15). Still, the chemical differentiation between different AMG metals has to be verified by PIXE, or by another multi-element technique if it cannot be excluded that other AMG metals are present in the tissues. The sensitivity of

the HgS/Se^{AMG} technique surpasses any other known technique for mercury detection (16).

The purpose of the present study was to apply the AMG method to tissues from pilot whales, providing information on cellular and subcellular localisations of mercury.

MATERIAL AND METHODS

On July 20th, 2000, a group of 36 pilot whales was driven to the authorized whaling bay of Húsavík on Sandoy, in the Faroe Islands. The whales were beached and dispatched immediately. Tissue samples from ten whales were collected 1-3 hours after the killing. The kidney, liver and muscle tissue samples were immersed in phosphate-buffered formalin immediately after excision and were post-fixed for at least an additional two days at room temperature.

Fixed tissue blocks were placed in 30% sucrose overnight before being frozen with gaseous CO₂. Tissue sections, 30 µm thick, were cut on a cryostat. The sections were placed on Farmer-rinsed glass slides that had been dipped in gelatin (0.5% gelatin + 0.15% chromalun) and allowed to dry at room temperature. Finally, the sections were fixed (15 min in 96% ethanol, 2 min in 70% ethanol, 2 min in 50% ethanol), rinsed in distilled water 3×2 min, and coated with gelatin.

Small tissue blocks were made and embedded in Epon. Sections (3 µm) were cut and placed on Farmer-rinsed glass slides that had been dipped in a 0.5% gelatin solution and allowed to dry before use.

The slides were placed in a jar and the AMG developer poured onto them. The jars were then placed in a water bath set at 26°C and the whole set-up was covered by a hood. During development, the jars were shaken gently by an electric device. All sections were developed for 60 min. The AMG development was stopped by replacing the developer with a 5% sodium thiosulphate solution for 10 min and rinsing in distilled water for 30 sec (for details see below).

The silver lactate AMG developer

Preparation of AMG solutions

I Protective colloid: Dissolve 1 kg of non-refined acacia resin (crude gum arabic, also used by the liquorice industry) in 2 l deionized water by intermittent stirring over 5 days at room temperature (20°C). Filter the solution through layers of gauze and freeze suit-

able portions of the resulting colloid in plastic jars. Such jars can be stored for at least 1 year.

II Citrate buffer (pH 3.7): Dissolve 25.5 g citric acid monohydrate and 23.5 g sodium citrate dihydrate in deionized, or distilled water to make 100 ml.

III Lactate buffer (pH 3.8): Dissolve 31.5 ml of 50% sodium lactate and 6 ml of 90% lactic acid in deionized, or distilled water to make 100 ml.

IV Hydroquinone: Dissolve 0.85 g hydroquinone in 15 ml deionized, or distilled water. Prepare just before use.

V Silver ion supply: Dissolve 0.11 mg silver lactate in 15 ml deionized water. The solution should be protected from light by wrapping tin foil around the vial.

VI AMG stop bath: A 5% sodium thiosulphate solution.

VII Farmer solutions: 10% (9 parts 10% sodium thiosulphate and 1 part 10% potassium ferricyanide) for cleaning of glass ware. 1% (9 parts 1% sodium thiosulphate and 1 part 1% potassium ferricyanide) for cleaning of the section surface.

Finally, mix solution I (60 ml), solution II or III (10 ml), and solution IV (15 ml), carefully in a 100 ml Farmer cleaned beaker. Add solution V immediately before use (for details, see 16, 17).

EM re-embedding technique

Semi-thin Epon sections are placed on a glass slide and AMG developed. After thorough rinsing of the section surface, place a drop of unpolymerized resin on the semi-thin section to be studied in the electron microscope, and place a blank resin block on top. After 24 hr at 60°C, remove the block from the glass slide by placing the preparation on a 90°C hot plate for about 30 sec. After trimming, cut ultrathin sections and place on a grid.

RESULTS

The HgS/Se^{AMG} technique visualises mercury as brown, or black AMG silver grains (Figs 1, 2). Histochemical analyses demonstrate that mercury accumulated massively in kidney tubules, in contrast to the largely unstained glomeruli (Figs 1A and B). The hepatocytes, as well as the liver macrophages, exhibited high mercury uptake (Figs

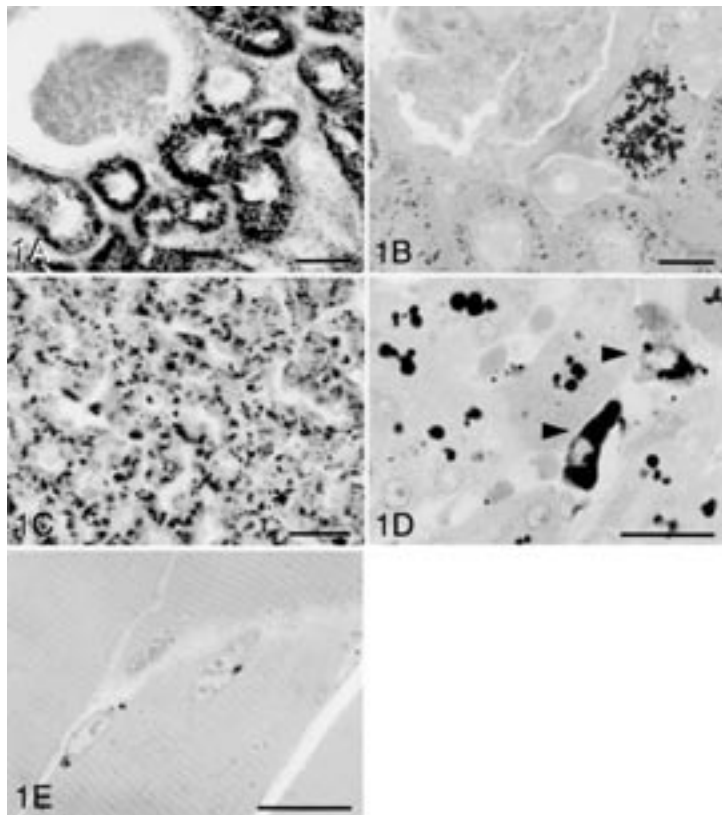


Fig. 1. Sections were AMG-developed for 60 min and counterstained with toluidine blue. A: Kidney cryo-section; 30 μm thick. Kidney tubules exhibit high Hg^{AMG} staining. Glomeruli are almost unstained. Scale bar = 50 μm . B: Kidney epon section; 3 μm thick. Same staining pattern as A. The apparent difference in staining intensity between 1A and 1B is a consequence of the thickness of the tissue sections. Scale bar = 30 μm . C: Cryo-section; 30 μm thick. The liver contains large deposits of mercury. Scale bar = 50 μm . D: Liver epon section; 3 μm thick. Hepatocytes contain large AM silver grains. Two heavily stained macrophages (Kupffer cells) are seen (arrowheads) in the lumen of a vessel. The vessel wall contains AM silver grains. Scale bar = 20 μm . E: Muscle tissue; epon section; 3 μm thick. Only scant amounts of mercury are seen around the nuclei of the striated muscle cell. Scale bar = 20 μm .

1C and D). The muscle tissue accumulated only scant amounts of mercury, primarily around the nuclei of the striated muscle cells (Fig. 1E). The vessel walls contained mercury in all 3 kinds of tissue (Fig. 1D). At the ultrastructural level, mercury was found to accumulate both intracellularly in lysosomes (Fig. 2), and extracellularly in the basement membranes of vessels. The relatively poor morphological quality is a consequence of the tissue damage caused by the delayed fixation.

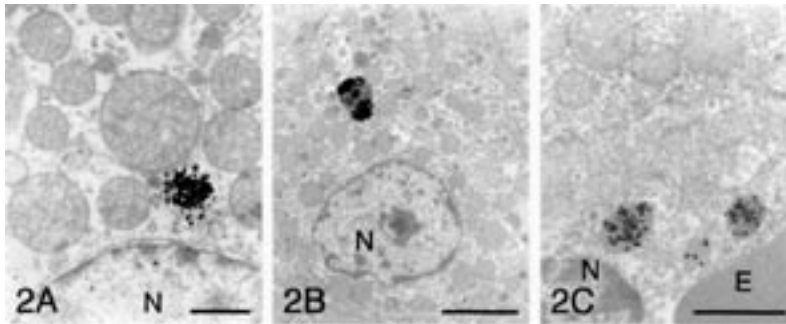


Fig. 2. Electron micrographs of kidney and liver tissue, AMG-developed for 60 min. N is the abbreviation for nucleus. Intracellular mercury was found exclusively in lysosomes. A: Kidney cell; scale bar = 1 μm . B: Hepatocyte; scale bar = 4 μm . C: Liver macrophage (Kupffer cell). Part of an erythrocyte (E) is seen. Scale bar = 2 μm .

Multi-element analysis (PIXE) of sections proves that the sections contain no other AMG detectable metals but mercury. The concentrations of mercury in the samples, expressed in parts per million (ppm) dry weight, were: Muscle 1.0 - 1.9 ppm, kidney 5.0 - 8.4 ppm, liver 8.4 - 11.8 ppm. The PIXE data corresponded well with the histochemical staining patterns and intensities.

DISCUSSION

The specificity of the HgS/Se^{AMG} technique has been established previously, and isolated AMG silver grains in sections from mercury-exposed animals have been shown to contain mercury (16, 18):

Cryo-sections are suitable for screening tissues to obtain an overview of mercury-stained areas (Figs 1A, 1C); dark-field microscopy, especially at the lower end of the detection limit of this method, can be very useful in order to determine the existence of the AMG grains in the tissue (19). Epon sections (Figs 1B, 1D, 1E), however, give detailed histological information, e.g. whether the AMG grains are located intra-, or extracellularly, and make ultrastructural analyses and the ensuing subcellular tracing of mercury possible.

The demonstration of mercury uptake using the HgS/Se^{AMG} technique has previously been shown to correspond to the morphological/pathological changes observed in rats intoxicated with organic mercury, suggesting that AMG could be useful as a prognosticator of possible target organs and mercury-induced dam-

ages (20). The present findings of high levels of mercury in liver and kidney tissue correlate with previous findings in marine mammals, such as pilot whales in the Faroe islands (21, 22) and beluga whales from arctic Alaska (23), as well as sledge dogs from Greenland (24), and laboratory Wistar rats (16, 25, 26), and suggest that pilot whales may contain a heavy load of mercury also in their nervous system as is the case with rats and dogs (24, 27). Thus, the possibility that mercury has a significant impact on the viability of pilot whales cannot be excluded.

In conclusion: This is the first time that AMG has been used for tracing mercury in pilot whales, and mercury tracing in whales at the EM level has, to our knowledge, never been reported previously. Mercury accumulates in the same organs of pilot whales as in experimental animals, like rats, and in naturally exposed terrestrial animals, like dogs. It might therefore be feared that the uptake of mercury by the nervous system, as well as its accumulation in the liver and kidney, add to the environmental pressure on the pilot whales.

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