THE LESIONS OF CHRONIC METHYLMERCUY POISONING
IN THE HARP SEAL (Pagophilus groenlandicus)

S.V. Tessaro and K. Ronald
Department of Zoology
College of Biological Science
University of Guelph
Guelph, Ontario, Canada
Abstract

Histological investigations on six harp seals (*Pagophilus groenlandicus*) determined the effects of chronic methylmercuric chloride (MMC) exposure at two select dosages. Two seals were exposed to 25.0 mg MMC/kg body weight/day; one died on day 20, the other on day 26, of exposure. Acute gastritis and cloudy swelling of hepatocytes were noted in these seals and death was attributed to renal failure. Clinical signs and histopathological examinations did not reveal neurological dysfunction. Two seals exposed to 0.25 mg MMC/kg body weight/day developed subtle renal lesions but were free of hepatic and neurological lesions by the time they were euthanized. Mercury levels in select tissues were determined. Total mercury accumulated in the nervous system in the following order: spinal cord < cauda equina and dorsal root ganglia < brain. There were no clear differences in the topographical distribution of mercury in the brain. The largest amount of total mercury accumulated in the liver. The highest rate of demethylation occurred in the kidneys. The high rate of demethylation coupled with the high accumulation of nephrotoxic inorganic mercury could explain the impairment of the kidneys prior to other organ systems, notably the central nervous system. The results obtained from this species, a marine mammal, differ from most studies of chronic organomercurial poisoning in mammals.
Introduction

There has been considerable concern expressed over the presence of mercury in the biosphere and the hazards of this element and its compounds to plant and animal life. Organic and inorganic forms of mercury occur naturally in the environment (Goldwater 1971). Heavy production and widespread utilization of mercury substances by man have further contributed to the bio-availability of these compounds (Nobbs 1972). Mercurials may reach aquatic systems either by direct pollution or by geochemical and atmospheric cycles (D'Itri 1972).

There has been some debate about whether or not mercury accumulates in organisms along food chains. It has been shown that, at least under some circumstances, fish can concentrate environmental mercury where only low levels of mercury exist (Johnels and Westermark 1969), and that the greatest portion of their total mercury load consists of the highly toxic methylmercuric form (Friberg and Vostal 1972). Piscivorous species such as seals are therefore potentially the most susceptible links in aquatic food chains at which organomercurial poisoning may occur.

High environmental mercury levels have been recorded for several species of seals (Sergeant and Armstrong 1973, Heppleston and French 1973, Smith and Armstrong 1975, Jones et al 1975). It appears that seals can tolerate much higher concentrations of mercury compounds than many other animals (Hendriksson et al 1969). Heppleston and French (1973) have suggested that in some wild seals, mercury levels in the brain may be limited to a maximum figure. Although seals have a relatively high tolerance to organomercurial compounds, these same substances are readily absorbed by the digestive tract and have a very long biological half-life in these animals (Tillander et al 1973).
One case of apparent mercury poisoning in a wild seal has been reported (Helminen et al. 1968).

From these limited observations on mercury levels in seals, it may follow that seals react differently from other species and therefore closer, experimental examinations were in order. This study was undertaken to determine the pathological consequences of chronic, oral exposure to methylmercuric chloride (MMC) in the harp seal.

Materials and methods

Four immature (11 months old) and two adult (12 and 16 years old) harp seals were used to study the chronic toxicity of orally administered MMC. All seals had been in captivity for at least 10 months; clinical histories were unremarkable and the animals appeared healthy according to blood parameters and general behaviour. The six seals were placed in three groups: two immature females received 25.0 mg MMC/kg body weight/day; an immature male and adult female received 0.25 mg MMC/kg body weight/day; an immature female and adult female served as controls. MMC (Alpha Products, Beverly, Mass.) was weighed out daily and placed in gelatin capsules which were administered in the diet of supplemented whole herring (Ronald et al. 1970). Seals which would not feed voluntarily were force fed. Each group was kept in a separate tank supplied with continuously flowing, 10 C well water. One seal from each group was to be euthanized after 60 days, and the other after 90 days exposure.

Postmortem tissue samples included eyes, brain, spinal cord, heart, kidney, liver and spleen. Stomach tissue was obtained from one high dosage seal. All samples were fixed in 10 per cent. buffered
formalin followed by dehydration and paraffin processing. Sections from each tissue were stained with haematoxylin and eosin (H & E); Holmes' (1943) silver nitrate, and the Kluver and Barrera (1953) method for myelin and nerve cells were also used on nervous tissue. The periodic acid-Schiff stain (PAS) was also used on some sections of visceral organs.

Total mercury levels in tissue samples were determined by atomic absorption spectrophotometry (Gaskin et al 1972). Methylmercury determinations were done by the method of Uthe et al (1972).

Results

The high dosage seals became progressively more lethargic after the third day of MMC administration. One seal died on day 20, the other on day 26 of exposure. The animals exhibited frequent vomiting in the hour before death and died in convulsions. The vomit contained small clots of blood. Neurological signs were absent throughout the clinical period. The control and low dosage seals did not develop signs of poisoning.

Lesions were confined to the kidney, liver and stomach of the high dosage seals and to the kidney in the low dosage dosage seals. Lesions were not found in the central nervous system.

The high dosage seals

Acute gastritis was observed in the stomach tissue of a high dosage seal (Fig 1). The severity of the lesion varied topographically, but generally did not involve the entire depth of the mucosa. The gastric epithelium was completely eroded from the tops of most mucosal ridges but was intact in the depths of the foveolae. The surface mucus was
filled with cellular debris and strands of sloughed epithelial cells. Focal haemorrhages were noted. Edema was evident in the lamina propria though the extent of this also varied topographically. Polymorphonuclear leucocytes were the predominant macrophages. The pyloric portion was less affected than the body of the stomach. Cellular debris was evident in the lumen of the pyloric canal but erosion of the epithelium of the canal itself was considerably less common than in the body of the stomach.

Cloudy swelling was indicated in the livers of both high dosage seals. Hepatic necrosis was not evident.

The glomeruli in the renal cortex of the high dosage seals appeared normal but the convoluted tubules were extensively swollen and the tubular epithelium was thin or often absent (Fig 2). The lumina of these tubules contained sloughed epithelial cells and other debris. Mitotic figures were occasionally encountered in the tubular epithelium. The proximal convoluted tubules in the renal medulla contained both smooth and granular proteinaceous material as well as some cellular debris.

The low dosage seals

A subtle renal lesion was seen in the low dosage seals. Pale, brown cytoplasmic vacuoles were observed in the epithelial cells of the proximal convoluted tubules (Fig 3). These vacuoles were difficult to see in H & E preparations but were much more visible in PAS-stained tissue. The vacuoles were lightly and irregularly scattered inside cells of the tubular epithelium of the 60 day exposure seal but were considerably more concentrated in those of the animal which received MMC for 90 days. No other pathological changes were seen in the kidneys of these seals.

Total mercury levles were determined for the tissues sampled
(Table 1). Though the sample size was too small for detailed comparison, it appeared that total mercury levels were higher in the brain than in the dorsal root ganglia and cauda equina which, in turn, were higher than those in the spinal cord. Topographical differences in total mercury levels within the brain were not clear.

Methymercury levels in liver and kidney samples were determined and the portion of non-methylated mercury calculated by subtracting methylmercury values from total mercury values (Table 2). In the control seals, most of the kidney and liver mercury burdens were non-methylated. It was apparent that a substantial portion of the total mercury burden of the kidneys of the high dosage seals was non-methylated while that of the livers was methylated. This was also the case in the low dosage immature seal which was exposed to MMC for 90 days. The adult female seal which received a low dosage of MMC for 60 days showed a different distribution and conversion pattern of mercury in the kidney and liver from that of the other experimental animals, all of which were immature.

Discussion

In previous studies the caustic effect of large oral doses of methylmercury on the gut have been noted (Aaronson and Spiro 1973). The body of the stomach of the high dosage seal appeared to be more severely damaged than the pyloric region possibly because of its lower pH which may have facilitated greater absorption of the methylmercuric cation. Though the effects on the gut were notable, they were not sufficiently extensive to cause the deaths of the animals. The results of the histopathological investigation indicated chronic renal failure
as the cause of death in these seals. This was supported by the signs exhibited by the seals and by clinical chemistry. The blood urea nitrogen (BUN) values for the 20 and 26 day exposure seals were 245 and 165 mg\% respectively and serum electrolyte levels were abnormal (Ronald and Tessaro, in prep). The behaviour of the high dosage seals shortly before death could be attributed to the symptom complex of uremia (Boyd 1974).

Demethylation of methylmercury occurs at varying rates in several organs. While demethylation is extremely slow in the central nervous system (Syversen 1974), it occurs most rapidly in the kidney and liver (Aaronson and Spiro 1973, Lefevre and Daniel 1973, Fang and Fallin 1974). In the harp seal, it would appear that a substantially higher rate of demethylation occurs in the kidney than in the liver. It is suspected that the rapid accumulation of mercury compounds and high rate of demethylation in the kidneys coupled with the severe nephrotoxic action of mercuric ion, caused the kidneys to fail prior to other organ systems in the high dosage seals. Though total mercury accumulation was greatest in the liver, this organ did not show severe lesions possibly because of the low proportion of, and a high tolerance to, mercuric ion.

As in the high dosage seals, the low dosage seals exposed for 90 days showed both high accumulation, and demethylation rate in the kidney and a higher accumulation but much lower demethylation rate in the liver. The liver appeared normal while the kidney showed early signs of damage possibly associated with faulty tubular absorption. The 60 day exposure, low dosage seal also exhibited a renal lesion but the relative demethylation rates in the liver and kidneys were reversed when compared with the other experimental animals. Whether this represents an age difference or individual variation in the metabolism of methylmercury cannot be ascertained.
Renal lesions are probably not rare in methylmercury intoxication. Klein et al (1973) have written a comprehensive report on the renal effects of methylmercury and Fowler et al (1974, 1975, 1975a) have discussed the ultrastructural changes incurred from methylmercury exposure. Most of the literature on organomericurial poisoning is concerned with the profound clinical and histological changes which occur in the nervous system. The level of methylmercury causing neurological signs varies considerably amongst different species (Berglund and Berlin 1969). It appears that the harp seal nervous system has a relatively high tolerance to methylmercury. It is important to note that renal failure can precede neurological dysfunction when high dosages of MMC are administered to harp seals, and perhaps other species. In this situation, the standard clinical signs of methylmercury intoxication would be lacking and accurate diagnosis difficult.

Acknowledgements

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Literature cited


Table 1. Total mercury levels (ppm) in tissues of harp seals.

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>CONTROL SEALS</th>
<th></th>
<th>LOW DOSAGE SEALS</th>
<th></th>
<th>HIGH DOSAGE SEALS</th>
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<tbody>
<tr>
<td></td>
<td>60 day exposure</td>
<td>90 day exposure</td>
<td>60 day exposure</td>
<td>90 day exposure</td>
<td>20 day exposure</td>
<td>26 day exposure</td>
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<tr>
<td>Brain - frontal lobe</td>
<td>0.45</td>
<td>0.36</td>
<td>14.80</td>
<td>21.86</td>
<td>42.90</td>
<td>23.80</td>
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<tr>
<td>Brain - occipital lobe</td>
<td>0.27</td>
<td>0.19</td>
<td>11.50</td>
<td>14.86</td>
<td>26.20</td>
<td>22.90</td>
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<tr>
<td>Cerebellum</td>
<td>1.09</td>
<td>0.37</td>
<td>-</td>
<td>-</td>
<td>27.20</td>
<td>21.80</td>
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<td>Cervical spinal cord</td>
<td>0.05</td>
<td>0.07</td>
<td>1.80</td>
<td>3.00</td>
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<td>Thoracic spinal cord</td>
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<td>0.06</td>
<td>5.70</td>
<td>6.78</td>
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<td>Cauda equina (lumbar region)</td>
<td>0.33</td>
<td>0.23</td>
<td>6.06</td>
<td>9.66</td>
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<td>Cauda equina (sacral region)</td>
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<td>Dorsal root ganglia</td>
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<td>20.40</td>
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<tr>
<td>Whole blood</td>
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<td>0.10</td>
<td>9.93</td>
<td>13.16</td>
<td>28.80</td>
<td>30.30</td>
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Table 2. Levels of methylated and non-methylated mercury (ppm) in kidneys and livers of harp seals and the percentage of total mercury which was non-methylated.

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>CONTROL SEALS</th>
<th>LOW DOSAGE SEALS</th>
<th>HIGH DOSAGE SEALS</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>60 day exposure</td>
<td>90 day exposure</td>
<td>60 day exposure</td>
</tr>
<tr>
<td>KIDNEY</td>
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<td>methylmercury</td>
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<td>0.20</td>
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<td>non-methylated mercury</td>
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<td>0.72</td>
<td>17.90</td>
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<td>percentage of total Hg as non-methylated Hg</td>
<td>96%</td>
<td>78%</td>
<td>26%</td>
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<td>LIVER</td>
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<td>non-methylated mercury</td>
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<td>percentage of total Hg as non-methylated Hg</td>
<td>99%</td>
<td>88%</td>
<td>68%</td>
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Figure 1. Photomicrograph of stomach tissue of a high MMC dosage seal showing loss of epithelial cells, hyperemia and edema. H&E (x25)
Figure 2. Photomicrograph of the renal cortex of a high MMC dosage seal showing swelling of the convoluted tubules, damage to the tubular epithelium, and cellular debris and proteinaceous material in the lumena of the tubules. H&E (x63).
Figure 3. Photomicrograph of the renal cortex of a low MMC dosage seal showing intracellular vacuoles (arrows) in the epithelial cells. PAS. (x400)