Accumulation and Removal of Hg^{203} in Different Regions of the Rat Brain

R. F. BUTTERWORTH, M. GONCE AND A. BARBEAU

SUMMARY: *We have studied the brain regional distribution of methyl mercury following intravenous administration of CH_3 $^{203}HgCl$ in rat. Early peak levels were obtained in cerebellum, medulla oblongata and midbrain. The efficacy of removal of ^{203}Hg by different chelators is also region dependent. The most efficient chelator for brain mercury proved to be meso-dimercaptosuccinic acid.*

RÉSUMÉ: *Nous avons étudié la distribution régionale cérébrale du méthyl mercure à la suite de l'administration intraveineuse de CH_3 $^{203}HgCl$ chez le rat. Le pics de concentration les plus rapides furent observés dans le cervelet, le tronc cérébral et le mésencéphale. L'efficacité de la chélation du méthyl mercure par différents agents est également dépendante de la région observée. L'agent chélateur le plus efficace au niveau du cerveau fut l'acide méso-dimercaptosuccinique.*

From the Department of Neurobiology, Clinical Research Institute of Montreal.

Reprint requests to: Dr. André Barbeau, M.D., Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal, Quebec, Canada H2W 1R7.

INTRODUCTION

Damage to the central nervous system by alkyl organo mercury compounds is well known and extensive, but rather specific changes in cerebrum, cerebellum, and long spinal tracts have been described (Cavanagh, 1969). More recently (Jacobs et al., 1977), ultrastructural changes in the nervous system of rabbits poisoned with methyl mercury have been described. In addition to effects on dorsal root and Vth cranial nerve ganglion cells, certain specific cell types within the central nervous system were found to be susceptible. These included granule, stellate, and basket cells of the cerebellum and the small neurons of the cerebral cortex. This *selective vulnerability* of specific groups of neurons with sparing of adjacent, morphologically different groups is characteristic of a number of neurological disorders (it has been suggested, for example, that the Purkinje cell degeneration associated with carcinomatous cerebellar degeneration could result from release of a neurotoxic transmitter agonist by the tumour. (Brain and Wilkinson, 1965).

Little, however, is known concerning the regional affinity of brain for organomercurials nor about their rates of clearance by different brain areas. The present study was proposed to study:

a) The regional distribution of methyl mercury following intravenous administration.

b) The efficacy of the chelators D-penicillamine, N-acetyl-D,L,-penicillamine and 2,3-dimercaptosuccinic acid in the removal of methyl mercury in different regions of brain.

Results of these studies are presented and possible therapeutic implications for chelation therapy are discussed.

MATERIALS AND METHODS

CH_3 $^{203}HgCl$, specific activity 2.6 mCi/mg Hg, prepared by isotopic exchange (99% pure) was purchased from New England Nuclear; soluene, triton-X, PPO and POPOP from Packard Instrument Co.; D-penicillamine and N-acetyl-D,L,-penicillamine from Sigma Chemical Co., and 2,3-dimercaptosuccinic acid from Aldrich Chemical Co. All solvents and reagents were reagent grade and double distilled, deionised water was used throughout the series of experiments.

For the study of clearance of labelled mercury by different regions of the brain, a group of 25 male adult Sprague-Dawley rats (250-300 g) was anaesthetised and injected with 0.5 ml of CH_3 $^{203}HgCl$ in saline (equivalent of 4.0×10^7 cpm) into the jugular vein. At times ranging from 1 to 5 days following treatment, groups of 5 rats were sacrificed by decapitation, brains quickly removed on ice and dissected into the following regions: cerebellum, medulla oblongata, striatum, hippocampus, hypothalamus, midbrain and cerebral cortex, which was further dissected into three parts: frontal cortex, occipital cortex and remainder of cortex. Care was taken to remove all visible blood vessels from brain tissue. Each fragment of tissue was then weighed and solubilised in 10 vol. soluene overnight at room temperature. To 0.1 ml solubilised tissue was added 0.1 ml acetic acid, 0.8 ml distilled water and 12 ml of a mixture of triton-X, PPO and POPOP in toluene

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prepared as follows: to 2 lt. toluene (scintanalyzed grade) was added 1 lt. triton-X, 0.5 g POPOP and 16.5 g PPO and the mixture was stirred (magnetic stirring) until all components were dissolved. Samples were assayed for radioactivity content using a Packard model 3375 liquid scintillation counter.

RESULTS

Figure 1 shows the total brain accumulation of Hg^{203} from 1 to 5 days following intravenous administration. The brain concentration of Hg^{203} increased steadily up to the 4th day following administration and this was followed by a slight decline on the 5th day.

As can be clearly observed from Figure 2, clearance of Hg^{203} from brain is region-dependent. In regions such as the cerebellum and medulla oblongata, Hg^{203} levels had started to decline 2 days following administration, whereas in regions such as the hypothalamus, striatum and hippocampus, Hg^{203} levels increased steadily 2 days, 4 days and 5 days following administration, respectively.

Figure 3 shows the effect of administration of the chelating agents N-acetyl penicillamine (800 mg per kg), mesodimercaptosuccinic acid (200 mg per kg), and D-penicillamine (625 mg per kg) on Hg^{203} content of different regions of the rat brain following administration of $MeHg^{203}Cl$. As in the case of accumulation of Hg^{203}, its removal by chelation is found to be region-dependent.

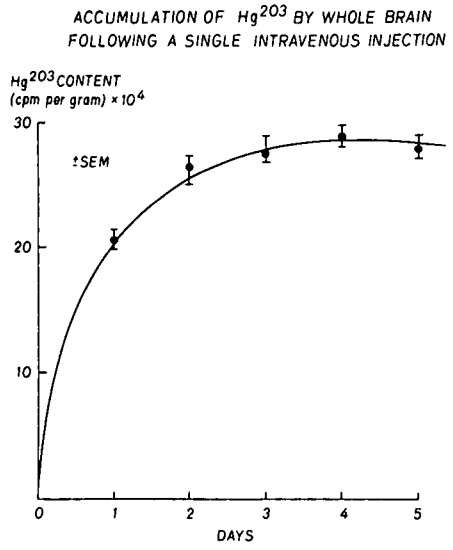

Figure 1 — Accumulation of Hg^{203} by whole brain, following a single intravenous injection.

DISCUSSION

1. Accumulation of Hg^{203} in Brain Regions Following i.v. Administration

In the rat, the first parts of the nervous system to be affected by methyl mercury intoxication are the posterior spinal roots followed by the peripheral nerves and the posterior columns (Hunter et al., 1949). In both man and in the rat, pathological changes in the central nervous system involving the granular layer of the cerebellum are seen and in the rat these changes closely follow the appearance of peripheral lesions. Both man and rat show ataxia and sensory loss with normal electrophysiological behavior of the motor nerves (Magos and Butler, 1976).

It has been reported (Norseth and Clarkson, 1970) that uptake and release of mercury by brain tissue is delayed, with peak brain levels not being reached until the sixth day following a single injection of methyl mercury. Our studies confirm these findings with, in our case, peak Hg^{203} levels being reached 4 days following a single intravenous injection of Hg^{203} as $Hg^{203}Cl$ (Fig. 1). In addition, we found that the delay in uptake of Hg^{203} is region dependent; peak levels in cerebellum, medulla oblongata and midbrain, for example, being attained sooner than those of hippocampus or cerebral cortex. It is rather difficult to relate these regional differences in rates of accumulation of Hg^{203} to published reports of the progress of neurological signs due to methyl mercury intoxication in the rat, as the literature is conflicting on this point. It is of interest, however, that a recent detailed report of the ultrastructural changes produced by methyl mercury intoxication in rabbits (Carmichael et al., 1975) showed that the widespread cerebellar changes preceeded changes in cerebral cortex by several days.

2. Removal of Hg^{203} from Regions of the Rat Brain by Different Chelating Agents

In a recent report (Friedman et al., 1976), it was shown that meso-

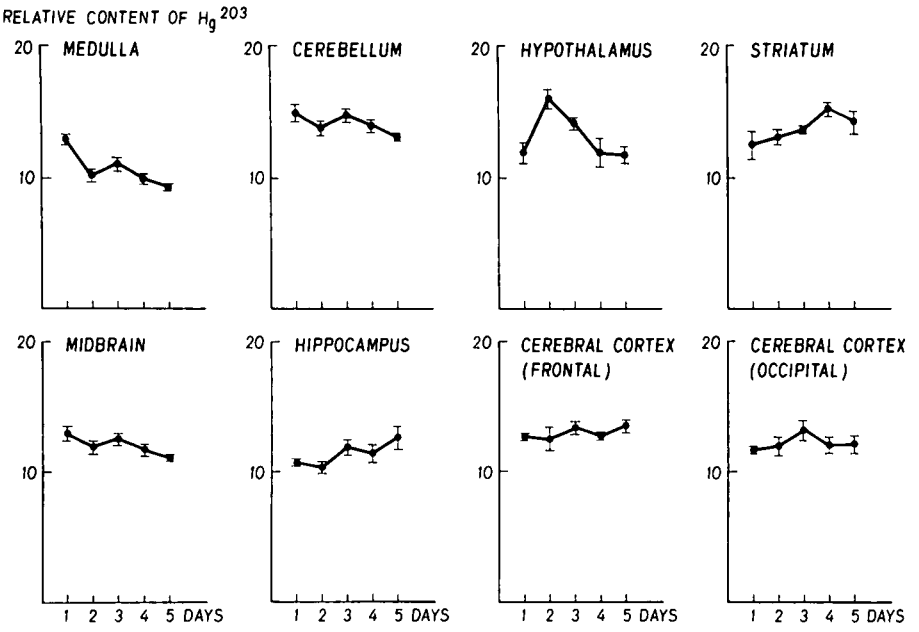

Figure 2 — Accumulation and Clearance of Hg^{203} by various regions of Rat Brain. Hg^{203} levels are expressed as per cent Hg^{203} per mg of brain (wet weight) as a fraction of total brain Hg^{203} content. Each point represents the mean of triplicate determinations of tissue Hg^{203} from at least 5 animals. Bars represent ±S.E.M. Brain dissection techniques are described in Methods.

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dimercaptosuccinic acid (DMS) in doses of 10-500 mg per kg was effective in removing mercury from mouse brain by up to 65% whereas D-penicillamine had either no effect or an adverse effect, depending on the dose used. In another report (Friedman & Corvi, 1975) comparing DMS to D-penicillamine as effective chelators, mice were treated with methyl mercury bromide followed by the chelator (100 mg per kg). Whereas DMS produced a 32% decrease in brain mercury concentration, D-penicillamine was without such effect. Similar results were obtained using the guinea pig.

In the case of N-acetyl-penicillamine (NAP), Aposhian in 1959 showed that NAP was the most potent derivative of penicillamine in protecting rats poisoned with mercury chloride. Since that time, toxicological studies have shown that doses of NAP up to 1,000 mg per kg in the rat did not result in any observable toxic reactions (Aposhian, 1959) and recently NAP has been shown to be a convenient, rapid, and safe means of mercury chelation in the treatment of 3 cases of accidental mercury poisoning in children (Aronow and Fleischmann, 1976).

From our results (Fig. 3) it is clear that the efficacy of removal of Hg^{203} by the different chelators is region dependent. Whereas D-penicillamine appeared to remove Hg^{203} from all regions of the rat brain to approximately the same extent (30-40% decrease in all cases), the N-acetylated derivative caused a 50% decrease in Hg^{203} in the occipital cortex, while the level in hypothalamus was actually found to *increase* by 19%. The consistently best chelator, found to cause decreases in Hg^{203} content of up to 60%, was the compound meso-dimercaptosuccinic acid.

It is well established for many species, including man, that methyl mercury affects certain special cell types in the central nervous system, including granule, stellate and basket cells of the cerebellum and small neurons of the cerebral cortex. Thus, from our data (Fig. 3) it is interesting to note that NAP was more effective in

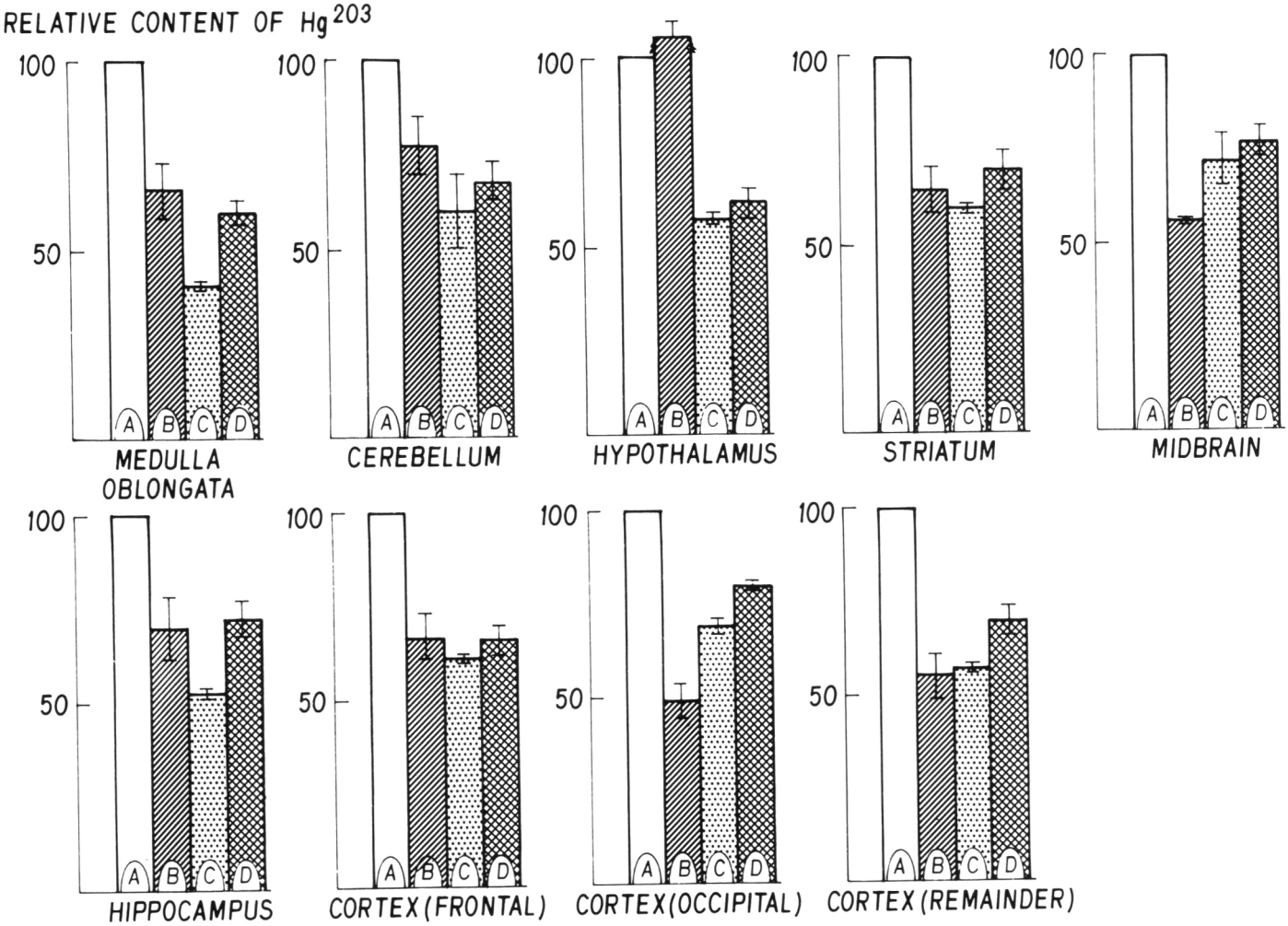

Figure 3—Elimination of Hg^{203} from specific Brain Regions by Different Chelating Agents. Values expressed as per cent ±S.E.M. of control values (mean of 5 experiments in each case). A: Control group B: Rats received N-acetyl penicillamine (800 mg per kg) C: Rats received meso-dimercaptosuccinic acid (200 mg per kg) D: Rats received D-penicillamine (625 mg per kg) For dosage schedules, routes of injection and details of vehicle used, see Methods.

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removing Hg^{203} from cortex than cerebellum, and DMS removed Hg^{203} more effectively from cerebellum and medulla oblongata than from the cerebral cortex. If these chelators are found to be sufficiently free of side effects to be used therapeutically as mercury chelators, it is conceivable that patients displaying predominantly cerebellar signs of mercury intoxication would benefit from DMS chelation therapy, whereas patients having more "cortical" signs and symptoms may derive greater benefit from the use of NAP.

Further work is required to understand fully the mechanisms involved in methyl mercury intoxication, including the toxin's effect on neurotransmitter metabolism in the central nervous system.

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