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Neurotoxicity Produced by Intracranial Administration of Methylmercury in Rats[1]

RUDY J. RICHARDSON AND SHELDON D. MURPHY

Department of Physiology, Kresge Center for Environmental Health, Harvard University, School of Public Health, Boston, Massachusetts 02115

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Neurotoxicity Produced by Intracranial Administration of Methylmercury in Rats. RICHARDSON, R. J. AND MURPHY, S. D. (1974). *Toxicol. Appl. Pharmacol.* **29**, 289–300. Methylmercuric chloride administered as a single intracranial injection in μg quantities produced a neurological syndrome in rats within 24 hr that resembled the effects produced by repeated sc injections of 10 mg/kg over a period of 7–14 days. Neuromuscular function evaluated semiquantitatively by graded performance in simple strength and coordination tests showed severe impairment at 24 hr in intracranially methylmercury-treated animals, with recovery taking place by 72 hr. Body weight decreased and recovered during a similar time course. Incorporation of tritiated leucine into brain protein was increased significantly at 24 hr as measured in vitro in brain homogenates and in vivo by administering the labeled amino acid ip. Incorporation returned to control values by 72 hr after the methylmercury injection. Residual brain mercury concentrations at 24 hr were about 5-fold lower than those accompanying overt neurological signs in rats produced by sc administration. Histological examination of brains from intracranially and subcutaneously dosed rats revealed that the lesions produced by the 2 methods were substantially different. Intracranial injection of methylmercury was found to produce an isolated neurotoxic syndrome similar in some respects to the neurotoxicity seen in systemic intoxications but dissimilar histopathologically.

It has been suggested that the appearance of neurological signs in methylmercury-intoxicated animals depends upon achieving a critical concentration of mercury in the central nervous system (CNS). Brain mercury concentrations of 20 and 17 μg/g fresh weight are reportedly required to produce neurotoxicity in mice and cats, respectively. Concentrations of about 40 μg/g were found in the brains of rats with severe neurological signs (Klein *et al.*, 1972).

By conventional routes of administration, the most satisfactory way to achieve critical brain concentrations and concomitant neurological signs in experimental intoxications with methylmercury compounds has been to administer repeated doses over a period of at least days or weeks. Attempts to shorten the latency period by increasing the dose result in severe injury to organ systems other than nerve tissue, and signs of neurotoxicity are often masked by an overall debilitation of the animal (Suzuki, 1969; Cavanagh and Chen, 1971a; Klein *et al.*, 1972).

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The present study was undertaken as an attempt to produce methylmercury neurotoxicity directly by injecting sufficient compound intracranially (icr) to achieve a critical brain concentration after a single dose. It was thought that this might permit studies of methylmercury neurotoxicity as an isolated phenomenon in the intact animal in experiments of relatively short duration. Preliminary experiments revealed that a single icr injection of 25 μg of methylmercuric chloride was capable of producing a neurological syndrome that resembled the nervous system effects produced by repeated sc administration of the compound. This result prompted us to study the effects of icr-administered methylmercury in more detail and to compare the intoxication produced by icr administration with that produced by the sc route.

Methylmercury intoxication in the rat has been correlated with loss of body weight, impairment of neuromuscular function, including the appearance of a characteristic hindlimb crossing reflex elicited by suspending the animal by the tail (Miyakawa and Deshimaru, 1969; Klein *et al.*, 1972); inhibition of brain protein synthesis as measured by the incorporation of labeled amino acids into proteins both in vitro (Yoshino *et al.*, 1966) and in vivo (Cavanagh and Chen, 1971b); attainment of a critical brain mercury concentration (Klein *et al.*, 1972); and neuronal degeneration in cerebral and cerebellar cortex as revealed by light microscopy of ordinary histological sections (Miyakawa and Deshimaru, 1969; Diamond and Sleight, 1972; Klein *et al.*, 1972). These correlates were adopted as criteria in the evaluation of the production of neurotoxicity by icr administration of methylmercury in the present study.

METHODS

Animals. Male Holtzman rats were used. The age of the rats is specified for each experiment described in Results. The animals were housed in air-conditioned rooms and supplied with food[2] and water ad libitum. Body weight was monitored daily during experiments.

Intracranial (icr) injections. An adaptation of the method of Clark *et al.* (1968) was used. A Hamilton microliter syringe of 50-μl capacity was fitted with a 27G stainless steel needle equipped with a polyethylene cuff to allow penetration to a depth of 4.5 mm. Injections were made perpendicular to the dorsal surface of the skull at a point 2 mm to the left of the midline and 2 mm caudal to a line connecting the anterior edge of the base of the ears. Methylmercuric chloride was administered as a total dose of 10–100 μg in 10 μl of 0.05 M Na_2CO_3 solution. Control rats received 10-μl injections of 0.05 M Na_2CO_3 solution. All animals were anesthetized with ether prior to injection. In 1 experiment, 25 μg of acrylamide was injected in 10 μl of distilled deionized water. Control rats received 10 μl of water.

Subcutaneous (sc) injections. Rats, 23 days of age at the beginning of the experiment, were given daily injections of methylmercuric chloride, 10 mg/kg, in a concentration of 10 mg/ml in 0.05 M Na_2CO_3. Control rats received injections of 0.05 M Na_2CO_3. Injections were continued for 7 consecutive days at a different location under the skin of the neck or back each day. Animals were sacrificed at 5 and 6 days after the final injection.

[2] Purina® Rat Chow, Ralston Purina Co., St. Louis, Missouri.

Neuromuscular function tests. These tests were adapted from those described by McNamara (1963).

Vertical rod tests. Rats were placed parallel to and facing the rod to enable them to grasp with all 4 limbs. Performance was graded by placing the rats both head upward (↑) and head downward (↓). The tests were scored as follows: 4 = firm grasp with all 4 limbs, controlled descent; 3 = less firm grasp, slides quickly down rod; 2 = less controlled descent, grasps with fewer than 4 limbs; 1 = grasp very weak, uncontrolled slide; 0 = does not grasp the rod, falls repeatedly.

Horizontal rod test (→). Rats were hung by the front feet from a horizontal rod. An escape platform was provided on the right side. The animals were trained to pull themselves up onto the rod and walk to the escape platform to avoid falling into cold water. Three trials on 3 successive days were usually sufficient to achieve perfect performance in untreated rats. This test was scored as follows: 4 = immediate mounting of the rod and walking to platform; 3 = able to mount the rod but hesitating or making false moves in proceeding to platform; 2 = able to mount the rod but failing to move toward platform, or, unable to mount the rod, but able to reach platform by "walking" underneath the rod; 1 = unable to mount the rod or to get to platform, but able to hang from the rod for at least 20 sec; 0 = falls repeatedly.

Each rat was given 3 trials on each of the tests. A fall during a trial resulted in a deduction of 1 point from the score attained on a successful trial. Thirty seconds was the maximum interval allowed for each trial.

Crossing reflex (χ). Rats were placed on a level surface and quickly picked up by the end of the tail. The degree of abnormal behavior when hung by the tail was scored as follows: 4 = indistinguishable from control animals; 3 = slight tendency to cross legs or twist body; 2 = twisting of body; 1 = twisting of body drastically, tendency to abduct legs; 0 = complete crossing of hindlimbs, sometimes accompanied by a radical contortion or twisting of the body.

Mercury analysis. Total mercury in tissues was determined by cold vapor atomic absorption spectrophotometry, using the method of Kopp *et al.* (1972), with the following modifications: Tissue homogenates, 10% (w/v) in 0.2% L-leucine, were used as samples. These homogenates could be stored for at least 2 weeks in Teflon-capped Pyrex tubes without incurring any loss of methylmercury. The sample aerator was equipped with a standard-taper 24/40 ground-glass joint to accept 125-ml Erlenmeyer flasks with 24/40 necks. Mercury standards were prepared by adding aliquots of methylmercuric chloride solutions in 0.05 M Na_2CO_3 to tissue homogenates from control animals. Standards were carried through exactly the same procedures as samples.

Incorporation of tritiated leucine into brain protein. In vitro incorporation of L-[4,5-^{3}H]leucine[3] was carried out using the procedure of Peterson and McKean (1969) for brain homogenates. Protein was determined gravimetrically after purification and drying and was then dissolved in 88% formic acid for liquid scintillation counting. For the in vivo incorporation, L-[4,5-^{3}H]leucine, specific activity 15 Ci/mmol, was diluted to 100 μCi/ml in isotonic saline and administered by ip injection at a dose of 100 μCi/kg. Animals were sacrificed 3 hr after injection. The same protein isolation and counting procedures as in the in vitro method were used.

Histology. Brains were fixed in neutral buffered formalin and embedded in paraffin;

[3] Schwarz/Mann, Orangeburg, New York.

TABLE 1

EFFECTS 24 HR AFTER INTRACRANIAL CH_3HgCl IN 32-DAY-OLD RATS

CH_3HgCl dose (μg)	Percent change in body weight	Neuromuscular function[a]				Brain protein synthesis in vitro (cpm/mg protein)[b]	Number of animals
		↓	↑	→	χ		
0[c]	+4.3 ± 0.1	3.8 ± 0.2	3.8 ± 0.2	3.0 ± 0.8	4.0 ± 0.0	393 ± 30	5
0[d]	+5.3 ± 0.1	4.0 ± 0.0	4.0 ± 0.0	3.4 ± 0.2	3.8 ± 0.2	394 ± 11	5
10	−3.0 ± 0.3	3.2 ± 0.6	3.2 ± 0.6	3.0 ± 0.8	3.4 ± 0.5	422 ± 57	5
20	−1.8 ± 0.1	3.4 ± 0.4	3.0 ± 0.2	2.6 ± 0.7	2.1 ± 0.4	481 ± 28[e]	5
30	−4.6 ± 0.1	2.4 ± 1.0	2.1 ± 0.8	2.0 ± 0.8	2.1 ± 0.3	519 ± 27[f]	5
40	−6.7 ± 0.2	2.4 ± 1.0	2.4 ± 0.6	2.4 ± 0.7	1.4 ± 0.2	587 ± 49[f]	5

[a] Scores range from 0 (complete impairment) to 4 (no impairment on vertical (↓ and ↑) and horizontal (→) rod and crossing reflex (χ) tests as described in Methods. Data are means ± SE.

[b] Incorporation of tritiated leucine into brain homogenates.

[c] Uninjected control; ether anesthesia only.

[d] Icr injection of 10 μl of 0.05 M Na_2CO_3.

[e] Significant difference from injected control ($p < 0.05$).

[f] Significant difference from control ($p < 0.01$).

coronal sections including frontal cortex, parietal cortex, brainstem, occipital cortex, and cerebellum were stained with hematoxylin and eosin and examined by light microscopy. In brains from icr-injected animals, care was taken to include a section containing the needle track.

Reagent-grade chemicals were used in all chemical procedures and aqueous solutions were prepared with deionized-distilled water. Data are reported as means ± SE. Student's t test was used throughout to determine the significance of differences between means; $p < 0.05$ was regarded as significant.

RESULTS

Neurological Impairment and Whole-Brain Protein Synthesis

Table 1 summarizes the effects at 24 hr after graded doses of icr-administered methylmercuric chloride, CH_3HgCl, on body weight, neuromuscular function, and brain protein synthesis, as measured by the incorporation of tritiated leucine into brain homogenates in vitro. Body weights decreased in all mercury-treated groups, while the Na_2CO_3-injected and uninjected groups had normal 24-hr weight gains. Scores on the vertical and horizontal rod and crossing reflex tests tended to decline with increasing doses. These findings suggested more severe neurological involvement as the dose was increased. Protein synthesis increased with increasing doses of methylmercury. The presence of neurological impairment concurrent with normal or increased protein synthesis in brain was inconsistent with the hypothesis that inhibition of protein synthesis is a primary event that leads to the eventual production of lesions and neurological signs (Yoshino *et al.*, 1966; Cavanagh and Chen, 1971b).

TABLE 2

EFFECTS 24 HR AFTER INTRACRANIAL CH_3HgCl IN 61-DAY-OLD RATS

CH_3HgCl dose (μg)	Percent change in body weight	Toxic signs	Brain protein synthesis in vitro (cpm/mg protein)	Number of animals
0[a]	+3.3 ± 0.8	None	405 ± 29	5
25	−3.8 ± 1.9	Some crossing reflex	528 ± 44[b]	5
50	−7.8 ± 1.1	Some crossing reflex; weakness; 1 death	535 ± 34[c]	4
100	−8.2 ± 2.6	Some crossing reflex; abnormal gait	627 ± 92[b]	5

[a] Icr injection of 10 μl of 0.05 M Na_2CO_3.
[b] Significant difference from control ($p < 0.05$).
[c] Significant difference from control ($p < 0.01$).

In order to confirm our initial observation of a dose-related stimulation of brain protein synthesis after icr injection of methylmercury, additional experiments were carried out in which incorporation of tritiated leucine into brain protein of rats was measured both in vivo and in vitro. The results of these experiments are shown in Tables 2 and 3. In these experiments, doses as high as 100 μg of CH_3HgCl continued to stimulate brain protein synthesis whether measured in vitro or in vivo. Weight loss and neurological deficit occurred in all mercury-treated groups.

TABLE 3

EFFECT OF INTRACRANIAL CH_3HgCl ON BRAIN PROTEIN SYNTHESIS *in vivo* IN 89-DAY-OLD RATS

CH_3HgCl dose (μg)	Brain protein synthesis in vivo (cpm/mg protein[a])	Number of animals
0[b]	150 ± 9	4
25	204 ± 12[c]	4
50	230 ± 19[d]	5
100	256 ± 6[d]	3

[a] Incorporation of tritiated leucine into brain protein 3 hr after ip injections of 100 μCi/kg of 100 μCi/ml concentration in isotonic saline.

[b] Icr injection of 10 μl of 0.05 M Na_2CO_3.

[c] Significant difference from controls ($p < 0.05$).

[d] Significant difference from controls ($p < 0.01$).

Protein Synthesis by Area of Brain

In the preceding experiments, neurological deficit produced by CH_3HgCl appeared to be related to an increase in the average protein synthetic activity of whole brain. It was of interest to determine whether increased protein synthesis occurred throughout the brain or represented a large effect in a local area. Groups of animals were given icr injections of 50 μg of CH_3HgCl in 10 μl of 0.05 M Na_2CO_3 into the left cerebral hemisphere as described in Methods. Control animals received 0.05 M Na_2CO_3 injections icr.

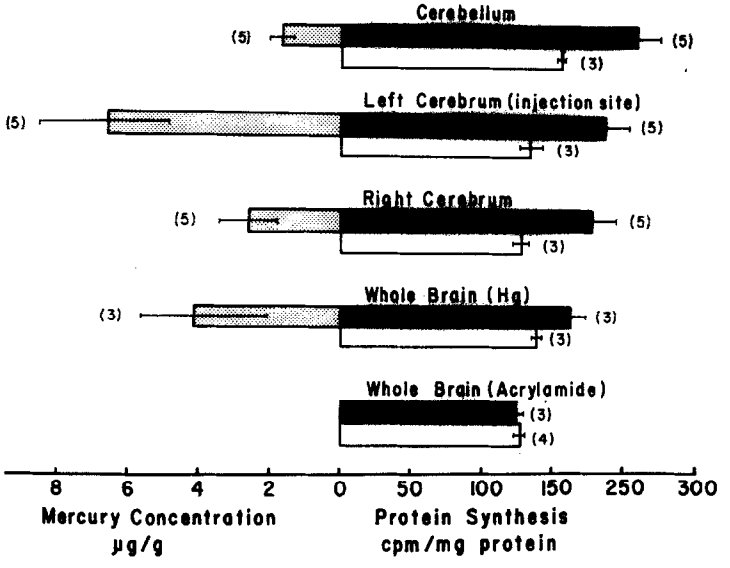

FIG. 1. Protein synthesis in vivo and mercury concentrations in brain segments 24 hr after intracranial CH_3HgCl or acrylamide. Groups of 57-day-old rats were given single intracranial injections of 50 μg CH_3HgCl in 10 μl 0.05 M Na_2CO_3 (shaded bars), or 10 μl of 0.05 M Na_2CO_3 (unshaded bars). Acrylamide, 25 μg in 10 μl H_2O (shaded bar), was given to 1 group for comparison. Corresponding controls received 10 μl H_2O (unshaded bar). Twenty-four hr after injection of CH_3HgCl, animals were injected ip with tritiated leucine and sacrificed 3 hr later.

For comparison, another group was given 25 μg of acrylamide, another neurotoxic chemical (Cavanagh, 1969), in 10 μl of water. Corresponding control animals received 10 μl of water. This dose of acrylamide is equivalent to 88 μg of CH_3HgCl on a molar basis. At 24 hr after the injections, all animals were dosed ip with tritiated leucine and sacrificed 3 hr later. Brains from the acrylamide groups and some of the mercury groups were left intact for homogenization, while the remaining brains were partitioned into cerebellum, right cerebrum, and left cerebrum and homogenized separately. Protein was isolated from each segment and counted and mercury was analyzed in aliquots of the homogenates. The results of this experiment are presented in Fig. 1. The striking feature of the data is the fairly uniform average degree of stimulation of protein synthesis in each brain segment from the CH_3HgCl treated animals, even though the mercury concentrations in each segment were quite different. Cerebellum exhibited the greatest, and in fact the only statistically significant ($p < 0.05$), increase in protein synthesis of the brain segments measured, but had the lowest mercury content. Stimulation was not as great in whole brain, which contained the brainstem, as in any of the separate segments in this experiment. The lack of effect with acrylamide is noteworthy from the points of view of refuting an hypothesis that any neurotoxic chemical injected directly into the brain might be expected to have an effect on protein synthesis. The mercury-treated animals in this experiment showed the signs of neurological involvement already described, but acrylamide-treated animals appeared normal at 24 hr. An average residual whole-brain mercury concentration of about 4 μg/g suggests that a considerable amount of mercury is removed from the CNS within 24 hr. If all the injected mercury were bound within the CNS, concentrations on the order of 20 μg/g would be expected.

Time-Course of Effects Following a Single Intracranial Injection

An experiment was conducted to follow the time-course of effects of a single icr dose of methylmercury over a period of 14 days. These results are shown in Table 4. Brain mercury concentration fell steadily over the 14-day period. Brain protein synthesis was significantly increased on the first day in mercury-treated animals but returned to control values by day 3 and did not change again. Body weight fell initially in the mercury-treated group, but began to recover by day 3 and increased thereafter, at a rate similar to control animals. Neuromuscular function was impaired at 1 day after methylmercury but showed a trend toward recovery over the 14-day period. These results support the idea (Suzuki, 1969) that neurological impairment in mercury intoxications is related to the concentration of mercury actually present in the brain at the time impairment is being evaluated. Protein synthesis stimulation appeared to be a relatively transient effect of methylmercury in this experiment.

Comparison of Effects of Icr and Repeated Sc Administration of Methylmercury

A direct comparison of histological sections from brains of animals with signs of neurotoxicity produced by icr injection and by repeated sc injections was carried out. Rats 22 days of age were given daily sc injections of 10 mg/kg of CH_3HgCl in 0.05 M Na_2CO_3 for 7 consecutive days. Five days after the final injection, severe signs of neurological impairment had developed in all the animals. At this point they were sacrificed and the brains submitted for histological examination. One day before the sacrifice of the sc-injected animals a cohort group was given 50 μg of CH_3HgCl icr in 10 μl of

TABLE 4

EFFECTS OVER A 14-DAY PERIOD FOLLOWING A SINGLE INTRACRANIAL INJECTION OF 50 μg OF CH_3HgCl IN 32-DAY-OLD RATS

Time after injection (days)	Group	Brain Hg concentration (μg/g)[a]	Brain protein synthesis in vivo (cpm/mg protein)[a]	Percent change in body weight from day zero	Neuromuscular function[b]				Number of animals[c]
					↓	↑	→	χ	
1	Exp.	4.53 ± 1.15 (8)	142 ± 4.4[d] (6)	−13.6 ± 1.5	1.6 ± 0.5	1.6 ± 0.5	1.2 ± 0.5	1.1 ± 0.1	36
	Con.	<0.10[e] (3)	103 ± 3.8 (6)	+2.3 ± 2.4	4.0 ± 0.0	4.0 ± 0.0	3.2 ± 0.2	3.5 ± 0.1	24
3	Exp.	2.50 ± 0.61 (9)	107 ± 4.4 (8)	−8.5 ± 2.8	2.4 ± 0.3	2.7 ± 0.4	2.1 ± 0.2	2.1 ± 0.2	27
	Con.	<0.10 (3)	97 ± 6.0 /6)	+14.6 ± 3.8	3.9 ± 0.1	3.9 ± 0.1	3.2 ± 0.2	3.1 ± 0.1	18
7	Exp.	1.57 ± 0.39 (7)	97 ± 6.4 (3)	+17.9 ± 3.6	3.3 ± 0.2	3.2 ± 0.1	2.9 ± 0.1	2.8 ± 0.1	18
	Con.	<0.10 (3)	104 ± 12 (3)	+36.7 ± 9.0	3.9 ± 0.1	4.0 ± 0.0	3.0 ± 0.4	3.6 ± 0.1	12
14	Exp.	0.28 ± 0.05 (9)	112 ± 10 (3)	+63.5 ± 3.2	3.2 ± 0.4	3.6 ± 0.3	2.5 ± 0.5	2.8 ± 0.3	9
	Con.	<0.10 (3)	114 ± 2.3 (5)	+82.7 ± 17.3	4.0 ± 0.0	4.0 ± 0.0	2.8 ± 0.5	3.4 ± 0.2	6

[a] Number in parentheses refers to the number of animals used for the measurement.

[b] Scores range from 0 (complete impairment) to 4 (no impairment) on vertical (↓ and ↑) and horizontal (→) rod and crossing reflex (χ) tests as described in Methods.

[c] Number of animals used in body weight and neuromuscular function measurements.

[d] Highly significant difference from control ($p < 0.01$).

[e] Hg concentration in control animals was always below the detection limit of 0.10 μg/1.

0.05 M Na_2CO_3 and sacrificed the following day, along with the sc-injected animals. Control animals, injected either icr or sc with 0.05 M Na_2CO_3, were included for comparison in each case.

Brains from the sc-injected control group had no significant lesions in the cerebral cortices, thalamus, midbrain, cerebellum, pons, or medulla. In the group that had received sc injections of methylmercury, there was acute neuronal necrosis with vacuolation of neuropil in layers III and IV of medial occipital cortex (occurring bilaterally) and in cingulum and globus pallidus. Some animals had the same type of lesion in frontal and parietal cortex as well. There were no significant lesions in any of the animals in cerebellum, pons, thalamus, and midbrain. The type and distribution of lesions was similar in all of the animals.

Icr-injected control animals had no significant lesions in the brain except in the section of left cerebrum containing the needle track. This appeared as a linear contusion with variable gliosis or adjacent polymorphonuclear inflammation. Brains from animals receiving methylmercury icr exhibited no significant lesions except for a region of total tissue necrosis, usually well circumscribed, in the left cerebral hemisphere including all cortical layers. The distribution of the lesion was variable. Fibrinoid necrosis of meningeal vessels was usually present. Histologically, the 2 treatments produced entirely different types and distributions of lesions.

To provide direct comparison with the chemical results obtained in the icr experiments, in vivo protein synthesis determinations and mercury analyses were carried out on cohort groups of sc-injected animals. The data in Table 5 shows that brain protein synthesis was slightly depressed but not significantly ($p > 0.05$). Mercury concentrations were about 5-fold higher than those found previously in animals showing neurological impairment after a single icr dose of methylmercury (Table 4).

TABLE 5

EFFECTS OF REPEATED SUBCUTANEOUS INJECTIONS OF CH_3HgCl IN RATS

Treatment group[a]	Brain Hg concentration (μg/g)	Brain protein synthesis in vivo	Body weight[b] (g)	Number of animals
Na_2CO_3	<0.10[c]	106.1 ± 6.0	160 ± 3	7
CH_3HgCl	22.8 ± 0.8	90.5 ± 6.8	72 ± 3	7

[a] 23-Day-old rats were given daily injections of CH_3HgCl, 10 mg/kg or 0.05 M Na_2CO_3 for 7 consecutive days and sacrificed 6 days after the final injection.

[b] Body weight on day of sacrifice.

[c] Hg concentrations in the control animals was always below the detection limit of 0.10 μg/g.

Effects of Methylmercury in Vitro on Brain Protein Synthesis

The effect of mercury on protein synthesis when added to the brain homogenate system in vitro as methylmercuric chloride is shown in Table 6. Low levels, corresponding to a brain mercury concentration of 2 μg/g did not significantly affect protein synthesis. Ten times this amount produced only about 20% inhibition in this in vitro system.

These results are comparable to the results in vivo shown in Table 5 where a brain mercury concentration of 23 μg/g attained after repeated sc dosing with methylmercury produced a mean inhibition of about 15%; apparent stimulation of brain protein synthesis occurred 24 hr after an icr injection of methylmercury, but after this initial response had subsided, residual brain mercury concentrations of 2.5 μg/g or less had no effect on protein synthesis (Table 4). No stimulation was noted when methylmercury was added in vitro.

TABLE 6

EFFECT OF ADDING CH_3HgCl TO BRAIN PROTEIN SYNTHESIS SYSTEM IN VITRO

CH_3HgCl concentration (M[a])	Concentration expressed as Hg (μg/g)	Protein synthesis (cpm/mg protein)
0[b]	0	637 ± 102
0[c]	0	559 ± 56
1.0×10^{-5}	2	563 ± 77
1.0×10^{-4}	20	452 ± 29
1.0×10^{-3}	200	281 ± 12[d]

[a] CH_3HgCl, H_2O, or 0.05 M Na_2CO_3 added as a 100-μl volume to the brain homogenate system used to measure brain protein synthesis in vitro as described in Methods. Final volume of incubation mixture was 1.2 ml. Concentrations refer to final concentrations in the incubation mixture.

[b] H_2O added.

[c] 0.05 M Na_2CO_3 added.

[d] Significant difference from Na_2CO_3 control ($p < 0.01$).

DISCUSSION

Icr administration of a single dose of methylmercury produced weight loss and neurological deficit in rats that resembled the neurotoxicity produced by multiple sc doses of the compound. A transient stimulation of protein synthesis in brain occurred after icr administration of methylmercury.

Miyakawa and Deshimaru (1969) reported disappearance of intracytoplasmic membrane structures as the earliest observable structural change in cerebellar granule cells following methylmercury poisoning in rats. Consistent with this ultrastructural finding is Yoshino's report (1966) of inhibition of protein synthesis by as much as 58% in brain cortex slices from rats treated with methylmercury. Yoshino gave methylmercury as the thioacetamide in a single large dose that was eventually lethal to the animals. Cavanagh and Chen (1971b) later reported a decrease in in vivo protein synthesis in cerebral cortex and cerebellum following multiple doses of methylmercury dicyandiamide that produced neurological signs but that were not lethal. The maximum inhibition observed was 26% in cerebral cortex and 21% in cerebellum, at day 5 after the last of 8 daily doses of 5 mg/kg given orally. By day 8 after the final dose, protein synthesis in cerebellum and cerebral cortex had returned to control values, but sciatic nerve protein synthesis, formerly depressed, was 142% of control.

Stimulation of in vivo protein synthesis in hepatocytes has been observed after treat-

ment of rats with methylmercury (Brubaker *et al.*, 1971). More recently, in vivo protein synthesis has also been found to increase in the brain after systemic dosing with methylmercury (Brubaker *et al.*, 1973).

Steinwall and Olsson (1969) have shown that high doses of methylmercury can disrupt the blood-brain barrier. Increased incorporation of a labelled amino acid into brain protein in vivo following treatment with methylmercury could be the result of blood-brain barrier dysfunction. However, this theory is discounted as an explanation of the results presented above, since the same dose-related increase in protein synthesis was observed after icr methylmercury administration when measured either in vitro or in vivo.

Injection of an organophosphorous cholinesterase inhibitor into the cerebrospinal fluid of rats increased the in vivo incorporation of labeled lysine into brain proteins (Clouet and Waelsch, 1963). The inhibitor was capable of phosphorylating and denaturing proteins generally, and it was argued that protein synthesis was accelerated in order to replace damaged protein. Mercury compounds could be expected to act similarly on the basis of their well-known ability to bind to and denature proteins (Webb, 1966).

Cavanagh and Chen (1971b) propose Schwann cell proliferation as the cause of increased protein synthesis in sciatic nerve from methylmercury intoxicated rats. Glial reaction and leukocyte infiltration could explain the increased amino acid incorporation in the area of the lesion produced by icr injection in our experiments, but the same degree of stimulation of incorporation in the opposite hemisphere and cerebellum, where no histologic changes were observed, cannot be satisfactorily explained on this basis.

Since Brubaker *et al.* (1973) found increased brain protein synthesis after systemic dosing with methylmercury, this phenomenon in brain tissue is apparently not dependent upon the icr route of administration. The increased protein synthesis in liver appears to be a true induction, associated with a proliferation of rough endoplasmic reticulum (Brubaker *et al.*, 1971). Changes in ribosome sedimentation profiles from brain were similar to those observed from liver after methylmercury treatment of rats, suggesting similar induction mechanisms in the 2 tissues (Brubaker *et al.*, 1973). Further work to elucidate the nature of this stimulation would be desirable, particularly in brain, where glial and neuronal components have been shown to have completely opposite protein synthetic responses to noxious stimuli (Hamberger *et al.*, 1970; Blomstrand, 1970).

The general approach of achieving a critical CNS concentration of mercury without introducing large amounts of the metal into the rest of the body might still be attempted, even though it appears that icr injection of methylmercury does not produce a good model of the histopathology typically seen with other routes of administration. Slow infusion of methylmercury through an intraventricular cannula might yield better results. Such an approach, in addition to providing an isolated neurotoxic syndrome within the intact animal, might also be used to study the clearance of bound mercury from the brain and turnover of mercury-binding proteins.

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