

BEHAVIORAL AND BIOCHEMICAL
CHANGES IN NEONATAL AND YOUNG RATS
FED METHYL MERCURIC CHLORIDE

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ABSTRACT

BEHAVIORAL AND BIOCHEMICAL CHANGES

IN NEONATAL AND YOUNG RATS FED

METHYL MERCURIC CHLORIDE

By

Elizabeth Post

Effects of methyl mercuric chloride (CH_3HgCl) on behavior were studied with male Sprague-Dawley rats in 3 separate experiments. Rats for experiments 1, 2 and 3 were respectively 15-, 21-, and 60- days old at the initiation of the experiments. The 15- and 21-day old rats were force fed a single dose of 2.0 mg CH_3HgCl in cocoa butter/100 g body weight. The 60-day old rats were force fed a single dose of 2.5 mg CH_3HgCl in 1,2-propanediol/100 g body weight. The results obtained from testing of these rats were compared with control rats fed either cocoa butter or 1,2-propanediol.

Body weights were measured at weekly intervals until sacrificing. Behavioral changes were measured in a T-maze and open field. Initially, the rats were trained for eight days in the T-maze, tested for five days, then retested after seven days. Between T-maze measurements, the rats were observed in the open field for five days,

and retested after an interval of seven days. Three days of extinction trials were performed on the seventh day following retesting in the open field. After one week, extinction was measured for one day. At the end of the behavioral tests, twenty rats (ten in each group) were decapitated, and the whole brain immediately frozen. Brain weight and cerebral DNA and RNA content were determined. Another ten rats (five in each group) were perfused in order to fix the brain *in situ*. Brains were then removed and stored in a balanced formalin solution. Parasagittal sections of the perfused brain were stained with hematoxylin and eosin and examined microscopically.

Significant differences ($P < 0.05$) in latency between mercury treated and control rats were observed during training in experiment 1 and during retesting in experiments 2 and 3. The number of correct responses was significantly different between the two groups of rats during test periods in the T-maze and extinction for experiment 3.

In the start box of the open field during the test period of experiment 1 mercury treated rats sniffed significantly more than the controls. The number of standing upright, circling, cleaning fecal bolli and urinations were however not statistically different between treated and control rats in all test periods for all experiments.

Besides those parameters measured in the start box, the number of areas traversed, time of inactivity, and latency were observed in the open field. Control rats traversed more areas than treated rats in experiments 1 and 3 during testing. Treated rats, in experiment 3, remained inactive longer than controls. No differences in inactivity were found between the two groups for the other two experiments. The number of standing upright, cleaning, and sniffing responses, fecal bolli, and urinations were similar between treated and control rats for all experiments. However, circling responses of the two groups of rats in experiment 3 were significantly different.

During retesting, control rats in experiment 3 crossed more areas, and were more active than treated rats, but no differences were observed between the two groups for experiments 1 and 2. Control rats, in experiment 2, took less time to enter the open field than treated rats. Latency periods for treated and control rats were similar in experiments 1 and 3. There was a slight difference ($P < 0.1$) in the standing upright responses between the two groups of rats in experiment 3. Both experiments 1 and 3 exhibited significant differences in circling responses between treated and control rats. There was no difference in cleaning, and number of fecal bolli and urinations. Control rats, in experiment 3, sniffed more often than treated rats, however, no differences occurred in the other two experiments.

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INTRODUCTION

Mercury is a natural element in the environment; thus a certain level of mercury can be found in rocks, soil, water, the atmosphere, and the biosphere. However, mercury, as inorganic or organic mercury, is a toxic substance. Despite this fact, man has used mercury in industry, medicine, and agriculture. Friberg and Jostei (4) state that mercury has been utilized in eighty types of industry in at least three thousand different ways. Up to thirty percent elemental mercury was once incorporated in a drug commonly used for the treatment of syphilis (4). In agriculture mercury has been found useful as a coating to protect grain against fungus (4).

Mercury has been dumped into rivers and lakes as industrial waste. This was not considered a hazard to the environment as mercury was believed to be an inert substance. Miller and Berg (33) showed that inorganic mercury can be converted to organic mercury by microorganisms, especially those present in the mud of lakes, rivers, and even aquaria. Imura *et al.* (34) found that methylcobalamin is an intermediary in the conversion of inorganic mercury to organic mercury compounds. The organic mercury in water has entered the food chain, and thus fish and shellfish can

contain an extremely high level of organic mercury.

Bache *et al.* (78) showed that the level of mercury and methyl mercury increased with the age of the fish.

The use of mercury in industry has resulted in cases of occupational poisonings, but more recently poisonings have occurred from the consumption of contaminated fish and seeds coated with mercury. At Minamata Bay in Japan, there were 121 cases with 46 deaths from 1953-61, and in Niigata in 1964-65 30 cases resulting in 6 deaths were reported. Outbreaks of mercury poisoning in Iraq occurred in 1956, 1961, and 1972 (11, 79). Eyl (11) reported that in 1960 several hundreds were diagnosed as suffering from mercury poisoning in West Pakistan. A year later several hundred more were poisoned. Forty-five Guatemalans were thought to have viral encephalitis from 1963-65, but autopsy revealed that it was mercury poisoning (11). In the United States, a family in New Mexico and a veterinarian in Texas were stricken in 1970 (11).

Pathological examination of cases of mercury poisoning from Minamata Bay and Niigata was undertaken by Takeuchi (10). There was a reduction in brain size of those who died of mercury toxicity, in comparison, to normal Japanese brain weights. Generally the brains were swollen, and the gray matter wasted away. In the cerebral cortex, usually nerve cells of the occipital lobe were destroyed. Damages varied in severity in the frontal, parietal, and temporal lobe. In the cerebellar cortex, there was a loss

of neurons in the granular layer. Recent investigations have shown degeneration and destruction of the sensory nerves in the peripheral nervous system. No changes have been observed in the optic nerve or retina. Berlin and Ullberg (75) used autoradiographs to show that mercury accumulates in the cerebellar cortex, occipital lobe, and calcarine fissure.

Examination of other cases of mercury toxicity has revealed loss, degeneration, or destruction of nerve cells in the calcarine fissure, granular layer of the cerebellar cortex, and occipital lobe. Logically, a loss of neurons will affect brain function, including changes in behavior. For these reasons, experiments were initiated to determine whether mercury will affect the behavior of animals. Furthermore, since younger animals, especially those still in the process of brain development, are more susceptible to mercury poisoning different aged animals were used in the present experiments. In order to verify the finding that brain size was reduced in mercury poisoning, the brain weight and cerebral DNA and RNA were also measured. Representative animals were also examined histologically to determine brain lesions.

LITERATURE REVIEW

Mercury in the Environment

Natural Sources of Mercury

Most rocks and soils contain up to 100 ppb mercury, especially near ore deposits, but generally the soil contains 60-80 ppb mercury; the atmosphere at ground level has up to 16 ppb, and water, except near areas of man-made contamination has less than 0.1 ppb (1). Joensuu reported that although the concentration of mercury in fossil fuels is low, the amount of fuel burned is large. He has calculated that the yearly consumption of 3×10^9 tons of coal in the United States will give off 3,000 tons of mercury (2).

Man-Made Sources of Mercury

Mercury has been used for years especially in agriculture, pulp and paper industries, and medicine, and each of these may contribute to the addition of mercury in our environment. In the Swedish report, Methyl Mercury in Fish, mercury is listed as an important ingredient in eye preparations, skin ointments, and diuretics (3). In industry, mercury is found as a discharge of the chloride-alkali industry, as a mildew proofing agent in oil, and in electrical apparatus, and latex for ship bottom parts. In

1960, the Swedish pulp and paper industry used as much as 15 tons of phenyl mercury as a slimicide for paper mill machinery and piping systems. In Sweden the yearly consumption of mercury as methyl and ethyl mercury for agricultural purposes has been estimated to be 4,500 kg mercury, or from 1940 to 1966 a total consumption of 80 tons. The primary use of mercury in U.S. agriculture has been as a seed dressing to protect the seed against pithium, rhizoctonium, and other weak soil parasitic fungi (5).

Consequences of Man's Use of Mercury

Many cases of poisonings from alkyl mercury compounds have been reported in the literature. Several reviews have been published listing cases of occupational exposure (3,4,6), and cases developing from the use of skin ointments containing mercury (3,4,7). One case was finally diagnosed as mercury toxicity after the hospital authorities found out that the girl had played with a ball of elemental mercury brought home from school (8).

In Japan it was proven that methyl mercury in fish and shellfish was responsible for the outbreak of Minamata Bay disease. The methyl mercury was formed from mercury waste from a nearby vinyl chloride factory. Hammond (1) stated that fish can concentrate methyl mercury through their food and directly through the gills so that their flesh contains thousands of times more mercury than the surrounding water. Hammond has proposed that if a 70 kg

man consumed more than 420 g of fish containing 0.5 ppm mercury a lethal dose of mercury could be accumulated.

Both the tuna and swordfish industries have been affected by the ban on some batches of fish found to have levels exceeding that set forth by the FDA, 0.5 ppm. Miller *et al.* analyzed seven fish captured 62-93 years ago and one swordfish caught 25 years ago. He compared them with some fish caught just recently. There was no difference in the mercury content of the tuna specimens. The results with the swordfish were too variable to be conclusive (9).

Symptoms of Organic Mercury Poisoning

Takeuchi described the symptoms associated with organic mercury poisoning, and related the degree of severity of the symptoms to the age of the subject (Table 1).

Table 1. Frequency of occurrence of various signs and symptoms in Minamata Disease (% of cases, ref. 10)

	fetal	children	adult
mental disturbance	100	100	71
ataxia	100	100	94
impairment of gait	100	100	82
disturbance of speech	100	94	88
hearing impairment	4.5	67	85
constriction of visual field	-	100?	100
disturbance in chewing and swallowing	100	89	94
brisk and increased tendon reflex	89	72	34
pathological reflex	54	50	12
involuntary movement	73	40	27-76
primitive reflex	73	0	0
impairment of superficial sensation	?	?	100
excessive salivation	72	56	24
forced laughing	27	29	-

One study of humans suffering from mercury poisoning indicated abnormal electrocardiograms (11). The subjects had prolonged QT intervals, ST segments, and T wave inversions. Symptoms which were similar to those observed in humans can be induced in animals. Miyokawa & Deschamaru conducted an experiment using rats. The animals became ataxic and incoordinated after given mercury. Another common symptom seen in the rat was crossing of the hind legs while being held by the tail (12). Morikawa produced symptoms of Minamata disease in cats (13). Three pregnant cats were used, and two exhibited neurological symptoms, and the other died at parturition. Eight baby cats were born, and two died at birth, four died soon after birth with no observable neurological symptoms, and two showed

neurological symptoms two weeks after birth. In another experiment by Morikawa, 21 cats were given four different organic mercury compound for several weeks. Cerebellar ataxia developed within 2-5 weeks. During the latter part of treatment the cats were apathetic, emaciated, and showed signs of panic (14). In another study rats exposed to mercury vapour did not eat for the first twenty-four hours after the treatment. Later ataxia, incoordination, and unsteadiness in gait appeared (15).

Treatment for Mercury Poisoning

Although there is a difference in the amount of mercury accumulated in the cerebrum, cerebellum, and brain stem with the type of mercury, inorganic or organic, to which the subject is exposed, the therapeutic management of the toxicity can be either ethylenediaminetetraacetic acid (EDTA), 2,3-dimercapto-1-propanol (BAL), penicillamines, or spironolactone (16). EDTA acts as a chelating agent, and thus will compete with the tissue for mercury. The action of BAL and penicillamines is based on their SH groups combining with mercury and competing with the tissue for the metal. In addition, BAL may have an effect on pyruvate oxidase activity in the brain. How this latter effect reduces toxicity is not known (17). The effectiveness of BAL is probably dependent on the dose and time of administration in relation to the onset of mercury poisoning. In a study conducted by Magos, 2 mg/kg

BAL given six days after treatment were not effective, but 8 or 16 mg/kg increased mercury excretion in the rat treated with HgCl (18). In this same study when 6 mg or 16 mg/kg were given five days after mercury treatment, urinary excretion of mercury increased. Six mg BAL given ninety minutes after mercury treatment increased the concentration of mercury in the urine by twenty-four percent during a period of 2 days (18).

Matsumoto *et al.* fed Wistar rats CH_3HgCl on the ninth and eleventh day of pregnancy. Half of the animals was given penicillamine hydrochloride four to five hours after mercury treatment. On examining the fetal brains, those rats that did not receive the penicillamine had more malformation of the cerebellum and degeneration of the neurons in the midbrain when compared to those that received penicillamine. Penicillamine also decreased the brain concentration of mercury (19).

Arena tested the effectiveness of BAL, D-penicillamine, D,L-penicillamine, and N-acetyl-dl-penicillamine against mercury poisoning. In this study, BAL removed the greatest amount of mercury from the GI tract than the other compounds (20).

Selye tested the effectiveness of spironolactone in preventing mercury poisoning. Female Sprague-Dawley rats with a mean body weight of 100 g were divided into two groups. Group 1 was not treated, group 2 received 10 mg spironolactone twice daily for the entire extent of the

experiment. On the fourth day both groups were administered a single dose of 400 μg HgCl_2 . All control animals died within 3 days after injection of HgCl_2 . At autopsy the kidneys of each of the control rats showed heavy cortical calcification with severe perirenal edema. All of the spironolactone treated animals were living and later examination of their kidneys revealed no lesions. Spironolactone possesses a thioacetate group which may introduce sulfur into the organism to detoxify mercury (21).

Biochemical Aspects of Mercury Poisoning

Mercury combines with thiol groups, and thus is capable of inhibiting enzymes containing thiol groups (22). Hughes described the mechanism as: $\text{CH}_3\text{HgCl} + \text{Prot-SH} \rightarrow \text{CH}_3\text{HgS-Prot} + \text{H}^+ + \text{Cl}^-$, where the protein SH represents the protein sulfhydryl group (23). Since hemoglobin contains a large number of SH groups, blood of poisoned individuals usually contains a high concentration of the metal.

The main effect of mercury is to disturb protein synthesis leading to a dysfunction in the cell (3). This was supported by Yoshino's work. Yoshino *et al.* found a decreased protein synthesis in the brain cortex of rats previously treated with mercury. Yoshino proved that during the latent period before the neurological symptoms appeared the incorporation of leucine- $\text{U-}^{14}\text{C}$ into brain protein decreased. After the neurological symptoms

developed, there was a decrease in oxygen consumption, in anaerobic lactate formation, and in succinate dehydrogenase activity, but an increase in glutamate dehydrogenase activity. These changes in enzyme activity were evident to the same degree in all parts of the brain analyzed by Yoshino (24). Another investigator reported that the incorporation of cytidine-³H into RNA was disturbed by the injection of methyl mercury. Mercury also has an affinity for amine, carboxyl, and hydroxyl groups. Mercury inhibits phenolsulphate conjugation, citrulline phosphorylation, oxidative mitochondrial phosphorylation, and serine biosynthesis (25).

Using Hg²⁰³ as phenyl mercury acetate and mercuric acetate, Ellis and Fang found the following percentage distribution in the kidney: 18-39% in nuclear fraction, 4-11% in mitochondrial fraction, 3-11% in microsomal fraction, and 50-71% in the soluble fraction of the cell sap (26). Takeuchi reported that lesions in mitochondria and lysosomes of mercury treated animals were a result of mercury accumulating in these cell fractions (10). Another study reported that mercury was found in the protein fraction of the cell; it is evenly distributed between the mitochondrial and microsomal fractions (3). However, Norseth (cited in Friberg & Vostel) reported that it was higher in the microsomes than the mitochondrial and lysosomes/peroxisomes (4).

Transport and Distribution

Mercury is absorbed in the GI tract, skin, and lungs (27). Ninety percent of the methyl mercuric chloride administered orally was absorbed within two hours (28). Six percent of methyl mercury dicyandiamide dissolved in water when placed on the skin was absorbed in five hours (4).

The main excretion routes for mercury are the feces, urine, sweat, milk and saliva. In one study it was reported that 80% of the organic mercury is excreted in the feces and 10% in the urine (29). Fifty percent of the total body burden of mercury ingested, is in the kidney. It is reabsorbed in the tubules and only about 10% of the total amount absorbed is excreted in the urine (Brown and Kulkarni, 25). There are differences in both retention and excretion of mercury depending on the compound involved. As a result some compounds such as methyl mercuric chloride are more toxic than other mercury compounds.

The distribution of mercury in the body varied with the mercury compound administered. Inorganic will preferentially be deposited in the kidney, liver and mucous linings of the body, whereas, organic mercury will be deposited in the central nervous system. Whether the dose is given singly or repeatedly will also affect the distribution of mercury in the body (4).

Biotransformation of inorganic mercury to organic mercury (30), and organic mercury to inorganic mercury has

been investigated (31). Clarkson reported that microorganisms are capable of volatilizing mercury from solutions of mercuric chloride (32). Jernelow (cited in Miller and Berg, ref. 33) showed that inorganic mercury is converted to methyl mercury by microorganisms present in the mud of Minamata Bay. He postulated that the following reactions occurred in the transformation (Figure 1).

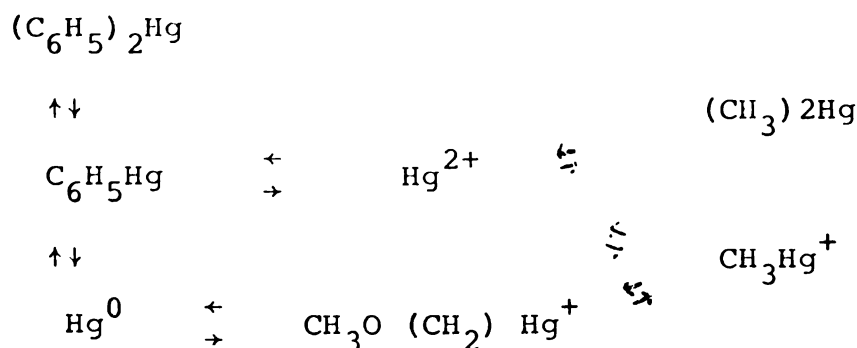


Figure 1. Transformation of mercury to its various forms.

Methylcobalamin will act in a non-enzymatic reaction to transfer its methyl group to mercury. Imuro *et al.* (34) found that in the presence of mild reducing agents such as zinc, ammonium chloride, or stannous chloride at a neutral pH dimethyl mercury was formed from inorganic mercury such as mercuric chloride. The latter may further react with dimethyl mercury to produce methyl mercuric chloride. Almost all of the inorganic mercury was methylated within five hours. Methylcobalamin is present in microorganisms, and mammalian tissues such as calf liver

and blood plasma and thus able to cause biotransformation of mercury.

Genetic Effects of Mercury

Mercury apparently affected the mitotic spindles of the cell (3). It doubled the number of chromosomes and dissociated individual chromosomes during mitosis. During polymerization, when the SH groups were oxidized to S-S bridges, mercury was bound to the SH group thus preventing polymerization. *In vitro* studies revealed that during anaphase mercury was bound to DNA, especially the nucleoside thymidine, and irreversibly denatured the DNA molecule. Sherfung *et al.* (35) measured by means of activation analysis the concentration of mercury in whole blood, red blood cells and plasma of 9 subjects regularly consuming contaminated fish as well as 4 control subjects. There appeared to be a correlation between the frequency of chromosome breaks in the red blood cells to the concentration of mercury. There was a disturbance of chromosomes and induction of polyploidy and other deviating chromosome numbers in the cell (4). *Allium cepa* roots treated with mercury illustrated c-mitosis or inactivation of the spindle fiber mechanism during cell division. Umada (cited by Friberg & Vostel, ref. 4) treated tissue cultures of *HeLa* cells with phenyl and ethyl mercuric chloride, and found c-mitosis. In another study, tradescantia were treated with methyl mercury, and the

spindle fibers were inactivated during meiosis. After exposure to mercury, drosophila exhibited non-disjunction of the chromosomes during meiosis. There was no evidence of crossing over, and there was only a small mutagenic effect (4).

Effects of Mercury on the Brain

Experiments revealed that in Wistar rats treated with mercuric nitrate, mercury was mainly accumulated in the kidney, liver, blood, and muscle. The concentration in the brain at 4 hours, 1 and 15 days after administration was 0.03 μg , 0.04 μg , and 0.03 μg respectively (Rothstein and Hayes, 36). Magos (37) found that within 30 seconds after administering either mercury vapour or mercuric salt to rats, mercury was detected in the blood; however, there was little change in other tissues until five minutes had elapsed. Mercuric chloride, however, took longer to enter the brain than into other organs. Other studies (30) showed that the concentration of mercury in the brain after administering alkyl mercury compounds did not reach its peak until the eighth day after injection.

Ulfvarson injected female rats with various aryl and alkyl mercury compounds at 10, 1, or 0.1% of LD_{50} . There was little difference in the concentration of mercury in the cerebellum and cerebrum for a specific mercury compound (38). Okinaka *et al.* (7) described three cases of encephalomyelopathy due to organic mercury poisoning. All

three cases resulted from treating a skin rash with methyl mercury thioacetamide. Two of the cases were analyzed for mercury concentration in various brain parts using the dithizone method. Mercury in brain parts ranged from 13 to 70 $\mu\text{g/g}$ of tissue. The concentration was higher in the cortex than the medulla, but there was little difference between the concentration of mercury in the cerebrum and cerebellum (Table 2).

Table 2. Concentration of mercury in the cerebrum and cerebellum ($\mu\text{g/g}$ of tissue).

Tissue	Case 1	Case 2
Cerebellar cortex	15-66	48.2
Cerebellar medulla	13-41	11.0
Cerebral cortex	18-79	22.1
Cerebral medulla	15-58	19.7

Fetal-infantile Minamata disease produces micro-encephalia in which the brain is reduced by two-thirds or even one-half in comparison to normal brains from individuals of the same age (Table 3). The decrease in brain weight indicates a decrease in cell number (10).

Table 3. Average brain weight in Minamata disease and its comparison with the normal.

<u>Brain weight of normal Japanese</u>			<u>Minamata disease</u>			
<u>Age</u> (yr.)	<u>Sex</u>	<u>Brain</u> <u>Weight</u> (g)	<u>Age</u> (yr.)	<u>Sex</u>	<u>Clinical</u> <u>Course</u>	<u>Brain</u> <u>Weight</u> (g)
1-2	female	1053	2.6	female	2.6 yrs.	650
3-5	female	1175	4	female	1.6 yrs.	700
			5	female	2.6 yrs.	950
6-9	female	1250	6.3	female	6.3 yrs.	630
			8	female	2.9 yrs.	810
			7	male	4 yrs.	600
20-29	female	1318	28	female	2.9 yrs.	1200
			29	female	53 da.	1150
30-39	male	1450	34	male	19 da.	1200
			34	male	96 da.	1110
40-49	male	1426	47	male	45 da.	1300
			49	male	85 da.	1200
50-59	female	1250	50	female	90 da.	1200
			58	female	60 da.	1050
	male	1417	52	male	100 da.	1430
			56	male	48 da.	1290
			57	male	1.4 yrs.	1450
			59	male	93 da.	1250
60+	male	1400	60	male	2.0 yrs.	1110
			61	male	76 da.	1410
			66	male	10.2 yrs.	1300
			78	male	9.9 yrs.	1000
			79	male	9.3 yrs.	1230

Classical pathological findings in mercury toxicity usually revealed some loss of neurons in the granular layer of the cerebellum, and degeneration or destruction of nerve cells in the layers of the cerebral cortex. Nerve degeneration and loss of glia cells occurred mainly in the cerebral cortex, calcarine area, precentral and postcentral cortical areas, superior temporal gyrus and frontal areas. Lamina 2 and 3 were the levels which mainly lost neurons, except where severe damage ensued. In that case, the first layer was the only one not affected. There were also some granule cells lost in the cerebellum. The anterior horn of the spinal cord has exhibited degeneration. Changes occurred both in the nucleus and perikarya of neurons (3).

One investigator injected adult dogs with methyl mercury thioacetamide, and found histological changes were predominant around the calcarine area with moderate disturbance in the temporal areas. In the cerebellar cortex, there was some loss of granule cells, but the Purkinje cells remained normal (39). In another study seven different organic mercury compounds were administered to 21 normal cats either by stomach tube or in the feed. The dose was 2-3 mg per kg body weight for 30-45 days. Within 2-5 weeks neurological symptoms had developed in some but not all the cats. Pathological findings were similar to those reported by other investigators. It was also noted that the brains were swollen, and in the white

matter there was some "loosening" of the nerve fibers. Perivascular loosening of the ground substance was noticed in the hippocampus, cerebral nuclei, and diencephalon. Some compounds were more destructive and diverse in their damage, yet all resulted in pathological damage to the classical areas (14). Berlin *et al.* reported that rats, monkeys, and rabbits exposed to mercury vapour showed a decrease in the density of cells in the grey matter of the cerebrum (15).

Histological examination of three human cases of mercury toxicity revealed loss and degeneration of nerve cells in the cerebral hemispheres primarily in the second and third layer (7). Only a slight decrease in the number of granule cells in the cerebellar cortex was observed, however, there was some loss of Purkinje cells. Hunter reported a case of a man who for fifteen years was continually exposed to mercury in a place where he worked. His brain was examined, and it was found that the frontal lobe was slightly atrophied. Gross convolutional atrophy was observed in the occipital areas, and both lateral lobes of the cerebellum. Another case of a young man, showed some swelling of the brain, and the axons were reduced in number, and frequently exhibited bulbous swellings. In the calcarine cortex, especially at level five, there was a loss of neurons. To a lesser extent there was a loss of neurons in the motor and sensory cortex, caudal part of the first temporal gyrus, and parastriatia region. In the

cerebellum, the white matter was porous. Basket cells and Purkinje cells were degenerated; but there was little change in the granule cells in the cerebellum (40).

Cases of human fetuses exposed to mercury during the sixth to eighth month of embryonation exhibited cortical lesions of the brain that were more widely dispersed and more severe than non-fetal infantile cases of Minamata disease. In contrast, adult-brain lesions were localized. This was reported by Takeuchi who compared the pathology of persons who had Minamata disease at different ages (Table 4, ref. 10).

Table 4. Comparison of pathology of Minamata disease among fetal, non-fetal and adult cases (No. of + signs indicates increased occurrence).

<u>Pathological feature</u>	<u>fetal</u> <u>infantile</u>	<u>non-fetal</u> <u>infantile</u>	<u>adult</u>
1. cortical disturbance of cerebrum	+++	+++	+++
2. cerebellar disturbance of granule cell	+++	+++	+++
3. central granule cell atrophy	+	++	+++
4. degree of granule cell disturbance	+++	++ - +++	+
5. hypoplastic changes of cytoarchitecture (a,b,c,d,e) *	+++	-	-
6. malformation of neurons	+++	-	-

*a-remaining matrix cells; b-nerve cells in cerebral medulla; c-abnormal cytoarchitecture; d-hypoplastic narrowing of granular layer in cerebellum; e-hypoplastic corpus callosum

Development of the Brain

DNA and RNA and Their Relation to Cerebral Weight

Total DNA in the brain reached adult level by the fourteenth day postnatally in the rat. By the thirteenth day RNA has reached adult level; and ribosomes, endoplasmic reticulum, and myelinated axons have appeared (41).

Altman stated that in the rat, DNA concentration declined after birth, increased from the fifth day to the fifteenth day, and then was reduced to adult levels of concentration (42). However, Winick and Noble (43) found that DNA in the brain increased sharply after birth to the twelfth day, then slowly decreased to an adult level by the twentieth day.

Zamenoff *et al.* found that there was a decrease in cerebrum weight with a corresponding decrease in DNA during malnutrition. In his study pregnant rats were maintained on a diet with one third the normal caloric value but with identical protein and vitamin content as in the diet of the controls. The dietary restriction was imposed from the tenth to twentieth day of pregnancy. Caesarian sections were performed on the twenty-second day to recover the pups. The undernourished dams had offspring with reduced cerebral weight and DNA concentration when compared with control litters (44). Another investigator studying the effects of neonatal malnutrition on the developing cerebrum, showed that the cerebral cortex of the animals had a thickness of 1,220 μ , and cells per unit

volume were increased from birth to 10 days of age. Up to thirty days of age, cellular density continued to increase for the malnourished animal and cortical thickness did not become normal until forty to fifty days of age (45). In contrast, in normal animals at ten days of age, the cortex has a mean width of 1,650 μ , and cellular packing density is greatly decreased.

In normal development, DNA concentration in cerebral cortex decreased by 77% between birth and ten days of age then gradually increased to adult values. In the cerebral cortex of rats subjected to neonatal malnutrition DNA decreased only by 24% between birth and twenty days of age. The level of DNA remained unchanged so that at sixty days it was 30% lower than controls (45).

When neonatal mice were fed a reduced amount of food, brain weight reductions correlated with decreases in body weight. The authors concluded that if nutritional deprivation occurred during the period of rapid growth it could lead to a suppression of mitosis or an irreversible reduction in the number of cells. The results indicated that though, there was a great decrease in DNA in the cerebellum, and some in the cerebrum, learning was not impaired (46).

Nomenclature of Brain Cells

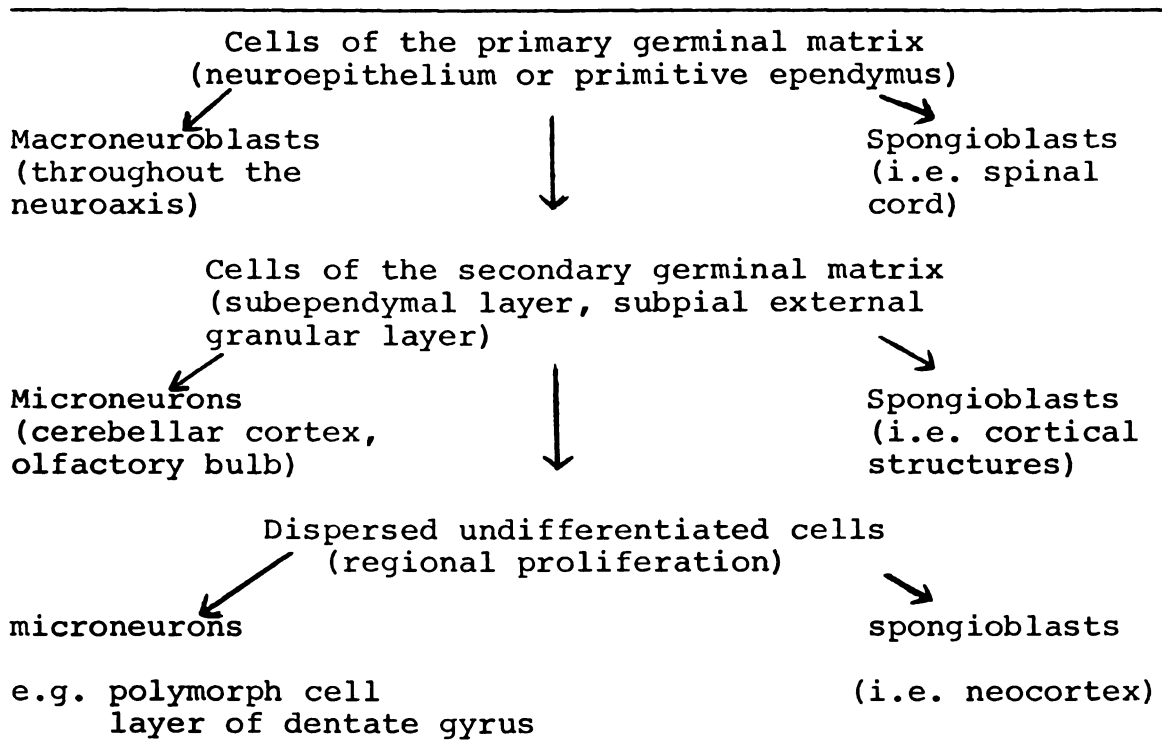
Brain tissue proper is composed of macroneurons, microneurons, neuroglia, and microglia. Macroneurons are

long axoned nerve cells which function as afferent elements of the nervous system. Microneurons are short axoned interneurons restricted to local integrating and modulatory functions. Astrocytes are the supporting elements of the brain which nourish nerve cells, as well. Oligodendroglia provide insulating myelin. Microglia are from mesenchymal origin, and are the scavenger cells under pathological conditions (42).

Development of the Brain

Altman had diagrammatically illustrated the development of all stages of nerve cells (Table 5). Proliferation of precursors of microneurons and neuroglia is essentially a postnatal phenomenon. The subependymal zone of the ventricles, and the subpial zone of the cerebellar cortex are the postnatal proliferative sites (47).

Table 5. Development of nerve cells in the brain according to Altman (47).



Altman (47, 48) discovered that the proliferation of the external differentiating cells of the cerebellum commenced soon after birth in the granular layer. The outer subpial zone contained round mitotic cells. The first cells to migrate and differentiate in the molecular layer were the basket cells. This activity occurred from the second to sixth day postnatally. Stellate cells were highly proliferative on the thirteenth day postnatally, and granule cells increased slowly from the sixth to thirteenth day after birth. The overall picture of cerebellar development indicated an accumulation of undifferentiated neurons in the first week and extensive

cell production and differentiation during the second week. By the end of the third week, the external granular layer is only one to two cells thick.

Other experiments by Altman (42, 48) showed that the greatest number of granule cells in the hippocampus developed postnatally. The cells formed prenatally, formed an outer zone in the granular layer. As cells were produced they were added to this layer, so the last cells to differentiate were at the base of the granular layer. Around the dentate gyrus of the hippocampus there was a high concentration of undifferentiated cells at ten days of age. This differentiation continued up to three months of age. The dorsal hippocampus was actively growing up to thirty days after birth. The hypothalamus exhibited proliferation up to fifteen days of age.

Altman has also investigated the development of the olfactory bulb using labelled thymidine (42, 47, 50). In the subependymal layer proliferating cells gradually migrate caudorostrally to the olfactory bulb. At 30 days of age the ependymal layer of the lateral ventricles had up to 30% of the cells that were labelled. Six days later 65% of the cells in the subependymal layer of the olfactory ventricles were labelled. Twenty days later these same labelled cells were a part of the first granule layer. From the olfactory bulb, the proliferating cells migrate to the corpus callosum and through the white matter to the neocortex. Rats given multiple injections of tritiated

thymidine during the first week of life, and killed three months later were found to have 30% of the cells in the dorsal cortex labelled. Some cells were labelled more intensely than others, indicating that a proportion of the cells were of postnatal origin. These cells were usually the neuroglia or microneurons (Altman & Das, 49, 50). Sugita (cited in Altman, 42) proposed that there was longitudinal growth of the forebrain for several days after birth. Altman had indicated that there was some degree of proliferation in the anterior forebrain (47). He had also studied the differentiation of neuroglia which primarily occurred after birth in the rat (51).

Relationship Between Brain Development and Behavior

A parallel exists between the development of the brain and behavioral development. Between the tenth to seventeenth day postnatally there is a marked acquisition of new motor and sensory capabilities; after this the process of socialization begins. Thus at twenty-one days of age the adult food seeking behavior has emerged, and weaning can take place (Dobbing, 41).

Morphologically, there are four stages of brain growth. During phase 1, the actual configuration of the brain is developed. This phase is completed by the third day of postnatal life in the rat. There follows a rapid increase in the size of the brain, growth of axons, dendrites, and the establishment of neuronal connections

(phase 11). The transition from the second to the third phase is gradual. The adult brain constitutes phase 111. Senile regression is phase IV, and the last phase. Dobbing points out that during periods of rapid growth the brain is vulnerable, however, since all areas of the brain do not develop simultaneously, the sections where the lesions may occur will thus vary (Dobbing, 41). At all stages of brain development, especially during phase I and II, damage to the brain will most likely produce behavioral changes.

The Learning Process

Learning

The limbic system or allocortex is concerned with the biological rhythm, sexual behavior, emotion, and motivation. Learning is the main function of the cerebral cortex which serves as a memory bank. Memory recalls events that have occurred immediately, in a few minutes or hours, and in the distant past. The temporal lobe consolidates learning, as well as stores events from the distant past. Specifically, the hippocampus allows the organism to obtain new knowledge, and retain old memories. New memories are not controlled by the hippocampus. The parietal lobe is concerned with sensory recognition. The frontal lobe deals with the "intelligence" of previous learning (52).

Altman states that the microneurons are responsible for neural "plasticity" or the substance of memory. That is, the acquisition of locomotor skills and the fixation

of behavior patterns relating to effective need-catering functions, but not the processes relating to cognitive instrumental functions. The need-catering functions are dependent on maturation or stage of development; they are highly resistant to extinction. Cognitive instrumental functions are easily altered by new experiences, and not dependent on microneurons which develop postnatally (Altman, 47).

According to Reynolds (53), aquisition of learning is an increase in the operants emitted. This increase is due to the increasing occurrence of a reinforcing stimulus, which can change a simple response into a more regular response.

Lashley found that rats with eighty percent of the cerebral cortex removed had no gross impairment in learning (cited by Thompson, 54). Thompson (54) examined this further by training albino rats to a position habit in a T-maze, and then removing up to ninety percent of the cerebral cortex. The author concluded that the neocortex, and limbic system were not necessary to mediate the learning response, but were important in establishing learning. The strength of the response was undiminished by the removal of the cerebral cortex. In another study (55) Fisher rats were injected with a carcinogen methylazoxymethanol. The carcinogen reduced the size of the brain mainly in the neo and paleocortex, brain stem, and cerebellum. Pathological examination revealed a

decrease in the number of neurons in the neocortex and hippocampus. The performance of the animals in the first set of problems in the Hebb-Williams maze was lower for the treated group than for the control group. However, the number of errors made by the treated group in the second set of problems decreased thus the damage was probably not permanent or other parts of the brain took over its function.

Segal *et al.* (56) was interested in the function of the hippocampus in classical aversive and appetitive conditioning. The hippocampus functions as a central processor influencing perceptual, as well as behavioral mechanisms. The experiment was designed to test two distinct behaviors. The results indicated a differentiation in the hippocampal system. The dentate gyrus augments conditioned stimuli leading to food reinforcement and inhibits a response from a stimuli preceded by an electrical shock (aversive stimuli). The hippocampal proper augments both stimuli.

Memory

Memory is the process of recalling or recognizing an event previously learned. In an experimental situation an animal regards one stimulus in preference to another because in the past it has been associated with a reward or avoidance of punishment. The actual process of storing and recording the learned association is the memory trace or

engam. Some neurophysiological, morphological, or biochemical changes occur with memory storage (41). Recent or short term memory is very labile, and dependent on an electrical current in the cortex and hippocampus. During behavioral stimulation the amount of RNA and protein synthesized are increased. This is accomplished by a specific mRNA. If protein synthesis is blocked, the fixation of recent memory is inhibited (57). One investigator inhibited protein synthesis by injecting rats with puromycin. With this treatment, long term storage was impaired, since changes in activity, and aversive conditioning in a T-maze indicated a decrease in learning ability (57).

In other studies to determine memory in relation to cholinesterase, rats were trained to perform a simple task in a Y-maze. The rats were given anticholinesterase either 30 minutes, 3 or 5 days after testing in the Y-maze, then retested in the maze. There was a loss of memory for the group receiving the compound 30 minutes after testing, but not for the group given it on the third day. For the group that received the drug five days after testing there was only slight recognition of the test. Thus there was an initial stage of vulnerability which was less than one day, and a latter stage from five days onwards. Forgetting was due to a reversal of the synaptic condition which underlies learning. The investigator next studied the effects of anticholinergics on memory. Anticholinergics

were administered 1, 3, 7, or 11 days after testing in the Y-maze. From one to three days after testing the anticholinergics blocked the receptor site or postsynaptic membrane thus inhibiting depolarization. From the seventh day after testing there was little effect on memory. The anticholinergic effect was the mirror image of the anticholinesterases. At the time of learning, a set of synapses altered their conductance. The postsynaptic endings became more receptive to acetylcholine up to a certain point. When this sensitivity began to decline forgetting occurred (58).

Extinction

Extinction is another form of learning. Synaptic connections initially formed in learning are either weakened or uncoupled in extinction, or it is another habit which is acquired (58). Deutch (58) concluded from his work with anticholinergics and anticholinesterases that extinction was a learning process of a separate habit that opposed the performance of the initially rewarded habit. Another means of explaining extinction is an operant which was previously reinforced, but is no longer rewarded. The response rate is low or completely eliminated, but the decline in response rate is gradual. The course of extinction varies with the previous experience of the organism. The schedule of reinforcement, magnitude of reinforcement, number of previous extinction experiences, and the magnitude of motivation are involved.

Behavioral Tests

Theory of the Open Field Test

According to Reynolds (47) emotion is a complex response involving both respondent and operant behavior. Hall (59) devised the open field as a test to measure emotionality in the rodent. He recognized the fact that during periods of emotional stress excited animals will defecate or urinate more. Emotional defecation and urination are defined as defecation and urination which cease upon repeated exposure to the situation which originally evoked the response. Candland and Nazz (60) further defined the indices of emotionality as activity, and defecation and urination. If the animal exhibited high activity or exploratory behavior, this indicated low emotionality. Activity decreased with repeated testing. He postulated that defecation could be a result of fear, establishing territorial rights, or replacing a strange odor with one that is familiar.

Ader *et al.* (61-63) in a series of experiments measured the corticosterone levels and adrenal weight in relation to diurnal rhythm and periods of stress. The author concluded that the behavioral characteristics of emotionality were not related to adrenocortical function. There was no difference in the behavior at those times of day when the steroid levels were at maximum and minimum points.

Open Field in Nutrition

The open field apparatus has been used extensively by investigators concerned with protein calorie malnutrition, and its effects on the learning ability of the neonate (Levitsky, 64; Cowley and Griesel, 65). Frankova and Barnes (66) measured horizontal (walking, running, and sniffing) and vertical (head up and stand up) responses with malnourished and control rats. The field they used was 27x34.5x5.6 cm and divided into six equal areas. The animal was observed for six minutes on the tenth, fourteenth, and three hundredth twenty-first days postnatally. More vertical movements were observed than horizontal responses indicating more activity in the square for the control rats. The exploratory drive decreased as a result of undernutrition in preweaning days. Another investigator tested the behavior of rats in the open field at 26 weeks of age. The rats were undernourished prenatally. The field was 122x122 square inches, and divided into six equal areas. Reaction time, entering into a complete square or half entrance, time in the center of field, and the number of fecal bolli were recorded. The authors concluded that there was a significant difference in the exploratory behavior of the progeny born of *ad libitum* fed dams versus those born to underfed dams. The difference was not due to the differences in body size (67).

T-maze Testing

The T-maze was originally designed by Watson and Yerkes to study sensory discrimination of animals. Simonson and Chow (68) measured the performance of progeny born to underfed mothers in a T-maze. Water was the primary reinforcement. After 121 trials, extinction was measured. During the tests, starting time, running time, error free (correct choice at the end of three choice points) retrace error, and the number of fecal bolli were counted. The results indicated an initial difference in starting and running time for the experimental rats. During the extinction trials the experimental animals continued to run, whereas the controls stopped running down the maze. The number of fecal bolli were 12.4 for the experimental rats compared to 2.7 for the controls.

The Effect of Mercury Poisoning on Behavior

Limited information is available concerning the effect of mercury on the behavior of animals. In one study, pigeons were trained to peck a key in a modified Skinner box. After a baseline response rate was maintained, eight pigeons were placed in a test chamber, mercury vapour was released into the test chamber at a rate of 17 mg/m^3 of air for two hours. This exposure procedure was repeated five times per week for thirty weeks. During these periods of exposure to mercury, the pigeons' response rates decreased. Immediately after exposure was terminated,

the response rates returned to the baseline. Control pigeons remained at the baseline response level throughout the experiment. Armstrong *et al.* concluded that the change in behavior was due to the weakening of stimulus control in performance (64). In another study, CFW mice were injected with methyl mercury hydroxide (1.5, 3, or 5 mg/kg ip) on day eight of pregnancy. Progeny of the mercury treated mice showed differences in open field, 2-way avoidance shuttle box, water runway performance, and spontaneous motor activity in comparison to saline treated controls (70). Another investigator (71) administered 2.5 mg CH_3HgCl /kg body weight in the drinking water of pregnant rats. The rat pups were cross fostered, and the dose was continued to all progeny up to 45 days after birth. Initial testing of gestational and postweaning treated groups revealed a learning deficiency; however, on retesting only the gestational group persisted in this deficiency. Evans and Kostyniak trained pigeons to peck a lighted disk in a Skinner box. The investigator gave $\text{CH}_3\text{Hg}^{203}\text{Cl}$ in 5 mMol Na_2CO_3 p.o. five times weekly for three to eleven weeks. Behavioral tests were given twice weekly, twenty four hours after treatment. The only change in behavior was the pause following food reinforcement (72).

MATERIALS AND METHODS

Animals

Three groups of male Sprague-Dawley rats aged 15, 21, and 60 days old were purchased from a local dealer, and used in three experiments. Each experiment consisted of thirty rats divided into two groups of fifteen each. In the first experiment, the rats were housed in Veterinary Research barn number 3. The second and third experiments were conducted in the Food Science Building. A 12 hour, day and night schedule was maintained for all animals.

All animals were given water *ad libitum*, and in all test periods except the T-maze and extinction trials the animals were given food *ad libitum*. They were fed the regular grain diet of our laboratory (Appendix I). The food reward given during the T-maze trials was in the form of 300 mg pellets obtained from the P. J. Noyes Company. Appendix II lists the composition of these pellets. During T-maze trials and extinction the animals were placed on a 2.5 hour feeding regime.

Method of Treatment and Dose of

Methyl Mercuric Chloride

The organic mercury compound, methyl mercuric chloride, (CH_3HgCl) was administered orally using cocoa butter as a

carrier for experiments 1 and 2 (15 and 21-day old rats respectively), but for experiment 3 (60-day old rats), 1,2-propanediol was used as the carrier. There were 12 mg methyl mercuric chloride added per gram of cocoa butter, and 7.5 mg methyl mercuric chloride per ml of 1,2-propanediol. The CH_3HgCl was given in single doses to the mercury treated rats. For experiments 1 and 2, the dose was 2.0 mg CH_3HgCl /100 g body weight, and for experiment 3 the dose was 2.5 mg CH_3HgCl /100 g body weight.

Structure of the T-maze

The T-maze consisted of a start box, running arm, and two goal boxes. The start box was 12x12x8 1/2 inches, the running arm was 47 1/2x12x8 1/2 inches, and the goal boxes were 18 1/2x12x8 1/2 inches each. A top view of the T-maze is illustrated in Figure 2. The T-maze was placed on a large plywood board elevated 20" from the ground by sawhorses. A 1/8 inch wire mesh screen covered the floor of the goal box, and extended 3 inches out from the door, and 10 inches along one half of the running arm.

Open Field Structure

A square box was constructed of plywood walls and floor. Inside the plywood square a thin sheet of metal transformed it into a circular field. The floor was varnished, and marked off into seven equal areas. The field was 30 inches in diameter, and 18 inches high, and the start box was 10 1/2x7 1/2x18 inches. A plexiglas

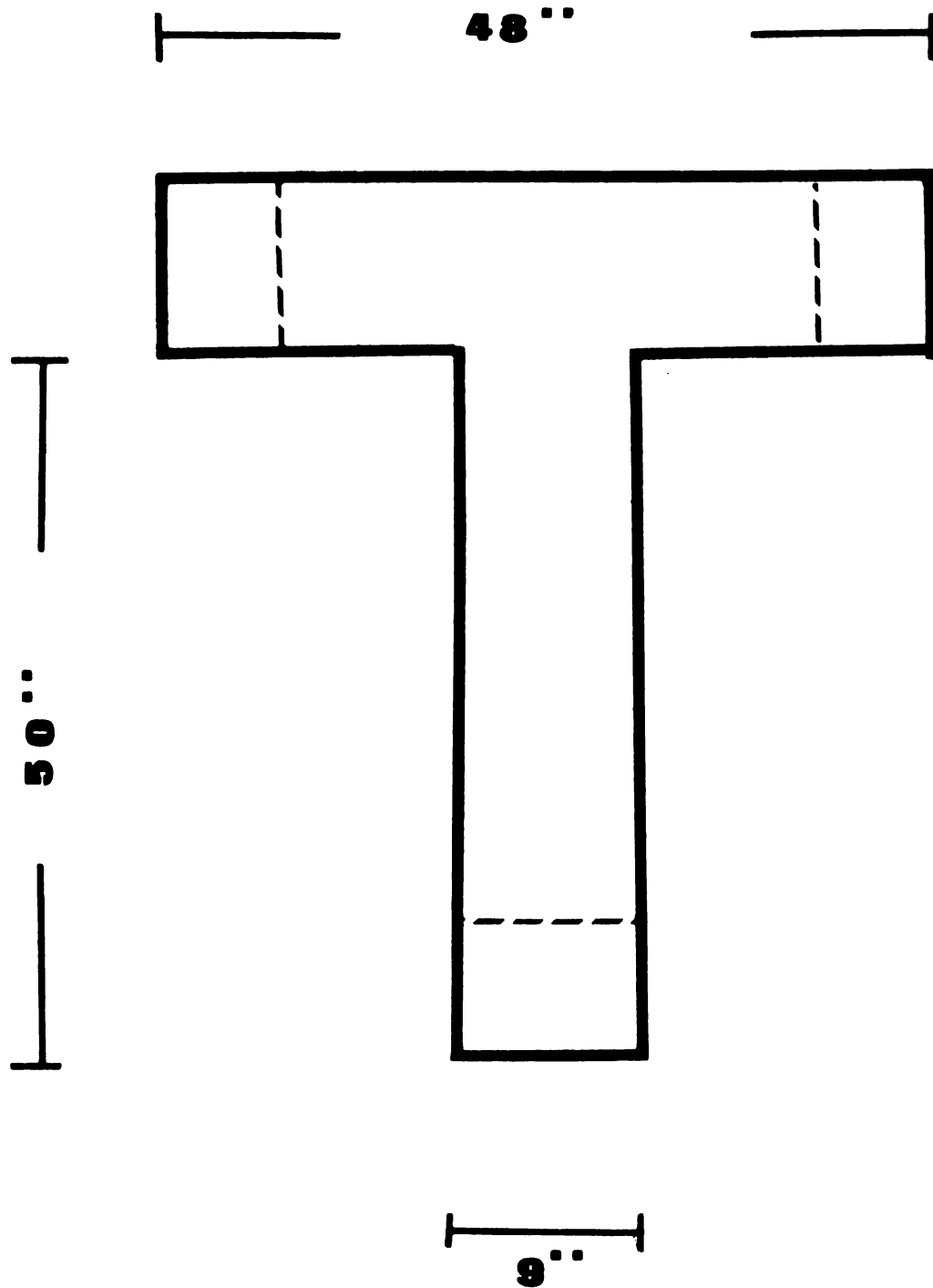
T - MAZE

Figure 2. Top view of the T-maze showing the dimensions of the running arm, and goal boxes.

guillotine door separated the open field from the start box. A detailed illustration of the apparatus is shown in Figure 3.

The open field was illuminated by a fluorescent light, and a mirror suspended over the field provided a clear picture of all movements of the rat under observation.

Behavioral Testing Procedure

The sequence and time of testing was identical for all experiments (Table 6). The only variable was the age of the rat when treated with mercury.

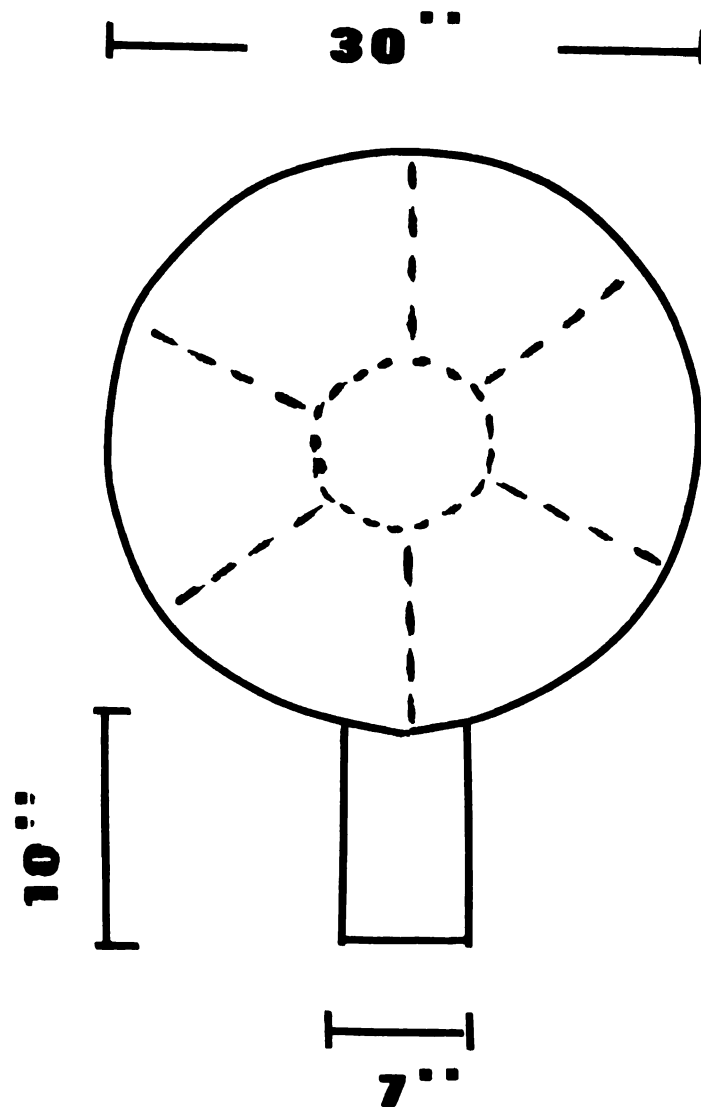
OPEN FIELD

Figure 3. Top view of the open field apparatus showing diameter of the field and length and width of the start box.

Table 6. Behavioral testing sequence (experiments 1, 2, 3)

Time after treatment (days)	Test
0-2	no testing
2-7	pretraining ¹
8-15	training ²
16-20	T-maze testing
21	no testing
22-26	open field testing
27	no testing
28-32	T-maze retesting
33	no testing
34-38	open field retesting
39-44	no testing
45-47	extinction testing in T-maze
48-53	no testing
54	extinction retesting
59-60	sacrifice

¹In experiment 3, the rats were allowed to investigate the T-maze on day 2, but the 2 1/2 hour feeding regime did not commence until day 4.

²For experiment 1, the rats had seven days of training instead of eight days.

Procedure for T-maze Testing

Testing consisted of ten trials per day per rat. The rat was placed in the start box for ten seconds, the door was opened, and the rat was allowed 60 seconds to start running down the maze. Once in the running arm, the animal was given 30 seconds to make a choice; errors consisted of a failure to make a choice in 30 seconds or entries into the wrong arm of the maze. A criterion for maximal performance was nine correct responses out of ten. The placement of the wire screen to indicate the correct

choice of arm was randomly arranged according to the Kellerman Series of Random Order (Appendix III). Food was the primary reinforcement, thus to eliminate the bias of smell as a clue to the correct goal box another food cup containing the food pellets, but covered with a wire screen, was placed in the incorrect goal box. Once in the correct goal box the rat was allowed 10 seconds to eat from the food cup. Latency to leave the start box, and the number of correct responses were measured in all periods in the T-maze. Intertrial intervals were 20 minutes.

Extinction trials were performed on the seventh day during which the rats were tested in the open field. In extinction, a criterion of three correct responses out of six was considered as satisfactory performance. The procedure was identical to testing and retesting in the T-maze except there was no food reinforcement for making the correct response. Latency and number of correct responses were again measured.

Procedure for the Open Field

The animal was placed in the start box for 60 seconds. During this period the number of times of defecating, urinating, standing upright, cleaning, and sniffing were recorded. The door was then opened and the animal allowed another 60 seconds to venture out of the start box into the open field. The latency period was recorded. If after 60 seconds the rat had not moved out, it was placed in the

middle of the open field. Once in the field they were observed for 5 minutes. Besides those parameters measured in the start box, inactivity and number of areas traversed were also measured in the open field.

All responses were recorded on a ten channel recorder manufactured by the Sanford Company Inc. Since the chart speed of the recorder was 2.5 mm/sec, this enabled the observer to record the frequency and length of time of each activity.

Brain Analysis

Animals whose tissues were to be analyzed for cerebral DNA and RNA were over etherized and decapitated. The head was placed on a cold surface provided by crushed ice while the brain was being removed. The brain was separated into cerebrum plus olfactory bulb, and cerebellum plus the remaining portion. The cerebellum thus included midbrain, pons, and medulla oblongata. The two brain portions were weighed and frozen until time of analysis.

Some of the animals not used for DNA and RNA determinations were used for histological examination. For this purpose the rat was lightly etherized and a sternal flap was made, and 100 mg of heparin in 1 ml saline were injected directly into the left ventricle through the apex of the heart for experiment 1. For experiments 2 and 3, EDTA (calculated on the basis of 1 mg/ml of blood assuming that the rats contained 7% blood) was used as the anticoagulant

and administered in the same manner as in the first group. Immediately after administration of the anticoagulant, a balanced salt solution, and then a balanced formalin solution, (Appendix IV), were administered by perfusion to fix the brain in site. The perfusion fluid after going through the brain and the body of the rat was allowed to escape the circulatory system by a cut in the right atrium. After one-half hour of perfusing, the head was removed and placed in formalin. At a later time, the whole brain was removed from the head and stored in formalin until histological examination was performed. A parasagittal section 0.5 mm lateral to the median line was made revealing the full length of the brain from olfactory bulb to spinal cord. The tissue slice was stained with hematoxylin and eosin.

DNA and RNA Determination Procedures

A modified Schneider, Schmidt, and Thannhauser method of nucleic acid analysis was used (Appendix V). The RNA levels were determined by the Mejbaum reaction (73 and Appendix VI), and the DNA concentrations by the Dische reaction (74 and Appendix VII).

Statistical Analysis

All behavioral data were analyzed on the model 3600 computer. An analysis of variance was performed with a 2x2 split plot design. If there was an interaction, a Duncan Multiple Range test was done to determine the day(s)

on which there was a significant difference between the control and mercury treated rats. A one way analysis of variance was made on the weight gain and biochemical data.

RESULTS AND DISCUSSION

Gross Observation and Weight Gain

Within half an hour after gavaging, the mercury treated rats in each experiment were lethargic and incoordinated in their gait. However, within 2-3 hours they appeared normal and had no visible neurological symptoms throughout the remainder of the experiment. Although food intake was not measured anorexia probably occurred in the mercury treated rats. This observation was reinforced by the body weight data which revealed that for several weeks, the treated rats weighed significantly less than the controls.

In experiment 1, the lower body weight of the treated rats lasted until the seventh week after gavage. From the seventh week to the time of sacrifice there was no significant difference in body weights between treated and control rats (Figure 4).

For experiment 2, the average initial weight of mercury treated rats was the same as that for the control group. From the second to the fourth week the treated animals weighed less. After this time, body weights became similar between the two groups of rats (Figure 5).

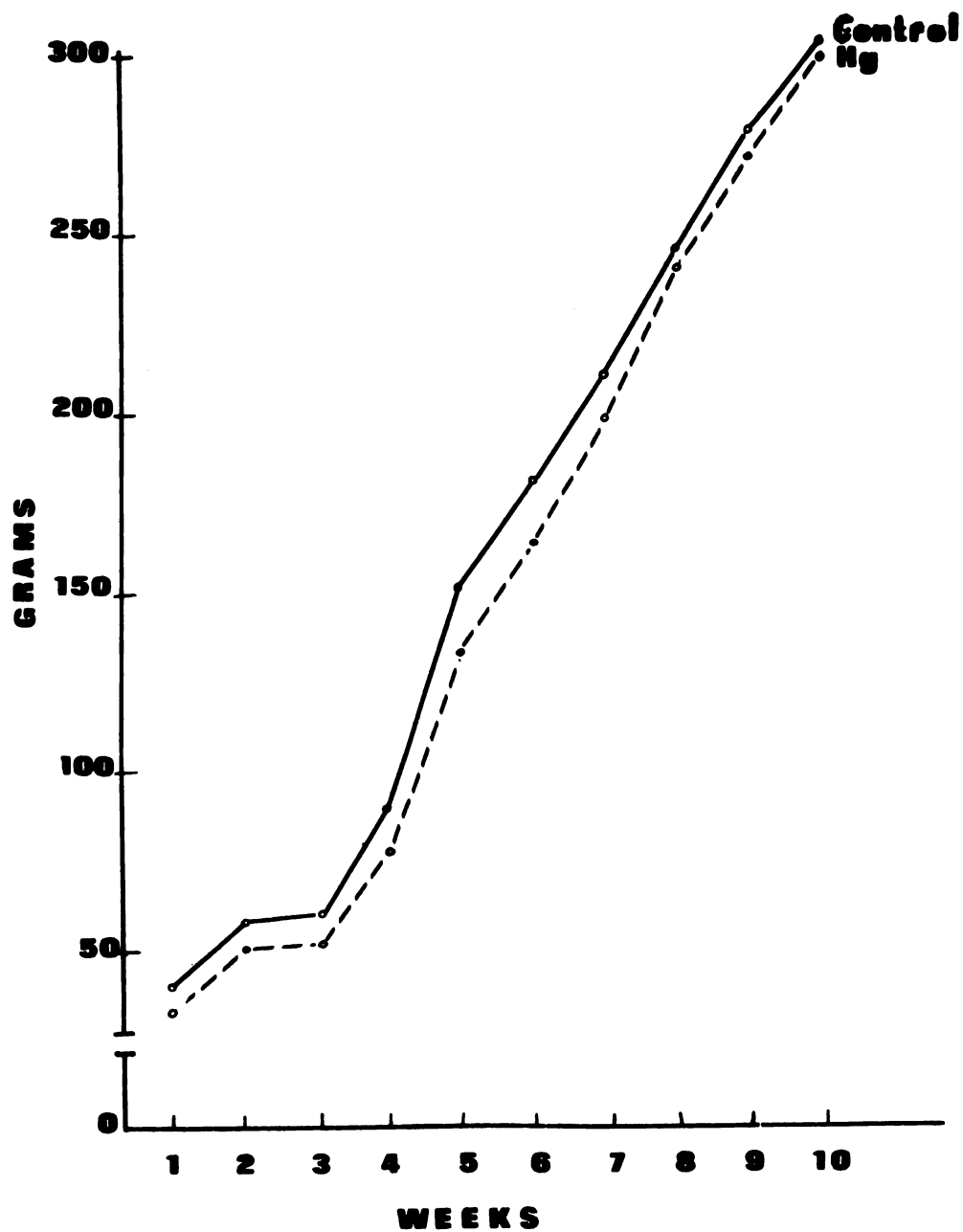


Figure 4. Body weights of mercury treated and control rats , experiment 1 .

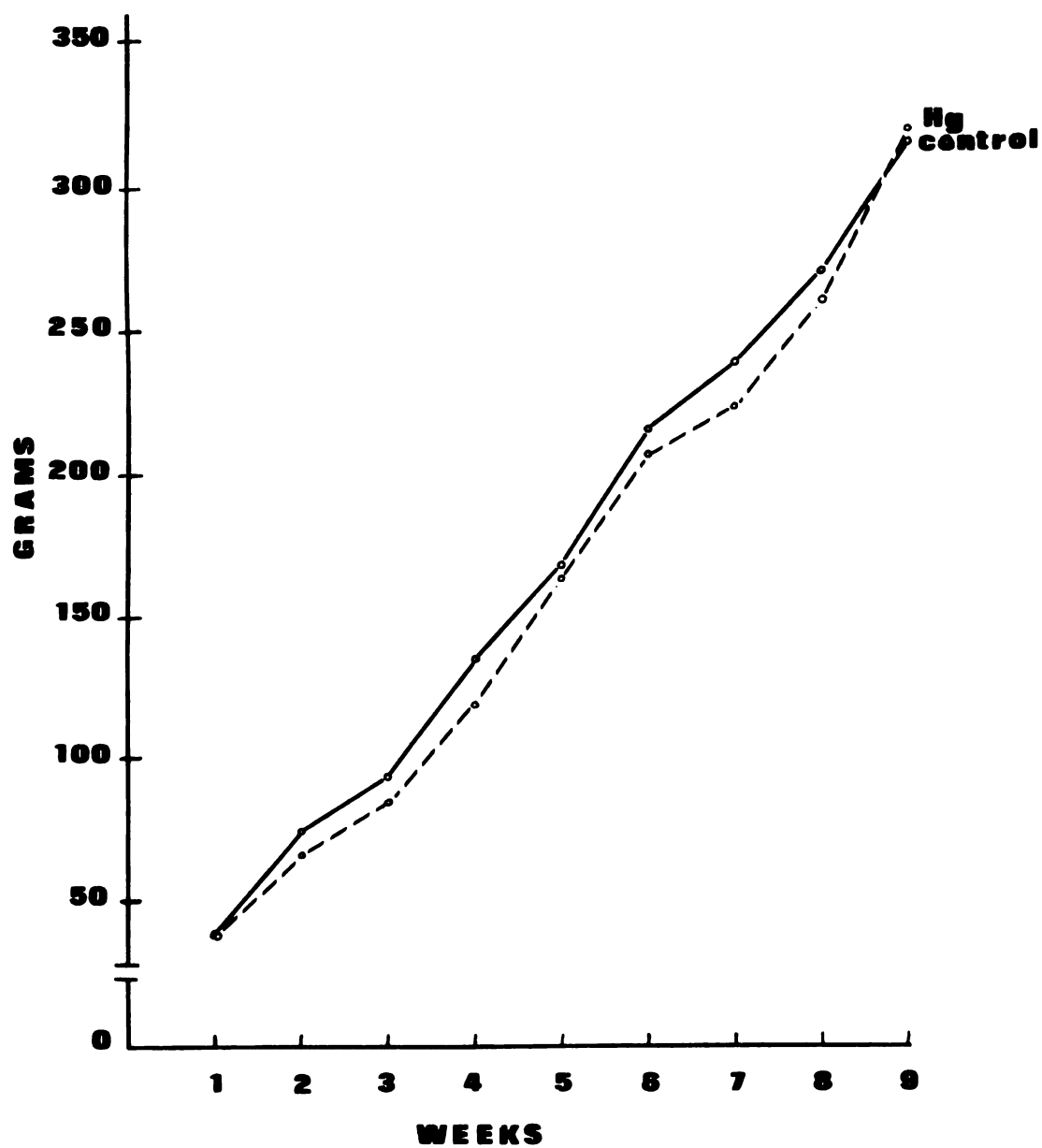


Figure 5. Body weights of mercury treated and control rats , experiment 2 .

For experiment 3, a more drastic effect was evident. Body weights of mercury treated rats were significantly lower than the body weights of control rats from the second to the ninth week (Figure 6). At the time of sacrifice, there was little difference between the two groups.

Training, Testing, and Retesting Periods
(Trials) in T-Maze

Treated rats, in experiment 1, took a shorter length of time than control rats to leave the start box on the first day of training. However, on subsequent days, including testing and retesting, latency did not differ between the two groups of rats (Table 7). The number of correct responses made in all periods (training, testing and retesting) by the treated rats and controls were similar (Table 8).

Latency during training and testing was similar between the two groups of rats, in experiment 2. The treated rats remained in the start box longer during the retest period (Table 9). The number of correct responses was similar between treated and control rats during all periods (Table 10).

Latency between mercury and control rats in experiment 3 was similar during the training and testing period. However, during retesting, the treated rats tended to be more latent in leaving the start box, in comparison, to controls (Table 11). On day 1 of training, treated rats

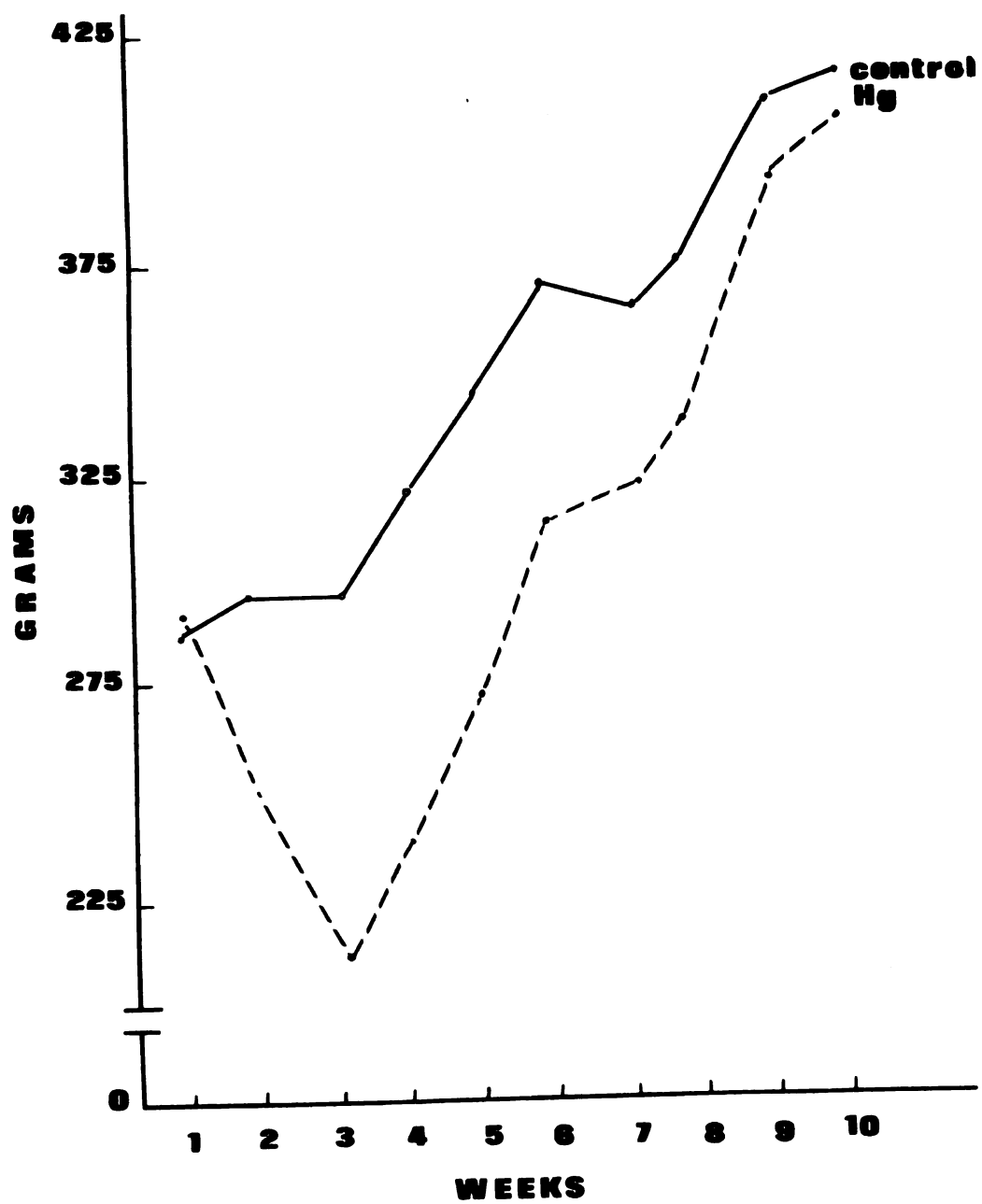


Figure 6. Body weights of mercury treated and control rats , experiment 3 .

Table 7. Latency in T-maze, experiment 1, (sec/rat).

Days	Training period		Test period		Retest period	
	Control	Hg	Control	Hg	Control	Hg
1	1.43	1.23	.98	.99	1.33	1.18
2	1.16	1.17	.92	.94	0.84	1.08
3	1.09	1.06	.98	.94	0.80	0.94
4	1.08	1.03	.79	.82	0.73	0.70
5	0.98	0.97	.58	.65	0.83	0.74
6	1.04	1.08				
7	0.93	0.99				
Average	1.10	1.08 ¹	.85	.91	0.90	0.93
± S.E.	0.06	0.04	.08	.05	0.11	0.09

± S.E. = ± standard errors

¹ On day 1, control rats took longer to leave the start box, $P < 0.04$.

Table 8. Correct responses in T-maze, experiment 1, (no./rat).

Days	Training period		Test period		Retest period	
	Control	Hg	Control	Hg	Control	Hg
1	4.0	4.8	9.5	9.3	9.6	9.3
2	5.7	4.9	9.6	9.7	9.7	9.8
3	8.3	7.5	9.9	9.9	9.5	9.7
4	8.1	7.5	9.9	9.9	9.1	9.7
5	9.1	8.4	10.0	10.0	9.3	9.5
6	9.3	9.0				
7	9.7	9.1				
Average	7.6	7.3 ¹	9.8	9.8	9.5	9.5
± S.E.	0.8	0.7	0.1	0.1	0.1	0.1

± S.E. = ± standard errors

¹ Significant interaction, $P < 0.05$.

Table 9. Latency in T-maze, experiment 2, (sec/rat).

Days	Training period		Test period		Retest period	
	Control	Hg	Control	Hg	Control	Hg
1	2.3	2.2	.98	.99	1.33	1.18
2	1.9	1.8	.92	.94	0.84	1.08
3	1.5	1.7	.98	.94	0.80	0.94
4	1.4	1.6	.79	.82	0.73	0.70
5	1.4	1.2	.58	.65	0.63	0.74
6	1.2	1.1				
7	1.2	1.1				
8	1.1	1.1				
Average	1.5	1.5	.85	.87	0.87	0.93 ¹
± S.E.	0.5	0.2	0.1	0.1	0.21	0.09

± S.E. = ± standard errors.

¹ Significant difference, $P < 0.01$.

Table 10. Correct responses in T-maze, experiment 2, (No./rat).

Days	Training period		Test period		Retest period	
	Control	Hg	Control	Hg	Control	Hg
1	4.3	4.9	9.5	9.3	9.6	9.3
2	5.5	5.5	9.6	9.7	9.7	9.8
3	5.9	9.8	9.9	9.9	9.5	9.7
4	6.7	7.1	9.9	9.9	9.7	9.7
5	7.9	8.3	9.9	10.0	9.1	9.5
6	8.5	8.3				
7	8.8	8.8				
8	9.3	9.6				
Average	7.1	7.8	9.8	9.8	9.5	9.6
± S.E.	0.6	0.6	0.1	0.1	0.1	0.1

± S.E. = ± standard errors.

Table 11. Correct responses (No./rat) and latency (sec/rat) in all periods, experiment 3.

Days	Correct response		Latency	
	Training period		Training period	
	Control	Hg	Control	Hg
1	2.3	4.2	21.0	15.4
2	5.2	5.2	15.5	7.9
3	7.5	7.0	7.8	3.6
4	8.4	8.8	2.5	1.1
5	9.2	8.8	1.6	1.9
6	9.2	9.7	1.3	1.2
7	9.2	8.8	1.3	2.8
8	10.0	9.2	0.9	2.9
Average	7.6	7.7 ¹	6.5	4.6
± S.E.	0.9	0.7	2.2	1.7
	Test period		Test period	
1	9.4	8.9	0.98	0.99
2	9.8	9.5	0.99	0.96
3	9.7	9.2	0.91	0.96
4	9.9	9.9	0.98	0.97
5	10.0	9.9	1.00	0.99
Average	9.0	9.5	0.97	0.97
± S.E.	0.2	0.1	0.02	0.01
	Retest period		Retest period	
1	8.5	8.9	1.05	1.13
2	7.4	8.5	.82	1.26
3	8.5	10.0	1.55	2.22
4	8.3	8.6	1.05	.95
5	10.0	8.9	.89	1.02
Average	8.6	9.2	1.07	1.32 ²
± S.E.	0.4	0.3	0.13	0.23

± S.E. = ± standard errors.

1 significant interaction, day 1, $P < 0.03$.

2 significant difference, $P < 0.1$.

chose the correct goal box significantly more often than control rats (Table 11). Nevertheless, the overall number of correct responses made by treated and control rats during training, testing and retesting was similar (Table 11).

Testing (day 1, 2, 3) and Retesting (day 4)
in Extinction Trials

When latency was measured in extinction trials there was no difference in performance between mercury treated and control rats for all experiments. This occurred in both test and retest trials (Table 12). The number of correct responses observed for the two groups of rats was similar during testing and retesting for experiments 1 and 2. However, the number of correct responses was higher for the treated rats in experiment 3 during testing, in comparison, to control rats. When the animals were retested, the number of responses became similar between the two groups of rats (Table 13).

There was no substantial increase in latency on each successive day of extinction trials, except for rats in experiment 3. The number of correct responses did not decrease substantially in experiments 1 and 2. Correct responses decreased for controls from 3.1 to 1.7, and for treated rats from 3.5 to 1.5 on day 3 to day 4. Theoretically in extinction, the number of correct responses should decline as the primary reinforcement was removed.

Table 12. Latency in extinction, experiments 1, 2, and 3 (sec/rat).

Days	Experiment 1		Experiment 2		Experiment 3	
	Control	Hg	Control	Hg	Control	Hg
1	1.6	0.8	2.6	1.5	3.3	3.4
2	0.8	1.5	2.6	2.6	1.9	2.5
3	1.8	1.3	1.8	2.3	5.8	6.5
Average	1.4	1.2	2.3	2.1	3.7	4.1
± S.E.	0.3	0.2	0.3	0.3	1.1	1.2
4	1.1	1.5	2.9	4.1	30.9	34.2
Average	1.3	1.3	2.5	2.6	10.5	11.6
± S.E.	0.3	0.2	0.2	0.5	6.9	7.6

± S.E. = ± standard errors.

Table 13. Correct responses in extinction in experiments 1, 2, and 3, (No./rat).

Days	Experiment 1		Experiment 2		Experiment 3	
	Control	Hg	Control	Hg	Control	Hg
1	5.4	5.5	5.7	5.7	3.5	4.6
2	5.3	5.2	5.1	5.2	3.3	3.7
3	5.3	5.1	4.5	4.4	3.1	3.5 ₁
Average	4.3	5.3	5.1	5.1	3.3	3.9 ₁
± S.E.	0.0	0.1	0.3	0.4	0.1	0.3
4	4.7	4.9	4.3	3.5	1.7	1.5
Average	5.2	5.3	4.9	4.7	2.9	3.3
± S.E.	0.2	0.1	0.3	0.5	0.4	0.9

± S.E. = ± standard errors.

1 significant difference, $P < 0.1$.

Extinction was a new learning experience for the rats. If this lack of reinforcement was not recognized by the rat, the animal would continue to choose the correct goal box. When this fact was realized by the rat, latency should increase. Results indicated that extinction did not occur in experiments 1 and 2, as latency was variable and increased only slightly, and no significant changes were observed in the number of correct responses. Rats in experiment 3 showed a decrease in the number of correct responses, and increased latency on day 4 of extinction. These animals were extremely wary, and this probably accounts for the results of extinction rather than the fact that extinction took place.

Considering latency as a function of age at time of treatment all rats in experiment 1 were in the start box a shorter length of time than rats in experiments 2 and 3. Latency was ten times as high for all rats in experiment 3, in comparison to experiment 1, and five times higher than experiment 2. Fewer correct responses were made in experiment 3 than in the other two experiments.

Criterion for Performance in T-Maze

There was no difference in the rate at which treated and control rats in experiments 1 and 2 reached criterion. However, control rats in experiment 3 reached criterion at 4.8 days compared to 6.2 days for the mercury treated rats (Table 14). During extinction trials no differences were

found for the rat in reaching the criterion for all experiments (Table 15).

Table 14. Number of test days to reach criterion in T-maze for all experiments (day/testing period/rat).

	Experiment 1	Experiment 2	Experiment 3
Control	4.5	7.6	4.8
Hg	5.7	6.6	6.2 ¹

¹significant differences, $P < 0.01$.

Table 15. Number of test days to reach criterion in extinction trials for all experiments (day/testing period/rat).

	Experiment 1	Experiment 2	Experiment 3
Control	3.7	3.6	2.5
Hg	3.7	3.5	3.5

Open Field

Testing in the Start Box

Standing upright responses were similar between the two groups of rats in experiments 1 and 2 (Table 16). On day 2, control rats in experiment 3 stood upright significantly more than treated rats (Table 16). There were no differences in circling responses (Table 17), and cleaning responses (Table 18) for treated and control rats in each

experiment. Treated rats in experiment 1 sniffed more on day 4 than controls (Table 19), but no differences were observed in experiments 2 and 3 (Table 19).

Testing in the Open Field

Latency was similar between the two groups of rats in experiments 1 and 2 (Table 20), however, control rats in experiment 3 remained in the start box longer than treated rats (Table 20). Similar number of areas were traversed by control and treated rats for experiment 1 as well as experiment 2 (Table 21); in experiment 3, controls crossed significantly more areas during all testing days than treated rats (Table 22). Inactivity was similar for rats in experiments 1 and 2 (Table 23), but in experiment 3 inactivity for treated rats was 27.0 seconds compared to 4.7 seconds for controls (Table 24). Standing upright (Table 25), and cleaning (Table 18) were similar for treated and control rats, in all experiments. Treated rats in experiment 1 circled more often on day 4 than control rats (Table 26). Circling responses between the two groups of rats in experiment 2 were similar (Table 26). Treated rats, in experiment 3, circled more on each day of testing than control rats (Table 26). There were no differences in sniffing (Table 27), number of fecal bolli (Table 28), and urinations (Table 29) when comparing the results between treated and control rats for all experiments.

Table 16. Standing upright responses in start box, experiments 1, 2, and 3, (No./60 sec/rat).

Days	<u>Test period</u>		<u>Retest period</u>	
	Control	Hg	Control	Hg
Experiment 1				
1	10.1	10.3	11.0	10.4
2	8.1	8.7	11.3	11.1
3	7.2	7.5	12.0	13.4
4	7.2	7.6	10.8	10.3
5	7.9	9.5	12.8	11.8
Average	8.1	8.7	11.6	11.4
± S.E.	0.5	0.5	0.4	0.6
Experiment 2				
1	5.3	6.7	8.5	9.0
2	4.9	5.1	7.7	9.1
3	7.6	7.1	7.8	8.2
4	7.2	8.5	9.1	9.4
5	9.1	7.0	9.1	9.7
Average	7.0	6.9	8.5	9.1
± S.E.	0.7	0.5	0.3	0.3
Experiment 3				
1	7.3	7.7	7.9	7.8
2	8.7	6.5	7.8	8.3
3	8.2	6.5	8.8	8.6
4	6.9	7.0	8.2	9.4
5	6.4	6.7	8.5	10.3
Average	7.5	6.9	8.2	8.9
± S.E.	0.4	0.2	0.2	0.4

± S.E. = ± standard errors.

1 significant difference on day 2, $P < 0.1$.

Table 17. Circling responses in start box, experiments 1, 2, and 3, (No./60 sec/rat).

Days	Test period		Retest period	
	Control	Hg	Control	Hg
Experiment 1				
1	4.3	3.5	4.9	4.0
2	3.3	3.8	4.3	3.9
3	2.2	3.0	3.7	3.8
4	2.9	3.1	3.6	3.0
5	3.3	3.0	4.3	4.3
Average	3.2	3.3	4.1	3.8
± S.E.	0.3	0.2	0.2	0.2
Experiment 2				
1	2.8	3.2	3.4	3.2
2	3.1	3.4	3.7	3.4
3	2.3	2.9	3.3	3.5
4	2.7	3.1	3.3	4.2
5	2.5	3.1	3.4	4.2
Average	2.7	2.9	3.4	3.6
± S.E.	0.1	0.2	0.1	0.2
Experiment 3				
1	1.2	1.1	2.9	2.9
2	2.0	2.4	2.7	3.5
3	2.0	1.3	2.7	3.4
4	2.2	1.9	4.5	3.8
5	2.5	2.1	4.3	4.6
Average	1.9	1.7	3.4	3.6
± S.E.	0.2	0.2	0.4	0.3

± S.E. = ± standard errors.

Table 18. Cleaning responses in start box and open field for all test periods, experiments 1, 2, and 3 (No./observation time/rat).

Days	Test Period				Retest Period			
	Start box		Open field		Start box		Open field	
	Control	Hg	Control	Hg	Control	Hg	Control	Hg
Experiment 1								
1	0.0	0.0	0.64	0.66	0.0	0.0	1.90	1.60
2	0.1	0.0	1.14	1.26	0.0	0.2	2.00	1.50
3	0.4	0.1	1.07	1.73	0.0	0.1	1.30	1.50
4	0.3	0.1	0.92	1.60	0.0	0.0	0.92	1.50
5	0.3	0.3	1.00	0.93	0.0	0.0	1.50	1.50
Average	0.2	0.1	0.95	1.23	0.0	0.1	1.50	1.50
± S.E.	0.07	0.05	0.09	0.20	0.0	0.2	0.20	0.02
Experiment 2								
1	0.1	0.1	1.8	2.3	0.1	0.0	0.33	0.40
2	0.1	0.1	1.9	2.3	0.1	0.2	1.60	1.33
3	0.1	0.2	2.5	2.2	0.0	0.1	1.60	1.13
4	0.3	0.2	2.3	2.5	0.1	0.1	1.06	1.33
5	0.3	0.1	3.3	4.7	0.1	0.1	0.93	1.36
Average	0.2	0.1	3.3	2.8	0.1	0.1	0.98	1.11
± S.E.	0.05	0.4	0.3	0.02	0.02	0.03	0.14	0.18

Table 18 (Cont'd)

Experiment 3										
1	0.0	0.0	0.0	0.31	0.08	0.0	0.0	0.46	0.85 ¹	
2	0.0	0.0	0.0	0.31	0.46	0.1	0.0	1.70	0.61 ¹	
3	0.1	0.1	0.1	0.85	1.46	0.0	0.1	1.20	1.11	
4	0.1	0.0	0.0	0.92	1.08	0.0	0.0	0.77	0.77	
5	0.0	0.0	0.0	0.69	0.85	0.0	0.0	0.85	0.85	
Average	0.04	0.02		0.62	0.79	0.02	0.02	0.99	0.84	
± S.E.	0.02	0.02		0.13	0.24	0.1	0.02	0.21	0.08	

± S.E. = ± standard errors.

¹ Significant difference on day 2, $P < 0.1$.

Table 19. Sniffing responses in start box, experiments 1, 2, and 3 (No./60 sec/rat).

Days	Test period		Retest period	
	Control	Hg	Control	Hg
Experiment 1				
1	9.1	9.7	12.0	11.5
2	8.7	10.1	12.4	13.1
3	8.1	7.4	12.4	13.9
4	7.8	8.4	12.2	11.5
5	8.9	10.5	13.1	13.1
		1		
Average	8.5	9.2	12.4	12.7
± S.E.	0.3	0.6	0.2	0.5
Experiment 2				
1	9.9	10.3	10.5	10.2
2	11.2	9.6	10.5	9.5
3	9.9	9.5	10.6	10.5
4	9.3	8.9	10.2	11.3
5	8.5	8.9	11.2	12.5
Average	9.8	9.5	10.6	10.7
± S.E.	0.4	0.3	0.2	0.5
Experiment 3				
1	7.1	8.4	9.9	9.3
2	8.2	7.5	9.5	9.9
3	9.4	8.6	8.4	9.2
4	8.9	9.9	8.5	9.7
5	9.8	8.6	9.6	10.4
Average	8.7	8.6	9.2	9.7
± S.E.	0.5	0.4	0.3	0.3

± S.E. = ± standard errors.

1 On day 4, treated rats sniffed more than controls, $P < 0.06$.

Table 20. Latency in open field during test and retest periods, experiments 1, 2, and 3, (sec/rat).

Days	Test period		Retest period	
	Control	Hg	Control	Hg
Experiment 1				
1	19.3	12.7	13.1	13.7
2	24.8	19.8	13.3	12.6
3	23.5	22.7	9.3	16.5
4	22.8	24.6	9.9	14.1
5	21.5	19.4	10.6	12.7
Average	22.4	19.9	11.2	13.9
± S.E.	0.9	2.0	0.8	0.7
Experiment 2				
1	15.0	18.7	9.4	16.7
2	15.9	14.9	14.3	27.1
3	15.7	22.2	14.6	16.2
4	9.5	11.1	9.6	18.2
5	11.8	12.3	14.6	20.8
Average	13.6	15.9	12.5	19.8
± S.E.	1.3	2.1	1.2	2.0
Experiment 3				
1	34.4	17.0	35.5	33.4
2	40.1	26.6	39.7	46.4
3	41.0	19.9	34.8	39.1
4	17.8	17.6	32.6	31.2
5	33.2	36.7	44.9	33.3
Average	33.3	23.6	37.5	36.7
± S.E.	4.2	3.7	2.2	2.8

± S.E. = ± standard errors.

1 Treated rats had longer latency in start box than control rats, $P > 0.01$.

2 Control rats remained in start box longer than treated rats, $P < 0.08$.

Table 21. Areas traversed in open field during test and retest periods, experiments 1 and 2 (No./5 min/rat).

Days	Test period		Retest period	
	Control	Hg	Control	Hg
Experiment 1				
1	84.5	76.1	101.5	103.3 ¹
2	83.9	86.5	115.1	104.1 ¹
3	87.9	88.4	114.6	108.9 ¹
4	85.1	80.0	115.4	95.0 ¹
5	85.2	70.8	118.2	103.1 ¹
Average	85.3	82.2	112.9	102.8
± S.E.	0.7	2.3	2.9	2.2
Experiment 2				
1	70.8	66.8	81.4	78.8
2	75.1	76.4	101.6	110.6
3	83.4	84.3	101.4	97.2
4	94.8	87.0	83.5	80.5
5	79.2	78.1	82.1	90.0
Average	80.7	78.5	90.0	91.5
± S.E.	4.1	3.5	4.7	5.4

± S.E. = ± standard errors.

1 Significant differences on days 2, 4, and 5, control > mercury treated, $P < 0.01$.

Table 22. Areas traversed on testing in open field,
experiment 3, (No./5 min/rat).

Days	Control	Hg
1	72.3	73.1
2	73.3	65.1
3	78.7	57.0
4	69.3	54.3
5	68.2	52.5
Average	72.4	60.4 ¹
± S.E.	1.8	3.8

± S.E. = ± standard errors.

¹ Areas traversed by control rats were greater for all test days, $P < 0.02$.

Table 23. Inactivity in open field during testing and retesting, experiments 1 and 2, (sec/rat).

Days	Test period		Retest period	
	Control	Hg	Control	Hg
Experiment 1				
1	3.9	3.9	8.5	8.2
2	6.4	6.3	10.3	8.0
3	8.8	9.1	11.1	8.4
4	10.4	10.4	8.8	9.5
5	9.1	11.5	12.9	13.5
Average	7.7	8.2	10.3	9.5
± S.E.	1.2	1.4	0.8	1.0
Experiment 2				
1	5.8	3.5	1.9	1.8
2	8.8	7.6	0.8	1.3
3	8.1	6.9	1.4	1.8
4	6.7	6.7	1.9	2.4
5	2.5	3.6	2.3	2.9
Average	6.4	5.7	1.8	2.1
± S.E.	1.1	0.9	0.3	0.3

± S.E. = ± standard errors.

Table 24. Inactivity in open field during testing and retesting, experiment 3, (sec/rat).

Days	Control	<u>Test period</u>	Hg
1	3.1		18.4
2	4.4		28.1
3	7.9		27.7
4	3.0		28.4
5	5.1		32.7
Average	4.7		27.0 ¹
± S.E.	0.9		2.4

<u>Retest period</u>		
1	4.2	10.1
2	5.1	22.8
3	5.2	21.3
4	8.4	20.1
5	10.3	20.3
Average	6.6	18.9 ²
± S.E.	1.2	2.3

± S.E. = ± standard errors.

1 Treated rats were less active than controls for each day of testing, $P < 0.001$.

2 Inactivity of mercury treated rats was more for all days of retesting, $P < 0.03$.

Table 25. Standing upright responses in open field during testing and retesting, experiments 1, 2, and 3 (No./5 min/rat).

Days	<u>Test period</u>		<u>Retest period</u>	
	Control	Hg	Control	Hg
Experiment 1				
1	32.8	35.3	39.7	39.0
2	31.3	31.9	40.7	37.3
3	34.6	34.2	39.1	42.6
4	32.5	33.5	36.7	38.9
5	33.1	33.1	40.3	38.3
Average	32.9	33.6	39.3	39.2
± S.E.	0.5	0.6	0.7	0.9
Experiment 2				
1	17.3	19.8	34.6	34.8
2	23.0	26.4	35.9	35.5
3	29.0	30.5	27.2	30.4
4	33.1	35.1	26.9	25.1
5	31.1	33.5	26.4	26.8
Average	26.7	29.1	30.2	30.5
± S.E.	2.9	2.8	2.1	2.1
Experiment 3				
1	33.8	33.8	40.9	33.3
2	34.7	27.6	37.1	31.0
3	34.2	27.8	43.5	31.2
4	33.5	28.2	35.6	34.3
5	30.2	24.7	39.7	32.7
Average	33.3	28.4	39.4	32.5 ¹
± S.E.	0.8	1.5	1.4	0.6

± S.E. = ± standard errors.

¹ Control rats stood upright more frequently than treated rats, $P < 0.06$.

Table 26. Circling responses in open field during testing and retesting, experiments 1, 2, and 3 (No./5 min/rat).

Days	<u>Test period</u>		<u>Retest period</u>	
	Control	Hg	Control	Hg
Experiment 1				
1	4.5	3.1	3.6	6.1
2	1.8	3.0	4.7	5.2
3	5.1	4.1	5.9	5.7
4	3.8	5.7	5.5	7.2
5	4.7	3.9	6.7	6.1
		1		2
Average	3.9	3.9	5.2	6.1
± S.E.	0.6	0.5	0.5	0.3
Experiment 2				
1	1.1	1.1	4.2	4.8
2	2.0	2.4	4.7	3.8
3	1.9	1.3	6.1	3.5
4	2.3	1.9	8.6	7.7
5	2.3	2.1	6.8	7.0
Average	1.9	1.7	6.1	5.2
± S.E.	0.2	0.2	0.8	0.9
Experiment 3				
1	2.8	3.5	4.2	4.9
2	5.2	5.2	3.7	4.9
3	3.2	5.2	6.1	5.3
4	4.5	6.4	5.3	6.7
5	3.7	5.9	6.0	5.6
		3		
Average	3.9	5.2	5.1	5.7
± S.E.	0.4	0.5	0.5	0.3

± S.E. = ± standard errors.

1 Significant difference on day 4, $P < 0.008$.

2 Significant difference on day 1, $P < 0.06$.

3 Significant differences on each day of testing, $P < 0.05$.

Table 27. Sniffing responses in open field during testing and retesting, experiments 1, 2, and 3 (No./5 min/rat).

Days	<u>Test period</u>		<u>Retest period</u>	
	Control	Hg	Control	Hg
Experiment 1				
1	36.8	35.3	47.0	47.1
2	35.3	38.9	48.9	46.1
3	39.9	40.7	47.6	50.3
4	37.9	39.1	45.9	46.8
5	41.0	40.6	51.3	48.9
Average	38.2	38.9	48.1	47.9
± S.E.	1.0	1.0	0.9	0.8
Experiment 2				
1	21.3	23.6	39.9	42.6
2	27.1	31.9	38.8	42.0
3	32.7	34.5	35.5	37.0
4	36.6	38.0	38.0	36.3
5	34.5	37.1	38.3	41.2
Average	30.5	33.0	38.1	39.8
± S.E.	2.8	2.6	0.7	1.3
Experiment 3				
1	38.0	38.5	47.2	39.1
2	42.2	37.4	44.3	40.5
3	39.7	37.9	51.5	41.2
4	40.2	39.1	44.8	44.0
5	36.2	36.2	49.3	41.8
Average	39.2	37.8	47.4	41.3 ¹
± S.E.	1.0	0.5	1.4	0.8

± S.E. = ± standard errors.

¹ Significant differences, Control>treated rats, $P<0.05$.

Table 28. Total number of fecal bolli in open field,
experiments 1, 2, and 3, (No./6 min/rat).

Days	Test period		Retest period	
	Control	Hg	Control	Hg
Experiment 1				
1	4	0	0	0
2	1	0	0	0
3	4	0	2	0
4	3	2	0	0
5	2	4	0	0
Average	2.8	1.2	0.4	0.0
± S.E.	0.6	0.8	0.4	0.0
Experiment 2				
1	0	2	2	1
2	1	3	1	2
3	0	0	2	0
4	1	2	0	1
5	0	0	0	1
Average	0.4	1.4	1.0	1.0
± S.E.	0.3	0.6	0.5	0.3
Experiment 3				
1	0	0	0	0
2	0	0	0	0
3	0	0	2	0
4	1	0	1	0
5	0	1	0	0
Average	0.2	0.2	0.6	0.0
± S.E.	0.2	0.2	0.4	0.0

± S.E. = ± standard errors.

Table 29. Total number of urinations in open field during testing and retesting, experiments 1, 2, and 3 (No./6 min/rat).

Days	Test period		Retest period	
	Control	Hg	Control	Hg
Experiment 1				
1	0	0	1	1
2	0	0	0	0
3	0	0	1	1
4	0	1	0	0
5	0	0	0	3
Average	0.6	0.2	0.4	1.0
± S.E.	0.6	0.2	0.3	0.6
Experiment 2				
1	0	0	2	0
2	0	0	0	0
3	0	0	0	1
4	0	0	1	0
5	0	0	0	0
Average	0.0	0.0	0.6	0.2
± S.E.	0.0	0.0	0.4	0.2
Experiment 3				
1	4	1	0	2
2	2	1	0	0
3	3	2	0	1
4	1	1	0	0
5	0	0	1	0
Average	2.0	1.0	0.2	0.6
± S.E.	0.7	0.3	0.2	0.4

± S.E. = ± standard errors.

Retesting, Start Box

When standing upright (Table 16), circling (Table 17), cleaning (Table 18), and sniffing (Table 19) were measured, no significant differences were observed between treated and control rats for all experiments.

Retesting in Open Field

Latency was similar between the two groups of rats in both experiments 1 and 3 (Table 20). Treated rats, in experiment 2, had a significantly longer latency period than control rats on all retest days (Table 20). Control rats, in experiment 1, traversed more areas on days 2, 4, and 5 than mercury treated rats (Table 21). Areas traversed by treated and control rats in experiment 2 were the same (Table 21). Control rats of experiment 3 crossed significantly more areas than the treated rats (Table 30). Control rats remained as inactive as treated rats in both experiments 1 and 2 (Table 23), however, in experiment 3 mercury treated rats were more inactive than controls on each day of retesting (Table 24). Standing upright (Table 25), cleaning (Table 18), and sniffing (Table 27) were similar between the two groups of rats in both experiments 1 and 2. In experiment 3, control rats stood upright more frequently, $P < 0.06$, than treated rats on all days of retesting (Table 25). Mercury treated rats circled significantly more than controls in experiment 1 (Table 26). No significant differences were observed in the number of circling

Table 30. Areas traversed in open field during retesting, experiment 3, (No./5 min/rat).

Days	Control	Hg
1	74.4	59.9
2	77.5	60.5
3	80.0	58.6
4	68.7	59.9
5	70.2	61.2
Average	74.2	60.0 ¹
± S.E.	2.1	0.4

± S.E. = ± standard errors.

¹ For each day during retesting mercury treated rats crossed less areas than the controls, $P < 0.03$.

responses of treated rats, in comparison, to control rats in experiments 2 and 3 (Table 26). Controls, in experiment 3, cleaned themselves significantly more on day 2 than treated rats (Table 18). More sniffing responses were observed for control rats in experiment 3 than mercury treated rats. No differences were observed in the number of fecal bolli (Table 28), and urinations (Table 29) between the two groups of rats for all experiments.

Table 31 summarizes the data accumulated from the behavioral tests. Little changes in performance between mercury treated and control rats were noted in the T-maze. In the open field the older rats exhibited more effects from the mercury than the two younger groups of rats. There was no change in behavior for the 21 day old rats, and only minor changes on certain days for the rats gavaged in the fifteenth day postnatally.

Since the level of dose of mercury was greater for the older rats (experiment 3) more statistically significant differences were observed between the two groups of rats than in experiments 1 and 2. In the present study there could have been a loss of part of the dose with the 15 and 21 day old rats, because the rats avoided swallowing the allotted quantity of mercury mixed in cocoa butter. However, based on visual observation the loss should only have amounted to a little more than 5 percent of the dose. Another important factor which may have a bearing on dose response relationship is the length of time required to

Table 31. Results of two-way analysis of variance on behavioral tests, experiments 1, 2, and 3. (N.S. = no significant difference and + signs = significant differences between control and mercury treated rats, + = $P < 0.01$, ++ = $P < 0.05$, +++ = $P < 0.1$).

Activity	Experiment 1	Experiment 2	Experiment 3
<u>T-maze</u>			
<u>training period</u>			
1. correct response	N.S.	N.S.	N.S.
2. latency	++	N.S.	N.S.
<u>test period</u>			
1. correct response	N.S.	N.S.	+++
2. latency	N.S.	N.S.	N.S.
<u>retest period</u>			
1. correct response	N.S.	N.S.	N.S.
2. latency	N.S.	+	+++
<u>extinction test</u>			
1. correct response	N.S.	N.S.	+++
2. latency	N.S.	N.S.	N.S.
<u>extinction retest</u>			
1. correct response	N.S.	N.S.	N.S.
2. latency	N.S.	N.S.	N.S.

Table 31. Cont'd.

Activity	Experiment 1	Experiment 2	Experiment 3
<u>Open field</u>			
<u>test period</u>			
1. areas traversed	+	N.S.	++
2. inactivity	N.S.	N.S.	+
3. latency	N.S.	N.S.	+++
4. standing upright	N.S.	N.S.	N.S.
5. circling	+	N.S.	+
6. cleaning	N.S.	N.S.	N.S.
7. sniffing	N.S.	N.S.	N.S.
8. fecal bolli	N.S.	N.S.	N.S.
9. urinations	N.S.	N.S.	N.S.
<u>retest period</u>			
1. areas traversed	N.S.	N.S.	++
2. inactivity	N.S.	N.S.	++
3. latency	N.S.	+	N.S.
4. standing upright	N.S.	N.S.	+++
5. circling	++	N.S.	+++
6. cleaning	N.S.	N.S.	N.S.
7. sniffing	N.S.	N.S.	++
8. fecal bolli	N.S.	N.S.	N.S.
9. urinations	N.S.	N.S.	N.S.

Table 31. Cont'd.

Activity	Experiment 1	Experiment 2	Experiment 3
start box of open field			
<u>test period</u>			
1. standing upright	N.S.	N.S.	N.S.
2. circling	N.S.	N.S.	N.S.
3. cleaning	N.S.	N.S.	N.S.
4. sniffing	+	N.S.	N.S.
5. fecal bolli	N.S.	N.S.	N.S.
6. urinations	N.S.	N.S.	N.S.
<u>retest period</u>			
1. standing upright	N.S.	N.S.	N.S.
2. circling	N.S.	N.S.	N.S.
3. cleaning	N.S.	N.S.	+
4. sniffing	N.S.	N.S.	N.S.
5. fecal bolli	N.S.	N.S.	N.S.
6. urinations	N.S.	N.S.	N.S.

administer the mercury. Since the rat avoided completely swallowing the cocoa butter containing the mercury it took as long as ten minutes to force the rat to swallow the dose. This was accomplished by repeatedly returning the pieces of cocoa butter to the mouth and allowing them to melt therein. In the 60 day old rats the cocoa butter was not used; the mercury compound was dissolved in 1,2-propanediol. Furthermore, the dose was increased from 2.0 to 2.5 mg/100 g body weight. Both the increased dose and perhaps the administration of the dose in a few seconds may have enough influence to cause changes in behavior in the rat. As further evidence of the fact that more mercury was administered to the 60 day old rats, the body weights of the mercury treated rats was significantly different from control weights up to the last week before sacrificing (Figure 6) whereas, body weights of the other two experiments became similar to controls much earlier (Figures 4 and 5).

Age appeared to influence the number of responses which were made in the open field. The 60 day old rats were less active than the younger rats, as evidenced by the number of areas traversed, inactivity, and latency. No differences were measured in standing upright and sniffing responses between the rats treated at different ages. Twenty-one day old rats circles less than the 15 day old rats, which in turn circled less than the 60 day olds. At retesting the circling response rats became similar.

In general, all parameters measured in the start box increased in rate of response from test to retest period. In the open field the number of sniffing and standing upright responses and areas of traversed increased, latency and inactivity decreased for all rats from test to retest period. All rats, except control rats in experiment 3, circled more on retesting than during testing. Mercury is slowly accumulated in the brain, in contrast, to other organs in the body, thus there is a latent period between exposure to mercury and the onset of neurological symptoms (Berlin & Ullberg, ref. 75). An interval of seven days was provided between observations in the open field to determine whether there was a change in behavior with time. It is assumed that with repetition, as the animals were observed for two five day periods, that response rates would decrease, and latency would increase (60). Generally the results were reversed from that which was expected.

Biochemical Results

There was no statistical difference in cerebral weight between control and mercury treated rats for all experiments (Table 32). The remaining brain tissue weight was not significantly different between the two groups of rats for experiments 1 and 2. However, control rats in experiment 3 had cerebellum plus medulla oblongata, mid-brain and pons which weighed less than that of the mercury treated rats (Table 32). This difference in weight could be an experimental error which occurred at decapitation.

Table 32. Analysis of brain sections: brain weight, DNA and RNA content.

	<u>Weight of cerebrum</u> (g)	<u>Weight of remaining brain tissue</u> (g)	DNA		RNA	
			<u>Total</u> (mg)	<u>Total/weight fresh tissue</u> (mg/g)	<u>Total</u> (μg)	<u>Total/weight fresh tissue</u> (μg/g)
<u>Experiment 1</u>						
Control	1.356±0.066	0.483±0.026	0.201±0.109	0.1563±0.068	258.9±28.2	190.6±19.6
Hg	1.403±0.069	0.491±0.028	0.201±0.079	0.1445±0.022	271.9±43.5	188.7±41.7
<u>Experiment 2</u>						
Control	1.282±0.044	0.276±0.079	0.1134±0.051	0.1115±0.079	212.7±31.6	217.8±30.7
Hg	1.277±0.062	0.282±0.067	0.1598±0.042	0.1625±0.056	222.9±28.2	219.8±25.2
<u>Experiment 3</u>						
1						
Control	1.353±0.055	0.393±0.067	0.2955±0.108	0.2430±0.035	407.4±28.2	300.0±12.9
Hg	1.307±0.122	0.447±0.045	0.3031±0.056	0.2333±0.031	373.8±62.0	292.0±58.5

± indicates standard deviation.

1 Significant differences in cerebellum, midbrain, medulla oblongata, and pons weight, P<0.05.

The spinal cord may not have been always severed from the brain at the same place. There might also have been some dehydration caused by a loose fitting container cap. This did occur in experiment 2 which explains the lower value for that group in weight of remaining brain tissue.

DNA and RNA contents were no different in the cerebrums of control rats, in comparison, to mercury treated rats. Zamenoff found that in the right and left cerebral hemispheres which were stored for seven days in a deep freezer, a total DNA content of $432 \pm 16.4 \mu\text{g}$ (76). In our experiments, brains were kept frozen from 1 month to 4 months from the time of sacrifice to analysis. Zamenoff (76) also used a different technique for extracting DNA from the brain tissue. Samples were centrifuged for 40 minutes at $18,000 \times g$, in contrast, to our method which was 15 minutes at $35,000 \times g$. He extracted DNA from the brain tissue four times in a boiling water bath, while only one hot extraction was called for in the Schmidt Thannhauser method. Munro (77) mentioned that unless brains were quickly placed in boiling water and then stored at freezing temperatures there would be a loss of DNA.

Histological Results

Histological examination of parasagittal sections of the whole brain revealed no lesions present in the brain of either mercury treated or control rats for all experiments. This was expected for experiments 1 and 2 but not for

experiment 3 since in the latter experiment several parameters were statistically different between control and treated rats. In all experiments, gross observations indicate that brain damage should be minimal. It is interesting to note that in the present studies particularly experiment 3 that behavioral tests can pick up the effect of mercury and not histologic examination.

SUMMARY

Methyl mercuric chloride was administered at 2.0 mg/100 g body weight in cocoa butter to 15- and 21-day old male Sprague-Dawley rats, and 2.5 mg/100 g body weight in 1,2-propanediol to 60-day old rats. Performance and behavior of the rat were measured in a T-maze, open field, and extinction trials in a T-maze. At the end of the behavioral tests some rats were sacrificed and brain weight, and cerebral DNA and RNA were determined. Other rats were perfused intracardially to fix the brain *in situ* and a parasagittal section of the perfused brain stained with hematoxylin and eosin.

No great differences were found between treated and control rats when they were tested in the T-maze and in extinction for all three experiments. No differences were observed for the 21-day old rats in the open field, and only minor differences on certain days for the 15-day old rats. However, for the 60-day old rats, during testing, controls had longer latency period than treated rats on each day of testing. They also crossed more areas than treated rats on all days of testing and retesting. Mercury treated rats remained inactive longer than controls for both periods of observation. The latter rats stood

upright and sniffed more often but circled less frequently than the treated rats on all retest days.

No differences in cerebral weight, DNA, and RNA were observed between mercury treated and control rats, in all experiments. Cerebellum plus remaining brain tissues weighed more for the mercury treated rats than controls in one of the experiments. Histological examination of the perfused brain showed no lesions in either treated or control brains, for all experiments. The change in behavior detected in the 60-day old rats was thus not reinforced by the biochemical and histological results. The dose may have been low enough to produce behavioral changes, but not permanent histological damage in the brain.

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APPENDICES

APPENDIX I
COMPOSITION OF GRAIN RATION (in %)

APPENDIX I

COMPOSITION OF GRAIN RATION (in %)

ground shell corn 60, soybean meal 28, alfalfa meal 20, fish meal 25, dried whey 25, ground limestone 16, dicalcium phosphate 17.5, iodized salt 5 lb., pro-gen 0.5 lb., prostrep-20 0.25 lb. Supplementary minerals and vitamins were added to provide per kg of diet: Mn 169, Fe 215, Ca 83, Zn 40, Cu 13, Co, 4, K 2, choline chloride 31.8, calcium pantothenate 0.25, riboflavin 0.15, niacin 1.5, B₁₂ 0.3, α tocopherol acetate 0.9, menadione 0.1, D L methionine 22.7, vitamin A palmitate (5,000,000 IU/g) 1.8 g, vitamin D 0.125 g, ascorbic acid 4.5.

APPENDIX II

COMPOSITION OF 300 MG FOOD PELLETS (%)

APPENDIX II

COMPOSITION OF 300 MG FOOD PELLETS (%)

Lab animal food	65 %
flour	6.5
zein	2.0
dry milk	7.0
gelatin	3.5
acacia	1.0
glucose	7.5
calcium phosphate	1.0
stearic acid	1.5
water	5.0

Kcal/g 4.3

moisture	5.0
ash	6.3
ether extract	4.9
protein	25.2
fiber	3.3
carbohydrates	55.3

APPENDIX III

KELLERMAN SERIES OF RANDOM ORDERED SIDES

APPENDIX III

KELLERMAN SERIES OF RANDOM ORDERED SIDES

day	order
1	R R R L L R L R L L
2	R R R L L R L L R L
3	R R L R L R R L L L
4	R R L R R L R R L L
5	R R L R L L L R R L
6	R R L L R R L R L L
7	R R L L R R L L R L
8	R R L L R L R R L L
9	R R L L R L L R R L
10	R R L L L R R L R L
11	R R L L L R L R R L
12	R L R R L R R L L L
13	R L R R L L R R L L
14	R L R R L L L R R L
15	R L R L L R R R L L

APPENDIX IV

FORMULA FOR BUFFERED FORMALIN SOLUTION

APPENDIX IV

FORMULA FOR BUFFERED FORMALIN SOLUTION^a

Commercial formalin	100 ml.
Distilled water	900 ml.
Sodium acid phosphate monohydrate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$)	4.0 g
Disodium phosphate anhydrous (Na_2HPO_4)	6.5 g

^aLillie's neutral buffered formalin (1948)

APPENDIX V

SCHNEIDER, SCHMIDT, THANNHAUSER METHOD FOR

DNA AND RNA DETERMINATIONS

APPENDIX V

SCHNEIDER, SCHMIDT, THANNHAUSER METHOD FOR DNA AND RNA DETERMINATIONS^a

A Preparation of samples (in the cold)

1. Weigh tissue.
2. Dilute with ice cold water to make 20% tissue homogenate.
3. Homogenize for 2 minutes in Waring Blender in cold room.

B Removal of acid-soluble compounds (in the cold)

1. Place 1 ml of 20% homogenate in centrifuge tube and add 2.5 ml of cold 10% TCA.
2. Centrifuge in the cold and discard supernatant.
3. Resuspend percipitate in 2.5 ml cold 10% TCA.
4. Centrifuge in the cold and discard supernatant.

C Removal of phospholipids (in room temperature)

1. Resuspend percipitate in 1 ml water and 4 ml 95% ethanol.
2. Centrifuge and discard supernatant.
3. Resuspend percipitate in 5 ml of 95% ethanol.
4. Centrifuge and discard supernatant.
5. Extract percipitate three times with three portions of 3:1 ethanol:ether, each time discarding the supernatant.

D Removal of RNA

1. Resuspend percipitate in 2 ml of 1 N KOH and maintain in water bath or oven at 37° C for 16-20 hours.
2. Neutralize solution (D1) with 6 N HCL.
3. Add equal volume of 5% TCA.
4. Centrifuge and save supernatant in volumetric flask.
5. Resuspend percipitate in 5 ml 5% TCA.
6. Centrifuge and add supernatant to D-4.

Appendix V (Cont'd)

E Removal of DNA

1. Resuspend precipitate in 5 ml of 5% TCA and heat in 90° C water bath for 15 minutes.
2. Cool and centrifuge and save supernatant in another volumetric flask.
3. Resuspend precipitate in 5 ml 5% TCA.
4. Centrifuge and add supernatant to step E-2.

F Concentration of DNA and RNA

1. Dilute each of the supernatants in step D-4 and E-4 to 10 ml with 5% TCA.
2. Determine RNA by orcinol reaction for pentose.
3. Determine DNA by diphenylamine reaction for desoxypentose.

^aAs outlined in *Methods of Biochemical Analysis*, Vol. I
Edited by David Glick, Intersciences Publishers, Inc.,
New York, 1954.

APPENDIX VI

DETERMINATION OF RNA BY ORCINOL REACTION

APPENDIX VI

DETERMINATION OF RNA BY ORCINOL REACTION^a

Orcinol reagent: 1 g orcinol is dissolved, immediately before use, in 100 ml CHCl_3 containing 0.84 g FeCl_3 .

1. Two ml nucleic acid extract is mixed with 3 ml of orcinol reagent.
2. Mixture is heated in a vigorously boiling water bath for 20 minutes.
3. The intensity of the green colour is read at 660 $\text{m}\mu$.

^aMejbaum, W. Z. physiol. Chem. 258:117.

APPENDIX VII

DETERMINATION OF DNA BY DIPHENYLAMINE REACTION

APPENDIX VII

DETERMINATION OF DNA BY DIPHENYLAMINE REACTION^a

Diphenylamine reagent: 1 g of purified diphenylamine is dissolved in 100 ml of reagent glacial acetic acid and 2.75 ml of concentrated sulfuric acid.

1. One ml nucleic acid extract is mixed with 2 ml diphenylamine reagent.
2. Mixture is heated in a boiling water bath for 10 minutes.
3. The intensity of the blue colour is read at 600 mμ, the wavelength of maximum absorption.

^aSchneider, W. S. 1957 Methods in Enzymology III:680.

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