THE EFFECTS OF METHYLMERCURY CHLORIDE ON BRAIN PROTEIN AND BEHAVIOR IN THE POSTNATAL DEVELOPING ANIMAL

A DISSERTATION

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We hereby recommend that the dissertation prepared under our supervision by Francine Joiner entitled The Effects of Methylmercury Chloride on Brain Proteins and Behavior in the Postnatal Developing Animal

be accepted as fulfilling this part of the requirements for the Degree of Doctor of Philosophy.

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CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

Environmental pollutants in the form of additives, wastes, drugs, pesticides, and fungicides have become the inheritance of a progressively industrializing world. The gradual buildup of such substances and their deleterious effects are in great need of more careful evaluation. According to Spyker (1975), the fetal animal ranks highest on the list of susceptibility. Exposure of pregnant animals has resulted in defective offspring, although the mother may be left unharmed. Some of the effects are not observable grossly, but are evident in behavioral tests (Joiner, 1975; Hughes et al, 1975; Olson et al, 1975; Salvaterra et al, 1973; Zenick, 1974). The recognition of subtle effects of mercury poisoning has added impetus to the search for the molecular mechanism of this environmental disease.

Norseth (1969) reported cytoplasmic incorporation of mercury without mercurial permeation of the nuclear envelopment. However, single stranded scissions in cellular DNA, decreased uptake of ^{3}H-thymidine and ^{3}H-uridine, and retarded cell multiplication have been found (Nakazawa et al, 1975), indicating nuclear involvement. Although

mercury is known to inhibit the mitotic cycle at low levels of exposure (Chang et al, 1976), prolonged exposure to mercury or exposure to higher levels of mercury promotes mitosis. This dual inhibitory and stimulatory effect on cell growth and tissue development was directly related to the mitotic index.

Changing levels of RNA in conjunction with mercury poisoning have been reported. Although a continuous decrease in RNA content in spinal ganglionic neurons was found (Chang et al, 1972) after methylmercury poisoning, a moderate increase in RNA was found in anterior horn neurons. The increase in RNA was attributed to a reparative neuronal response to cellular damage. Also, ribosomal disorganization was found to be an early event following methylmercury poisoning (Carmichael and Cavanagh, 1976). Interference with ribosomal function was attributed to mercurial affinity for the more than 50 sulfhydryl groups found in mammalian ribosomes.

Toxicity studies have revealed many varied results of mercury exposure. Growth reduction, increased relative kidney weight, and histochemical changes in kidney enzymes of weanling rats administered 2.5 ppm methylmercuric chloride orally were reported by Verschuuren et al (1976). No effect on fertility or lactation was found. Viability

of offspring of treated animals in the F1 and F2 generations was impaired. Weight reduction, reduced lymphocyte count, and increased neutrophil count in the F1 were also reported. Increases in weights of kidneys, heart, spleen, brain, and thyroid were found. Mercury was found concentrated in the blood, hair, kidney, liver, and brain. Slight decreases in neonatal weight but not in brain weight were reported (Sobotka et al, 1974). Early eye-opening in neonates from methylmercury treated dams was attributed to compressed nervous system development. Teratogenic effects of mercury were reported (Scharpf et al, 1973) when mercury was administered on days 6-19 of gestation, and high mercury concentrations in the pituitary of both exposed mothers and their pups were reported (Garcia et al, 1974).

Changes in the rates and levels of protein synthesis as a result of mercury poisoning have also been observed. After administration of methylmercury dicyandiamide (5 mg/kg for 8 days) by stomach tube (Cavanagh and Chen, 1971), amino acid incorporation decreased in the cerebellum, cerebral cortex, spinal cord, liver, and spinal ganglia. The sciatic nerve increased in amino acid incorporation. This result was attributed to increased Schwann cell reactivity in Wallerian

degeneration. An increase of in vitro incorporation of amino acids into protein during Wallerian degeneration was previously reported (Takahashi et al, 1961). A decrease in protein synthesis in brain slices was found after mercury administration (Yoshino et al, 1966). Enhanced protein synthesis in the brain was found (Brubaker et al, 1973) during the pretoxic and latent stages of mercury intoxication with 10 mg/kg/day for 7 days with methyl-mercury hydroxide.

Olson et al (1975) observed diminished learning capacity in second generation rats. Impaired ability to learn an active avoidance response in adulthood after mercury exposure at 28, 35, and 42 days of age also has been reported (Hughes et al, 1975). Zenick (1974) reported permanent learning disabilities in animals exposed to mercury during gestation or directly post-weaning, but not in animals exposed during the nursing period via mother's milk. Fetal and immature animals are more vulnerable to mercury exposure than adults (Spyker, 1975). The central nervous system of rats is especially vulnerable during the early postnatal period. The first 21 days after birth; the "critical period", is a time of very active metabolism in the brain (Himwich, 1973). Although most of the neurons are present in the cortex at birth, however, many are not

completely developed in the cerebellum. Also at this time there is a great deal of growth and arborization in dendrites and axons. Axonal-dendritic connections are being made at this time, and the blood-brain-barrier is being formed. Three to nine days after birth has been designated as the period of glial cell synthesis. The period of time between 10 and 20 days encompasses the active period of myelination.

Interference with metabolic processes during this active period may involve interruption of these discrete and yet overlapping events in development. Events that may be affected during this period are interference with formation of certain types of nerve cells, suppression of the maturation and synapsing of these cells, disruption of assembly of neural connections or receptor sites, interference with myelination, and cell loss.

The present study investigated the hypothesis that mercury exposure during the postnatal developmental period (1-21 days) would have an immediate effect on brain protein synthesis followed by a long-range effect on behavior. It was hypothesized that observable changes in the rates of incorporation of amino acids into brain proteins might give insight to rates of protein synthesis and protein accumulation. It was hypothesized that subsequent

behavioral examination of adult animals previously injected during a developmental phase of life would yield valuable knowledge about irreversible changes incurred during the developmental period.

CHAPTER II

MATERIALS AND METHOD

Animals

Male and female rats of the Sprague-Dawley strain were bred and offspring selected by date of birth. In order to encompass the critical period, males and females, odd days of age ranging from one day to twenty-one days, were chosen for the study.

Injection of Methylmercury Chloride

All animals were injected intraperitoneally with 8 mg of methylmercuric chloride per kg of body weight or with a comparable volume of saline. A total of 440 animals for the behavioral tests and 88 animals for the protein synthesis test were used in the study. Each group was composed of four animals treated and four animals control per age group for protein incorporation measurement. Ten animals treated and ten animals control of each sex and each age criteria were used for the behavioral tests.

Groups and Conditions

All animals for behavioral tests were housed in standard littering cages with littermates and mother until twenty-one days of age. At this time animals were weaned, sexed, and housed with animals of the same age, sex,

and treatment group. All animals were fed with standard laboratory chow (Purina) and water ad libitum. A reversed light-dark cycle of ten hours light, fourteen hours dark was enforced.

Animals injected for the behavioral study were allowed to reach 135 $\pm$ 25 days of age. At this time all animals were weighed, observed for presence of righting reflex (RR) and hind-limb-crossing phenomenon (HLC) (Klein, R. et al, 1972), and tested for five days in a water T maze. Following this testing period, all animals were tested for behavior in the open field, for sexual behavior, and for fertility.

Animals employed in protein studies were housed with mothers and untreated littermates in the twelve hour period between mercury injection and U-C-14-leucine injection.

Apparatus

A water T maze was constructed of wood. The stem was 145 cm long and 27.5 cm wide. The alleyways were 71.25 cm long, and 27.5 cm wide, and 38 cm deep. The completed structure was covered with a 12' x 12' sheet of thick plastic and filled with water to a depth of 35 cm. Water was maintained at ambient temperature.

Two identical sexual behavior arenas were used in testing sexual behavior of treated and control males. The arenas were plexiglass-fronted, semicircular enclosures

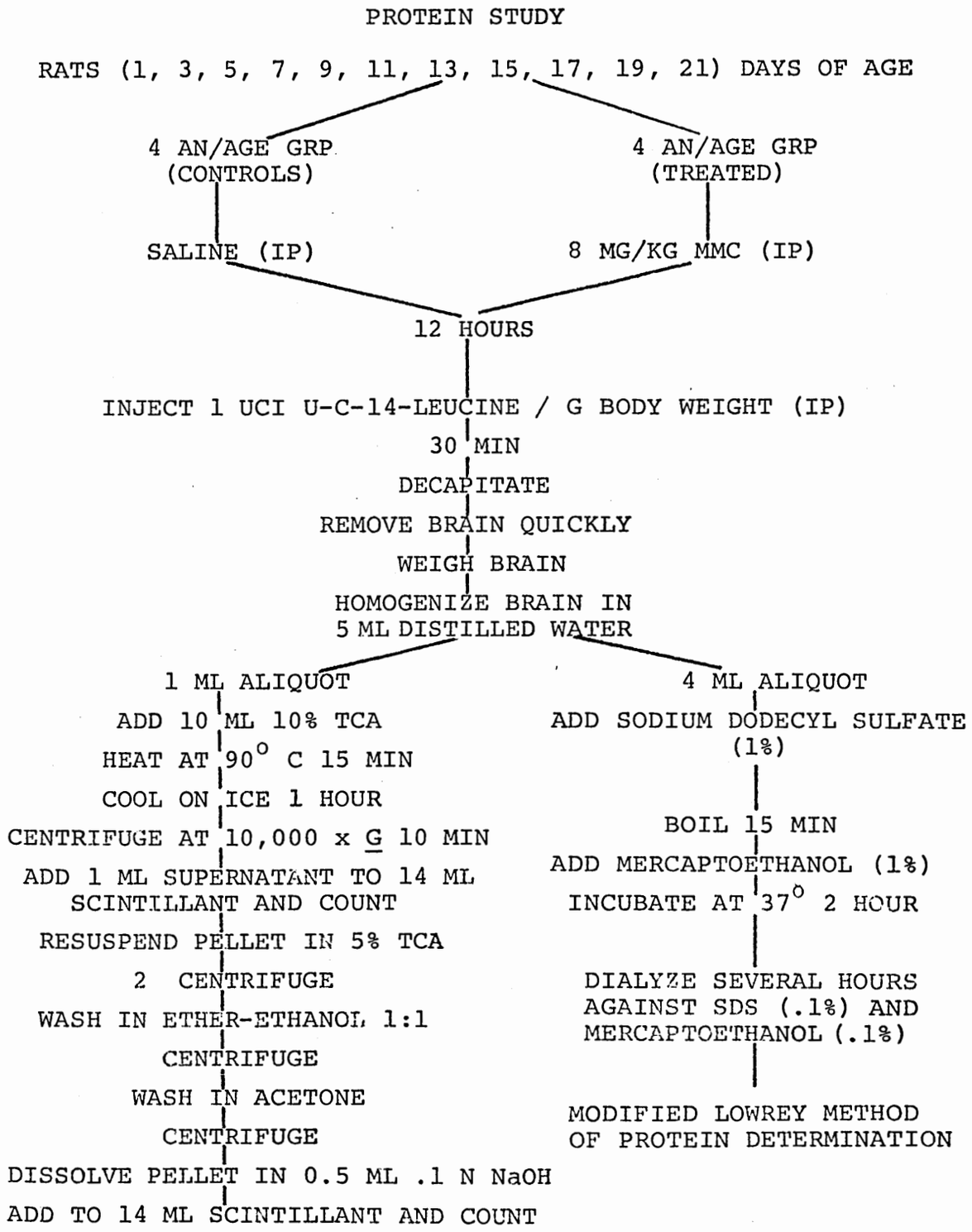
having a height of 28 cm and a radius of 31 cm. The arenas were placed side by side on a table illuminated by two red 25 W light bulbs suspended 1.5 m in front of the arenas.

The open field apparatus was composed of a circular field 81 cm in diameter surrounded by a 33 cm high wall. The field was divided by two concentric circles and lines radiating from the center into 12 truncated triangular-shaped areas surrounding a central circular area.

Procedure

Four animals at the age designated by each treatment group were injected with 8 mg/kg methylmercuric chloride or a comparable volume of saline (Figure 1). After an interval of 12 hours, each animal was injected intraperitoneally with 1 μCi of U-C-14-leucine (Amersham-Searle, Arlington Heights, Illinois 60005) per 7 g of body weight and allowed 30 minutes for incorporation. At this time the animals were killed by decapitation and the brains were quickly removed and weighed. Each brain was homogenized in a ground glass tissue homogenizer in 5 ml distilled water. An aliquot of 1 ml was reserved for measurement of incorporation of labelled leucine into trichloroacetic acid (TCA) precipitated protein and the remainder was reserved for future studies.

Figure 1. Procedure for injection regime and extraction of protein from brain homogenate.


PROTEIN STUDY
RATS (1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21) DAYS OF AGE
4 AN/AGE GRP (CONTROLS)
4 AN/AGE GRP (TREATED)
SALINE (IP)
8 MG/KG MMC (IP)
12 HOURS
INJECT 1 UCI U-C-14-LEUCINE / G BODY WEIGHT (IP)
30 MIN
DECAPITATE
REMOVE BRAIN QUICKLY
WEIGH BRAIN
HOMOGENIZE BRAIN IN 5 ML DISTILLED WATER
1 ML ALIQUOT
ADD 10 ML 10% TCA
HEAT AT 90° C 15 MIN
COOL ON ICE 1 HOUR
CENTRIFUGE AT 10,000 x G 10 MIN
ADD 1 ML SUPERNATANT TO 14 ML SCINTILLANT AND COUNT
RESUSPEND PELLET IN 5% TCA
2 CENTRIFUGE
WASH IN ETHER-ETHANOL 1:1
CENTRIFUGE
WASH IN ACETONE
CENTRIFUGE
DISSOLVE PELLET IN 0.5 ML .1 N NaOH
ADD TO 14 ML SCINTILLANT AND COUNT
4 ML ALIQUOT
ADD SODIUM DODECYL SULFATE (1%)
BOIL 15 MIN
ADD MERCAPTOETHANOL (1%)
INCUBATE AT 37° 2 HOUR
DIALYZE SEVERAL HOURS AGAINST SDS (.1%) AND MERCAPTOETHANOL (.1%)
MODIFIED LOWREY METHOD OF PROTEIN DETERMINATION

The 1 ml of brain homogenate was added to 10 ml 10% TCA, heated at 90° C for 15 minutes, cooled on ice for 1 hour, and centrifuged once each in ether-ethanol (1:1) (10 ml) and acetone (10 ml). The pellet was then dissolved in 0.5 ml of 0.1 M NaOH, added to 14 ml Aquasol (New England Nuclear). The external standards ratio method of correction was used.

The remaining 4 ml aliquots of brain homogenate for each treatment group were processed for specific activities. Sodium phosphate buffer (pH 7.0) (10 ml) containing sodium dodecyl sulfate (SDS) (1%) was added to the protein samples and boiled for 15 minutes. Sodium phosphate buffer (pH 7.0) (10 ml) containing mercaptoethanol (1%) was added and incubated for two hours at 37° C and centrifuged at 10,000 x g. The supernatant was dialyzed for several hours against sodium phosphate buffer (pH 7.0) containing 0.1% mercaptoethanol. A 0.5 ml sample was added to 5 ml Aquasol and counted. Another 0.5 ml sample was processed for determination of protein concentration according to a modified Lowry method (Begum, 1974). The counts per minute obtained from the TCA precipitate were normalized to counts per minute per gram of brain. In order to set up an index of metabolic activity, the counts per minute per gram of brain were related to the specific activities

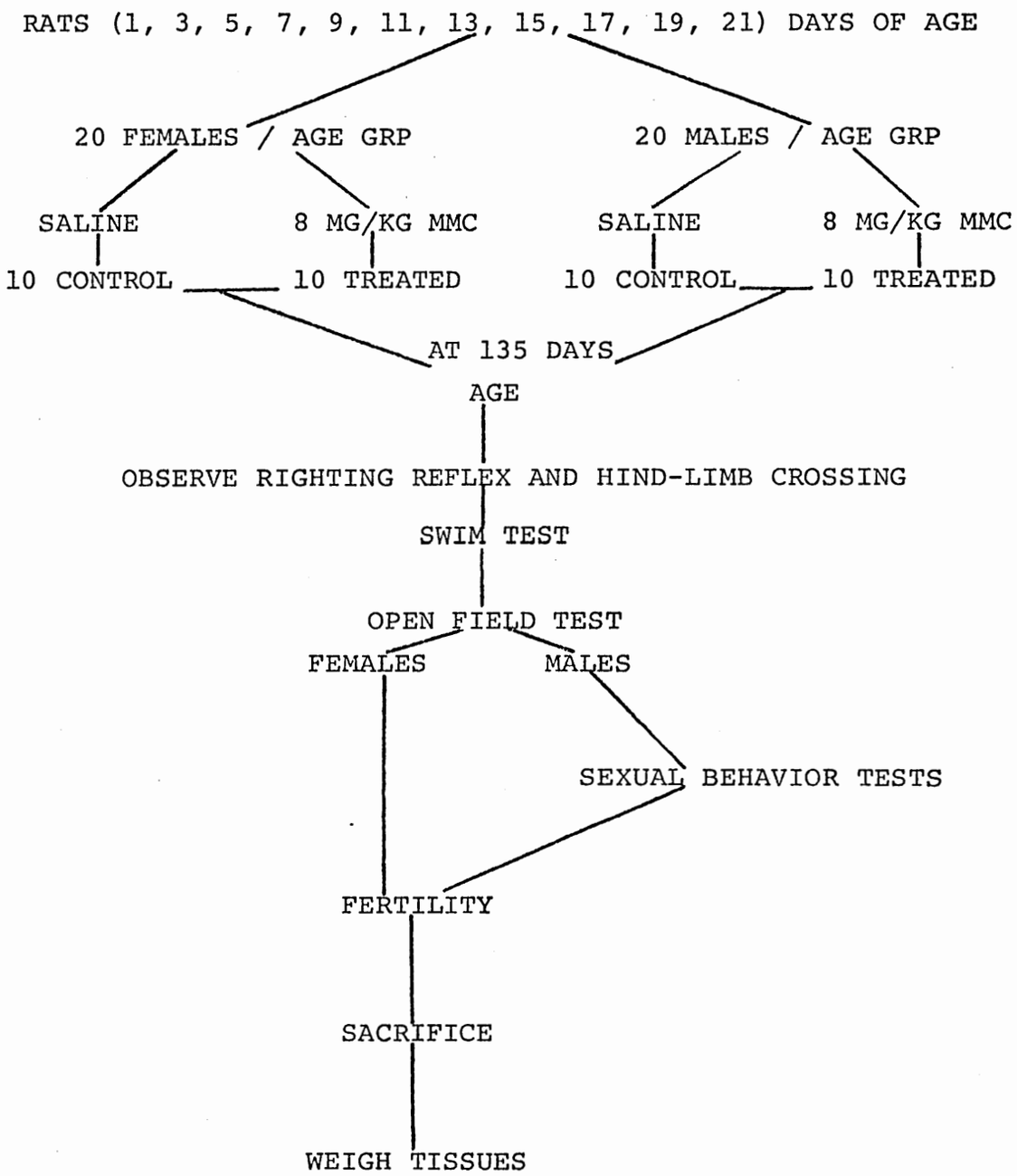
obtained from the SDS extracted samples. For example, if a specific activity of 100 counts per minute per 10 µg was obtained, this value was related mathematically to the counts obtained for the TCA precipitate (example: 25 counts per minute). The TCA counts per minute were multiplied by the µg of protein from the specific activity and divided by the counts from the specific activity $\frac{(25 \times 10)}{100}$. Therefore, counts per minute were eliminated, leaving µg of accumulated protein (2.5 µg) in the TCA precipitate.

Ten males and ten females (controls) and ten males and ten females (treated) for each age group were tested behaviorally (Figure 2) beginning at 135 ± 25 days of age. Each animal was held upside down and dropped from a height of 62 cm in order to test for righting reflex (RR). Each animal was suspended by the tail to test for presence or absence of hind-limb-crossing phenomenon (HLC). Each animal was scored negatives or positives for these two criteria (RR and HLC).

In each water maze trial, the animal was placed in start position and allowed to swim to an escape ramp at the end of one of the arms of the T maze. On the first day of testing, escape ramps were placed at both arms of the maze allowing the animal to choose his route of escape. Each animal was given three trials on the first day of testing

Figure 2. Procedure for injection regime and behavioral study.

BEHAVIORAL STUDY



thereby showing his direction of preference. The side opposite the side of choice was denoted the side to be used for the remaining four days of testing. On days 2-5, each animal was given four trials per day with the escape ramp placed on the test side. Escape latency in seconds was measured beginning with entry at the start position and ending when the animal touched the escape ramp. Animals were scored errors when they entered the wrong arm of the maze and were confined and caused to swim there for 15 seconds.

The open field behavioral test, beginning 14-21 days after initiation of the swim test, included placement of the animal into the central circular area and observation of his activity for a period of two minutes. Number of defecations, number of areas entered with both front feet (ambulations), and the number of times the animal stood on his hindlegs (rearings) were recorded.

Males to be observed for sexual behavior, beginning 7-14 days after the open field, were placed in one of the two sexual arenas and given 5-7 minutes to adapt to the enclosure. At the end of the adaptation period, a stimulus female was introduced into the arena to begin a 15 minute sexual behavior test.

Two males were observed simultaneously by one experimenter from a distance of 1.5 m. Mount frequency (number of mounts without intromission during the 15 minute period), mount latency, (time from introduction of the female to the first mount), intromission frequency (number of intromissions during the observation period), intromission latency (time from introduction of the female to intromission), and ejaculation latency (time from the introduction of the female to ejaculation) were the measurements of sexual behavior. The animals were observed until they achieved one ejaculation or for a period of 15 minutes, whichever was the shorter period of time. Open field and sexual behavior were tested in the second third of the dark cycle.

Immediately after the sexual behavior test, animals were paired with females of the same treatment group or with a colony animal of the same age. Males were killed on completion of the 21 day fertility test. Females that failed to litter or could not be palpated as pregnant within 7 days of removal from the male were caged with a colony male of similar age for 7 days. Females that did not litter by 21 days after completion of the second mating were killed at that time. Litter size at birth and 10 days of age was recorded. Individual weights of offspring were

determined at 10 days of age. Females that littered were killed along with their offspring 10 days after giving birth. At sacrifice, brain, pituitary, testes, ovary, kidney, and liver weights of adults were recorded. Weights of litters at 10 days were also recorded.

Statistical Analyses

Results of the protein incorporation study were analyzed by Student's t-Test and one-way analysis of variance (ANOVA). All other data were analyzed by two-way ANOVA and Newman-Keul's Range Test. Enumerative data (errors in the swim test, all open field data, and mounts, intromissions, and ejaculations) were transgenerated ($\sqrt{n+1}$) in order to manipulate zero values.

CHAPTER III

RESULTS

Protein Analysis

Measurements of incorporation of U-C-14-leucine into TCA precipitable protein were corrected to give counts per minute per gram of wet weight brain (Figure 3) and estimated accumulation of protein in µg per gram of wet weight brain (Figure 4).

Figure 3 shows the rate of incorporation of U-C-14-leucine over the critical period of brain development (1-21 days after birth). The shape of the resulting control developmental curve corresponded well with those found in the literature (Himwich, 1973) with a peak of incorporation at birth followed by a decrease at three days, a smaller surge of incorporation from 7-9 days and decreasing at 11 days. Another peak occurred between 11 and 15 days, followed by a very large peak between 15 and 21 days. The curve of the treated animals was similar in shape with the exception of a lower minimum at 3 days, a larger peak between 11 and 17 days, followed by a smaller peak between 17 and 21 days. All treated values were significantly different from controls at .05 level (Student's t-Test).

Figure 4 showed a large accumulation of protein per gram of brain at 1 and 3 days with a gradual decrease

Figure 3. Rates of incorporation of U-C-14-leucine into TCA precipitable protein of whole brain. Each point represents a mean of four animals $\pm$ 1 S.E.

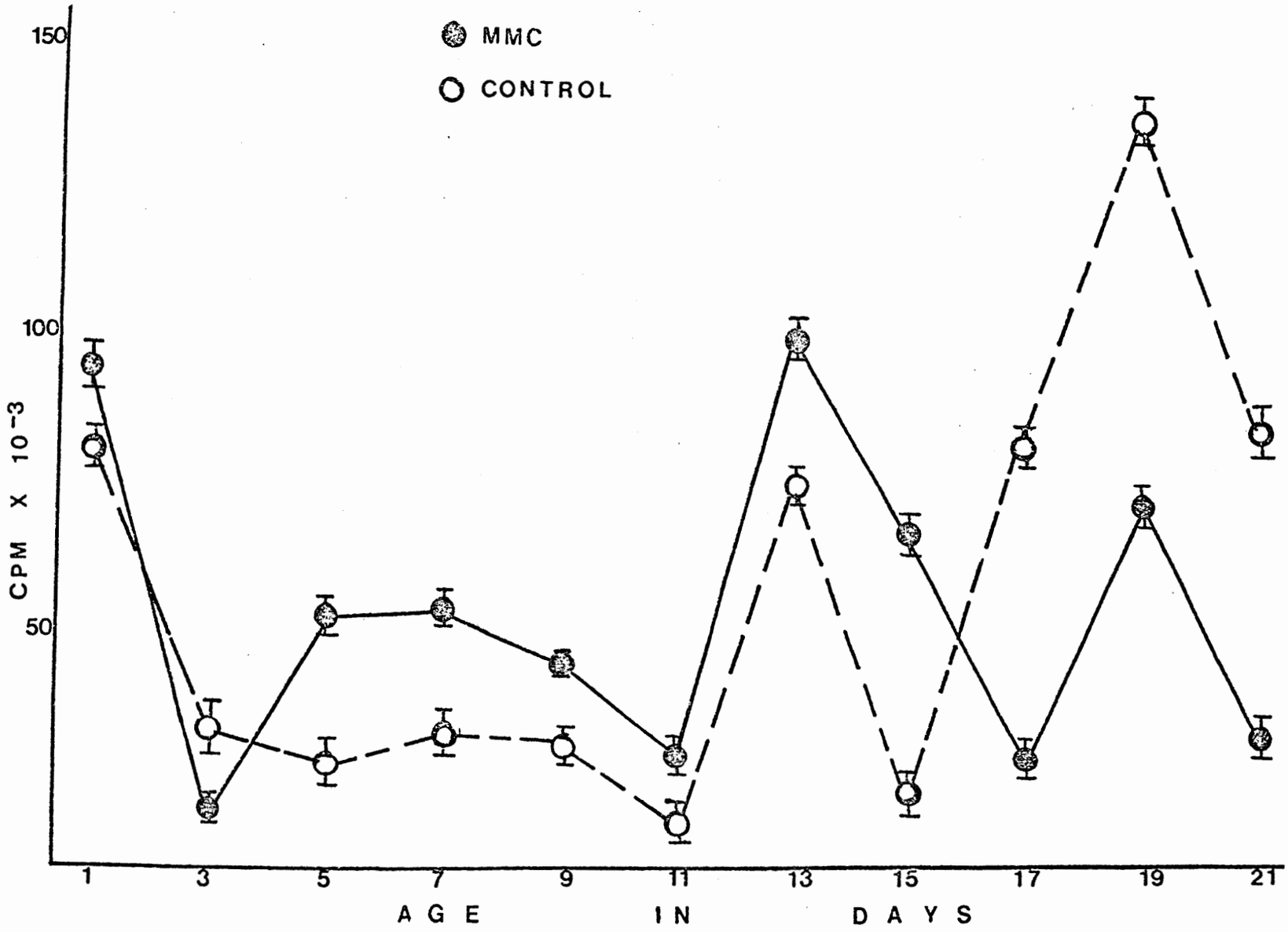
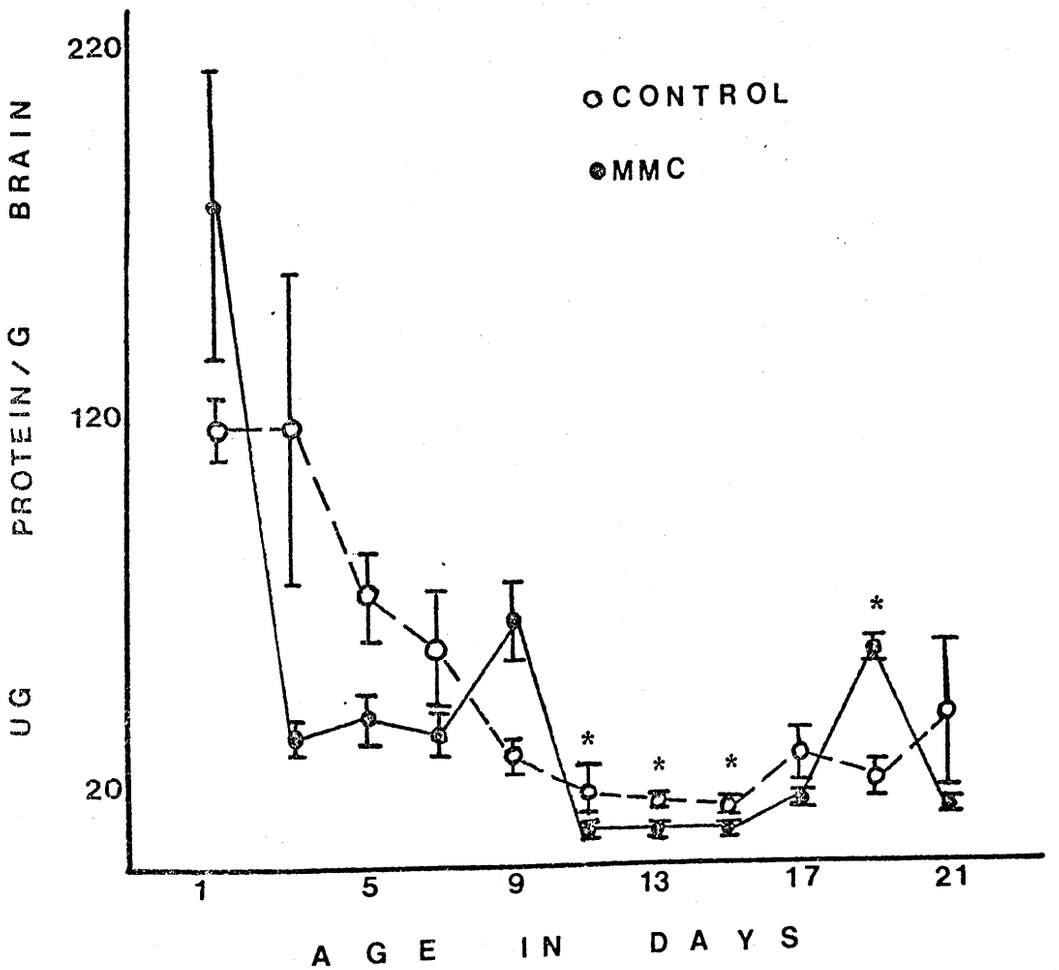

MMC
CONTROL
150
100
50
CPM X 10⁻³
1
3
5
7
9
11
13
15
17
19
21
AGE IN DAYS

Figure 4. Accumulation of protein, µg per gram of wet weight brain. *Significant at .05 level: one-way ANOVA. Each point represents a mean of four animals ± 1 S.E.


220
120
20
UG PROTEIN / G BRAIN
CONTROL
MMC
*
*
*
*
1
5
9
13
17
21
AGE IN DAYS

and leveling off as ages increased to 21 days. Mercury animals had increases in accumulation at ages 1, 9, and 19, and decreases in accumulation at the remaining ages. Differences were significant at 11, 13, 15, and 19 days of age (one-way analysis of variance [ANOVA], $P < .05$).

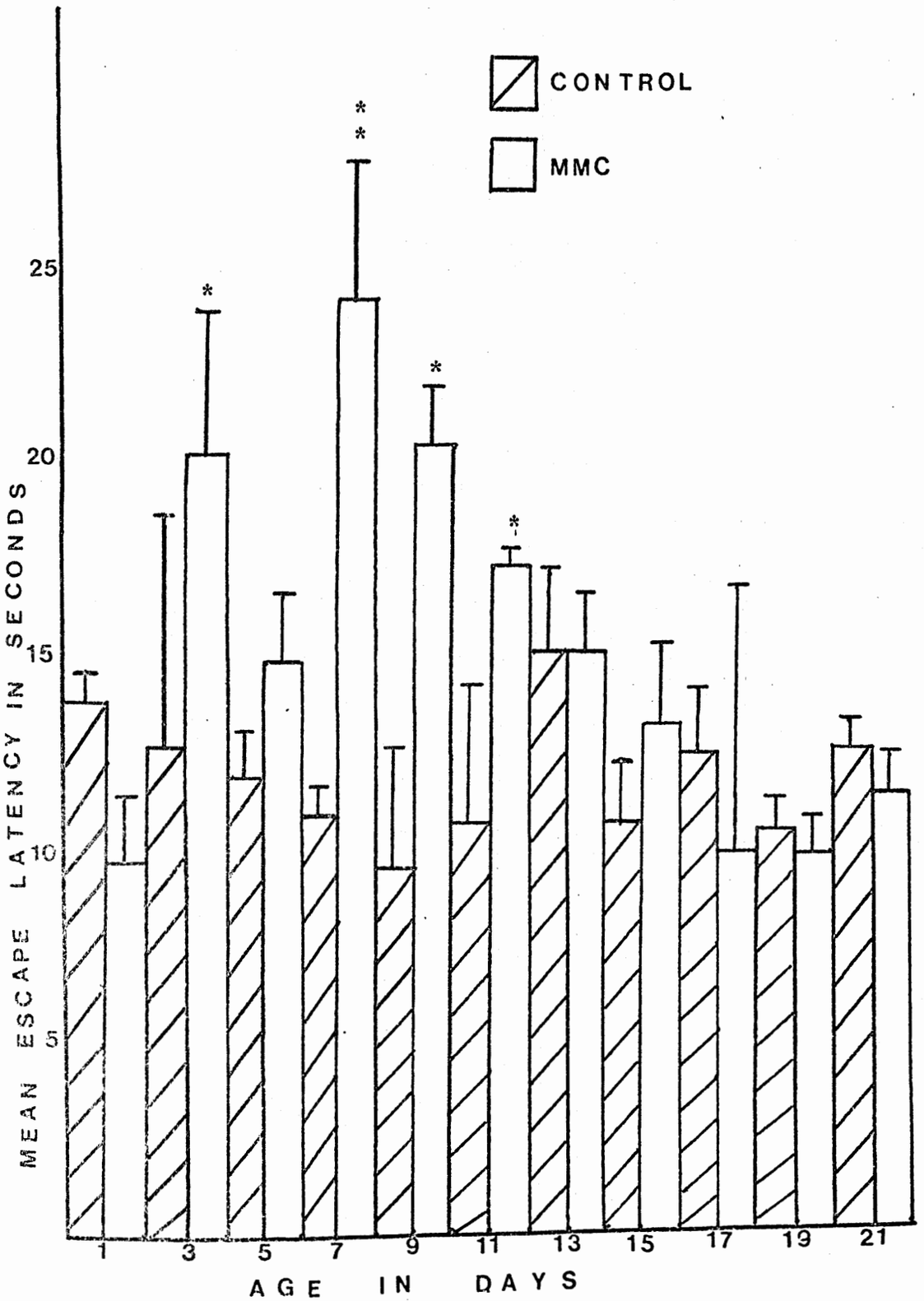
Righting Reflex and Hind-Limb-Crossing Phenomenon

Impairment of righting reflex and presence of hind-limb-crossing were not frequently observed. Impaired RR was observed in 17 animals, with more in the control group than in the treated group. Only two incidences of HLC were observed, one in a control animal and the other in a treated animal.

Swim Test

Females. Females had significant increases in mean escape latency (Figure 5) at 3, 7, 9, and 11 days of age when compared to controls of the same ages (Newman-Keul's Range Test). The two-way ANOVA indicated a different response between age groups ($P=.05$) and an overall significant difference between treated and control animals ($P=.01$). A significant increase in mean errors (Table 1) (Newman-Keul's Range Test) was observed at the 7 day injection age in comparison with controls. However, the two-way ANOVA indicated no overall significant differences.

Figure 5. Escape latency in swim test (females). *Significant at .05 level, **Significant at .01 level: Newman-Keul's Range Test. Each figure represents mean of 10 animals + 1 S.E.


CONTROL
MMC
MEAN ESCAPE LATENCY IN SECONDS
25
20
15
10
5
1
3
5
7
9
11
13
15
17
19
21
AGE IN DAYS

Males. Males had significant increases in mean escape latency (Figure 6) at injection age 11 and decreases in mean escape latency at age 21 (Newman-Keul's Range Test) when compared to controls. The two-way ANOVA indicated a different response between age groups (P=.0001) and an overall significant interaction between age and treatment (P=.02). Significant decrease in mean errors in comparison to controls (Newman-Keul's Range Test) was seen only at age 7 (Table 1) and was not measurable by the two-way ANOVA.

Open Field

Females. Tables 2a and 2b compile the results of the open field tests of females. Two-way ANOVA indicated significant differences between ages and between treated and control animals (P=.05) for mean rearings and mean outer ambulations. A significant interaction (P=.05) between ages and treatment was found for mean outer ambulations. No significant differences were found for mean inner ambulations, mean face washes, or mean defecations (two-way ANOVA). Results from Newman-Keul's Range Test indicated significant increases in mean rearings at injection age 7 and decreases at injection age 13 in comparison to controls. Decreased mean ambulations in the outer circle were observed for the 15 day injection groups

Figure 6. Escape latency in the swim test (males). *Significant at .05 level, **Significant at .01 level: Newman-Keul's Range Test. Each figure represents mean of 10 animals + 1 S.E.

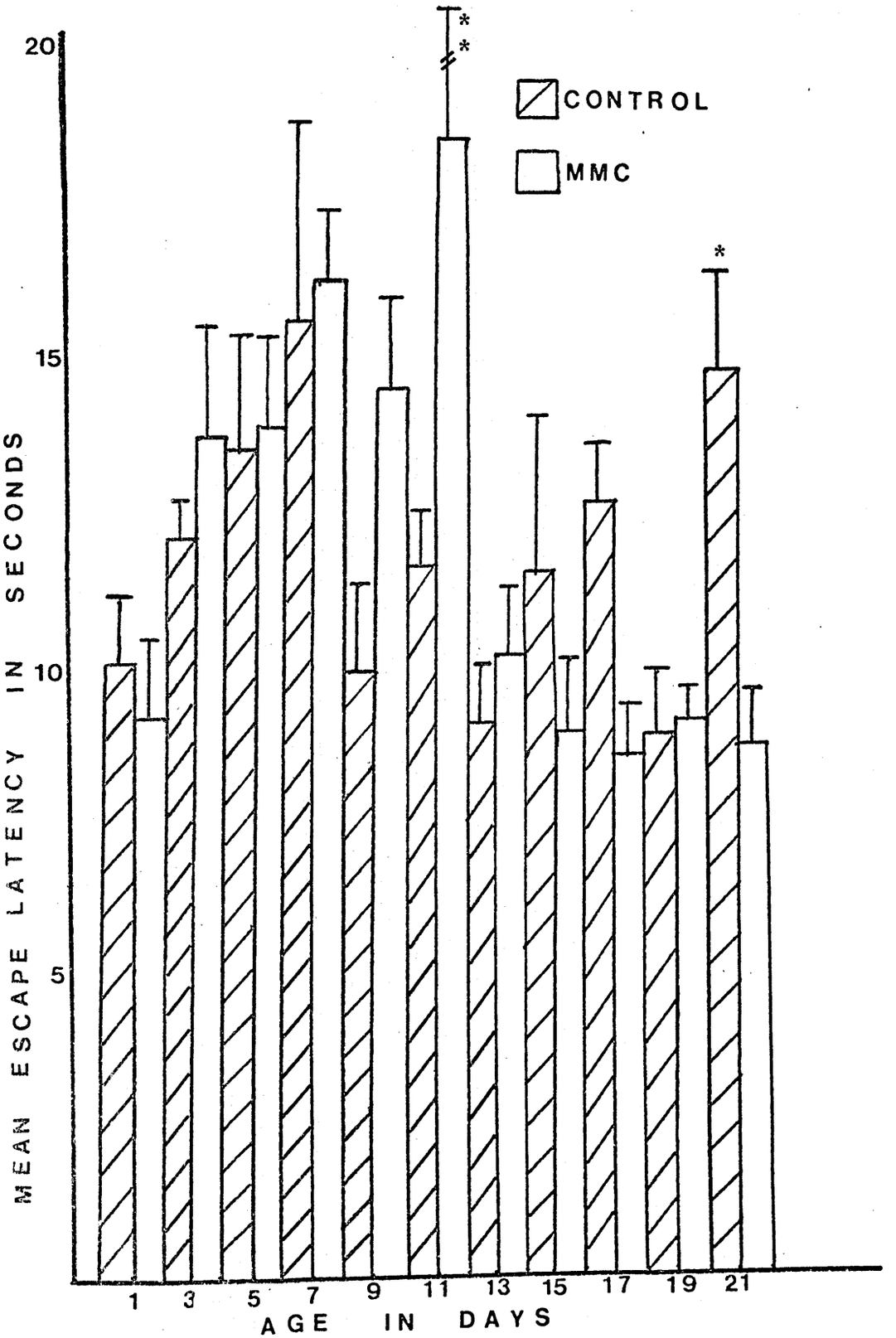

20
15
10
5
MEAN ESCAPE LATENCY IN SECONDS
CONTROL
MMC
*
*
*
1
3
5
7
9
11
13
15
17
19
21
AGE IN DAYS

Table 1. Mean[a] number errors in swim test.

Age at injection (days)	Females		Males	
	Control	MMC	Control	MMC
1	9.3	7.3	3.7	6.1
3	5.6	7.1	5.1	6.3
5	7.3	6.6	7.9	7.6
7	6.6	10.5*	9.3	5.5*
9	5.7	5.5	6.5	5.7
11	6.8	8.6	6.6	5.9
13	7.6	9.6	8.6	6.5
15	7.8	8.4	8.6	7.8
17	3.9	5.7	6.2	4.4
19	6.8	7.1	6.4	7.8
21	5.8	7.9	6.3	7.5

*Significant at .05 level: Newman-Keul's Range Test.

[a]Each figure is a mean of 10 animals.

Table 2a. Open field data (females): Mean[a] rearings (R), outer ambulations (O), and inner ambulations (I).

Age at injection (days)	R		O		I	
	Control	MMC	Control	MMC	Control	MMC
1	3.3	4.8	18.1	22.9	4.9	4.8
3	8.5	5.4	26.9	20.0	3.1	5.4
5	7.1	9.3	20.8	17.9	3.9	5.8
7	10.6	17.5*	17.3	32.9*	6.1	13.0*
9	6.4	4.6	20.3	18.4	4.2	4.2
11	5.9	4.8	26.3	20.0	7.0	3.0
13	12.5	5.6*	27.0	16.0	7.3	3.1
15	7.0	5.3	23.6	9.8*	5.4	2.3
17	7.2	5.3	23.4	28.2	6.6	9.4
19	4.4	5.3	22.1	24.0	3.1	7.6
21	7.7	3.5	32.9	19.4	9.8	3.6*

*Significant at .05 level: Newman-Keul's Range Test.

[a]Each figure is a mean of 10 animals.

Table 2b. Open field data (females): Mean[a] face washes (FW) and defecations (DEF.).

Age at injection (days)	FW		DEF.	
	Control	MMC	Control	MMC
1	0.2	0.3	0.2	0.0
3	0.7	0.1	0.0	0.0
5	0.4	0.4	0.0	0.3
7	0.4	2.6**	0.0	4.2**
9	0.4	0.1	0.1	0.5
11	1.2	0.1*	0.5	0.3
13	0.0	0.2	0.0	0.0
15	0.6	0.2	0.0	0.0
17	1.3	0.2**	0.0	0.0
19	0.0	0.4	0.0	0.3
21	1.1	0.2**	0.1	0.0

*Significant at .05 level.**Significant at .01: Newman-Keul's Range Test.

[a]Each figure is a mean of 10 animals.

and increases for the 7 day groups while significant decreased mean ambulations in the inner circle were observed for the 21 day injection groups and increases were observed for the 7 day group. Significantly increased mean face washes were observed in the 7 day group while decreases were observed for the 11, 17, and 21 day injection groups in comparison to controls. Significantly increased mean number of defecations were observed for the 7 day treated groups when compared to controls.

Males. Tables 3a and 3b compile male open field behavior. A two-way ANOVA for male open field behavior indicated no significant overall changes for open field measures with the exception of a difference ($P=.05$) in response between ages for mean ambulations in the outer circle. Results for Newman-Keul's Test indicated relatively increased mean rearings for 5, 17, and 21 day treated animals and decreased mean rearings for 1 and 11 day animals in comparison to controls. Significant decreases in mean ambulations in the outer circle were observed for 11 and 21 day animals. Increased mean ambulations in the inner circle were observed for 3 and 5 day animals and significant decreases were seen for 13 day animals in comparison with controls. No significant differences between treated and controls were found for face washes. Defecations for 15 day animals were significantly decreased.

Table 3a. Open field data (males): Mean[a] rearings (R), outer ambulations (O), and inner ambulations (I).

Age at injection (days)	R		O		I	
	Control	MMC	Control	MMC	Control	MMC
1	6.3	2.8*	11.1	12.0	3.9	3.5
3	3.4	6.0	20.1	16.6	2.3	5.2*
5	4.3	8.0*	15.2	14.1	2.0	6.7**
7	4.1	2.2	10.2	11.1	3.3	3.8
9	4.8	4.7	12.0	13.9	4.5	3.7
11	7.7	3.5**	14.8	7.5**	5.2	3.6
13	8.1	7.0	12.7	14.1	6.3	3.5*
15	5.8	6.4	9.6	14.1	5.3	3.6
17	2.1	5.3*	13.5	19.5	3.8	3.2
19	3.2	2.9	12.3	11.0	3.0	2.6
21	2.5	6.5**	14.1	6.8**	3.2	3.6

*Significant at .05 level. **Significant at .01 level: Newman-Keul's Range Test.

[a]Each figure is a mean of 10 animals.

Table 3b. Open field data (males): Mean[a] face washes (FW) and defecations (DEF.).

Age at injection (days)	FW		DEF.	
	Control	MMC	Control	MMC
1	0.16	0.17	1.4	0.8
3	0.25	0.25	1.4	0.8
5	0.17	0.25	1.1	1.5
7	0.08	0.23	1.1	1.4
9	0.74	0.39	0.6	0.8
11	0.08	0.17	1.5	2.8
13	0.23	0.44	[illegible]	0.6
15	0.32	0.44	1.9	0.5*
17	0.17	0.00	0.2	0.0
19	0.08	0.00	0.4	0.4
21	0.08	0.00	0.0	0.5

*Significant at .05 level: Newman-Keul's Range Test.

[a]Each figure is a mean of 10 animals.

Sexual Behavior Tests

The data collected in Table 4 express mean number of mounts, intromissions, and mean number of animals ejaculating per treatment group. A two-way ANOVA showed differences (P=.007) between ages for number of mounts, differences between ages (P=.009) and differences between treated and controls (P=.027) for intromissions, differences between ages (P=.0105), differences between controls and treated (P=.0004), and an interaction between ages and treatment (P=.0383) for number of animals ejaculating. No significant differences between treated and controls for mount latency (Figure 7) or intromission latency (Figure 8) were observed. However, differences between ages (P=.0038) for ejaculation latency (Figure 9), differences between control and treated (P=.0004) for ejaculation latency, and interaction between ages and treatment (P=.04) for ejaculation latency were observed.

Newman-Keul's Test showed no significant differences in number of mounts for treated and control. Mean number of intromissions for treated was decreased at the 5 day and 9 day injection levels when compared to controls. Mean number of treated animals ejaculating was decreased for groups 5, 7, and 9 in comparison with controls. When compared to controls, intromission latencies were

Table 4. Mean[a] number of mounts, intromissions, and number of animals ejaculating.

Age at injection (days)	Mounts		Intromissions		Ejaculations	
	Control	MMC	Control	MMC	Control	MMC
1	0.6	0.6	1.4	1.4	0.3	0.1
5	3.6	3.9	3.2	0.2*	0.5	0.0**
7	2.7	4.7	5.8	2.9	0.5	0.1*
9	3.6	1.0	5.3	0.1*	0.6	0.0**
11	4.2	2.6	5.7	2.8	0.4	0.3
13	2.5	3.5	5.0	4.7	0.4	0.2
15	3.6	2.7	4.2	3.8	0.4	0.6
17	2.9	2.7	6.7	4.8	0.6	0.5
21	2.8	5.7	5.9	8.6	0.5	0.7

*Significant at .05 level. **Significant at .01 level: Newman-Keul's Range Test.

[a]Each figure represents a mean of 10 animals.

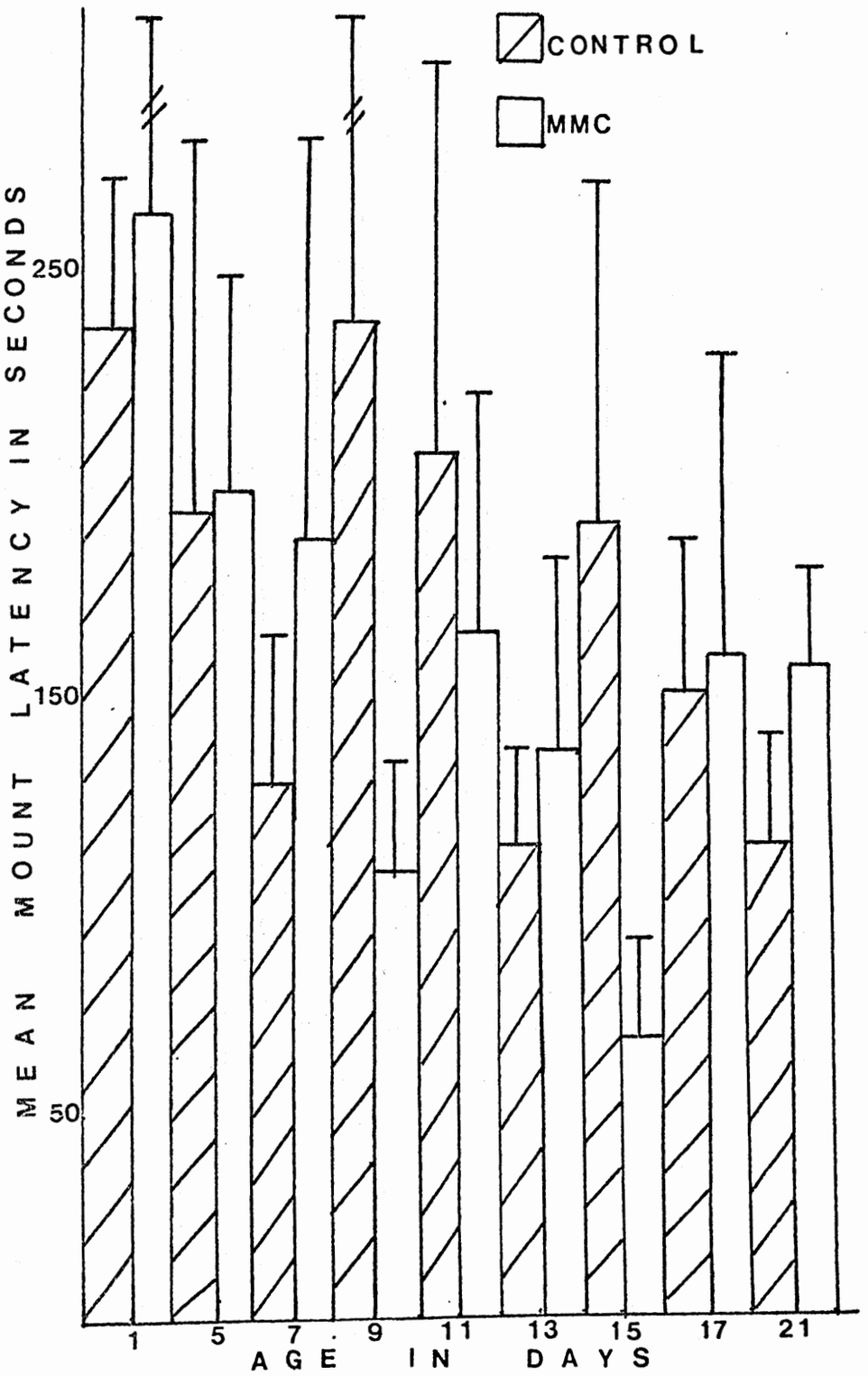

CONTROL
MMC
MEAN MOUNT LATENCY IN SECONDS
250
150
50
1
5
7
9
11
13
15
17
21
AGE IN DAYS

Figure 7. Mount latency in seconds. Each figure represents mean of 10 animals + 1 S.E.

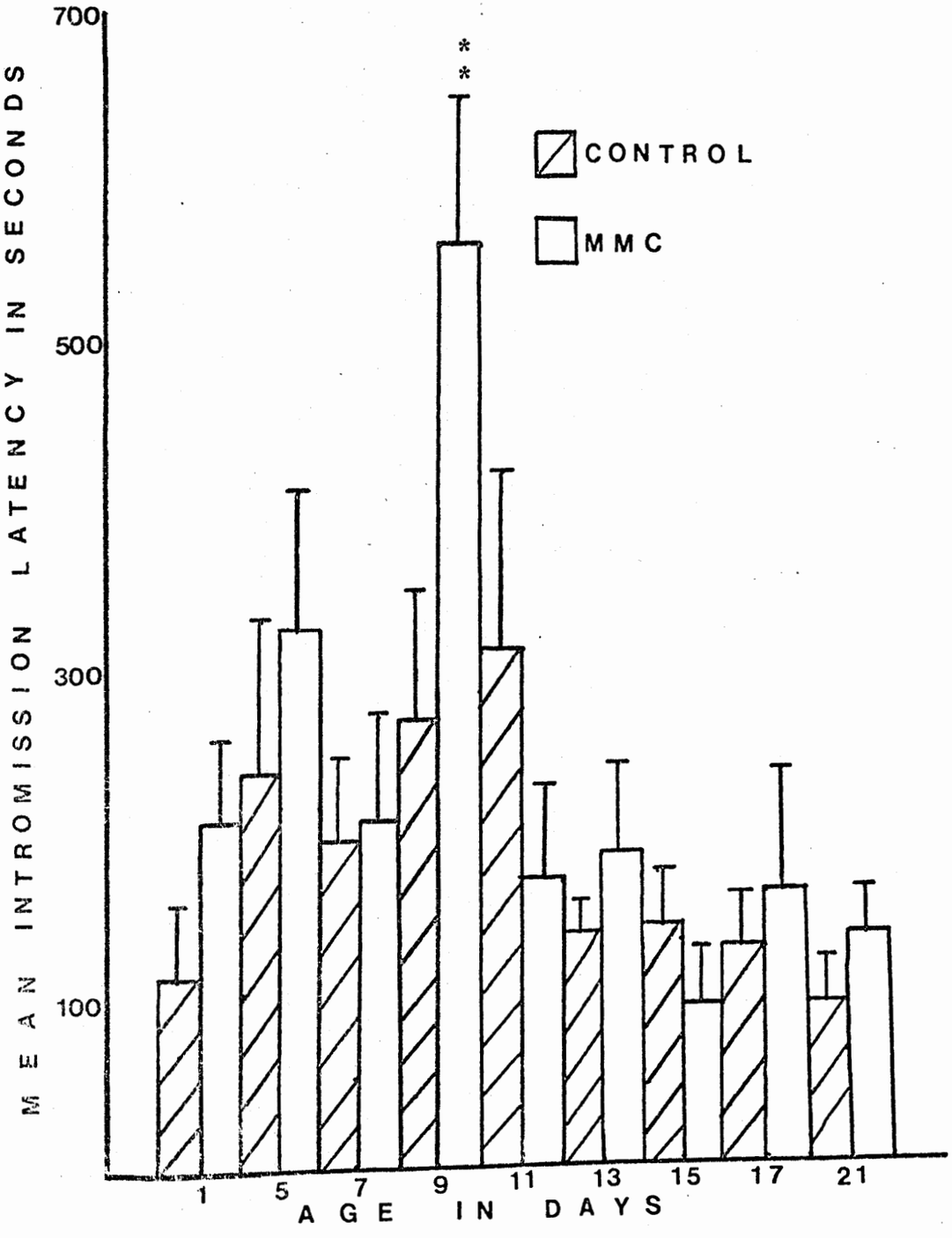

700
500
300
100
MEAN INTROMISSION LATENCY IN SECONDS
**
CONTROL
MMC
1
5
7
9
11
13
15
17
21
AGE IN DAYS

Figure 8. Intromission latency in seconds. **Significant at .01 level: Newman-Keul's Range Test. Each figure represents mean of 10 animals + 1 S.E.

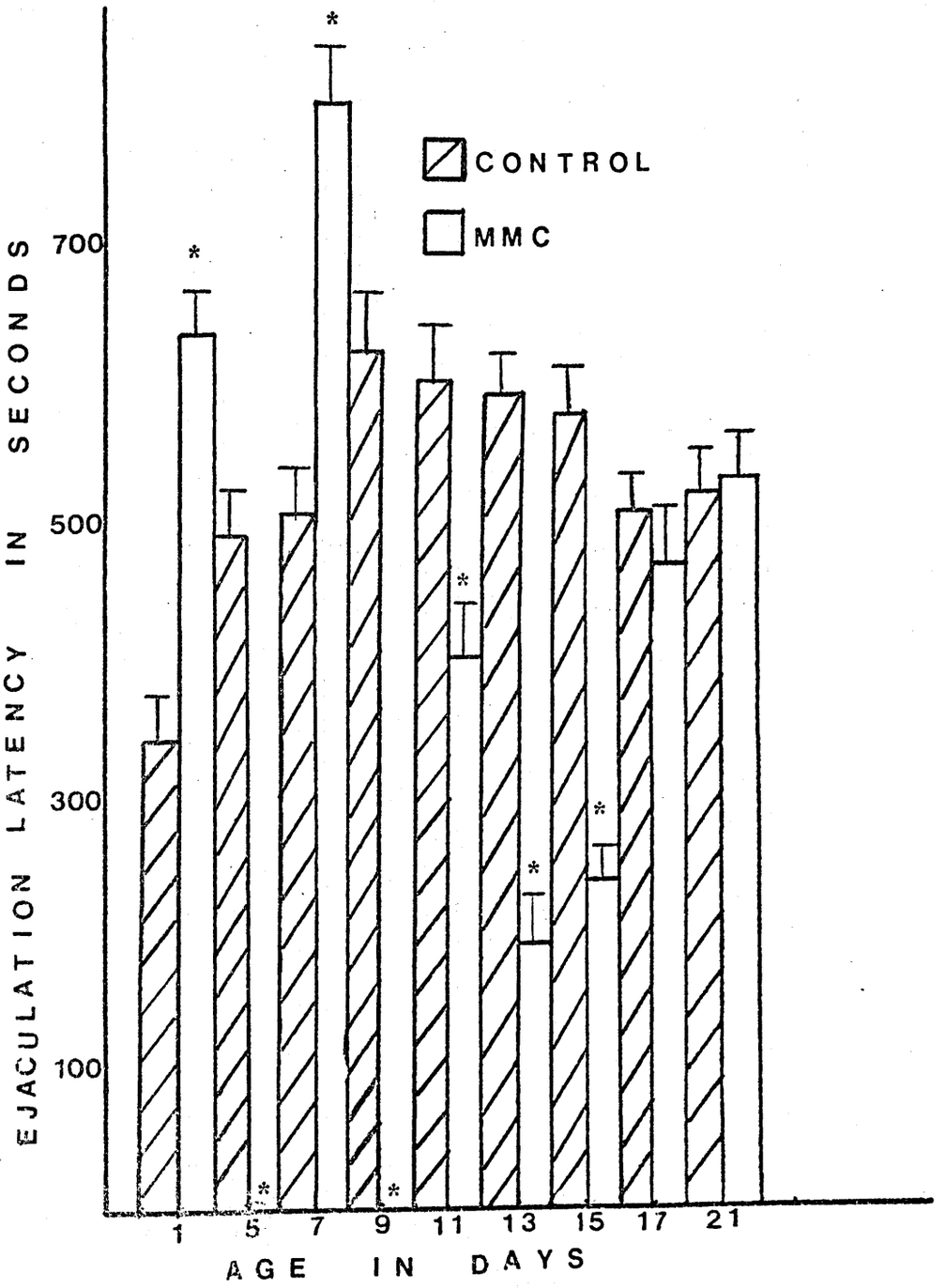

CONTROL
MMC
EJACULATION LATENCY IN SECONDS
700
500
300
100
1
5
7
9
11
13
15
17
21
AGE IN DAYS

Figure 9. Ejaculation latency in seconds. *Significant at .05 level: Newman-Keul's Range Test. Ages 5 and 9 had no ejaculations, therefore, no ejaculation latencies. Each figure represents mean of 10 animals + 1 S.E.

increased for the 9 day treated animals. Mean ejaculation latencies were significantly increased for treated animals in treatment groups 1, 5, 7, and 9, and decreased significantly for treated animals in groups 11, 13, and 15 in comparison with controls of the same ages.

Fertility

Fertility data were expressed as percent siring litters (Figure 10), mean litter size at birth (Figure 11), and percent offspring born dead (Figure 12). Percent treated animals siring litters was not significantly less than percent of controls. Mean litter size at birth for treated animals was lower than controls at injection ages 1, 5, 7, 15, 17, and 19 and higher at ages 13 and 21.

Tissue Weights

Females. Wet weights of female tissues are contained in Tables 5a and 5b. Results of a two-way ANOVA showed no significant overall differences for ovary, kidney, pituitary, or body weights.

Results for brain weights showed a significant ($P=.01$) difference between ages, a significant ($P=.01$) difference between ages, and a significant ($P=.01$) interaction between ages and treatments. Also, a significant ($P=.02$) interaction between ages and treatment was found

Figure 10. Percent animals siring litters.

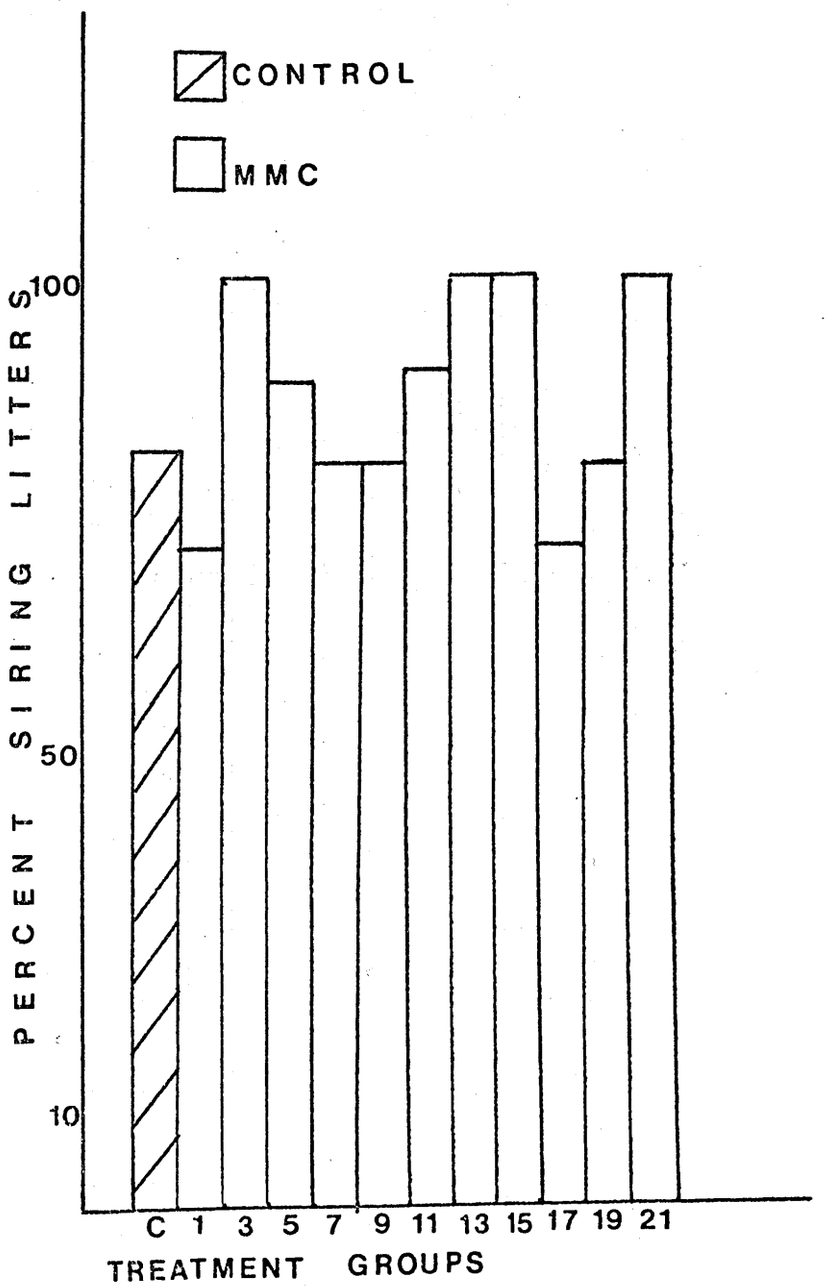

CONTROL
MMC
PERCENT SIRING LITTERS
100
50
10
C 1 3 5 7 9 11 13 15 17 19 21
TREATMENT GROUPS

Figure 11. Mean litter size at birth.

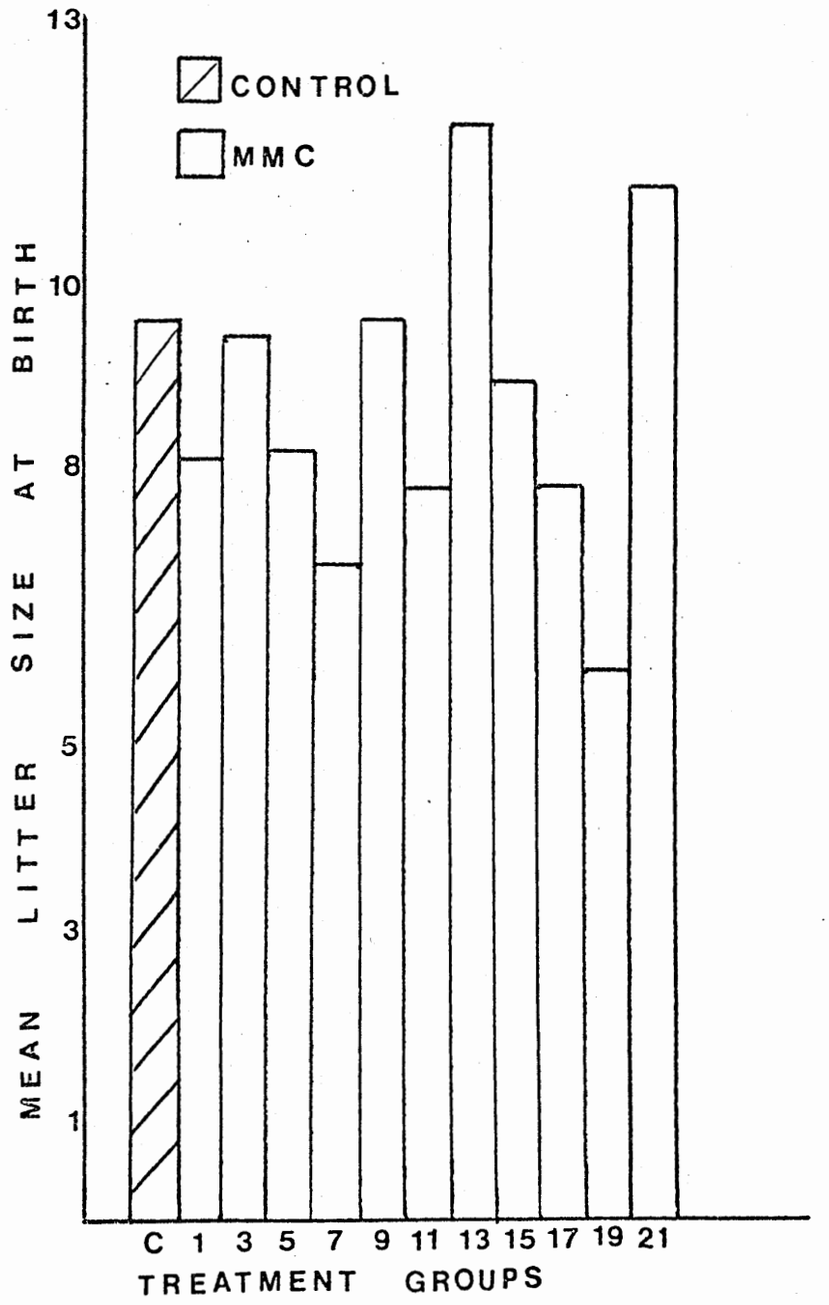

13
10
8
5
3
1
CONTROL
MMC
MEAN LITTER SIZE AT BIRTH
C 1 3 5 7 9 11 13 15 17 19 21
TREATMENT GROUPS

Figure 12. Percent pups born dead.

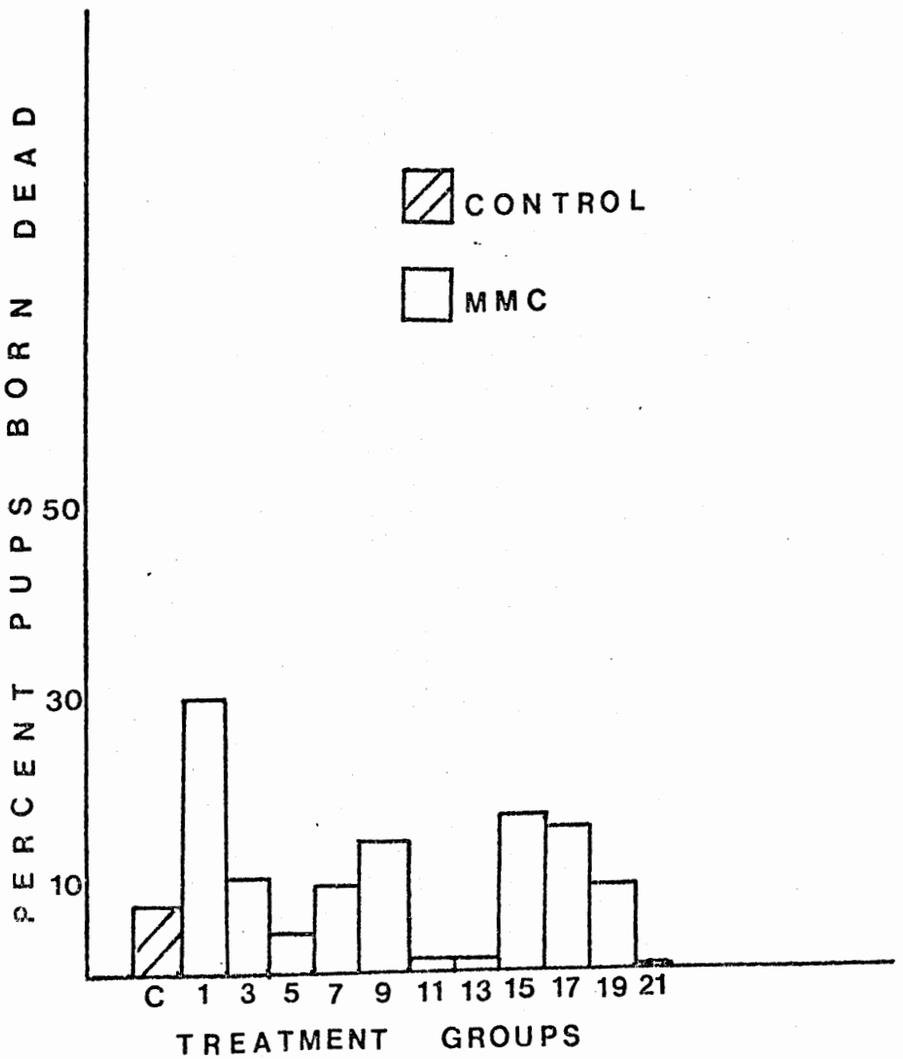

PERCENT PUPS BORN DEAD
50
30
10
CONTROL
MMC
C 1 3 5 7 9 11 13 15 17 19 21
TREATMENT GROUPS

Table 5a. Mean[a] tissue weight ± S.E.M. (females).

Age at injection (days)	Ovary (mg)		Kidney (g)		Liver (g)	
	Control	MMC	Control	MMC	Control	MMC
1	89 ± 3.0	66 ± 2.6**	1.87 ± .04	1.97 ± .04	12.15 ± 1.1	13.21 ± 1.2
5	80 ± 2.8	92 ± 5.0**	1.81 ± .04	1.93 ± .04	12.16 ± 1.1	14.19 ± 1.2
7	75 ± 3.3	77 ± 2.8	1.83 ± .05	1.78 ± .04	12.05 ± 1.3	11.69 ± 1.1
9	91 ± 3.0	71 ± 2.7**	1.91 ± .04	1.84 ± .05	13.78 ± 1.2	11.41 ± 1.1
11	89 ± 3.0	68 ± 2.6*	1.81 ± .04	1.87 ± .04	13.23 ± 1.2	12.79 ± 1.3
13	88 ± 3.8	87 ± 3.0	1.95 ± .06	2.11 ± .05	12.92 ± 1.5	14.76 ± 1.2
15	88 ± 3.8	91 ± 3.0*	1.95 ± .06	1.91 ± .04	12.92 ± 1.5	12.95 ± 1.1
17	86 ± 2.9	86 ± 3.5	1.76 ± .04	1.86 ± .05	12.61 ± 1.1	12.92 ± 1.4
21	73 ± 2.7	80 ± 2.8**	1.90 ± .04	1.91 ± .04	13.83 ± 1.2	13.35 ± 1.2

*Significant at .05 level. **Significant at .01 level: Newman-Keul's Range Test.

[a]Each figure represents a mean of 10 animals.

Table 5b. Mean[a] tissue weight ± S.E.M. (females).

Age at injection (days)	Brain (g)		Pituitary (mg)		Body Weight (g)	
	Control	MMC	Control	MMC	Control	MMC
1	1.68 ± .41	1.67 ± .36	13.58 ± 1.2	11.80 ± 1.1	307.7 ± 5.5	297.9 ± 5.2*
5	1.66 ± .41	1.63 ± .39	13.13 ± 1.1	11.59 ± 1.1	286.3 ± 5.4	293.4 ± 5.2
7	1.62 ± .34	1.64 ± .39	13.59 ± 1.4	12.16 ± 1.2	285.9 ± 6.4	277.6 ± 5.0*
9	1.69 ± .41	1.61 ± .42	13.20 ± 1.1	11.66 ± 1.2	314.0 ± 5.6	275.9 ± 5.5**
11	1.63 ± .40	1.61 ± .38	11.77 ± 1.1	12.35 ± 1.1	286.8 ± 5.1	293.8 ± 5.4
13	1.61 ± .52	1.62 ± .40	12.65 ± 1.5	11.81 ± 1.1	297.4 ± 7.7	309.8 ± 5.6**
15	1.61 ± .52	1.66 ± .41	11.69 ± 1.6	13.01 ± 1.1	299.3 ± 7.1	315.4 ± 6.7**
17	1.62 ± .40	1.66 ± .49	12.54 ± 1.1	11.00 ± 1.3	289.3 ± 6.0	282.8 ± 6.9
21	1.67 ± .41	1.67 ± .41	12.11 ± 1.1	12.27 ± 1.1	290.1 ± 5.4	292.7 ± 5.7

*Significant at .05 level.**Significant at .01 level: Newman-Keul's Range Test

[a]Each figure represents a mean of 10 animals.

for liver weights. Newman-Keul's Test indicated significant decreases in mean weights for ovaries of treated animals for 1, 9, and 11 day injection animals and increases in mean ovary weights from 5, 15, and 21 day animals in comparison with controls. No significant differences between control and treated could be found (Newman-Keul's Test) for mean kidney, liver, brain, or pituitary weights when compared to controls. Mean body weights of treated animals were decreased at ages 1, 7, and 9 and increased at ages 13 and 15 when compared to controls of the same ages.

Males. Male weight data was collected in Tables 6a and 6b. A two-way ANOVA indicated differences between ages ($P=.002$) for testes weights, differences between control and treated ($P=.03$) for kidney weights, differences between control and treated ($P=.007$) for liver weights, differences between ages ($P=.04$) for brain weights, an interaction ($P=.0001$) for brain weights, and differences between ages and treatments ($P=.01$) for body weights. Newman-Keul's Test indicated significantly ($P<.01$) decreased mean brain weights in comparison to controls for group 7 animals. Mean body weights of treated animals were increased for ages 1, 5, 13, 17, and decreased for ages 7, 9, 11, 15, and 21 when compared with controls.

Table 6a. Mean[a] tissue weight in grams ± S.E.M. (males).

Age at injection (days)	Testis		Kidney		Liver	
	Control	MMC	Control	MMC	Control	MMC
1	3.57 ± .06	3.56 ± .06	2.61 ± .05	2.99 ± .05	12.93 ± 1.1	14.83 ± 1.2
5	3.68 ± .06	3.83 ± .07	2.44 ± .05	2.79 ± .06	12.74 ± 1.1	15.11 ± 1.9
7	3.71 ± .06	3.73 ± .06	2.84 ± .05	2.68 ± .05	13.34 ± 1.5	14.80 ± 2.7
9	3.77 ± .07	3.61 ± .06	2.85 ± .06	2.69 ± .05	14.61 ± 1.3	11.35 ± 2.4
11	3.54 ± .06	3.70 ± .06	2.64 ± .05	2.66 ± .05	12.90 ± 1.1	13.86 ± 1.4
13	3.78 ± .06	3.97 ± .06	2.69 ± .05	3.13 ± .05	13.27 ± 1.2	15.70 ± 1.3
15	3.66 ± .06	3.65 ± .06	3.06 ± .06	2.71 ± .06	14.16 ± 1.2	12.30 ± 1.2
17	3.69 ± .06	3.68 ± .07	2.64 ± .05	2.73 ± .06	12.28 ± 1.1	14.69 ± 1.4
21	3.69 ± .06	3.55 ± .06	2.69 ± .05	2.53 ± .05	13.26 ± 1.2	12.93 ± 1.1

[a]Each figure represents a mean of 10 animals.

Table 6b. Mean[a] tissue weight ± S.E.M. (males).

Age at injection (days)	Brain (g)		Pituitary (mg)		Body Weight (g)	
	Control	MMC	Control	MMC	Control	MMC
1	1.76 ± .42	1.75 ± .46	10.68 ± 1.0	9.69 ± 0.9	399.8 ± 6.3	426.8 ± 5.9*
5	1.74 ± .42	1.82 ± .45	9.93 ± .9	10.22 ± 1.1	399.9 ± 6.3	428.4 ± 6.9*
7	1.79 ± .42	1.57 ± .40**	10.42 ± 1.0	9.86 ± 1.0	435.8 ± 6.0	401.8 ± 6.3*
9	1.81 ± .40	1.71 ± .41	11.41 ± 1.1	10.38 ± 1.0	438.0 ± 7.0	408.8 ± 6.4*
11	1.77 ± .42	1.68 ± .41	11.11 ± 1.1	10.33 ± 1.0	402.7 ± 6.4	388.3 ± 7.5*
13	1.75 ± .40	1.75 ± .40	10.63 ± 1.0	11.29 ± 1.0	426.7 ± 6.2	439.6 ± 6.3*
15	1.79 ± .42	1.79 ± .45	11.01 ± 1.0	10.88 ± 1.1	425.9 ± 6.5	408.0 ± 6.7*
17	1.73 ± .42	1.77 ± .50	10.02 ± 1.0	9.59 ± 1.2	399.1 ± 6.3	455.0 ± 8.7*
21	1.76 ± .42	1.78 ± .42	11.22 ± 1.1	10.00 ± 1.1	415.0 ± 6.4	397.7 ± 6.3*

*Significant at .05 level.**Significant at .01 level: Newman-Keul's Range Test.

[a]Each figure represents a mean of 10 animals.

CHAPTER IV

DISCUSSION

For many years, researchers have repetitively published reports of the dual inhibitory and stimulatory effects of mercury. Although the original results reported were considered to contain discrepancies in technique, conditions, and method, repeated replication of published results suggested a true duality in the effects of both organic and inorganic mercury in living systems and in tissue culture.

For example, formation of an increased number of dense granular lysosomes containing mercury has been reported (Fowler et al, 1975). This process was stimulated at doses of 3 and 5 ppm, but inhibited at higher doses (10 ppm). A continuous decrease in RNA content in spinal ganglia neurons after methylmercury poisoning and an initial decrease in RNA content followed by increases with inorganic mercury have been found (Chang et al, 1972). A duality in cell volume was also reported after both methylmercury and inorganic mercury administration. Initial increases in cell volume were followed by decreases in cell volume.

Indications of dual inhibition and stimulation of methylmercury on cell growth and tissue development have been reported (Chang et al, 1976). Mitosis was found to be inhibited by low levels of mercury and promoted by higher levels or by prolonged exposure. Decreases in amino acid incorporation in cerebellum, cortex, spinal cord, liver, and spinal ganglia, and increases in sciatic nerve have been reported (Cavanagh and Chen, 1971). It has been found (Bull and Lutkenhoff, 1975) that methylmercury enhanced the reductive phase of the redox state of nicotinamide adenine dinucleotides and cytochrome intermediates at low doses and inhibited them at higher doses. Enhanced protein synthesis in brain followed by a decrease was reported (Brubaker et al, 1973). This decrease was directly related to the amount of mercury that had reached the tissues.

In almost every parameter observed in the current study, both inhibitory and stimulatory results were found (Table 7). Instead of varying doses as some other studies did (Chang et al, 1976), the current study varied age at injection. According to other reports (Katzman, 1972), dilute mercuric chloride given intravenously causes increased permeability of the blood-brain-barrier. The blood-brain-barrier is being formed during the age regime

Table 7. Summary of Statistically Significant Results.*

Ages		1		3		5		7		9		11		13		15		17		19		21	
Sex		M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Protein	incorporation			D		I		I		I		I		I		I		D		D		D	
	accumulation											D		D		D				I			
Swim Test	errors	I						D	I														
	latency				I				I		I	I	I									D	
Open Field	rearings	D				I			I			D			D			I				I	
	ambulations (outer)								I			D					D					D	
	ambulations (inner)			I		I			I					D									D
	face washes								I				D						D				D
	defecations								I				D						D				D
Weights	ovary		D				I				D		D				I						I
	brain							D															
	body	I	D			I		D	D	D	D	D		I	I	D	I	I				D	
Sexual Behavior	intromissions					D				D													
	ejaculations					D		D		D													
	intromissions latency									I													
	ejaculation latency	I				I		I		I		D		D		D							

*D = Decrease; I = Increase

of injection for this study. Therefore, perhaps the inhibitions or stimulations in protein synthesis rates and accumulation were a reflection of the development of the blood-brain-barrier during the critical period of development. Since all uptake of amino acids into the brain is regulated by the blood-brain-barrier, and the barrier is immature at this time, the amount of amino acids and mercury penetrating into the brain may well be increased at certain ages of injection and decreased at other ages.

Protein Analysis

The information in Figure 3, rates of incorporation of U-C-14-leucine into TCA precipitable protein, may be interpreted in at least two ways. Initial observation of the control curve versus the treated curve shows both enhanced and decreased rates of incorporation. All treated points from 1-15 days, with the exception of a reduced rate of 3 days, show a trend of enhanced rate of incorporation of treated animals. Those animals from 17-21 days have severely decreased rates of synthesis when compared to controls.

With further scrutiny, the treated curve may be viewed as compressed in size when compared to controls.

The incorporation rates of the treated animals were advanced early in the developmental curve compared to those of the controls with the largest peak of incorporation beginning 4 days earlier than the control and prematurely falling. The largest peak of synthesis of the treated never reached the height of the large peak for the controls. Similar results were found (Kovacs et al, 1969) when the effects of thyroid hormone (T3) on protein synthesis in developing brain were studied. The advancement of maturation was supported by early appearance of innately organized behavior. This early enhancement and subsequent early fall in rates of protein synthesis may have caused premature termination of cell proliferation occurring during this critical period of brain development. Early termination and stunting of the surge of protein synthesis during this postnatal period is said to cause a deficit in the number of microneurons in specific brain regions developing during that time span. Eayrs (1968) found the thyroxine-treated animals to be advanced in maturation early in life and to have impaired responses to adaptive behavioral tests later in life.

An analogy may be drawn between the compression and overall decrease in rates of protein synthesis during development for both thyroxine-treated and methylmercury

treated animals. Sobotka et al (1974) reported earlier eye-opening and enhanced neonatal development of motor coordination of the offspring of female rats treated with methylmercury. This enhancement was an indication of compressed central nervous system development.

Figure 4 (estimated accumulation of protein) showed both enhanced and decreased accumulation of protein. The values for one day and 3 day animals were variable, due possibly to the immaturity and high water content of early neonatal rat brain. A trend of decreased protein accumulation was evidenced from 1-21 days as a result of methylmercury treatment with the exception of increases at 1, 9, and 19 days. The decrease in accumulation was particularly evident during most of the period of glial cell synthesis (3-9 days after birth) and during most of the active period of myelination (10-21 days after birth). The newly synthesized protein may have been degraded by the degenerative effects of methylmercury, thereby causing a decrease in accumulation of protein. Degenerative effects of mercury have been reported by Fehling et al (1974) and Lehotzky and Mezaros (1974) in the form of fragmented sheets of myelin and demyelination.

The effects of methylmercury on rates of protein synthesis and protein accumulation stressed disturbances

during the period of glial and microneuron proliferation. If premature termination of glial cell synthesis and microneuron synthesis occurred during this period, the total number of cells would be decreased. Therefore a reduction in number of cells could be responsible for the decrease in protein accumulation. Observed decreases in protein accumulation could further be related to disturbed behavioral events viewed in adult animals.

For example, during this period, microneurons are formed especially in the granular layer of the cerebellum, the olfactory lobes, and in the hypothalamus. Sexual behavior requires a complex sequence of events modulated by sensory, motor, and hormonal factors. The impairment of adult male sexual behavior of methylmercury treated animals served as an indication of this interference during the developmental process. Motor impairment in the swin test, emotional adaptive changes in the open field, and organ weight changes could also be related to changes in protein synthesis rates and accumulation during development.

Motor Ability and General Observations

Observation of adult animals (injected while 1-21 days of age) revealed no obvious impairment of general motor abilities. This was further evidenced by lack of

interference with the righting reflex and by absence of the hind-limb-crossing phenomenon reported by Klein et al (1972). Only one treated group differed from controls with regard to general appearance. Several of the 1 day treated group had extremely distended abdomens. When opened, a grossly enlarged cecum was revealed, although no tumors were evidenced. The entire enlargement was engorged with food, suggesting decreased gut motility.

Swim Test

Treated females showed a trend of increased mean escape latencies in the swim test for age groups 3-11. Males were less severely affected with increases in escape latency only for treated group 11. Males had faster swimming times in group 21, treated. Treated animals showed increases in number of errors for two age groups, 1 day males and 7 day females. When grossly observed, swimming behavior for treated animals appeared normal. Treated animals were indistinguishable from controls with the exception of the treated animals with distended abdomens. These animals were predominately found in group 1, males. Because of the cumbersome growth, these animals had to swim awkwardly with the distention floating upward, causing the animal to swim on its side. However,

escape latencies for these animals were as low as or lower than those of controls. Excess errors were observed in these animals.

Zenick (1974) reported permanent learning deficits in animals exposed to methylmercury during gestation and postweaning. No differences could be found between nursing animals and controls. Zenick's nursing animals were exposed to methylmercury via mother's milk instead of directly by injection. However, in the current study, with direct exposure of animals during the nursing period of 1-21 days after birth, permanent effects were found on escape latencies. Exposure during the period of 1-11 days disturbed some developmental sequence related to swimming behavior and left learning capabilities (expressed by number of errors) unimpaired except in two groups. The most vulnerable group for males was day 1 and for females was day 7. The slower swimming times for treated expressed retarded reaction times to the problem or impaired swimming abilities.

Open Field

Testing of animals in the open field allowed observation of treated and control animals in an adaptive situation. Enumeration of rearings revealed differences

between treated and controls for two groups for females and for five age groups for males. Both increases and decreases were observed. Increased rearings were observed for 7 day females and 5, 17, and 21 day males. Decreased rearings were observed for 13 day females and 1 day 11 day males.

Outer ambulations were decreased for 15 day females and 11 and 21 day males. Increased outer ambulations were observed for 7 day females. Inner ambulations were decreased for 21 day females and 13 day males. Increased inner ambulations were observed for 3 and 5 day males and 7 day females. Face washes were increased for 7 day females and decreased for 11, 17, and 21 day females, and not affected in males. Defecations were increased for 7 day females and decreased for 15 day males.

Although no consistent trends in open field data could be established, disparity between treated animals and controls was observed. Increases in means of rearings, face washes and defecations could be interpreted as evidence for increased emotionality in these treated animals. Decreases in these parameters may reflect a flatness or slowness of response in the treated animal.

More ambulations in the outer circle were measured for controls than in the inner circle. The treated

animals that had fewer outer ambulations were not reacting as the controls did in attempting to seek the darker area close to the walls of the apparatus. Those animals with higher than normal outer ambulations and decreased inner ambulations showed inability to adapt to the apparatus and venture back out into the inner circle.

Sexual Behavior Tests

Sexual behavior of treated males was severely affected by methylmercury. Number of mounts and mount latency were not affected. However, number of intromissions and number of animals ejaculating were severely decreased for 5, 7, and 9 day animals. Ejaculation latencies were increased for groups 1-9 with 5 day and 9 day animals not achieving ejaculations during the 15 minute test period. On the other hand, 11-15 day animals had significantly decreased ejaculation latencies. It was concluded that methylmercury administration to developing animals caused strongly observable effects on adult male sexual behavior.

Fertility

Although sexual behavior was disturbed in treated animals of groups 1 through 15, only two groups of these males were found to be different from controls with regard

to fertility measures. Treated animals in groups 1 and 17 were the only ones having decreased percentages of siring litters. The animals in groups 1-15 were less able than controls to adapt to the test situation but they were able to sire litters.

No consistent differences between treated and controls were found for the parameter mean litter size at birth. However, more treated groups (7) had smaller litter sizes than controls while two treated groups exceeded controls in litter size (groups 13 and 21). Indication of effect on offspring of treated animals was reinforced by the effects found on percent pups born dead. The offspring of one day treated animals were the most affected with 30% born dead. Since there is extensive study in the literature (Verschuuren, 1976) on effects of mercury on offspring of treated animals, in the current study, further tests were not done. It was concluded in the present study that mercury treatment during the critical developing period caused permanent effects on viability of offspring of treated animals.

Tissue Weights

The only female tissue weights markedly affected by methylmercury were those of ovaries. However, there was

no consistent pattern of effect. Increases in ovary weights were found for treated groups 5, 15, and 21. Decreases were found for treated groups 1, 9, and 11. These values could not readily be related to effects on fertility with the exception of group 1 animals. Decreased ovary weights corresponded with increased number of pups born dead for group 1 females. Both male and female body weights were disturbed. However, both increases and decreases were observed.

The ages most affected in all parameters in decreasing order were groups 7, 11, 9, (5, 15, and 21), (1 and 13), 17, 3, and 19. The behavioral parameters most affected by methylmercury were rearings in the open field, escape latency in the swim test, and male sexual behavior. In every measured parameter, female and male reactions were significantly different. Escape latencies for females were affected for more age groups than for males while mean number of rearings were more affected for males than for females.

The purposes of the present study were to measure the effects of methylmercury on protein synthesis and to determine if acute changes in early postnatal protein synthesis could be related to changes in adult behavior. The purposes were fulfilled by the experiments employed.

The disturbances in rate of protein synthesis and accumulation of protein were mirrored in behavioral changes in the swim test, open field, and sexual behavior tests. Although slower swimming, increased errors for a few animals, and changes in the open field behavior were found, the most dramatic effect measured was that on male sexual behavior.

The results of this study suggest that alterations in protein synthesis during the early postnatal period of development may cause permanent effects on adult behavior. These observed effects included increased emotionality or flattening of emotional response, reduced adaptive ability, and interference with complex behavioral responses (sexual behavior). Disturbances in tissue and body weights and in viability of offspring were also observed.

CHAPTER V

SUMMARY

1. The purposes of the experiment were:
   a. To investigate the acute effects of methylmercury administration on rates of protein synthesis and protein accumulation in a developmental period of active metabolism (1-21 days after birth).
   b. To observe behavioral changes in adult animals that were exposed to mercury when young.
   c. To relate the acute effects on protein synthesis to long-lasting behavioral effects.
2. Male and female Sprague-Dawley rats, ages 1-21 days, odd days only, were injected with 8 mg/kg methylmercuric chloride.
3. Four males and four females treated and ten males and ten females control were injected for each age group for protein measurements. Twelve hours after injection, these animals were injected with U-C-14-leucine (1 μCi/7g body weight) and allowed 30 minutes for incorporation. The animals were then killed and incorporation of amino acids into trichloroacetic acid (TCA) precipitable protein were measured.

4. Ten females and ten males treated and ten females and ten males controls were injected (8 mg/kg methylmercuric chloride for behavioral studies and allowed to reach 135 days of age. A battery of behavioral tests and observations including swim testing, open field, sexual behavior tests, fertility tests, and measurements of tissue weights was carried out.
5. Results: Treated animals had compressed developmental protein synthesis curves, and both increased and decreased rates of protein synthesis and protein accumulation. Treated animals also showed increased mean errors and increased mean escape latencies in the swim test. Open field behavior of treated, especially rearing, was disturbed. Adult male sexual behavior was severely disturbed; fertility of most of these animals was unimpaired. Viability of offspring and weights of some tissues (ovary, brain, and whole body) were disturbed.
6. Conclusions: Methylmercuric chloride given during the critical period of development has both acute effects on protein synthesis and permanent and measurable behavioral effects in adult animals that were treated while young. The metabolic disturbances in development caused by methylmercury may be potentiating

or degenerating, depending upon the age at injection and the parameter measured.

7. Future study might include:
   a. Electrophoresis of whole brain protein in order to determine changes in individual classes of brain proteins as a result of mercury administration.
   b. Investigation of protein synthesis changes in specific brain areas after mercury exposure.
   c. Study of changes in formation of myelin proteins after mercury exposure.

CHAPTER VI

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CHAPTER VII

VITA

Francine Elaine Lancaster Joiner was born February 25, 1947, in Belton, Texas. In 1965, she graduated with honors from Temple High School, Temple, Texas. In 1973, she graduated with honors from Texas Woman's University with a B.A. in Psychology and a minor in Biology. Her M.S. in Biology, minor in Psychology, was obtained in May 1975, from this university. Her master's work was supported by a Mary Hufford Scholarship and by a graduate teaching and research assistantships. The first two years of her doctoral work were supported by a State Doctoral Fellowship. In the fall of 1976, she joined the faculty of Texas Woman's University as Instructor of Biology, coordinator for Anatomy and Physiology laboratories.