Pharmacology Biochemistry and Behavior, Vol. 2, pp. 709–713. ANKHO International Inc., 1974. Printed in the U.S.A.

Behavioral and Biochemical Consequences in Methylmercury Chloride Toxicity[1]

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(Received 4 April 1974)

ZENICK, H. *Behavioral and biochemical consequences in methylmercury chloride toxicity.* PHARMAC. BIOCHEM. BEHAV. 2(6) 709–713, 1974. – The present experiment was designed to assess the developmental period(s) during which exposure to methylmercury chloride (MMC) would result in permanent learning deficits in rats. In addition, the mercury (Hg) content of the brain at these different stages was measured. Offspring (30 days of age) of mothers exposed during gestation and offspring exposed directly to MMC for nine days after weaning exhibited the greatest learning deficits on a water escape T maze. These deficits persisted through a retest session 21 days later. Biochemical analysis of brain Hg content indicated that Hg need not be present for these learning deficits to occur.

Mercury toxicity Learning deficits Biochemical correlates

ALTHOUGH the deleterious effects of mercury (Hg) poisoning have become increasingly apparent in recent years, there have been few experiments examining the effects of Hg toxicity on behavior. Armstrong *et al.* [1] found changes in the operant behavior (multiple FR60, FI15 schedules of reinforcement) of pigeons exposed to high levels (17 mg/m^3) of Hg vapor. Beliles *et al.* [2], employing the same task, did not find any decrement in performance when the level of Hg vapor was reduced to threshold limit value of 0.1 mg/m^3.

Beliles *et al.* [3] found an increase in escape latencies and a decrease in avoidance responding in rats exposed to high Hg vapor levels (17 mg/m^3) for 30 days. In addition, they observed an increase in the severity and duration of reflexive fighting behavior. Decrements in conditioned responses have been reported in cats [7] and rabbits [5]. Salvaterra *et al.* [9] found a significant decrease in rearings in mice receiving 5 and 10 mg/kg methylmercury both at one and three hours after injection. By 72 hours, all groups had recovered. In the only study employing a complex learning task, Hellberg and Nystron [6] found an increased reaction time on learning set responding in monkeys exposed to methylmercury for two months. However, there was no significant difference in error scores when compared to control counterparts.

While both inorganic and organic Hg may exert detrimental effects, the organic mercurial, methylmercury, has the most devastating influence on the integrity of the central nervous system [10]. Moreover, data on man and rat indicate that more than 90% of the methylmercury chloride (MMC) entering the body is absorbed, unchanged, by the intestine [4]. For these reasons, MMC was used in the present study.

There is little evidence available regarding the critical developmental periods during which exposure to Hg results in behavioral deficiencies. Spyker *et al.* [11] exposed mice to methylmercury dicyandiamide (i.p.) on the seventh or ninth day of gestation and observed significantly altered behavior in the offspring in an open field apparatus and on a swimming task. However, no assessment has been made of the relative toxicity of Hg exposure during gestation, nursing, and early postweaning.

Researchers examining the effects of Hg on behavior, have not investigated, but rather assumed that the presence of Hg in the brain was responsible for the observed behavioral decrements. The present study was designed to assess the development period(s) during which exposure to MMC would result in permanent learning deficits in the rat. In addition, the Hg content of the brain at these different stages was measured.

METHOD

Animals

The animals were derived from the breeding of 35 female, Holtzman albino rats (Holtzman, Madison, Wisconsin) with males of the same strain. Vaginal lavages were taken to confirm the presence of sperm and the onset of pregnancy. The mothers were caged individually, with

[1] Supported in part by USPHS, NIH Grant RR-00285.

ad lib food and water. At weaning, the offspring were ear-punched for later identification and housed by groups.

Apparatus

A water escape T maze, constructed of galvanized iron and painted with a flat black enamel paint was used. The stem of the maze was 86.36 cm long and 15.24 cm wide. The alleyways were 30.48 cm long and 15.24 cm wide. The water in the maze was 30.48 cm deep and maintained at 25.5°C.

Groups and Conditions

Five pregnant rats were assigned to each group. Litter size was manipulated at birth to insure that each mother nursed 8 offspring. Equal numbers of each sex were assigned to the behavioral and biochemical investigations.

Water consumption and weight gains were noted daily for mothers and offspring and averaged weekly for each rat. These weekly averages were used to compute the amount of MMC to be added to the drinking water to achieve an approximate dosage of 2.5 mg Hg/kg daily water intake. Water bottles were situated so that only the mothers could drink during gestation and nursing.

Three developmental periods were defined: gestation, nursing, and postweaning. For the gestation (onset of pregnancy to parturition) and nursing (birth to 21 days) intervals, MMC was administered to the animals' mothers. For the postweaning interval, (21 days of age to 30 days of age) MMC was administered directly to the offspring. Seven groups of animals were used. (Pilot studies had indicated that groups receiving MMC for two or more developmental periods only duplicated the results obtained from the groups employed in the present study.) Group G received MMC only during gestation; Group N received MMC only during nursing; and Group PW received MMC only during the postweaning interval. Control animals (Group C) were never exposed to MMC. Since the Group G animals could be exposed to MMC from both gestation administration and from any residual carryover to the nursing interval, two cross-fostered groups were used. Group CFN were born of control mothers and nursed by animals that received MMC during the gestation interval. Group CFG received MMC during gestation and were nursed by control mothers. Thus, Group CFN animals received only what MMC carried over from gestation to nursing while Group CFG received MMC during gestation with no carryover to nursing. Group S served as a state dependent learning control group (see procedure).

Procedure

Ten animals per group were employed in the learning task (a male and female were randomly selected from each of the 5 litters assigned to each group). Testing began at 30 days of age, when MMC administration was terminated for those animals still on MMC treatment (e.g. Group PW). This testing age was set after biochemical analyses indicated that, when MMC was continuously administered to 21-day old rats, Hg reached an asymptotic level in the brain in 9 days.

On each test trial, animals were placed in the start position and allowed to swim to an escape ramp at the end of the arm of the T maze. The rats were placed under a warm air fan during the 30 min inter-trial interval. On Day 1 of testing, 3 pretraining trials were given with escape ramps at the ends of both maze arms so that the maze could be exited from either arm. The acquisition task was given on Days 2, 3, 4, and 5. On each of the 5 daily trials, a single escape ramp was placed on the non-preferred side for each animal (i.e., on the side chosen one or fewer times during the 3 pretraining trials). A wrong turn was scored when the animal turned in a direction inconsistent with escape. Thus, if the animal entered the wrong arm (entry past forelegs) or turned in the stem and headed back toward the start, an error was scored. Escape latency measurement started when both of the animal's forelegs were past a mark 10.16 cm from the start of the maze and stopped when the animal touched the escape ramp.

In order to assess the duration of treatment effects, the rats were retested 21 days after the completion of original training, with 5 trials a day for 4 days on the same task. The retest date was set after biochemical analyses indicated that brain Hg levels of 30-day old rats who had received MMC for 9 days, returned to baseline levels within 21 days of termination of MMC treatment. At the retest period, 10 additional control rats (Group S) were run for the first time on the pretraining and acquisition tasks. Their performance served to assess the state dependent effects of MMC [8]. If MMC did produce state dependent learning then the postweaning group (PW) that has MMC still present during acquisition (drugged) would be expected to show little transfer of training to the retest when MMC has been dissipated from the system (non-drugged). In the absence of a permanent effect of MMC, Group PW's performance in retest would be like that of animals learning the task for the first time (Group S).

Biochemical Analysis

Six offspring per group were sacrificed at the end of gestation, nursing, and on the pretraining day (30 days of age). Six additional animals in the postweaning group were sacrificed on the first day of retest (51 days of age) to insure that there were no detectable amounts of Hg in the brains. The rats were decapitated, and the brain mass lying posterior to the olfactory lobes and anterior to the first cervical vertebra was excised within 25 sec. All samples were weighed and then frozen dry until analyzed.

For the analysis, the brains were first digested by refluxing for 2 hr in a solution composed of 7.5 ml of 98% sulfuric acid, 7.5 ml of 70% nitric acid and 15 ml of distilled water. At the end of digestion, 5% potassium permanganate was added to oxidize the Hg to mercuric ions. The solution was heated in a water bath for 20 min at 55°C. Five ml of 1.5% hydroxylamine hydrochloride were added to remove any excess permanganate, followed by the addition of 5 ml of 10% stannous chloride to reduce the Hg to metallic form. An aerator was inserted into the solution, the bottle stoppered, the Hg vaporized, and circulated in the analyzer system. Measurement (%T) was made with a Coleman MAS-50 flameless atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

Water consumption and weight and weight gains did not differ among mothers or among their offspring, suggesting no debilitation attributable to these factors.

TABLE 1

F VALUES FOR ESCAPE LATENCY AND ERROR DATA DURING PRETRAINING, ACQUISITION AND RETEST

		Pretraining	Acquisition		Retest	
	df	Escape Latency	Errors	Escape Latency	Errors	Escape Latency
Groups (G)	5	F<1	24.35*	17.70*	46.64*	54.40*
Days (D)	3		46.32*	46.31*	66.61*	111.35*
G × D	15		1.23	1.15	1.24	1.46

*$p \leq 0.001$

Behavior

The major findings from analyses for pretraining, acquisition, and retest are summarized in Table 1. There were no significant differences in escape latencies among groups on the pretraining trials, indicating no differences in swimming ability among the rats.

The experimental design conformed to a 4 (days) × 6 (groups) repeated measures analysis of variance. On the acquisition task, significantly poorer performances in terms of both errors and escape latencies ($p \leq 0.001$) were observed in groups receiving treatment during either gestation (Groups G, CFG) or during the 9 day postweaning period (Group PW). This trend ($p \leq 0.001$) continued through the retest session suggesting a permanent learning deficit. Groups C, N, and CFN did not differ from each other in either errors or latencies during acquisition or retest. Since error and latency data parallel one another, only error data is presented in Fig. 1. Since Groups C and CFN do not differ and Groups G and CFG do not differ, the potential carryover from gestation to nursing appears to be of little importance.

The state dependent control group's (Group S) performance on the retest session mirrored that of the control (Group C) during the acquisition task. Furthermore, Group S's performance on Days 1, 2, and 4 of the retest session was significantly better ($p \leq 0.05$ for errors and escape latency) than the postweaning group (Group PW). Thus the deficit shown by Group PW during retest probably does not reflect a state dependent transfer failure.

When all scores were collapsed across days, there was a significant day to day improvement in learning during acquisition and retest ($p \leq 0.01$ for errors and escape latencies). Comparisons of day to day performance (Least squares difference [14]) revealed a significant improvement each day ($p \leq 0.01$ for errors and escape latencies) during acquisition and retest. The group × day interaction was not significant, indicating a similar rate of learning across groups. Thus the gestation and postweaning groups' poorer performances may be attributed in part to their inability to overcome initial learning deficits.

Differences in memory capabilities could have also contributed to the observed deficiency. This difference would best be reflected by the degree of savings between groups that occurred in the 21 day interim prior to retest. This hypothesis was tested by running a 2 (days) × 6 (groups) repeated measures ANOVA comparing the errors on Day 4 acquisition, trial 5 vs. errors on Day 1 retest, trial 1. All groups made significantly fewer errors ($p \leq 0.01$, $df = 5$, $F = 11.72$) on Day 4 acquisition than Day 1 retest, with the same performance hierarchy across groups existing as that present in acquisition and retest (C, N, and CFN better than PW better than G and CFG). There was also a significant day × group interaction ($df = 5$, $F = 4.67$, $p < 0.01$) with Groups C, N, and CFN showing significantly greater savings than Group PW which was in turn better than Groups G and CFG (Method of Least Squares Difference). These means and differences are reported in Table 2. Thus differences in memory capability also contributed to the learning deficits in Groups G, N, and CFG. Poor transfer of training may, in fact, account for performance differences on Day 1 acquisition if Groups G, PW, and CFG fail to transfer the pretraining experience as completely as Groups C, N, and CFN.

Biochemistry

Results of biochemical analyses are summarized in Table 3. The highest amount of Hg recovered was in offsprings sacrificed at birth, whose mothers received MMC during gestation. This finding confirms the reports of Suzuki *et al.* [12] and Tejning [13] that Hg crosses the placental barrier.

Group PW (exposed to MMC for 9 days prior to testing) was the only group with detectable amounts of in the brain at the time of testing (3.9 μg Hg/gm brain weight). The amount recovered in Group PW agrees favorably with the asymptote disclosed by pilot work (4.1 μg Hg/gm brain weight). No group had detectable amounts of Hg in the brain at the time of retest. This finding suggests that the permanent learning deficits exhibited by Groups G, PW, and CFG resulted from structural/functional damage enacted during earlier periods of exposure to MMC rather than to the actual presence of Hg. This is further reflected by Group S's better performance than either Groups G, PW, or CFG during retest.

The amount of Hg in the brain of rats exposed during nursing (Group N – 0.255 μg; Group CFN – 0 μg) was not significantly different from the control Group C. It may be

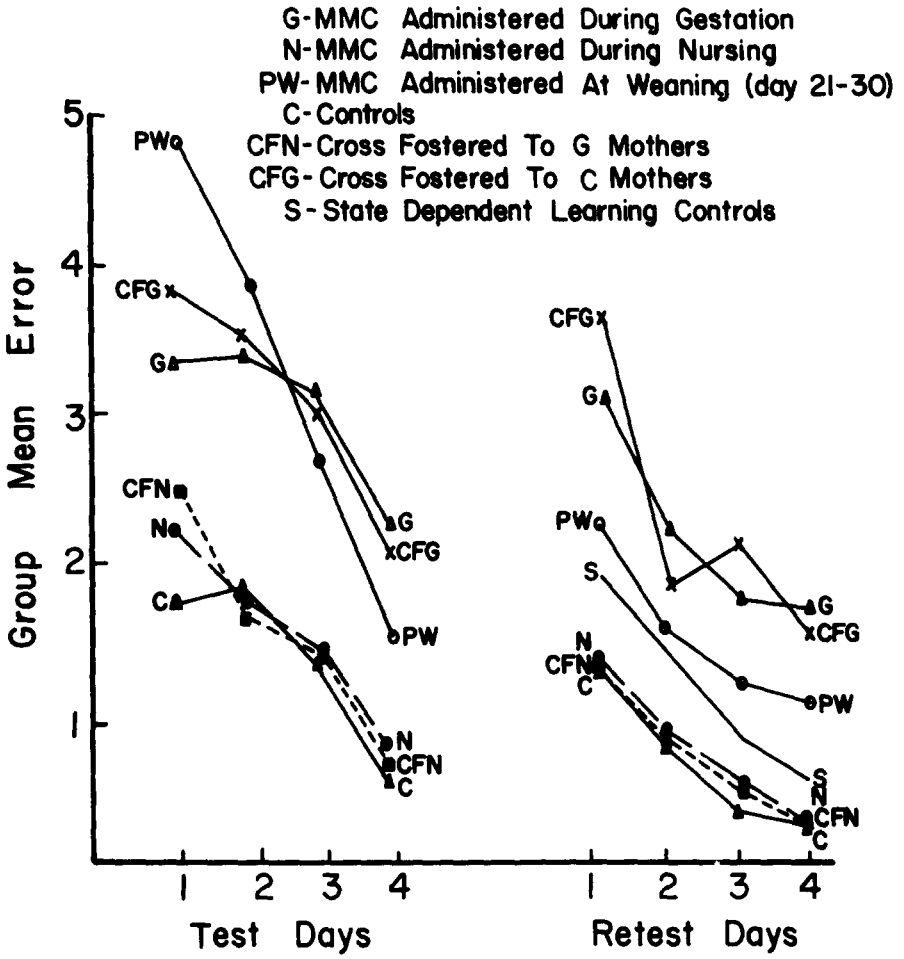

FIG. 1. Group mean errors for daily test-retest sessions.

TABLE 2

GROUP MEAN ERROR FOR DAY 4 ACQUISITION, TRIAL 5, VS DAY 1 RETEST, TRIAL 1

Group	Day 4 Acquisition Trial 5	Day 1 Retest Trial 1		Savings*
G	1.1	5.7	=	−4.6
N	0.5	1.7	=	−1.2
PW	1.0	4.0	=	−3.0
C	0.3	2.9	=	−2.6
CFN	0.5	2.6	=	−2.1
CFG	1.4	5.7	=	−4.3

*A difference of 1.12 between group savings score is significant $p<0.05$ (Method of Least Squares Difference).

TABLE 3

MERCURY IN THE BRAIN (μg/gm BRAIN WEIGHT) AT THE END OF EACH DEVELOPMENTAL PERIOD

Group	Developmental Period: Gestation Amount Recovered	Developmental Period: Nursing Amount Recovered	Developmental Period: Pretraining Amount Recovered
G	7.07	0.4939	0
N	0	0.2551	0
PW	0	0	3.9
C	0	0	0
CFN	0	0	0
CFG	6.26	0.352	0

that there is very little MMC in the mother's milk, or the Hg may exist in a form which cannot readily cross the offspring's blood brain barrier. In either case, exposure during nursing was not sufficient to create any learning deficits in Groups N or CFN.

Although the dosage, route of administration, task, etc. employed in this study do not mirror other research in this area some of the results are comparable. The behavioral and biochemical data indicate that MMC-treated groups (G, PW, and CFG) differ from nontreated groups (C, CFN) and that the nursing only animals of Group N cannot be distinguished from the nontreated groups. The behavioral impairment is permanent; especially potent in animals whose mothers were treated during gestation [11], and persists in the absence of detectable levels of mercury in the brain.

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