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Postnatal Behavioral Effects in Mice After Prenatal Exposure to Methylmercury[1]

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HUGHES, J. A. AND Z. ANNAU. *Postnatal behavioral effects in mice after prenatal exposure to methylmercury.* PHARMAC. BIOCHEM. BEHAV. 4(4) 385–391, 1976. – CFW mice were injected with methylmercury hydroxide (1, 2, 3, 5 or 10 mg/kg as mercury) on Day 8 of gestation. Mice treated with 3, 5 or 10 mg/kg averaged 1/3 fewer pups than controls. Pups from these treated animals weighed less than controls and the weight differences persisted through weaning but were no longer significant at 56 days of age. Mice exposed to methylmercury *in utero* showed significant differences from controls in their behavior in a 2-way active avoidance shuttle box and in a punishment situation but not when tested in an open field, a water escape runway or a conditioned suppression paradigm. Neither the mothers nor progeny of the mice exposed prenatally to methylmercury showed behavioral deficits.

Methylmercury Prenatal exposure Postnatal Behavior

THE toxicity of inorganic compounds of mercury has been known since antiquity [18] and postnatal poisoning by organic mercury since the nineteenth century [10,11]. It was not, however, until the middle of the present century that a case of intoxication *in utero* by an alkyl mercurial was first reported [12]. Subsequent clinical and experimental investigations have revealed that alkylmercury compounds are readily transferred across the placental barrier to become concentrated in the fetus at levels as high as 2 or more times those seen in the mother [4, 7, 16, 19, 23, 26, 35]. Reduced litter size in experimental animals and birth of progeny with teratogenic, neurologic and behavioral defects in both man and animals have resulted from exposure of the pregnant mother to methylmercury. Severe deficits have been noted to occur in the young of mothers who are themselves asymptomatic, suggesting that the developing organism may be more sensitive than the adult to organic mercury poisoning.

Most clinical and experimental studies concerned with prenatal methylmercury intoxication have dealt with the appearance of relatively gross neurological symptoms. Recent studies, however, indicate that subtle behavioral deficits can result from congenital exposure to low doses of methylmercury without the appearance of other neurologic dysfunctions [14, 20, 27, 28, 30, 32, 36].

The purpose of the present studies was to extend such observations by injecting low doses of methylmercury during gestation and subsequently studying the performance of the offspring in a variety of learning and motor tasks.

METHOD

Animals

The animals used in these experiments were mice of the CFW strain (Carworth, Division of Becton, Dickonson and Co., New York, N.Y.).

Mating procedure. Eight to 10 week old, individually marked, virgin females were selected at random from a stock colony and placed, 2 to a cage, with experienced male breeders. Next morning, each female was examined for the presence of a vaginal plug and, if present, the mouse was considered to be pregnant and was placed into an individual nesting cage for the duration of the gestation period (91 to 96 control mice so scored actually went on to bear litters, a success rate of 95%).

Dosage procedure. Eight days after the appearance of the vaginal plugs, the pregnant females were assigned at random to experimental or control groups and the appropriate freshly prepared treatment solutions were administered to them by peroral injection. The eighth day of gestation was selected because it corresponds with the beginning of the period of maximal susceptibility of the developing rodent brain to teratogenic agents [34]. Methylmercury hydroxide (Nor-Am Agricultural Products, Woodstock, Illinois) was diluted with 0.9% sterile saline to give dosage levels of 0.0, 1.0, 2.0, 3.0, 5.0 and 10.0 mg Hg/kg body weight as methylmercury in concentrations to allow one tenth ml of solution to be injected per 10 g of the animal's body weight. Control mice were similarly treated with an equivalent volume of saline. Once

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injected, a mouse was returned to its home cage and, apart from changes of food and bedding, it was undisturbed until it had delivered its pups.

Treatment of litters. The number of pups in a newly delivered litter was counted but the litter was otherwise unmolested for the first 24 hr. The litter was then removed from its mother, the pups individually weighed, reduced in size (when possible) to 4 pups, and returned to its own or to a foster mother.

Fostering of the litters entailed returning the pups of treated mothers to control mothers or to mercury-treated mothers which had received the same dose as the biological mothers and pups of control mice to mercury treated mothers or to other control mothers.

The pups were weighed weekly until weaning at 21 days of age. With the exception of the 24 hr weights in which all of the pups in a litter have been included, only those litters with 4 pups living at the end of 3 weeks were used in the calculations of the mean weights.

At weaning each pup was individually coded, sexed, weighed, and placed into an individual clear plastic cage. Behavioral testing was begun when the pups attained an age of 56 days. In selecting the animals for testing, care was taken to include no more than 1 animal from a given litter in any one test. This was done in order to avoid a cluster effect [1]. After selection, the mice were coded in such a manner as to make the experimenter blind to the nature of their treatment.

Behavioral Apparatus and Procedures

Open field. This test was selected because it has been shown to be effective in demonstrating differences between rodents due to a variety of factors including the effects of handling, foster rearing, isolation, prenatal administration of drugs, prenatal irradiation, prenatal maternal stress, malnutrition and strain of biological or foster parents [3,21]. Using such a procedure, Spyker, Sparber and Goldberg were able to uncover differences in performance between mice exposed to methylmercury dicyandiamide *in utero* and control mice. The procedure is simple, relatively reliable and inexpensive. Basically a measure of spontaneous motor activity and eliminatory behavior, it has also been thought to measure emotionality, fear, wall seeking behavior and exploratory behavior [3, 15, 21].

The apparatus was a slightly modified version of that used by Southwick and Clark [29]. It was composed of an open box painted flat black with side walls 24 cm high and enclosing a square floor 75 cm to a side. The floor was divided into twenty-five 15 cm squares. A removable guillotine door with an attached roof section was situated in one corner of the open field and served as a starting chamber. Illumination was provided by means of a red 25 watt bulb suspended 91.4 cm above the center square.

A mouse was introduced into the starting chamber through a side door in one of the walls. At the end of 1 min the guillotine door and its attached roof section was removed and a 5 min period of observation initiated. Behaviors measured included the time taken by the mosue to leave the start square and enter another (latency), the number of times the mouse entered outer squares, inner squares or the center square and the number of times the mouse reared upon its hind feet. At the end of the trial period, the animal was placed into a shuttle box for subsequent testing in that apparatus. The number of fecal boli deposited during the trial period was then counted, and the box cleaned with a damp sponge.

Shuttle box. Active avoidance procedures have been used to demonstrate differences between inbred strains of mice [5] and to show effects after *in utero* exposure to drugs [21]. We reasoned, therefore, that the technique might be capable of uncovering differences in mice prenatally exposed to methylmercury and their controls.

In this paradigm the animals were presented with initially neutral stimuli (light and sound) followed by a noxious unconditioned stimulus in the form of an electric shock. The task of the animals was to associate the previously neutral stimuli with the shock and to learn to avoid the shock by moving to a safe side of a 2 compartment shuttle box. The shuttle boxes used were basically the same as Warner's 2 compartment model [33] and are commercially available from BRS/LVE (Mouse Toggle Floor, Model 156-02). Each shuttle box was situated in a sound attenuating outer chamber. After introduction of a mouse into the shuttle box, its spontaneous activity was measured by counting the number of times it crossed from side to side for a period of 5 min. The start of the experimental session was then signalled by a white noise from a speaker in the center of the roof of the isolation chamber and generated by an audio generator. A session was comprised of 100 trials spaced 1 min apart. At the end of each min a clicking noise and a white light served simultaneously as the conditional stimulus (CS). Should the mouse cross to the other (safe) side of the shuttle box during the presentation of these warning stimuli, it would have avoided being shocked and a new trial was initiated. If, after 5 sec of the CS, the mouse had not yet crossed to the opposite chamber of the box, a 0.10 mA constant current scrambled shock was delivered to its feet by means of a BRS-Foringer Model SC-901 shock scrambler and Model SG-901 shock generator which were connected to the floor bars. The time taken by the mouse to escape the shock was then recorded and a new trial was begun. The criterion for the termination of training was 11 consecutive avoidances for most mice. A malfunction in a timing ring caused the criterion to be 7 consecutive avoidances in the cases of those mice exposed *in utero* to 5.0 mg Hg/kg as methylmercury hydroxide and then crossfostered to saline control mothers and those mice whose grandmothers had been treated with 5.0 mg Hg/kg as methylmercury hydroxide.

Conditioned suppression. First described by Estes and Skinner [13], the conditioned suppression procedure consists of presenting a neutral conditional stimulus (CS) for a brief period of time and terminating it with an aversive unconditional stimulus such as a foot shock. When superimposed upon a positively reinforced behavioral baseline such as lever pressing for food, the presentation of the CS typically results in a reduction of the baseline responding. The technique has been employed by many investigators to assess the effects of pharmacologic agents upon fear and anxiety [9] and it seemed reasonable to suppose that it might detect differences between mice exposed to methylmercury while *in utero* and mice similarly exposed to saline.

Eighteen mice were used, each from a different litter. Nine were born of mothers treated with 5.0 mg Hg/kg as methylmercury hydroxide on Day 8 of gestation and 9 were from mothers similarly treated with saline. When 56 days of age, the mice were deprived of water for 23 hr daily and trained to press a lever for water (0.021 ml) on a vari-

able ratio 30 schedule of reinforcement using standard electromechanical equipment and experimental chambers. Once stable lever pressing performance had been obtained, a conditioned suppression paradigm was superimposed. Each session consisted of 4 daily trials during which a 2 min CS was presented (a clicking sound) and which was terminated by a 0.5 mA scrambled constant current AC shock delivered by way of the floor bars and which served as an unconditional stimulus (UCS). Trials were spaced 2, 10, 19 and 30 min from the start of the session. On Day 11 extinction trials were begun in which the CS was presented without the UCS. The extinction trials were continued until the performance of the mice was no longer significantly different from that of the period prior to the introduction of shock.

The magnitude of the response suppression was measured using the ratio B/A+B [2] in which A is the number of responses emitted during the 2 min period prior to the CS and B is the number of responses emitted during the presentation of the CS. Ratios were calculated for each trial and then these were averaged to give a single ratio per mouse per day.

Punished behavior. In this paradigm mice were punished by response contingent electric shock of a positively reinforced drinking response. The mice could avoid being shocked by not running into a compartment and drinking from a spigot wired to deliver an electric shock. The procedure was chosen to gauge whether differences between treated and control mice could be detected in a situation in which an active response is punished. The experiment would also give evidence as to whether there were motor deficits between treated and control mice by measuring the time taken to leave the start compartment and enter the drinking compartment.

The device used for this paradigm consisted of a rectangular Plexiglas runway 61 cm long, 8 cm wide and 15 cm high. At the distant end of the runway a stainless steel drinking tube protruded from the center wall. Underneath the tube the floor of the apparatus was covered with a stainless grid. A removable guillotine door with an attached roof section was placed at the opposite end of the runway enclosing a start chamber. The device was divided into 4 equally spaced areas by means of black lines painted on the walls. The start chamber, which took up half of area 1, was painted flat black and the rest of the runway was clear. Mice were deprived of water for 23 hr and then introduced into the start chamber. At the end of 1 min, the guillotine door and its attached roof section were removed, giving access to the water spout. The time taken by the animals to enter compartment 4 (containing the water spout) was measured along with the total time spent in compartments 1 and 4 and the number of times each mouse drank. At the end of the 5 min trial period each mouse was returned to its home cage and given water ad lib for 30 min. The runway was cleaned with a damp sponge prior to the testing of each animal. After a week of collecting baseline information, the water tube and the grid beneath it were connected to a Lehigh Valley Electronics Model 1643 AC shocker adjusted to deliver 30 volts. When a mouse stepped onto the grid and attempted to drink, it completed the normally open circuit and a shock was delivered. The animal could avoid being shocked by not touching the electrified spout. After two days the shock circuit was inactivated and extinction trials were run until performance was no longer significantly different from baseline.

Water escape. A common symptom of methylmercury poisoning in humans is lack of motor coordination [4, 16, 26]. In this procedure mice, submerged in a water trough, had to orient themselves within the trough and swim to an escape ramp at its distal end. The task was thus a measure of locomotor ability and also a measure of learning – in that over repeated trials the mice would be expected to decrease their escape latencies. Reading [25] used the test to demonstrate differences in the maternal environments of inbred mice and we felt it might be an adequate measure for finding differences in mice caused by exposure to methylmercury. This was especially the case since Spyker, Sparber and Goldberg [32] reported differences in a swimming test between mice treated *in utero* with methylmercury and control mice.

The apparatus consisted of a rectangular plastic trough 1 meter long and 15 cm wide which was filled to a depth of 13 cm with water held at a temperature of 20°C. Fifteen cm above one end of the trough was a starting box which contained a trap door and a removable roof. At the other end was a wire mesh escape ramp leading out of the water. An electric eye was situated at the interface of the water and the ramp and served to record an animal's escape.

A mouse was placed into the starting chamber and 1 min later the trap door opened plunging the animal into the water. At the same time an electric timer was set into operation. Its operation ceased when the mouse crossed the electric eye beam as it climbed onto the escape ramp. Each mouse was given 3 trials in the device. Mice that had not escaped within 60 sec or who were in jeopardy of drowning were removed from the water. Escape latency for these animals was considered to be 1 min.

RESULTS

Non-Behavioral Measures

Administration of methylmercury hydroxide to pregnant mice on Day 8 of gestation caused a dose-related decrease in the number of pups born per litter. These decreases were significantly different from control for the 3.0, 5.0 and 10.0 mg Hg/kg as methylmercury hydroxide doses (Table 1).

TABLE 1

MEAN NUMBER OF PUPS SURVIVING PER LITTER 24 HR AFTER BIRTH. MICE TREATED WITH METHYLMERCURY HYDROXIDE ON DAY 8 OF GESTATION ARE COMPARED WITH MICE WHICH RECEIVED ONLY THE EQUIVALENT QUANTITY OF SALINE

Dose in mg Hg/kg body weight	Mean number of survivors per litter, SEM Experimental
0	9.20±0.62†(N=66)
1	8.55±0.71 (N=11)
2	8.13±1.33 (N=8)
3	6.02±0.65 (N=23)*
5	5.54±0.16 (N=23)*
10	2.53±0.94 (N=12)*

*t-score obtained was significant at the 0.05 level (single tailed test).

†N denotes the number of litters in the group.

Since there were no significant differences between the number of survivors in the various saline control groups, the control results have been pooled.

The mean weight per pup per litter at 24 hr was lower than control in mice receiving 10.0 mg Hg/kg as methylmercury hydroxide and measures at 7, 14 and 21 days revealed significant weight differences in litters born of mothers who received 3.0 or more mg Hg/kg methylmercury hydroxide. These differences persisted through weaning, with mercury treated animals averaging 16% less weight than control mice. When reweighed at the time of behavioral testing (56 days) the weight differences were no longer significantly different from control (Table 2). Only those litters in which all 4 pups were alive at weaning have been included in the evaluation of weight data.

Table 3 depicts the results of a fostering experiment using mice treated with 5.0 mg Hg/kg as methylmercury hydroxide and saline controls. Litter weights were recorded at the time of fosterings and again at the time of weaning (21 days). There were no significant differences between the groups at the time that the fosterings were made. At weaning the mean weight of the litters in which both the biological and the foster mothers had been treated with methylmercury was lowest. A 2-way analysis of variance indicated that the weight differences could be attributed both to exposure of the fetus *in utero* and the treatment of the foster mother if the biological mothers had been treated with methylmercury ($F = 9.0745$, $p<0.05$ and $F = 8.0745$, $p<0.05$, respectively).

Behavioral Measures

Shuttle box. The results of this experiment have been summarized in Table 4. Mice whose mothers had been treated with 2.0 mg Hg/kg as methylmercury hydroxide averaged more trials to criterion than controls, but the difference was not statistically significant ($p>0.05$, t-test). The 3.0 and 5.0 mg Hg/kg as methylmercury hydroxide groups, however, averaged twice as many trials to criterion as their saline counterparts ($p<0.05$, t-test). These differences were already evident by the end of the first 100 trials in that the mercury treated mice were shocked significantly more often than their controls.

Five of the 18 mice whose mothers had been treated with 3.0 mg Hg/kg and 6 of the 19 mice whose mothers had received a 5.0 mg Hg/kg dose as methylmercury hydroxide failed to attain the criterion even after as many as 800 trials (all control mice had attained criterion within 400 trials). If these mice are excluded from the analysis and the means recalculated for the 2 groups, the 3.0 mg Hg/kg groups would have a mean of 197 ± 26 trials to criterion and the 5.0 mg Hg/kg mice a mean of 234 ± 3 trials. Both of these means are still significantly greater than their respective controls at 0.05 level of confidence.

Time to escape shock was not significantly different from control for any treated group with the exception of those mice whose mothers had been dosed with 3.0 mg Hg/kg as methylmercury hydroxide. These mice had a mean escape latency of 0.53 ± 0.04 sec compared with 0.42 ± 0.04 for the controls. Included within this group are the 5 mice which had failed to meet the criterion. These mice had a mean escape latency of 0.62 ± 0.08 sec while the successful mice in the group had a mean of 0.46 ± 0.04 sec, a value not significantly different from control. In the case of the 5.0 mg Hg/kg mice, examination of the mean

TABLE 2

MEAN WEIGHTS PER PUP PER LITTER 24 HR AFTER BIRTH, AND AT 1, 2 AND 3 WEEKS OF AGE. WITH THE EXCEPTION OF THE 24 HR WEIGHTS, ONLY DATA FROM LITTERS WITH ALL 4 PUPS ALIVE AT WEANING HAVE BEEN INCLUDED

Treatment mg Hg/kg	Weight Per Pup Per Litter (g ± SEM) 24 Hr	Week 1	Week 2	Week 3	Week 9
Pooled Controls	1.93±0.05 (N=74)	5.89±0.16 (N=70)	10.12±0.13 (N=70)	14.64±0.22 (N=70)	37.84±0.77 (N=70)
1 mg	1.91±0.07 (N=11)	6.11±0.17 (N=11)	9.93±0.19 (N=11)	14.75±0.35 (N=11)	37.63±0.69 (N=11)
2 mg	1.97±0.07 (N=8)	5.82±0.22 (N=7)	10.17±0.16 (N=7)	14.15±0.32 (N=7)	38.21±0.54 (N=7)
3 mg	1.87±0.00 (N=21)	4.75±0.10* (N=15)	8.68±0.20* (N=15)	12.72±0.31* (N=15)	36.95±1.01 (N=15)
5 mg	1.85±0.05 (N=27)	5.20±0.21* (N=17)	8.63±0.36* (N=19)	12.10±0.45* (N=19)	37.22±0.81 (N=17)
10 mg	1.82±0.03* (N=8)	4.67±0.18* (N=5)	8.35±0.39* (N=5)	12.24±0.39* (N=5)	37.46±0.35 (N=5)

*$p<0.05$ (t -test).
Since there were no significant differences between the various saline injected control groups, their values were pooled.

TABLE 3

MEAN WEIGHTS IN g OF THE FOUR CLASSES OF MICE USED IN THE FOSTERING EXPERIMENT AT THE TIME OF THE FOSTERING AND AT 21 DAYS OF AGE. EACH LITTER CONTAINED 4 PUPS AND THERE WERE 5 LITTERS FOR EACH TREATMENT

Treatment of Mother		Mean Weight of Pups at Fostering in g ± SEM	Mean Weight of Pups at 21 days of age
Biological	Foster		
5 mg Hg/kg	5 mg Hg/kg	1.88±0.15	12.75±0.76
5 mg Hg/kg	0.9% NaCl	2.00±0.06	15.48±0.51
0.0% NaCl	5 mg Hg/kg	2.04±0.08	15.56±0.52
0.9% NaCl	0.9% NaCl	2.03±0.08	16.00±0.36

TABLE 4

SUMMARY OF THE RESULTS OF THE TWO-WAY ACTIVE AVOIDANCE EXPERIMENTS. ALL VALUES ARE MEAN PLUS OR MINUS SEM

Dose to mother in mg Hg/kg	Number of avoidances in session one	Number of escapes in session one	Number of trials to criterion
2 mg (N=10)	16.60±4.70	78.70±7.21	252.20±56.93
Controls (N=10)	14.30±4.34	84.00±5.85	176.80±38.47
3 mg (N=18)	6.28±1.18*	93.17±1.44*	281.22±37.83*
Controls (N=14)	18.00±2.86	82.00±2.86	123.00± 3.24
5 mg (N=19)	7.72±1.98*	91.72±1.96*	318.00±35.49*
Controls (N=17)	13.41±2.30	86.59±2.30	176.50±14.82
5 mg (foster)‡ (N=14)	23.06±4.81	75.43±5.17*	186.00±27.30*
Controls (N=14)	30.57±3.23	60.43±6.05	115.86±10.04
5 mg (mothers) (N=19)	11.21±2.42	87.46±2.55	155.95± 9.73
Control (mothers) (N=17)	14.61±3.88	83.72±4.64	162.94±10.15
5 mg F_2 (N=14)‡§	31.53±4.74	64.33±5.30	155.67±22.69
Controls (N=11)	42.40±12.71	62.10±7.91	124.40±11.58

*Experimental mean was significantly different from the control mean (t-test, 0.05 level of confidence.)
†N refers to the number of animals in a group.
‡The criterion for those mice was seven consecutive avoidance responses.
§The grandmothers of these mice received the methylmercury injection.
Mothers of the experimental mice were dosed with the indicated amount of CH_3HgOH on day eight of pregnancy, mothers of the control mice received an equivalent amount of 0.9% NaCl solution. Testing was begun when pups were 56 days old.

escape latency revealed no significant differences from control for those treated animals which had attained the criterion or for those which had not.

Results of a fostering experiment involving mice treated with 5.0 mg Hg/kg as methylmercury hydroxide were similar to those obtained with the non-fostering mice. The experimental mice took significantly more trials to reach criterion and had fewer avoidances in the first session than their controls (Table 5). A 2-way analysis of variance revealed that the nature of the treatment given to the biological mother had a significant effect upon the number of trials to criterion, that the treatment given to the foster mother had little or no effect and that there was no interaction between the treatment given to the biological mother and that given to the foster mother.

Mothers of the non-fostered 5.0 mg Hg/kg mice and their controls were tested in the shuttle box just after their pups had been weaned. No significant differences could be detected in any of the variables measured.

Mice whose grandmothers had been treated with 5.0 mg Hg/kg as methylmercury hydroxide were not different from control mice with regard to their performance in the shuttle box.

Open field. No significant differences were detected between the mercury treated mice and their controls with regard to any of the measured parameters either before the session in the shuttle box or after it.

Conditioned suppression. No significant differences could be found. The animals were almost identical in their acquisition of the behavior magnitude of suppression, and subsequent course of extinction.

Punished behavior. Acquisition in this procedure, as measured by the mean latency to enter the compartment containing the water tube, was very rapid and there was no significant difference between the experimental and control mice. Significant differences did arise, however, during the extinction trials. In particular, the mercury treated mice began drinking sooner and more frequently than the control mice. On the ninth day of the experiment, the mean latency to enter compartment 4 was 58 ± 34 sec for the mercury treated mice whereas the control mice had a mean latency of 198 ± 44 sec. Significant differences were maintained through the 11th day of the experiment as is shown in Fig. 1a. The mercury treated mice also drank significantly more often on Days 8–10, with means of 2.00 ± 1.09, 3.11 ± 1.14, 3.22 ± 1.1 drinking bouts for the experimental mice compared with means of 0, 0.44 ± 0.44, 0.44 ± 0.44 for the controls (Fig. 1).

TABLE 5

MEAN NUMBER OF TRIALS TO CRITERION IN THE TWO-WAY AVOIDANCE SHUTTLE-BOX OF THE FOUR CLASSES OF MICE FROM THE FOSTERING EXPERIMENT. THERE WERE FIVE MICE IN EACH GROUP, EACH FROM A DIFFERENT LITTER

Treatment of Mother		
Biological	Foster	Trials to Criterion SEM
5 mg Hg/kg	5 mg Hg/kg	174.43±20.49
5 mg Hg/kg	0.9% NaCl	198.14±54.44
0.9% NaCl	0.9% NaCl	118.57±14.85
0.9% NaCl	5 mg Hg/kg	113.14±14.61

Water escape. Both methylmercury treated and control mice showed a monotonic decrease in time to escape the water trough in 3 trials. No significant differences were detected between the groups. The mean time to escape for 19 mice born of mothers treated with 5.0 mg Hg/kg as methylmercury hydroxide was 13.65 ± 3.44 sec compared with 13.89 ± 6.43 sec for 10 control mice.

DISCUSSION

Administration of methylmercury to pregnant mice pro-

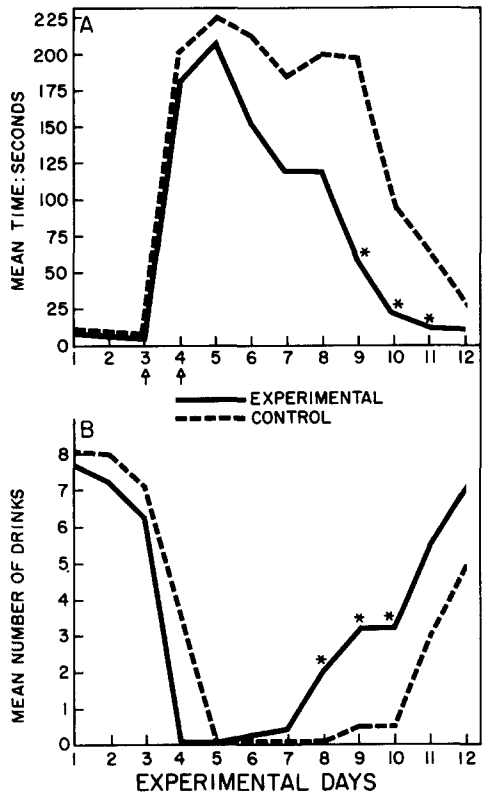

FIG. 1. (A) Mean time in sec to enter the compartment containing the water spigot during passive avoidance experiment. Shock circuit was active on Days 3 and 4. Solid line: Mice whose mothers had received 5.0 mg Hg/kg methylmercury hydroxide on Day 8 of gestation (n = 9); stippled line: Mice whose mothers received 0.9% NaCl on Day 8 of pregnancy (n = 9). Asterisk denotes a significant difference at the 0.05 level of confidence (Mann-Whitney U-test). (B) Mean number of drinks taken by methylmercury treated and control mice during passive avoidance experiment.

duced a number of effects when the dosage administered was 3.0 mg Hg/kg or higher. The mercury treated mice had fewer pups per litter, the affected pups weighed less than controls during the nursing period and were different from controls in both an active and a passive avoidance situation.

With the exception of the mice treated with 10.0 mg Hg/kg as methylmercury hydroxide, experimental pups weighed the same as controls 24 hr after birth. Subsequent determinations, however, revealed significantly less weight gain in pups exposed to 3.0 mg Hg/kg as methylmercury hydroxide or more. When it is considered that these mercury treated mice came from litters which averaged 1/3 fewer pups than their controls, it is evident that in terms of total litter weight the mercury treated mice actually weighed less than the controls (i.e. in terms of biomass). Had the number of pups in the mercury treated litters been the same as in the control litters, the weight per pup of the treated mice may well have been less than the control weight. Reduced litter sizes in the treated mice probably provided the opportunity for relatively more nutrition per pup, thereby allowing them to approximate the weights of the control mice. That smaller litter sizes tend to contain larger pups has been known for some time [8] and the mean weight per pup of 3 litters in the present study, each with less than 4 pups per litter, was 2.46 g, lending some support to the notion that the added nutritional advantages afforded the pups in the smaller litters may have offset to some extent the deleterious effects of the compound.

While the transfer of alkylmercurials through the mother's milk has been reported [17], it is difficult to believe that the quantities present in the milk of mice treated with a single dose of 3.0 or 5.0 mg Hg/kg as methylmercury hydroxide on Day 8 of gestation were of sufficient magnitude to account for the decrements in weight seen in pups 5 weeks later. Ostlund [24] found the biological half-life of methylmercury injected into mice to be 8.4 ± 2.0 days and 12.6 ± 4.1 days for doses of 3.0 and 5.0 mg Hg/kg, respectively, and Garcia *et al.* [17] have reported that the percent of dose of $^{14}CH_3HgCl$ in one gram of rat's milk was 0.02% one day after force feeding a dose of 30 uci. Perhaps the mercury affected the quantity or quality of the mother's milk. Yang *et al.* [35] found smaller pups than control at weaning after the forced feeding of single doses of methylmercury chloride. They attributed the smaller size to poor lactation and performance of the mothers rather than to the mercury in the milk. A deficit in quantity or quality of the milk might also serve to explain why, in the present study, the differences in weight were no longer evident after 5 weeks of feeding on lab chow.

The clearest behavioral differences between the young of methylmercury treated mice and their controls were seen in their behavior in the shuttle box and in the passive avoidance task. Both the 3.0 mg and the 5.0 mg Hg/kg as methylmercury hydroxide treated mice produced progeny which took significantly more trials to reach the criterion (when they reached it at all) than did their saline treated counterparts. Significant differences could be detected as early as the first session of 100 trials in the shuttle box with the mercury treated mice producing fewer avoidances than control mice. The nearly identical performance between experimental and control mice in a number of measures of motor ability (time to escape from water trough, ambulation in the open field, time to escape from shock in the shuttle box, time to enter compartment containing the water spout in the punishment procedure) suggest that these differences reflect a learning deficit rather than a motor impairment.

The fostering procedure indicated that the behavioral deficit was due to exposure of the mice *in utero* and was not significantly influenced by the nature of the treatment given to the mother which reared the pups. Examination of mice whose grandmothers had been dosed with methylmercury revealed no differences from control suggesting that defect is restricted to the generation in which the exposure occurs and testing of the mothers treated with methylmercury under the same conditions as their progeny revealed no differences from control, reaffirming the observation that exposure of the fetus *in utero* to methylmercury can cause profound effects while leaving the mother asymptomatic.

In the punishment situation both the treated and control mice acquired the response on the first day that the shock circuit was activated. Significant differences between the animals were revealed during the extinction procedure. The mercury treated mice entered the water compartment more

often, stayed in it longer and began drinking more often and sooner than the saline controls.

Our open field results differ from those of Spyker, Sparber and Goldberg [32] in that they found significant differences between the progeny of 129 SvSl mice treated with a single dose of 5.36 mg Hg/kg as methylmercury dicyandiamide on Days 7 or 9 of pregnancy and the progeny of control mice. Specifically, the offspring of the methylmercury treated mice took significantly more time to leave the start box, urinated less, defecated less and displayed a backing phenomenon in which the mice would take 3 or more steps in a row in a backwards direction. Procedural or strain differences between the mice may account for this lack of correspondence between our results. Strain differences in relation to mercury retention and embryolethality have been reported [22,31].

In summary, relatively small doses of methylmercury administered once during gestation can lead to deficits in progeny when tested in specific test situations. Such deficits could not be inferred from gross observations of the animals, since the initial weight difference between treated and control animals had disappeared at maturity, and no other obvious behavioral abnormalities were present. Since the test situations in these experiments involved not only a learning component, but also an emotionality factor (response to electric shock) it is difficult to determine whether a single or both factors led to these deficits. The fact that in the 2-way avoidance the mercury animals had the same escape latency as the controls, suggests that pain thresholds were unaffected and perhaps the emotional response to pain remained relatively unimpaired. This of course does not exclude the possibility that the learning of emotional responses remains unaltered.

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