Decreased Learning Capacity in Rats Exposed Prenatally and Postnatally to Low Doses of Mercury

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The hazards of exposure of excessive amounts of mercury are well documented. However, the effects of more subtle alterations from low levels of mercury have only recently been considered (SPYKER et al 1972, SOBOTKA et al 1971, BERGLAND et al 1969). The most damaging and permanent effects of mercury toxicity are seen in the developing embryo. Methylmercury easily crosses the placental barrier where it concentrates in the fetal tissue, and in high amounts in the developing central nervous system.

The increased amounts of mercury now being found in fish (RIVERS et al 1972, WESTÖÖ 1969)present a potentially hazardous situation. Inhabitants of countries where fish constitute the main source of protein could be subjected to unobservable damaging effects from mercury ingestion.

Our research was designed to assess the effects of low doses of mercury on the maturation and learning ability in the offspring of treated mother rats, and to evaluate the differences between two different diets of mercury in fish, one of natural origin and one artificially contaminated with methylmercury hydroxide.

METHODS

Subjects and Rearing Conditions

Pregnant rats (Holtzman Rat Company of Madison, Wisconsin.) on Day 1 of gestation were utilized. Day 1 sperm positive analysis was done by the suppliers. Fifty-nine offspring from thirteen of the mothers served as the subjects in the main experiment and participated in the four behavioral tasks; swimming, righting reflex, motivational analysis and learning ability. The study was conducted in four replications with each experimental group of mothers randomly divided into one of three study groups:

Group 1. "2 PPM mercury (marlin[1]) rats." This group was fed a specially prepared diet consisting of a mixture of 13.4% by weight

[1]Pacific Blue Marlin, caught in Jan. 1971 off the coast of Hilo, Hawaii, weighing approximately 496 lbs. After evisceration, it was cut into 4 pieces and sent frozen to Madison, WI where it was kept frozen until use.

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lyophilized and powdered Pacific Blue Marlin, *Makaira ampla*, and 86.6% Rockland Rat and Mouse Chow in mash form (Comstock Seed and Feed, Madison, Wis.). The final total mercury concentration was 2 PPM per kg diet.

Group 2. "2 PPM mercury (tuna) and methylmercury hydroxide rats". The animals were fed a mixture of 13.4% "Geisha" brand albacore tuna, *Thunnus alalunga* (distributed by Nozaki Associates, Inc., New York, N. Y. and packaged in solid water pack in 7 oz. cans); also lyophilized and powdered, and 86.6% rat food. The methylmercury hydroxide was added to give the desired 2 PPM total mercury per kg diet as in the first group.

Control Group. The control rats were fed the standard rat diet, Rockland Rat and Mouse Chow.

The experimental groups of mothers were fed their specially prepared diets from Day 1 of gestation and were maintained *ad libitum* on food and water during their duration in the experiment. The offspring from the mothers were maintained in the same experimental condition as their mothers. Food and water for the offspring were continuously available until age 42 days, when feeding was restricted so as to bring body weights down to between 80% and 85% of normal *ad libitum* values, in preparation for motivational and maze testing. Of the fifty-nine offspring, twenty were in Group 1, nineteen were in Group 2 and twenty were in the control group. Approximately equal numbers of males and females were in each study group.

Behavioral Testing

Two different behavioral testing times were employed in the study; early developmental testing with the rat pups tested on postnatal Days 7 through 17 and motivational and learning tests on Days 45 through 68. The early developmental tests consisted of two tasks: a swimming test (SHAPIRO et al. 1970) and a righting reflex task (EAYRS et al. 1955). During the early testing, a record of the pup's weight was recorded on Days 7, 10, 13, and 16.

The motivational test was a locomotion test conducted on the symmetrical maze apparatus and designed by DAVENPORT et al. (1973). The test was designed to investigate motivational differences between the three study groups. Such differences would have a direct bearing on the results of the last test, the symmetrical maze learning task. The motivational testing began on Day 45. Three days prior, Day 42, all the animals were reduced to between 80% and 85% *ad libitum* feeding weight in order to magazine train the animals to the automatic pellet dispenser. After training, a random half of the rats in each study group were returned to *ad libitum* feeding conditions and the remaining half were maintained at the 80% to 85% free feeding level.

Following the motivational testing, the rats which were fed *ad libitum* were again reduced to 80% to 85% of free-feeding weight.

All the rats were tested on the three practice problems (P-2, P-3, and P-4) and four test problems (T-6, T-8, T-9, and T-10) in the symmetrical maze series (DAVENPORT et al. 1970). Further description of the behavior tasks follows:

Swimming Test. The swimming task was conducted in an aquarium half-filled with lukewarm water. Daily, beginning on Day 7, the rat pups were separated from their mothers and placed in a drying cage. Each pup was dropped into the aquarium from about 25 cm. The rat was then given a score on his performance. DORCEY's (1972) scoring method was used in preference to that employed by SHAPIRO et al. (1970). An arbitrary score of "0" through "4" was assigned on each trial, "0" representing lack of swimming ability and "4" mature swimming ability.

Righting Reflex. Prior to the swimming task, each rat received three righting-reflex trials on Days 7 through 17. On each trial, the rat was held upside down by the neck and lower back and dropped approximately 30 cm onto a cotton pad. The righting reflex was considered present if the rat landed on all four legs, receiving a score of "1". Otherwise is was scored as "0".

Motivational Test. The locomotion test of motivation was conducted on the symmetrical maze apparatus but with all the barriers of the maze field removed. In this way, the maze functioned as an automated runway; this is equivalent to the procedure of practice problem P-1 of the maze series (DAVENPORT et al. 1970). After the rats reached the 80% to 85% ad libitum weight, they were run on 18 to 23 hours of food deprivation. The animals were magazine-trained according to the methods outlined by DAVENPORT et al. (1970) prior to the locomotion tests. Each rat was tested daily for eight days; 12 consecutive trials per day. Locomotion on all trials was rewarded by a single 45-mg food pellet.

Maze Learning. Upon completion of the motivational testing, the fed rats were again reduced to between 80% and 85% of free-feeding weight in preparation for maze testing. When all the rats were at their reduced weights (Day 60), the practice and test problems were administered at the rate of one problem per day; twenty to twenty-three hours after their daily feeding. The procedures for the symmetrical maze and the symmetrical maze itself are described fully by DAVENPORT et al. (1970). Departures from the standard procedure in the present research include 1 - rather than 2 - pellet rewards (Noyes 45 mg food pellets) and the use of test problems T-6, T-8, T-9, and T-10 only.

Physiological and Anatomical Procedures

Upon completion of the main experiment, the rats in the last replication of the experiment were sacrificed at sixty-eight days of age for determination of mercury content of the brain, liver, and kidney and for organ-to-body-weight ratios of the brain, liver, kidney, lung and heart[2]. Gross examination of the organs was completed before mercury analysis.

Heavy Metal Analyses

Additional analyses of the two fish samples (the marlin and tuna) were done to eliminate possible confounding effects from other heavy metals. Copper, arsenic, lead, cadmium and selenium were analyzed by Galbraith Laboratories, Inc., Knoxville, Tennessee.

RESULTS

Physical Development

The early record of animal weight on Days 7, 10, 13 and 16 showed significant differences in weight between the Group 1 and the control group on Day 13 only. An overall analysis of variance showed the Group 1 animals to be significantly smaller ($p <$ .05) than the control rats. There were no significant differences in the animals' weights on Days 7, 10 or 16 or when the animals were again weighed at 42 days of age. There were no differences in the age of eye opening in the three groups. No differences in physical appearance were seen in any of the animals in the study.

Early Behavioral Maturation

Retarded maturation was observed in the Group 1 rats (2 ppm Hg marlin rats) with respect to swimming behavior and righting reflexes. In the swimming task (Figure 1) the Group 1 rats were inferior to the controls: analysis of variance of the summed scores over Days 7 through 15 showed significantly slower development of swimming ability by the Group 1 rats ($\underline{p} < .05$). (See Figure 1).

No significant differences were seen between the Group 2 rats (2 ppm Hg tuna and CH_3HgOH) and the controls.

In the righting reflex test variation was such within each group's score that no significant differences were found ($\underline{F} = 1.1$). However, a day by day analysis of the scores on Days 14 through 17 with an overall analysis of variance showed the Group 1 rats to be inferior to the controls and Group 2 rats on Day 15 ($\underline{F} =$ 6.29, $\underline{df} = 2/57$, $\underline{p} < .01$) and Day 16 ($\underline{F} = 11.99$, $\underline{df} = 2/57$, $p < .001$). Days 14 and 17 were not significant ($\underline{F} = 1.82$ and $\underline{F} = 1.00$ respectively).

[2] The analysis for the selected organs as well as all analysis of mercury concentrations in the diet preparations were done on a Perkin-Elmer Atomic Absorption Spectrophotometer Model 303. The method used is outlined in the Assoc. Off. Analytical Chem. Jour. 10, 37-57 (1965)

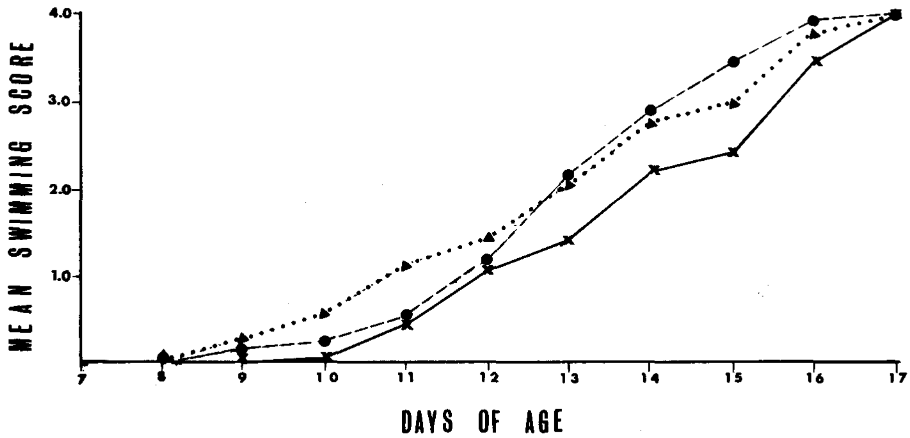

Figure 1. Mean swimming scores for Group 1 (represented by a solid line with x's), Group 2 (represented by a dashed line with circles) and controls (represented by a dotted line with triangles) on postnatal days 7 through 17.

Motivational Tests

The motivational tests were employed to analyze for motivational differences between the three test groups. In addition to the locomotion speed each day for each of the test groups, several other variables were analyzed: high vs low hunger rats, the locomotion speed on the first four days vs the last four, the effect of the treated group vs the high or low hunger condition, and whether any interactions occurred. The only significant effect of the analysis was the faster running by the high- than by the low-hunger rats ($\underline{F} = 43.68$, $\underline{df} = 1/54$, $p < .001$). There were no interactions between the several variables.

Maze Learning

The symmetrical maze testing produced evidence of a learning deficit in the Group 1 rats. An analysis of variance showed significant differences between the total errors of the Group 1 rats and controls ($\underline{p} < .01$). There were also significant differences between the Group 1 rats and Group 2 ($\underline{p} < .01$) with the Group 2 animals being far superior to Group 1. An overall mixed design analysis of variance showed significant effects of problems ($\underline{F}$ =

14.48, df = 3/168, $p < .001$); there were no interactions. (See Figure 2).

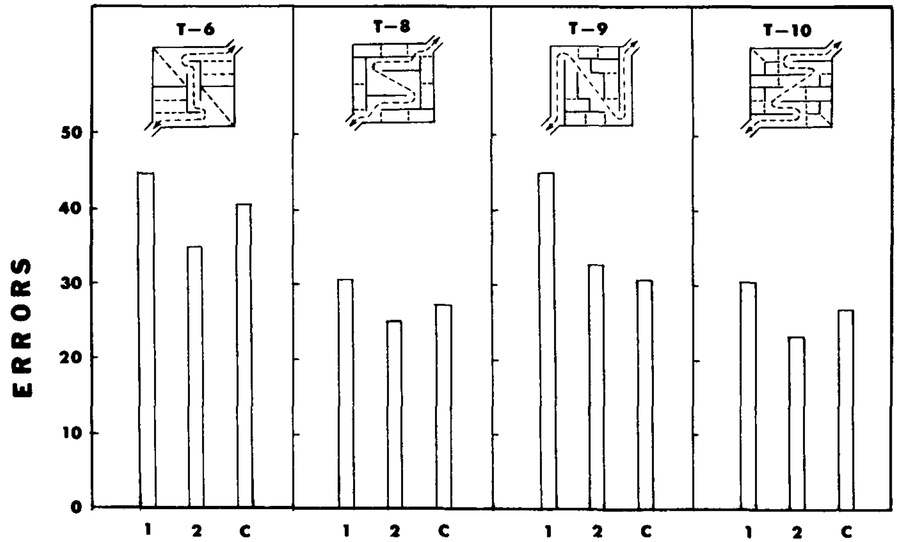

Figure 2. Means of the symmetrical maze error scores for Group 1, Group 2 and controls on the maze problems (T-6, T-8, T-9 and T-10) administered on Days 60 through 68. The maze patterns are shown by the inserts. Each bar graph within each test problem division represents one of the experimental groups: "1" represents Group 1, "2" represents Group 2 and "C" represents the control group.

Physiological and Anatomical Measures

There were no important differences between the three test groups when the weights of the brain, liver, kidney, lungs and heart were expressed as proportions of total body weight.

Analysis of the total mercury content of the brain, liver, and kidney showed substantial differences in the mercury concentrations between the three groups. The three groups ranked as follows in order of increasing mercury levels: controls, Group 1, Group 2. A comparison of Group 2 rats and the Group 1 animals revealed a mercury increase of 7.3 times in the brain of Group 2 over Group 1, 5.8 times in the kidney, and 4.8 times in the liver. A similar comparison between the Group 1 rats and the controls showed the Group 1 animals to have 4.0 times the mercury level of the controls in the brain, 8.7 times the mercury level in the kidney and 11.7 times the level in the liver.

A gross examination of the organs revealed no obvious morphological abnormalities of any of the animals in the three groups.

Heavy Metal Analyses

Analyses of copper, arsenic, lead, cadmium and selenium revealed that only cadmium differed significantly in concentration in the two fish (Cd = 0.00667 ppm in the tuna diet and Cd = 0.0667 ppm in the marlin diet). However, this difference should have no significant effect on the animals since all the levels of the heavy metals were by far within allowable minimum daily uptake standards.

In summary, the 2 ppm diet composed of Pacific Blue Marlin had deleterious effects on the behavior maturation and learning ability of second generation rats. These findings are somewhat surprising in view of the current literature on the chemical form of mercury in fish, especially the marlin (RIVERS et al. 1972). More research is needed into subtle effects on organisms of naturally occurring mercury contamination and the toxicity of mercury in combination with other metals and elements before the question of the health hazards of mercury in fish can be answered.

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