Barbiturate Potentiation in Mercury Poisoning[3]

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There has emerged in recent years much interest in mercury toxicity and considerable speculation about the danger it represents to the environment (GOLDWATER 1971). To date, a relative paucity of information describing the behavioral and/or drug effects of methyl mercury exist, eventhough there is general agreement that the central nervous system is altered early in toxicity (FRIBERG et al 1970).

Recently POST et al (1973) described the effects of methyl mercuric chloride on rats in open field and T-maze tests. These authors found significant differences between control and mercury treated rats in 1 out of 3 experiments after a dose of 2.5 mg methyl mercury chloride/100 g body weight. A study with an avian species (ROSENTHAL et al 1972) has shown that chicks exhibited significantly slower detour learning time when methyl mercury dicyandiamide was injected in eggs prior to hatching. It remains to be seen what effects methyl mercury might have on the physiological action of a barbiturate such as sodium pentobarbital. Furthermore, since previous studies (PARIZEL et al 1969, GANTHER et al 1972, WELSH et al 1973) have also shown that selenium is effective in reducing mercury toxicity, two experiments were conducted to determine the effects of increasing levels of dietary methyl mercury on barbiturate induced sleeping time and the effects of selenium-mercury interaction on the above parameter.

Barbiturate potentiation (BP) is a nonspecific test known to be influenced by a wide variety of drugs capable of inhibiting enzymatic activity or having central nervous system activity (CARR 1963). The known ability of mercury to inhibit a variety of enzymes and its strong influence on the central nervous system suggested that it might have an effect on barbiturate induced sleeping time which thereby, could be an early indication of mercury toxicity.

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MATERIALS AND METHODS

In Experiment I, forty 3 week-old male Coturnix quail were divided into four groups of ten birds each. A semi-purified diet (FOX et al 1964) was supplemented with 0, 4, 12 and 24 ppm Hg as methyl mercuric chloride and fed to various groups for the first 21 days of the experiment. Data on weight changes and mortality were recorded throughout the experiment. On day 21, four birds from each group were sacrificed for mercury analysis of liver samples (UTHE et al 1970) and all remaining birds were placed on the control diet with no supplemental mercury.

Barbiturate potentiation was measured by the induced sleeping time tests. Each bird was injected subcutaneously in the region over the point of origin of the biceps femoris with a dose of 50 mg/kg bodyweight sodium pentobarbital at a concentration of 6 mg/ml. Duration of anesthesia was measured from the time the birds lose their righting reflex to the time they regained it as judged by their ability to right themselves rapidly twenty times in succession. The BP tests were done on each bird after 7, 14 and 21 days of mercury exposure and 21 and 49 days of withdrawal from mercury feeding.

In Experiment II eighteen 3 week-old male quail were randomly distributed into two groups. Group 1 received the previously described basal plus 30 ppm Hg while group 2 received the basal plus 30 ppm Hg and 0.4 ppm supplemental selenium (Se) as sodium selenite making the total Se content of the 2 diets 0.2 and 0.6 ppm Se, respectively. Barbiturate potentiation tests were conducted after 0 and 7 days of feeding the test diets. Birds were then sacrificed and the concentration of Hg in the brain of each bird was determined by atomic absorption spectrophotometry. Treatment effects in all studies were analyzed for statistical significance by the students "t" test.

RESULTS AND DISCUSSION

Table I shows the values obtained in Experiment I for the BP test. There was a significant increase in BP in the groups receiving 12 and 24 ppm Hg respectively after 7 days of exposure and a similar increase in the 4 ppm Hg group after 14 days of mercury feeding. Although the BP values obtained for the 24 ppm Hg group again increased significantly between 14 and 21 days of exposure, all other treatment groups remained relatively constant during this period. At this time all groups were given the control diet with no supplemental mercury added. After 7 weeks of feeding the control diet, BP times were still greater than controls. No gross symptoms of mercury toxicity were observed in any of the experimental animals except the 24 ppm Hg group during the third week of exposure to mercury. A 30% mortality was observed in the 24 ppm Hg group while no other mortalities were recorded in the other groups. Liver mercury levels closely reflected dietary levels as shown in Table II.

In Experiment II, selenium supplementation prevented the significant increase in BP time observed in the birds fed mercury alone (Table III). Again no differences were observed in weight gains, mortality or other gross symptoms of toxicity during the 7 day exposure period. Mercury levels in the brain tissue of birds (Table III) receiving mercury without additional Se were significantly greater

TABLE I.

Barbiturate potentiation time (min.) of quail fed methyl mercuric chloride

Exposure (d)	Dietary Level of Hg (ppm) 0	4	12	24
7	29.7 ± 5.3[a]	31.8 ± 5.3[a]	41.2 ± 7.6	45.2 ± 13.5[b]
14	27.8 ± 5.8[a]	43.7 ± 15.6[b]	44.7 ± 11.6[b]	43.7 ± 9.4[b]
21[d]	30.4 ± 7.1[a]	45.3 ± 11.7[b]	42.5 ± 15.3[b]	85.6 ± 39.7[c]
42	31.6 ± 5.9[a]	51.0 ± 16.6[b]	48.8 ± 12.8[b]	- [e]
70	34.5 ± 3.3[a]	52.6 ± 16.5[b]	54.0 ± 8.7[b]	-

[a,b,c] Means (± S. D.) on the same line bearing different superscripts are statistically different by at least (P .05).

[d] Mercury exposure ceased at 21d.

[e] All birds dead due to toxicity.

TABLE II.

Cumulative mercury consumption (Mg/100g bodyweight) and liver mercury levels of quail fed methyl mercuric chloride

Exposure (d)	Dietary level of mercury (mean + S. D.) 0	4	12	24
7	0[a]	0.41 ± 0.04	1.17 ± 0.09	2.18 ± 0.23
14	0	0.79 ± 0.13	2.17 ± 0.21	4.08 ± 0.44
22	0	1.11 ± 0.11	3.18 ± 0.29	6.60 ± 1.53
21d Liver Hg (ppm)	0	11.27 ± 2.20	30.04 ± 4.90	54.42 ± 9.20

[a] Mercury level not detectable.

than in birds receiving added selenium. The latter observation in consistent with the observation of WELSH et al (1974) that selenium supplementation decreases retention of mercury in the Japanese quail.

Mercury can now be added to the list of materials which are known to potentiate barbiturate activity. It is known to be a potent enzyme inhibitor and may inhibit up to 75% of liver enzyme activity (KOSMIDER et al 1969). Mercury also showed characteristics of a neurotoxic material when it was given at the highest dietary level since it produced loss of coordination as well as disturbances in the basic reflexes such as righting reflex and balancing ability. Sodium pentobarbital seems to intensify this toxic aspect of mercury at a much lower dietary level of mercury than usually observed which suggests some direct physiological potentiation in addition to any possible enzyme inhibition. These results support the contention that dietary environmental contaminants can influence drug activity.

TABLE III.

Barbiturate potentiation time (min.) and brain levels of mercury (Experiment II.).

Treatment	Exposure time (days)		Brain Hg (ppm)
30 ppmHg	43.0 ± 7.9	58.7 ± 11.3[a]	18.05 ± 2.0[a]
30 Hg + 0.4 ppmSe	43.2 ± 8.6	45.3 ± 14.4[b]	15.19 ± 2.4[b]

[a,b] Means (± S. D.) within the same column bearing different superscript are significantly different by at least (P .05).

SUMMARY

Barbiturate potentiation was observed in Japanese quail fed dietary levels of 4, 21, and 24 ppm Hg as methyl mercuric chloride. Gross symptoms of mercury poisoning were not observed until after BP was observed. After the initial increase in BP time was observed in the birds receiving 4 and 12 ppm mercury, there followed a plateau in response until those birds receiving 24 ppm Hg began to show gross symptoms of toxicity and the, BP time markedly rose again. Pronounced BP persisted 7 weeks after removal of mercury from the diet reflecting the long biological half-life of mercury as the methyl derivative. Selenium effectively prevented BP after 7 days exposure to toxic levels of methyl mercury chloride. Therefore, results suggest that early toxic effects of mercury can be observed readily by BP and that this environmental contaminant may influence drug activity or increase the sensitivity of the nervous system to sodium pentobarbital.

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