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Influence of Acetaldehyde, Dietary Protein, Carbon Tetrachloride and Butylatedhydroxytoluene on the Toxicity of Methylmercury in Rats

Marvin A. Friedman, L. Richard Eaton, and William Bailey

Department of Pharmacology, Medical College of Virginia, Health Sciences Division, Virginia Commonwealth University, and MCV/VCU Cancer Center, Richmond, Va. 23298

ABSTRACT

The influence of environmental or dietary factors on the toxicity of methylmercury (MeHg) was investigated due to possibilities that humans exposed to methylmercury may have been sensitized. Groups of 8 rats were exposed to 0, 20 or 40 ppm MeHg in a semisynthetic diet and fed 0.5% BHT, 5% protein (instead of 15%), or injected with 250 mg/kg CCl_4 or acetaldehyde. In control rats neurotoxicity occurred at 4 weeks and 9 weeks with 40 and 20 ppm MeHg, respectively. Mortality was observed at 6 weeks with 40 ppm and 1 rat died in week 9 with 20 ppm MeHg. Acetaldehyde injected rats died at week 4 and 6 when fed 40 or 20 ppm MeHg. Neurotoxicity was observed in week 3 and 5 in these groups, respectively. Treating rats with the low protein or BHT accelerated neurotoxicity and mortality by 1 week with 40 ppm MeHg. These agents had killed all test animals within 7 weeks at 20 ppm MeHg. Neither acetaldehyde nor BHT influenced 0 ppm MeHg controls while 5% protein induced precipitous weight loss. In the case of CCl_4, the rats lived longer in combination experiments than one would have expected from the individual treatments.

INTRODUCTION

Recently our laboratory has been involved in studies on the influence of dietary substances on the toxicity in rats of methylmercury. The original observations on which our studies have been based show antagonistic effects of dietary seleneum with inorganic and organic mercurials (GANTHER et al, 1972; STOEWSAND et al, 1974; POTTER et al, 1974; IWATA et al, 1973; OHI et al, 1976; STILLINGS et al, 1974). These studies also demonstrated that tuna contained a protective factor. Due to the temporal relationships between these studies and the presence of seleneum in tuna, a case has been made that the protective factor in fish is seleneum. Our laboratories have shown that swordfish also contains this protective factor and contains a large amount of seleneum (FRIEDMAN et al, in press). However, even more recently, our laboratories have shown that treatment with piperonyl butoxide, a pesticidal synergist which inhibits liver mixed function oxidase activity, synergizes methylmercury toxicity (FRIEDMAN and EATON, in press). The conclusion drawn from these studies was that a non-metalic substance, can act in a greater than additive

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fashion to increase the sensitivity of rats to methylmercury.

It was the purpose of the studies reported here to determine whether other non-metallic dietary changes would influence the sensitivity of rats to methylmercury. In order to determine this, rats were exposed to low protein diets and diets containing butylatedhydroxytoluene (BHT). Additional groups were injected with acetaldehyde and carbontetrachloride as these substances were too volatile to include in the diet.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing between 100 and 115 grams were used in these studies. These rats were fed ad libitum a semisynthetic diet described previously (FRIEDMAN et al, in press). Rats were housed 2 per cage in clean air cages equipped with automatic watering and self-flushing. Methylmercury chloride (MeHg) was dissolved in DMSO, bound to casein and added to diets as the casein preparation at levels of 0, 20 and 40 ppm. Each experimental group consisted of 8 rats. Rats were observed daily and weighed weekly. Each animal was observed for neurotoxicity manifesting itself as crossed hind legs. Neurotoxicity is recorded on graphs as those animals demonstrating this sign plus those animals which have died. Feeding studies were carried out for 10 weeks.

Modifications were made to the control diet for some of the test groups. Diets were made 0.5% in BHT by adding 30 grams of BHT to 15 kg diet. Diets were made 5% in casein by replacing casein with sucrose. Due to volatility, rats were injected with acetaldehyde. Each rat received 25 mg acetaldehyde in 0.1 ml DMSO 3 times a week throughout the studies. Similarly rats were injected 25 mg CCl_4 twice the first week and weekly thereafter in 0.1 ml DMSO.

RESULTS

The influence of acetaldehyde on the body weights of rats fed 0, 20 or 40 ppm MeHg is shown in Figure 1. In the absence of dietary MeHg, the acetaldehyde had no significant influence on body weight. However, when rats were fed 20 ppm MeHg in combination with acetaldehyde, decreased body weight in comparison to 20 ppm controls was seen. There was no significant effect over the first 4 weeks. The rats no longer gained weight after the fourth week and the 3 rats remaining at week 7 precipitously lost weight. In the case of the controls fed 20 ppm MeHg, there was no change in body weight after the fifth week. Rats fed 40 ppm MeHg, gained weight the first 3 weeks and then progressively lost weight thoughout the rest of the study. In contrast, rats fed 40 ppm MeHg and treated with acetaldehyde gained weight the first week and then remained constant throughout the rest of the study.

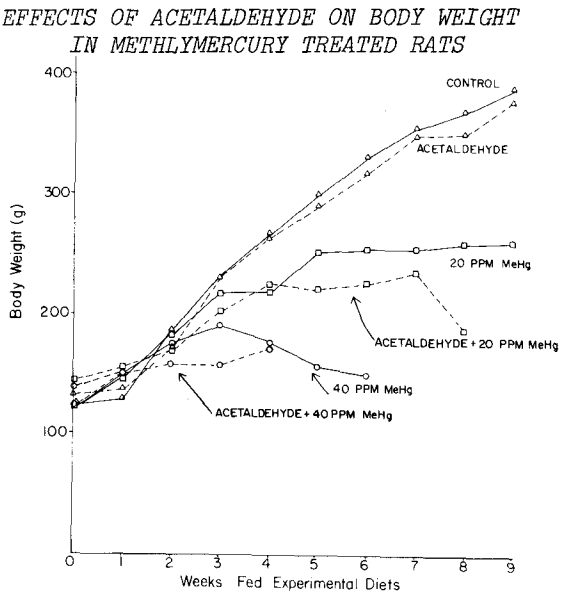

FIGURE 1. Groups of 8 rats were injected 3 times a week with with 25 mg acetaldehyde and body weights determined weekly.

The influence of acetaldehyde on mortality and the appearance of toxic signs are shown in Figures 2a and 2b. As can be seen in Figure 2a acetaldehyde markedly accelerated mortality. While half of the acetaldehyde 40 ppm MeHg treated rats were dead on week 4, there was no mortality in the 40 ppm alone rats. This represents a 1.3 week acceleration of toxicity. This syngergism was even more noteworthy at the lower mercury level. While only one 20 ppm control died in this study (at week 9), half of the acetaldehyde rats were dead at week 6. The magnitude of the effect of acetaldehyde, therefore, appears dose related being of greater magnitude at low doses of MeHg. Similar results were seen with neurotoxicity (Figure 2b). As with mortality, neurotoxicity at 40 ppm MeHg was accelerated by 1.2 weeks. In the case of 20 ppm MeHg the acetaldehyde accelerated toxicity by 4 weeks.

The influence of low protein, BHT and CCl_4 on body weights are shown in Figures 3a, 3b, and 3c. None of the treated rats grew as rapidly as their corresponding controls. The control, low protein rats (Figures 3a) lost weight through the first 3 weeks before adapting and gaining weight through the rest of the study. The BHT and CCl_4 rats gained weight inconsistently through the study and were generally similar to each other. It is noteworthy that ascites fluid was noted in several CCl_4 treated rats complicating interpretation of their body weights. Including 20 ppm MeHg in the diet markedly changed the growth curves (Figure 3b). The controls grew through the first 5 weeks and plateaued. The low

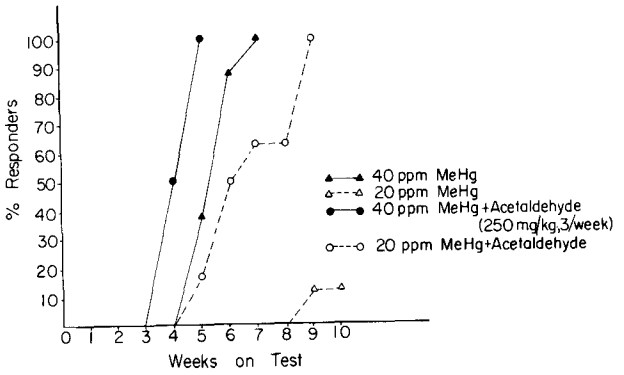

FIGURE 2a

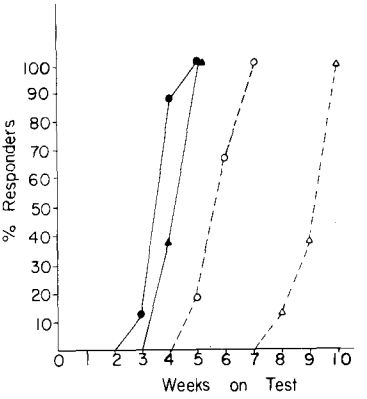

FIGURE 2b

FIGURE 2. Groups of 8 rats were injected 3 times a week with acetaldehyde and fed either 0, 20, or 40 ppm methylmercury. Figure 2a represents the cumulative mortality while Figure 2b represents neurotoxicity.

protein fed rats lost weight throughout the study. The BHT fed rats gained weight through the first 4 weeks and then slightly lost weight throughout the rest of the study. Rats injected with CCl_4 exhibited the most marked response. They gained weight at approximately the same rate as controls through the first 5 weeks. This was followed by a precipitous weight loss simultaneous with the appearance of MeHg toxicity. At 40 ppm, a highly toxic level of MeHg, the rats fed the low protein diet lost weight each week of the study (Figure 3c). The BHT fed rats reamined constant with a slight decrease in body weight at week 5. The CCl_4 injected rats showed slight weight gain through the fourth week followed by a noteworthy decrease in week 5.

Mortality in each of these regiments is shown in Figures 4a and 4b. In control rats fed 40 ppm MeHg, median time to mortality was approximately 5.2 weeks (Figure 4a). However, by week 5 all BHT and low protein rats were dead. In the case of 20 ppm MeHg(Figure 4b) mortality was also markedly accelerated. All rats fed low protein diets were dead at the end of the sixth week. In the case of the BHT, half the rats were dead at the end of the sixth week while subsequently only one more animal in this group died (during the eighth week). The interpretation of CCl_4 data is difficult as there were control rats killed by the treatment. In Figure 4b, it can be seen that 1 rat died in the first week, 2 in the third week, and 2 in the fourth week. None of the remaining 3 rats died through the rest of the study. Dietary MeHg at 20 ppm protected from this CCl_4 toxicity as only one rat

EFFECTS OF BHT, CCl_4, AND LOW PROTEIN ON BODY WEIGHT IN METHYLMERCURY FED RATS

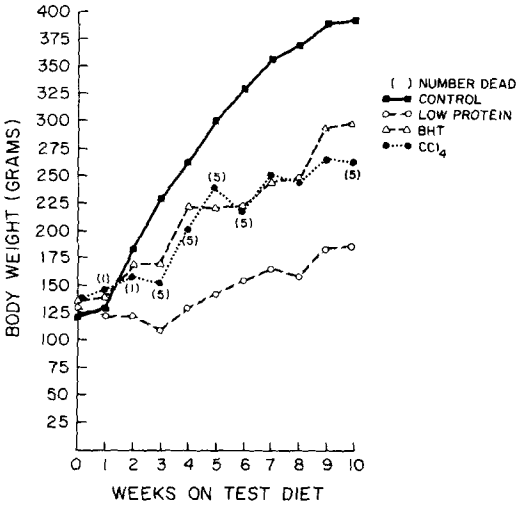

FIGURE 3a

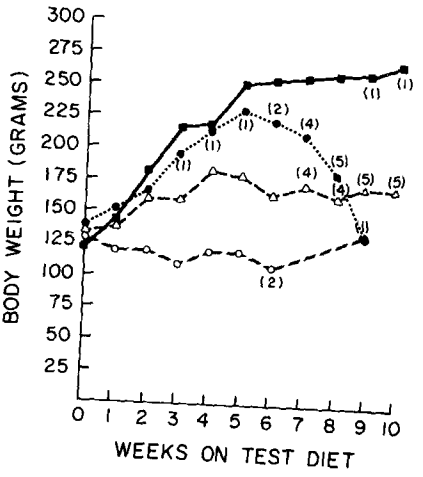

FIGURE 3b

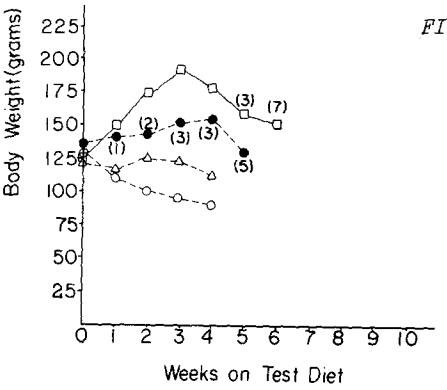

FIGURE 3c

FIGURE 3. Groups of 8 rats were injected with 25 mg CCl_4 or fed 0.5% of BHT or a 5% protein diet for 10 weeks. Control rats are shown in Figure 3a. Rats fed 20 ppm methylmercury are represented in Figure 3b while 40 ppm fed rats are represented in Figure 3c.

died during the first 5 weeks. Rats died throughout the rest of the study but of MeHg (not CC14) toxicity as will be shown later. This protective effect of MeHg was not clear with 40ppm MeHg (Figure 4a) probably due to the elevated mercury toxicity.

Neurotoxicity in these rats is shown in Figures 5a and 5b. In the case of rats fed low protein and BHT in combination with 40 ppm MeHg (Figure 5a), neurotoxicity preceded controls by 1

EFFECTS OF BHT, CCl_4 AND LOW PROTEIN DIET ON MORTALITY INDUCED BY METHYLMERCURY

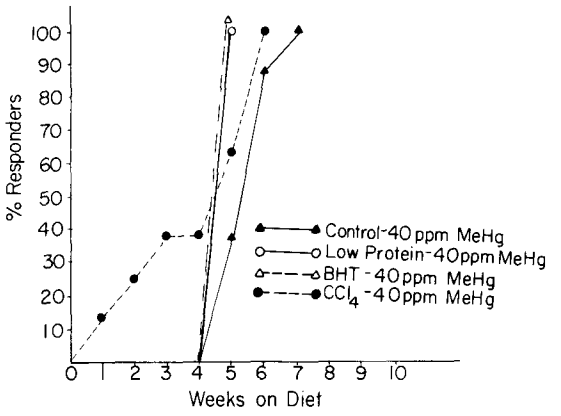

FIGURE 4a

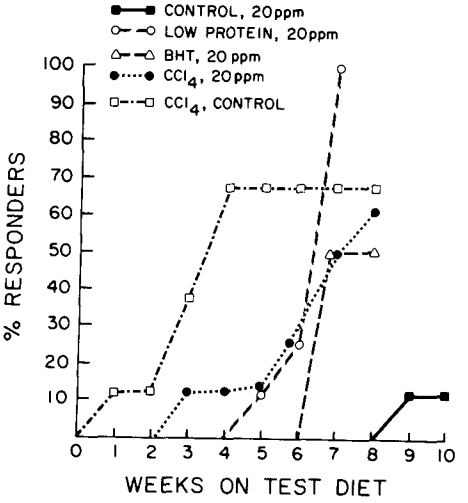

FIGURE 4b

FIGURE 4. Groups of 8 rats were injected with CCl_4 or fed 0.5% BHT or 5% protein for 10 weeks. Figure 4a represents the mortality induced when 40 ppm methylmercury was included in the diet while Figure 4b represents 20 ppm methylmercury.

week implying identical interpretations. At 20 ppm MeHg, the neurotoxicity in rats fed the low protein diet parallels that of the 20 ppm controls only accelerated by 4 weeks (Figure 5b). In rats fed BHT with 20 ppm MeHg, there was a responder at 3 weeks (an early death) followed by a response curve preceding the control curve but about half a week later than the low protein curve. In the case of the control CCl_4 the neurotoxicity solely represents the lethality in the controls (Figure 5b). By comparing the neurotoxicity and mortality curves, the effects of CCl_4 on crossed hind legs can be determined. In weeks 6, 7, and 8, there were 1 out of 6, 1 out of 4, and 3 out of 3 rats showing crossed hind limbs. The curve generated by intergrating these observations parallels control curve preceding it by 2 weeks.

EFFECTS OF BHT, CCl_4 OR LOW PROTEIN DIET ON NEUROTOXICITY INDUCED BY METHYLMERCURY

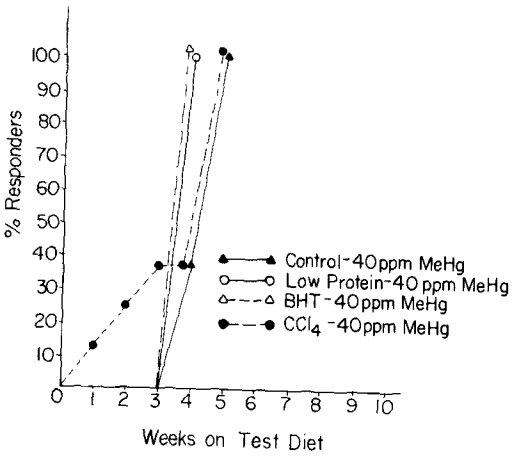

FIGURE 5a

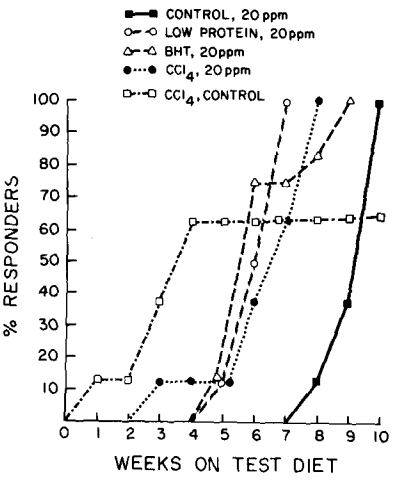

FIGURE 5b

FIGURE 5. Groups of 8 rats were injected with CCl_4 or fed 0.5% BHT or 5% protein. Figure 5a represents groups fed 40 ppm methylmercury while Figure 5b represents 20 ppm methylmercury.

DISCUSSION

Data presented in this communication demonstrate that the organic constituents of rat diets will markedly influence the toxic responses to methylmercury. In the simplest case, injections of acetaldehyde markedly synergized all responses to methylmercury while showing no outward toxicity signs of its own. Acetaldehyde is a well studied cardiotoxinand produced no change in body weight in rats under test. Therefore, this was a case of an apparently non-toxic dose of a chemical synergizing methylmercury. Furthermore, the acetaldehyde appeared more synergistic at the lower dose of methylmercury. The synergy appears more marked when mortality is considered rather than neurotoxicity because the acetaldehyde treated rats went on to die very quickly after the onset of neurotoxicity while there was a longer latency period with the controls. The mechanism of this synergy is not clear. Acetaldehyde was not selected for any biochemical characteristics but rather because it was produced in Japan at the time of the Minamata poisoning.

The synergy observed with BHT was entirely unexpected. Since vitamin E has protective activity against methylmercury poisoning, BHT, also an antioxidant, was expected also to possess this

antagonistic effect. Similarly, BHT is an inducer of liver mixed function oxidases. Inhibition of liver mixed oxidase activity by piperonyl butoxide increases methylmercury toxicity (FRIEDMAN and EATON, in press). Since BHT is an antioxidant and an inducer of liver mixed function oxidase, it was expected to antagonize methylmercury. However, at both 20 and 40 ppm methylmercury there was a marked synergy. The relationship between this synergy and decreased food intake is not clear. On the one hand, decreased food intake represents decreased methylmercury exposure. On the other hand, there are protective factors in the diet such as protein and vitamin E which may decrease the methylmercury toxicity.

Almost identical results were seen with the low protein diet. In this case methylmercury intake was severely diminished as the rats found this diet unpallatable. However, control rats gained weight on this low protein diet. One must conclude that dietary protein is highly protective against methylmercury toxicity.

The CCl_4 situation was complicated by mortality in the control-CCl_4 group. Five of the 8 rats died in this group within 3 weeks (Figure 4b). Methylmercury at both 20 ppm and 40 ppm protected from this effect. This can be attributed to an inhibitory effect of methylmercury on mixed function oxygenase activity (GOLDSMITH and SOARES, 1975; ALVARES et al, 1972). This mixed function oxygenase activity is necessary for CCl_4 toxicity. However, in contrast there appeared to be a potentiation of the methylmercury toxicity by CCl_4. This was anticipated as CCl_4 like piperonyl butoxide, is an inhibitor of mixed function oxidase activity.

The pharmacological basis of these synergisms is the subject of on-going research in our laboratories. However, many possibilities can be eliminated. Increased dietary methylmercury intake can be eliminated as a consideration as food intake if anything was decreased, and intestinal absorption in the rat is almost complete. This leaves consideration of demethylation reactions, plasma binding, blood-brain partitioning, and presence of false receptors in the brain. However, it has become crystal clear from these studies that there is not a direct relationship between dietary mercury levels and toxicity in the rat. In the case of the dietary protein and acetaldehyde studies the practical relevance is clear. In Minamata and Niigata, the pollution sources were synthesizing acetaldehyde. Possible contamination of the environment (air, food or water) with this organic pollutant may have markedly accentuated the human response to organic mercury. In the case of the Iraqi poisonings, Iraq being a developing country had protein shortages which were documented by the WHO. Therefore, the mercury toxicities may have been highly potentiated in these two situations. It then becomes very important to understand the mechanism of this response in order, perhaps, to treat the disease or even more importantly to prevent it.

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