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Phenobarbital Protection Against Methyl Mercury Nephrotoxicity (38746)

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(Introduced by R. L. Dixon)

Exposure to low doses of methyl mercury for extended time periods has previously been reported to cause a renal proximal tubular nephropathy (1) involving swollen mitochondria, dense lysosomes and the smooth endoplasmic reticulum (SER). These effects were ameliorated by simultaneous exposure to the pesticide dieldrin (2) a stimulant of protein synthesis and some enzyme systems associated with the endoplasmic reticulum (ER). It has been reported by Norseth (3) that mercury derived from methyl mercury exposure is extensively bound to the liver microsomal fraction. The above findings suggest interaction between the endoplasmic reticulum and mercury. The possibility of decreasing renal methyl mercury toxicity by utilizing a classic stimulant of endoplasmic reticulum systems such as phenobarbital has received little attention. This investigation was undertaken to evaluate the effects of phenobarbital on ultrastructural alterations in kidneys of rats exposed to low levels of methyl mercury. Attempts were made to correlate these observations with kidney, blood, and urine levels of both methyl- and inorganic mercury.

Materials and Methods. Forty-eight male Charles River rats were divided into four groups of 12 animals each. The first group received 5.0 ppm mercury as methyl mercury hydroxide (MMH) in distilled drinking water for 2 or 4 wk. A second group received MMH plus an intraperitoneal injection of sodium phenobarbital (PB) at a dose of 75 mg/kg once every 5 days for a total of three or six injections, respectively during the course of the experiment. The third group received only injections of phenobarbital while the fourth served as controls. The animals received laboratory chow and were housed in barrier isolation rooms. Six animals in each group were killed after 2 wk of exposure and the remainder at 4 wk. Average daily water consumption for all groups was 25 ml, yielding an approximate daily mercury dose of 0.7 mg/kg in treated animals.

Twenty-four hr urine samples were collected by placing six animals per group in metabolism cages on the day before sacrifice. The animals from each of the groups were killed by cervical dislocation and their right kidneys fixed for morphologic evaluation as described previously (2). The left kidney, blood and urine aliquots of each were analyzed for both methyl and inorganic mercury by gas chromatography (4–5). Numerous physiological studies (6–10) have reported that renal blood in several species including the rat is about 0.27 ml per g of kidney tissue. In order to approximate the amount of methyl and inorganic mercury actually in renal tissue, milliliter blood concentrations for each animal were multiplied by 0.27 and this figure was subtracted from the total kidney concentration of that animal.

The Kruskal Wallis test (19) was used to test for statistical differences in urine volumes and renal or urinary levels of mercury among the four groups. If the groups were significantly different, then pairwise comparisons were made by the Mann Whitney U test (10).

Results. Histologic sections of kidneys from MMH treated animals showed moderate swelling of proximal tubule cells by light microscopy at 4 wk but not 2 wk. Kidney sections from all other treated ani-

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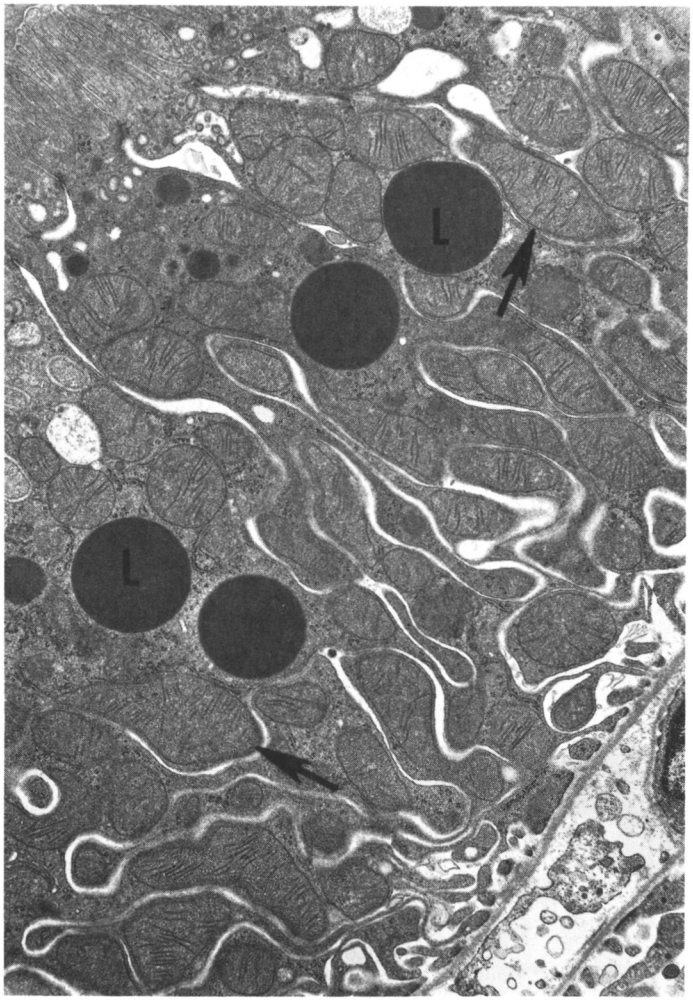

FIG. 1. Proximal tubule cell from a kidney of a male rat given 5.0 ppm MMH in the drinking water for 4 wk. Note swollen mitochondria (arrows) and numerous dense lysosomes (L) (×7429).

mals were similar to controls. Electron micrographs revealed that more terminal portions of second segments (pars descendens) as well as entire third segments (pars recta) of proximal tubules from rats exposed to MMH had swollen mitochondria and dense granular lysosomes at 4 wk (Fig. 1). Tubule cells of animals given MMH plus phenobarbital had marked increases in smooth endoplasmic reticulum (SER) but there were few altered mitochondria and a great reduction in the number of dense granular lysosomes (Fig. 2). Tubule cells of rats given only phenobarbital exhibited normal architecture except for increased amounts of SER and numbers of microbodies (peroxisomes). Kidneys of control animals were unremarkable.

Urine volumes for the various groups are presented in Table I. The urine volumes for all groups were comparable except for the MMH plus PB groups which had somewhat higher urine volumes at 2 wk and lower vol. at 4 wk.

Kidney, blood, kidney levels minus the calculated blood contribution and urine levels of methyl and inorganic mercury are given in Table II. Concentrations of either form of mercury were near or below detection limits (0.2 ppm) by gas chromatography in control and PB treated animals. Total renal and renal concentrations of methyl

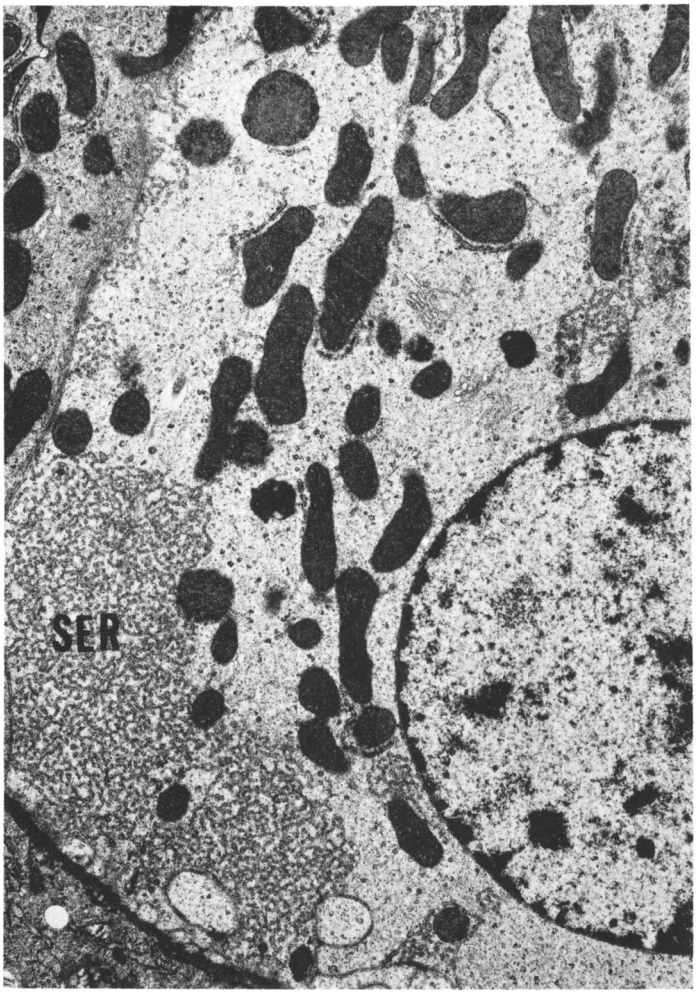

FIG. 2. Proximal tubule cell from a male rat given 5.0 ppm MMH in the drinking water for 4 wk but receiving injections of phenobarbital (75 mg/kg). The cell is unremarkable except for a large aggregate of smooth endoplasmic reticulum (SER) (×7429).

TABLE I. MEAN ± SEM 24 HR URINE VOLUMES (ml) IN METHYL MERCURY HYDROXIDE (MMH) PLUS PHENOBARBITAL (PB) TREATED RATS

	Urine volume 2 Wk
Control	6.28 ± 1.05
MMH	6.63 ± 0.85
PB	8.33 ± 1.02
MMH + PB	11.50 ± 1.12
	Urine Volume 4 Wk
Control	8.40 ± 0.86
MMH	9.78 ± 1.19
PB	8.22 ± 0.80
MMH + PB	6.68 ± 1.15

mercury corrected for blood levels in MMH treated rats did not change between 2 and 4 wk. Kidney methyl mercury levels (corrected for blood contribution) in the MMH plus phenobarbital group were significantly higher than those of animals treated with MMH alone at 2 wk ($P < 0.1$) although at 4 wk the effect of phenobarbital was not significant. Renal inorganic mercury concentrations were unaffected by PB treatment but were significantly higher ($P < 0.01$) for both groups at 4 wk in comparison with 2 wk.

Blood levels of methyl mercury were significantly greater ($P < 0.01$) at 4 wk in the MMH plus PB group in comparison to the

TABLE II$_2$ MEAN ± SEM KIDNEY, BLOOD, KIDNEY CORRECTED FOR BLOOD, AND URINE VALUES FOR CH_3Hg^{2+} AND Hg^{2+} IN METHYL MERCURY HYDROXIDE (MMH) PLUS PHENOBARBITAL (PB) TREATED RATS.

	Kidney	
	CH_3Hg^+ (mcg/gm)	Hg^{2+} (mcg/gm)
2 wk MMH	20.33 ± 3.36	7.75 ± 1.53
2 wk MMH + PB	31.45 ± 2.03[a]	9.30 ± 1.76
4 wk MMH	23.67 ± 3.19	13.09 ± 1.19[b]
4 wk MMH + PB	29.75 ± 6.48	17.05 ± 4.97[b]
	Blood	
	CH_3Hg^+ (mcg/ml)	Hg^{2+} (mcg/ml)
2 wk MMH	8.44 ± 2.36	4.92 ± 1.17
2 wk MMH + PB	11.76 ± 3.26	5.13 ± 1.81
4 wk MMH	29.90 ± 1.80	5.06 ± 2.72
4 wk MMH + PB	48.10 ± 3.60[c]	12.40 ± 3.90
	Kidney corrected for blood content	
	CH_3Hg^+ (mcg/gm)	Hg^{2+} (mcg/gm)
2 wk MMH	18.05 ± 3.41	6.42 ± 1.47
2 wk MMH + PB	28.27 ± 1.83[a]	7.92 ± 1.72
4 wk MMH	15.59 ± 3.32	11.72 ± 1.70
4 wk MMH + PB	16.76 ± 7.14	13.71 ± 5.48
	Urine	
	CH_3Hg^+ (mcg/ml)	Hg^{2+} (mcg/ml)
2 wk MMH	0.48 ± 0.28	0.098 ± 0.036
2 wk MMH + PB	0.40 ± 0.14	0.045 ± 0.025
4 wk MMH	0.51 ± 0.06	0.058 ± 0.041
4 wk MMH + PB	0.69 ± 0.14	0.326 ± 0.066[c, d]

[a] Significantly different from 2 weeks MMH at $P < 0.10$.

[b] Significantly different at $P < 0.01$ compared to corresponding 2 wk values.

[c] Significantly different at $P < 0.01$ compared to MMH treated only.

[d] Significantly different at $P < 0.05$ compared to all urine Hg^{2+} values.

MMH group. Inorganic mercury levels in blood were also higher in this group but this difference was not significant.

Urinary levels of methyl mercury were not statistically different between the two treatment groups for either time period. The levels of inorganic mercury were essentially the same among all groups except the group which received MMH plus PB for 4 wk. These animals had a 5.5-fold increase in the excretion of inorganic mercury (significant at $P < 0.05$).

Discussion. The exact mechanism by which phenobarbital protects against methyl mercury induced nephrotoxicity is unclear. Decreased renal toxicity in PB treated rats does not appear to be related to the levels of methyl and inorganic mercury in blood or renal tissue although urinary excretion of inorganic mercury is greater in these animals. These observations are consistent with the previous suggestion (2) that agents such as PB and dieldrin may exert a protective effect through an increase in intracellular "non-toxic binding sites" concommitant with the observed proliferation of SER. Binding of mercury to such sites might diminish its interaction with other cellular organelles such as mitochondria. Another related possibility is that PB increases the inducibility rate of metallothionein, which is considered to be an important detoxication pathway for mercury. Metallothionein which is a low molecular weight metal binding protein (12, 13) is thought to play a major role in the intracellular binding of renal mercury. It has been reported (14) to have a high affinity for inorganic but not methyl mercury. Further biochemical studies are needed to assess the effects of PB on the intracellular distribution of methyl and inorganic mercury with respect to these-possibilities.

The increased urinary excretion of inorganic mercury may represent either enhanced demethylation of methyl mercury and/or excretory capacity. Both processes are necessary (15) for the detoxication of methyl mercury by body viscera. Phenobarbital enhancement of inorganic mercury excretion by the kidney as demonstrated in this study, may be the more important protective effect

because inorganic mercury is highly toxic to renal proximal tubule cells (16, 17).

In conclusion, the above study indicates that phenobarbital protects against methyl mercury-induced nephrotoxicity but that this protection does not appear to be related to alterations in renal mercury levels. The potential therapeutic value of this approach should be explored further.

Summary. Phenobarbital injections to rats given a low oral dose level of methyl mercury for 2 or 4 wk decreased methyl mercury-induced ultrastructural alterations in kidney proximal tubule cells, increased urinary excretion of inorganic mercury and increased blood concentrations of methyl mercury. These effects were not seen after 2 wk of treatment but were highly significant after 4 wk.

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