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Effects of Methylmercury on Microsomal Mixed-Function Oxidase Components of Rodents

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SUMMARY

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Preliminary treatment of rats with methylmercury hydroxide (10 mg/kg/day for 2 days) decreased hepatic cytochrome P-450 content by 52%, type I substrate (piperonyl butoxide) binding spectra by 40%, and type II substrate (aniline and metyrapone) binding spectra by 59% and 66%, respectively. Decreased cytochrome P-450 levels were apparently caused by increased degradation of the fast-phase component of the biphasic CO-binding pigment degradation curve. When chlordane, which decreases the degradation rate of the fast-phase component, was administered in conjunction with methylmercury hydroxide, the net effect was a degradation rate similar to controls. A control experiment was devised to demonstrate that the biphasic degradation curves were not influenced by heme exchange during preparation of subcellular particles from control, chlordane-treated, or methylmercury-treated rats. Rats exhibited the greatest methylmercury-induced decrease in cytochrome P-450 content, followed by mice and guinea pigs. Male rat liver P-450 was decreased more than that from female rats. Methylmercury was converted in substantial quantities to inorganic mercury in rats, mice, and guinea pigs. Microsomal mercury levels were highest n guinea pigs, followed by mice and rats.

INTRODUCTION

Methylmercury causes extensive neurological damage following chronic or acute exposures (1-3). Recently it was reported that rats treated with methylmercury hydroxide exhibited decreased hepatic microsomal mixed-function oxidase activity (4-6). This apparently resulted from increased degradation of CO-binding pigment (7). In this study we report on microsomal hemoprotein turnover in smooth and rough endoplasmic reticulum as a result of methylmercury treatment. Chlordane, an organochlorine pesticide, induces mixed-function oxidase activity and thus causes the opposite effect of methylmercury. Chlordane also decreases degradation of the fast-phase component of the biphasic degradation curve of CO-binding pigment (8). When rats were exposed to methylmercury and chlordane simultaneously the effects of these agents were canceled, so that the mixed-function oxidase activity of this group was similar to controls (5). In this study we have investi-

gated the turnover of CO-binding pigment in rats treated with both methylmercury and chlordane to determine whether turnover rates would correspond to effects on mixed-function oxidase enzymes.

Recent reports have questioned the validity of turnover studies in which the heme moiety of hemoproteins is radioactively labeled. Radioactive heme may exchange nonenzymatically with unlabeled heme (9, 10). Also, the biphasic degradation curve (8, 11) of CO-binding pigment may be an artifact of radioactive heme bound nonspecifically by albumin and other proteins (12–14). However, Levin *et al.* (15) indicated that δ-aminolevulinic acid equivalents incorporated into microsomes and CO-binding particles are almost exclusively associated with the cytochromes following chromatography of ^{14}C- and ^{3}H-labeled microsomal hemoproteins. To determine whether the equilibrium of heme exchange, during preparation of subcellular particles, is influenced by MMH[1] and chlordane administration to rats, we devised an experiment to estimate the magnitude of exchange between cytochrome P-450, cytochrome b_5, and other heme molecules.

Our previous studies on the effects of MMH on liver microsomes have focused on male rats (4, 5, 7). Here we also report on the effects of this substance on female rats, mice, and guinea pigs. These studies include measurement of inorganic mercury and methylmercury bound to microsomes.

MATERIALS AND METHODS

Animals and preparations of subcellular fractions. Male and female rats (Charles River, 150–175 g; males were 6 weeks old and females 7 weeks old), male guinea pigs (Charles River, 200 g; 3 weeks old), and male mice (Charles River, 25–30 g; 5 weeks old) were used in the following experiments. Animals were housed according to the procedures of Klein and Herman (16). MMH (97%) was administered subcutaneously (10 mg/kg/day for 2 days) at 9 a.m.; control animals received deionized water on the same schedule. This treatment schedule was designed to coincide with previous studies (4, 5, 7) in order to evaluate the mechanism of action of MMH on microsomal enzymes. In some experiments chlordane (60% γ-chlordane) was administered orally (30 mg/kg/day for 2 days) at 9 a.m. in 0.2 ml of dimethyl sulfoxide whereas controls received 0.2 ml of dimethyl sulfoxide only. Animals were fasted for 17 hr prior to death and were killed by decapitation 24 hr after the last treatment. Microsomes were prepared from excised livers (7) in one set of experiments, and smooth and rough endoplasmic reticulum prepared according to Gram *et al.* (17) in a different set of experiments. Microsomal subfractions were subjected to digestion with lipase (steapsin of hog pancreas, Sigma Chemical Company, type II) to prepare CO-binding pigments, in the absence of cytochrome b_5 (11).

Incorporation of [^{3}H]ALA and heme exchange studies. Incorporation of [^{3}H]ALA (1.15 Ci/mmole) into CO-binding particles and cytochrome b_5 following intravenous injections of 10 μCi of the labeled precursor was investigated using the modified procedure (7) of Levin and Kuntzman (11). [^{3}H]ALA was injected 24 hr after the second MMH treatment. Heme exchange was examined by creating two artificial hybrid rat livers. The first (hybrid I) consisted of equal parts of chopped liver (each chopped section weighed approximately 200 mg), selected in a random manner from male rats which had and had not received [^{3}H]ALA. The second (hybrid II) was prepared by mixing in equal parts microsomal preparations from the same [^{3}H]ALA-treated and untreated rats. Labeled heme in each of the microsomal cytochromes after lipase digestion (cytochromes P-450 and b_5) was determined in each group of livers by the methods previously described (11). Radioactivity measurements in these experiments were expressed in disintegrations per minute per milligram of protein in cytochrome P-450 and b_5 preparations. If no heme exchange occurred, the specific activity in each of the hybrid microsomal cytochromes should be 50% of that found in the microsomal cytochromes of livers from the [^{3}H]ALA-treated animals. If heme exchange did occur, the

[1] The abbreviations used are: MMH, methylmercury hydroxide; ALA, δ-aminolevulinic acid.

magnitude of exchange could be quantified by the following formula: (50% − % ^{3}H in hybrid cytochrome) × 2. The hybrid cytochrome represents either cytochrome P-450 (CO-binding pigment) or cytochrome b_5. Variation from 50% of the specific activity of [^{3}H]ALA-treated livers in hybrid I should represent the maximum heme exchange which occurred between the microsomal cytochromes and other heptic heme-containing proteins. Variation from 50% of the radioactivity of [^{3}H]ALA-treated animals observed in the microsomal cytochromes of hybrid II should represent heme exchange between cytochromes P-450 and b_5. The flow chart in Fig. 1 illustrates the above procedure.

Other assays. Cytochrome P-450 was measured by its carbon monoxide difference spectrum following reduction with dithionite, and cytochrome b_5 was measured by its difference spectrum following reduction with NADH (18). Type I (piperonyl butoxide, >90%) and type II (aniline and metyrapone) subtrate binding spectra were obtained and quantified by previously described methods (19). Aminopyrine demethylation was determined by a modified procedure (20) of Orrenius (21), and the formaldehyde released was quantified by the Nash reaction (22). Total protein content was determined by the method of Lowry *et al.* (23). Methylmercury and inorganic mercury levels in various liver fractions were obtained by a modified procedure (24) of Westoo (25).

RESULTS

Effects of MMH on substrate-binding spectra. Cytochrome P-450 was decreased by 55% whereas decreases for substrate-binding spectra were 40%, 59%, and 66% for type I, type II (aniline), and type II (metyrapone), respectively (Table 1). The effects on the substrate-binding spectra and cytochrome P-450 content were maximal 24 hr after the last treatment. The effects diminished with time, so that by 7 days after the last treatment the magnitudes of the spectra and cytochrome P-450 content were similar to those of controls. Prior treatment with MMH did not cause a shift in symmetry or location of peaks and troughs of the binding spectra.

Incorporation of [^{3}H]ALA into rat liver CO-binding pigment. Data on the levels of [^{3}H]ALA equivlents present in smooth and rough endoplasmic reticulum and microsomes 0.5 and 24.0 hr after [^{3}H]ALA injections are summarized in Table 2. In these experiments the cytochrome P-450

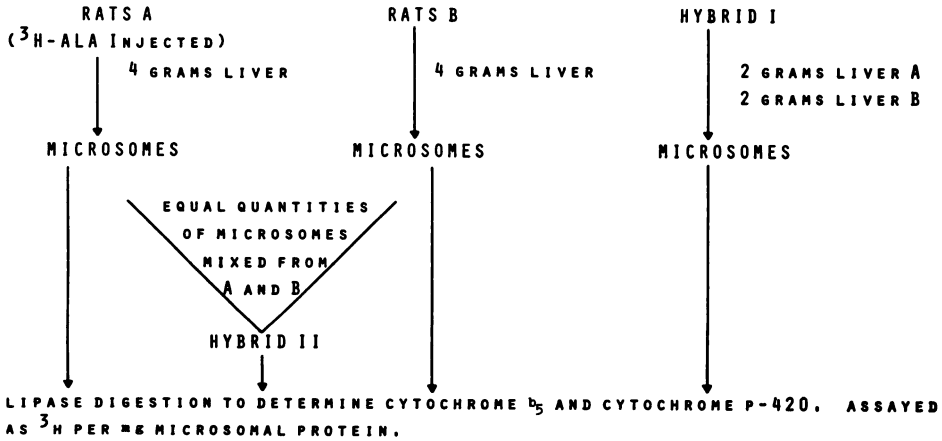

FIG. 1. *Procedure for detecting heme exchange between microsomal cytochromes and other hepatic hemoproteins during preparation of CO-binding particles*

Each group consisted of four male rats. Each rat in the group A received 10 μCi of [^{3}H]ALA in 0.5 ml of distilled water in the tail vein, and each rat in the group B received only 0.5 ml of distilled water. Labeled heme in each of the microsomal cytochromes after lipase digestion, (cytochromes P-420 and b_5) was determined in each group of livers by the methods previously described (11).

TABLE 1

Effects of MMH treatment on male rat liver microsomal cytochromes and substrate-binding spectra

Each value (mean ± standard deviation) was derived from at least nine animals, according to the procedure of Škrinjarić-Špoljar *et al.* (18).

Cytochrome	Control	MMH-treated	Change
	($\Delta A \times 10^4$)/*mg protein*		%
Cytochrome b_5[a]	337 ± 30	246 ± 31	−26.9
Cytochrome P-450[a]	635 ± 59	285 ± 45	−55.1
Type I (piperonyl butoxide)	303 ± 20	179 ± 28	−40.9
Type II (aniline)	272 ± 35	111 ± 11	−59.2
Type II (metyrapone)	782 ± 83	253 ± 27	−66.2

[a] ΔA represents 450–490 nm for P-450 measurements and 425–409 nm for b_5 measurements.

TABLE 2

Depletion of [^{3}H]ALA from rat liver CO-binding pigment of microsomes and microsomal subfractions

Each value represents the mean ± standard deviation derived from four animals.

Cell component	[^{3}H]ALA after injection (10 μCi)		[^{3}H]ALA[a] incorporated
	0.5 hr	24 hr	
	dpm/mg protein		% *maximum*
Control			
Microsomes	3749 ± 173	1391 ± 207	37.1
Smooth endoplasmic reticulum	4098 ± 209	1411 ± 174	34.4
Rough endoplasmic reticulum	3205 ± 350	1066 ± 113	30.1
MMH-treated			
Microsomes	3607 ± 296	605 ± 40	16.8
Smooth endoplasmic reticulum	4482 ± 543	733 ± 65	16.3
Rough endoplasmic reticulum	3850 ± 415	621 ± 116	16.2

[a] Maximal incorporation of [^{3}H]ALA into CO-binding pigment occurred 0.5 hr after injection into control and MMH-treated rats.

contents of livers were in the steady state between 24 and 48 hr after the last MMH treatment (Table 3). Maximum incorporation of radioactivity occurred 0.5 hr after injection, and the fast-phase portion of the degradation curve was complete by 24 hr (7). The half-life of the fast-phase component was 10 hr in control microsomes and smooth and rough endoplasmic reticulum, compared to 5.5 hr in MMH-treated microsomes and microsomal subfractions. Maximum incorporation of [^{3}H]ALA into hepatic CO-binding pigment of the smooth and rough endoplasmic reticulum and microsomes of control and MMH-treated rats was approximately the same (3000–4000 dpm/mg of protein). However, the disappearance of radioactivity was about twice as rapid in MMH-treated rats compared to controls. After 24 hr only 16% of the maximum incorporated radioactivity remained in microsomes and microsomal subfractions of the treated rats whereas the values were approximately 34% for controls. When rats were treated with chlordane, 43% of the maximum incorporated radioactivity in hepatic microsomal CO-binding pigment remained after 24 hr (Table 4). Simultaneous exposure to MMH and chlordane produced an additive effect, in which degradation of CO-binding pigment was slightly less than controls (Table 4).

Heme exchange studies. Our results for hybrid I indicate that heme exchange between microsomal cytochromes and other hepatic hemoproteins does not occur in

TABLE 3

Effects of MMH (10 mg/kg/day for 2 consecutive days) on hepatic microsomal cytochrome P-450 and aminopyrine demethylation in rats at various time intervals after second dose

Each value represents the mean ± standard deviation derived from four animals.

Days after second dose	Cytochrome P-450[a]			Aminopyrine demethylation		
	Control	MMH	Change	Control	MMH	Change
	($\Delta A \times 10^4$)/*mg protein*		%	*nmoles formaldehyde released/min/mg protein*		%
1	635 ± 73	308 ± 38	−51.5[b]	13.1 ± 1.3	5.6 ± 0.7	−57.4[b]
2	664 ± 37	305 ± 44	−54.0[b]	13.1 ± 1.7	6.1 ± 1.1	−53.5[b]
4	651 ± 55	437 ± 51	−33.9[b]	12.2 ± 1.9	7.5 ± 0.6	−38.7[b]
7	613 ± 70	571 ± 47	−6.8	12.7 ± 0.6	12.6 ± 1.3	−1.0

[a] ΔA represents 450–490 nm.

[b] Significantly different from controls ($p < 0.05$).

significant quantities during preparation of CO-binding pigments from control or treated rats. Exchange data for controls and rats treated with chlordane or MMH are given in Table 5. In control microsomes no detectable exchange occurred between cytochrome P-450 and cytochrome b_5, as evidenced by the fact that lipase-treated microsomes (cytochrome P-450) and the soluble fraction after lipase treatment (cytochrome b_5) of hybrid II both contained approximately 50% of the radioactivity of the [^{3}H]ALA-treated group. There might have been a small amount of exchange toward cytochrome b_5 in the MMH-treated and chlordane-treated groups. However, the difference in cytochrome b_5 radioactivity from controls is not significant ($p < 0.10$). This should not be sufficient to influence the biphasic degradation curve.

Effects of MMH on rat, mouse, and guinea pig microsomes. Male rat liver cytochrome P-450 and aminopyrine demethylation were decreased by 52% and 59%, respectively (Table 6). Male mouse liver P-450 and aminopyrine demethylation were decreased by only 29% and 35%, respectively. Decreases in guinea pig microsomes were 18% for P-450 content and 34% for aminopyrine demethylation. Control male mice had the highest level of oxidative activity, followed by male rats and male guinea pigs, and differences were significant, with $p < 0.05$. Decreases in microsomal cytochrome b_5 were not as great as with P-450, but the same species variations were exhibited. Glucuronyltransferase and microsomal protein levels were not significantly altered in any of the species tested (Table 6). Liver weights were not significantly changed in guinea pigs and rats, although mouse liver weights were decreased by 23%.

TABLE 4

Effects of chlordane and MMH treatment on depletion of [^{3}H]ALA from rat liver CO-binding pigment

Each value represents the mean ± standard deviation derived from an average of four animals.

Treatment	[^{3}H]ALA after injection (10 μCi)		[^{3}H]ALA incorporated
	0.5 hr	24 hr	
	dpm/mg protein		% *maximum*
Control	3456 ± 406	1130 ± 97	32.7
MMH	3610 ± 225	549 ± 163	15.2
Chlordane	2953 ± 424	1245 ± 119	42.2
MMH + chlordane	3147 ± 160	815 ± 183	25.9

Effects of MMH on microsomal enzymes of male and female rats. Cytochrome P-450 was decreased by only 34% in females, compared to the 52% decrease in males (Table 6). The difference for aminopyrine demethylase was not as great, with a 48% decrease observed in females and a 59% decrease in males. However, female cytochrome b_5 was decreased more than that of

TABLE 5

Heme exchange between cytochrome P-450, cytochrome b_5, and other hepatic hemoproteins during preparation of microsomal cytochromes from control, MMH-treated, and chlordane-treated rats

Each value represents the mean ± standard deviation derived from four male rats. Hybrid preparations were obtained by the procedures outlined in Fig. 1. Variation from 50% of the radioactivity of [^{3}H]ALA-treated livers in hybrid I should represent the maximum heme exchange which occurs between the microsomal cytochromes and other hepatic heme-containing proteins. Variation from 50% in the microsomal cytochromes of hybrid II should represent heme exchange between cytochromes P-450 and b_5. Cytochrome P-450 represents the radioactivity in CO-binding pigment obtained from the pellet of lipase-digested microsomes. The P-450 after this treatment is in the P-420 form (10). Cytochrome b_5 is represented by radioactivity in the supernatant from lipase digested microsomes.

Rats	Radioactivity of microsomal hybrid cytochromes compared to cytochromes from [^{3}H]ALA-treated rats	
	Hybrid I	Hybrid II
	%	%
Cytochrome b_5		
Control	48.2 ± 10.7	49.6 ± 7.6
MMH-treated	50.1 ± 9.1	55.3 ± 10.8
Chlordane-treated	50.3 ± 5.2	60.4 ± 15.8
Cytochrome P-450		
Control	46.2 ± 12.9	49.8 ± 6.8
MMH-treated	53.7 ± 13.1	50.1 ± 9.3
Chlordane-treated	46.8 ± 13.5	48.1 ± 8.1

males. Neither glucuronyltransferase nor liver weights were significantly altered in either sex.

Mercury levels in microsomes and whole homogenates. The amounts of mercury detected as methylmercury and inorganic mercury in liver microsomes and whole homogenates are illustrated in Fig. 2. Analysis was made by a combination atomic absorption gas chromatography procedure with a lower detection limit of 20 ppb in 1.0 ml. The gas-liquid chromatography techniques were used to evaluate both methyl and inorganic mercury. Analysis of our stock MMH solution revealed no detectable inorganic mercury. The extraction method and analytical procedure did not convert methylmercury to detectable amounts of inorganic mercury. Methylmercury was the only organic mercurial detected in liver microsomes and whole homogenates of MMH-treated animals. Mercury values illustrated in Fig. 2 were obtained by subtracting control levels of inorganic mercury and methylmercury from the corresponding liver fraction of treated animals.

Total mercury levels in microsomes were 5.6 μg/g of wet liver for mice, 5.2 for guinea pigs, 3.0 for female rats, and 1.4 for male rats. Examination of the breakdown of MMH shows that inorganic mercury concentrations are highest in guinea pigs and mice, followed by female and male rats (Fig. 2A). Methylmercury concentrations followed the same pattern, with the exception that guinea pigs had the highest levels and mice were second. Microsomal inorganic mercury as a percentage of total microsomal mercury was 41 in mice, 34 in guinea pigs, 20 in female rats, and 17 in male rats.

Species differences were not as great when mercury analyses were performed on whole homogenates. Values, in micrograms of mercury per gram of wet liver, were 65, 59, and 55 for female rats, guinea pigs, and male rats, respectively. However, the highest levels of inorganic mercury were detected in male rats, followed by female rats and guinea pigs, although these differences were not statistically significant (Fig. 2B). Methylmercury levels were highest in female rats, followed by guinea pigs and male rats. The proportion of inorganic mercury of total mercury was greatest in male rats, followed by female rats and guinea pigs.

Microsomal inorganic mercury as a percentage of total liver inorganic mercury was 0.8 for male rats, 2.4 for female rats, and 9.8 for guinea pigs. Microsomal methylmercury as a percentage of total methylmercury was 4.8 for male rats, 6.1 for female rats, and 9.3 for guinea pigs.

DISCUSSION

Our data indicate that depression of cytochrome P-450 content by MMH treat-

TABLE 6

Effects of MMH treatment (10 mg/kg/day for 2 days) on hepatic microsomal cytochromes and enzymes in male and female rats, male mice, and male guinea pigs

Values represent the means ± standard deviations derived from the numbers of animals indicated in parentheses.

Animals	Cytochrome P-450[a]	Cytochrome b_5[a]	Aminopyrine demethylase	Glucuronyl transferase	Microsomal protein	Liver weight
	($\Delta A \times 10^4$)/ *mg protein*	($\Delta A \times 10^4$)/ *mg protein*	*nmoles formaldehyde released/min/ mg protein*	*nmoles 1-naphthol conjugated/ min/mg protein*	*mg/g/wet/liver*	*g*
Male rats (6)						
Control	673 ± 35	361 ± 28	11.7 ± 2.5	109 ± 11	22.2 ± 1.4	7.2 ± 0.9
MMH	321 ± 38	266 ± 20	4.7 ± 0.4	116 ± 23	22.8 ± 3.3	7.2 ± 0.6
% change	−52.3[b]	−26.4[b]	−59.8[b]	+6.4	+2.7	0
Female rats (6)						
Control	452 ± 23	330 ± 43	6.0 ± 0.8	123 ± 8	16.2 ± 1.9	6.4 ± 0.7
MMH	297 ± 25	213 ± 15	3.1 ± 0.3	115 ± 11	18.6 ± 2.5	6.1 ± 0.3
% change	−34.3[b]	−35.5[b]	−48.3[b]	−6.5	+16.0	−4.7
Male mice (16)						
Control	780 ± 30	362 ± 40	13.6 ± 0.7	133 ± 18	17.6 ± 0.6	2.1 ± 0.2
MMH	548 ± 68	291 ± 35	8.9 ± 1.6	116 ± 7	18.3 ± 4.0	1.6 ± 0.2
% change	−29.3[b]	−19.8[b]	−34.6[b]	−12.8	+4.0	−23.8[b]
Male guinea pigs (6)						
Control	543 ± 70	414 ± 32	6.2 ± 0.9	157 ± 28	25.6 ± 1.7	5.1 ± 0.6
MMH	441 ± 28	389 ± 28	4.1 ± 1.1	156 ± 14	25.1 ± 2.9	5.0 ± 1.4
% change	−18.7[b]	−6.0	−34.1[b]	−0.6	−1.9	−2.0

[a] ΔA represents 450–490 nm for P-450 measurements and 425–409 nm for b_5 measurements.

[b] Significantly different from controls ($p < 0.05$).

ment is reflected by decreases in the activity of a wide spectrum of mixed-function oxidase enzymes. Both type I and type II substrate-binding spectra are substantially depressed in liver microsomes from treated rats. The specific activity of aminopyrine demethylase is decreased by approximately 50% in both smooth and rough endoplasmic reticulum (4). This correlates well with the 2-fold increase in the degradation rate of the fast-phase component of CO-binding pigment in whole microsomes and both microsomal subfractions. To investigate further the effects on cytochrome P-450, we treated rats simultaneously with MMH and chlordane. Chlordane, used alone, increased the half-life of the fast-phase component, in agreement with previous turnover studies (8). When MMH and chlordane were administered simultaneously, degradation of CO-binding pigment was only slightly faster than controls. Therefore, at the specified subacute dosage schedule, the effects of neither environmental agent predominated over the other. Mixed-function oxidase activity and levels of microsomal cytochromes were affected in a manner similar to the fast-phase component when MMH and chlordane were administered together (5).

Data presented here indicate that, during preparation of CO-binding pigments, exchange of heme between microsomal cytochromes and other hepatic hemoproteins is minimal. The experimental design used to measure exchange is valid, assuming that cytochromes P-450 and b_5 are the only microsomal hemoproteins. This assumption has been substantiated in rats by many reports (18, 26–29). It should be noted that this has not been studied in mice and guinea pigs. It is also assumed that the number of heme molecules in the heme pool of control, chlordane-treated, and MMH-treated rats is much greater than the number of ^{3}H-labeled molecules of cytochrome P-450 or cytochrome b_5. This assumption appears valid, since the maximum number of [^{3}H] ALA equivalents detected in CO-binding

FIG. 2. *Mercury levels in hepatic microsomes (A) and whole homogenates (B) following subcutaneous treatment of male rats, female rats, mice, and guinea pigs with MMH at 10 mg/kg/day for 2 days*

Hatched bars represent methylmercury, and unshaded bars represent inorganic mercury. Each value was obtained using the number of animals indicated in Table 5. Values were obtained by subtracting control levels of inorganic mercury and methylmercury from the corresponding liver fractions of treated animals. Control levels for organic and inorganic mercury were below 10 ppb (100 ng/g of tissue).

particles of entire livers averaged only 0.008 nmole, which represents a very small fraction of the total hepatic heme pool. Even if only a small portion of the total hepatic heme were transferable, it is unlikely that the size of the transferable heme pool could influence the exchange data reported here. To compensate for the fact that mixed-function oxidase enzymes are not uniformly distributed in the liver, portions of chopped liver were selected in a random manner when preparing hybrid I. If no exchange occurred during preparation, 50% of the radioactivity in microsomes from [^{3}H]ALA-treated rats would theoretically be detected in the hybrid cytochromes. According to our results, the small amount of exchange that might occur is not significantly affected by prior treatment of rats with MMH or chlordane, with the possible exception that heme exchange from cytochrome P-450 to cytochrome b_5 is slightly faster in MMH- and chlordane-treated rats. High standard deviations make results from this experiment difficult to interpret. Nevertheless the magnitude of exchange between the microsomal cytochromes could not be large enough to suggest that the biphasic degradation curve is an artifact of an exchange reaction occurring during preparation of CO-binding particles, in which the equilibrium is significantly altered by exposure to MMH or chlordane. These results, along with the recent reports that heme is not bound nonspecifically by other hepatic hemoproteins (15) and that recrystallized heme from [^{3}H]ALA-treated rats was not contaminated by labeled nonheme compounds (30), help to confirm the validity of turnover studies in which the heme moiety of hemoprotein molecules is radioactively labeled.

The effects of MMH on microsomal enzymes exhibit some interesting species and sex differences. Guinea pigs show little decrease in hepatic cytochrome P-450 levels following injections of MMH at 10 mg/kg/day for 2 days. Depression of male rat liver P-450 was 52% compared to only 18% for male guinea pigs. Possible explanations for the species variations, other than inherent differences in microsome-mercury interactions, are as follows. (a) Rats used in

the experiment were 6 weeks old, whereas guinea pigs were only 3 weeks old and could have been less susceptible because of an age factor. (b) Guinea pigs accumulated more mercury in the endoplasmic reticulum than did rats, which might have resulted in induction of the mixed-function oxidases. An inductive effect of MMH in rats has been observed following 17 daily doses.[2] Also, the dose-response curve of MMH in rats revealed that mixed-function oxidase activity was decreased by 50% at any dose between 2.5 and 10.0 mg/kg/day for 2 days (4), although microsomal mercury levels more than doubled through the dosage range. However, we found that guinea pig microsomes, like rat liver microsomes, were affected in a similar manner at 2.0 and 10.0 mg/kg dosage schedules even though hepatic mercury levels were 3 times higher at the higher dose. Therefore it appears unlikely that the species differences were caused by induction in guinea pigs by higher microsomal mercury levels. (c) There might be a latent period in guinea pigs before the microsomal enzymes are significantly affected. This does not appear likely, since guinea pig hepatic cytochrome P-450 levels were decreased by 18% 1 day after the last of two daily treatments, and by 16% 4 days after the last MMH treatment. The same explanations might be applicable to mouse hepatic P-450, which also was not decreased as much as in rats.

Our results on microsomal mercury levels following MMH injections were somewhat unexpected. An inverse relationship was observed between bound mercury levels and the magnitude of the depression of cytochrome P-450 content in the species tested. Male guinea pig and mouse microsomal mercury levels were approximately 4 times higher than those of male rats, and female rats had twice the levels of males. However, male rat liver microsomal enzymes were the most severely affected. This relationship held for total mercury, inorganic mercury, and methylmercury concentrations. These results, in addition to our heme degradation studies, indicate that the effect of MMH on microsomal enzymes is indirect and not due solely to enzyme inhibition. Alvares *et al.* (31) reached the same conclusion after studying the effects of methylmercury chloride on rat liver oxidative enzymes and cytochromes *in vitro*. Conversion of methylmercury chloride to inorganic mercury in rat liver has been reported previously (32, 33). These workers used isotopically labeled mercury and found higher percentages of total mercury in hepatic microsomes than reported here. However, they used a different strain of rats, treated the animals with methylmercury chloride rather than MMH, used a different treatment schedule, and prepared microsomes in a different manner.

[2] M. F. Folsom, unpublished observations.

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