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Correlations among the Changes in Hepatic Microsomal Components after Intoxication with Alkyl Halides and Other Hepatotoxins

DAVID E. MOODY, JACQUELINE L. JAMES, GARY A. CLAWSON, AND EDWARD A. SMUCKLER
Department of Pathology, University of California School of Medicine, San Francisco, California 94143

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SUMMARY

Male rats were given a single dose of 1 of 13 alkyl halides [2 of which produced lipid peroxidation (carbon tetrachloride and bromotrichloromethane) and 11 of which did not (chloroform, bromoform, dichloromethane, dibromomethane, 1,2-dichloroethane, 1,2-dibromoethane, 1-bromo-2-chloroethane, 1,1,2-trichloroethane, 1,2,3-trichloropropane, 1,2-dibromo-3-chloropropane, and 1,2-epoxy-3-chloropropane] or 4 other hepatotoxins (dimethylnitrosamine, thioacetamide, ethionine, and cadmium acetate). Eighteen hours later, hepatic microsomes were isolated and their protein, RNA, phospholipid, cytochrome P-450, cytochrome b_5, NADPH cytochrome *c* reductase, and fatty acid contents were determined. The cytochrome P-450 content decreased significantly after 14 of the treatments, and cytochrome b_5 and NADPH cytochrome *c* reductase contents decreased significantly after five and four of the treatments, respectively. The majority of the treatments resulted in a shift of the microsomal fatty acid content, characterized by increases in the relative contents of linoleic (12 treatments), palmitic (11 treatments), and oleic acids (9 treatments), and decreases in the relative contents of arachidonic (14 treatments) and stearic acids (9 treatments). The decreases in cytochromes P-450 and b_5 showed high degrees of correlation with the decrease in arachidonic acid ($r = 0.90$ and 0.86, respectively) and the increase in linoleic acid ($r = -0.80$ and -0.65, respectively). These strong correlations were also found when values for the rats given the four hepatotoxins were considered alone. However, slightly lower correlations were determined when the data for the four other hepatotoxins and/or the data for the lipid peroxidation-positive alkyl halides were omitted from the calculations. The possible relationship between changes in microsomal cytochromes and polyunsaturated fatty acids is discussed.

INTRODUCTION

Alkyl halides are employed as solvents, organic intermediates, and pesticides (1), leading to exposure of both consumers and industrial workers, and several alkyl halides, primarily halogenated methanes, have been identified as contaminants of chlorinated drinking water (2). These compounds have been shown to exert toxic responses in liver, kidney, and lung. They also have mutagenic properties and are carcinogenic in rodents (1). The exact mechanisms by which alkyl halides produce these acute or chronic injuries are not known.

The hepatic endoplasmic reticulum (microsomes) is often the initial site of injury after intoxication with alkyl halides and other hepatotoxins (3–6). This is potentially related to the high concentration of cytochrome P-450-dependent mixed-function oxidases in hepatic microsomes, as the alkyl halides are metabolized to "reactive metabolites" by this oxidase (7–10). Alterations in the microsomal components may arise from the direct effects of the toxin or its metabolites on macromolecules, or indirectly from effects on those factors which regulate the steady-state levels of the components. Other macromolecules within the same microenvironment may also be altered, either because they or their regulatory pathways are susceptible to the chemical or because of an interdependence among several components for the maintenance of their respective steady-state levels. Study of the responses of microsomal components to injury may prove to be a useful method of determining such similarities in susceptibility to injury, as well as illuminating the interrelationships of these macromolecules.

A decrease in the cytochrome P-450 content of hepatic microsomes has been noted in rats exposed to a number of short-chain (C_{1-3}) alkyl halides, including CCl_4 (11), halothane (12), $CBrCl_3$ (13), DBCP,[1] $CHCl_3$, DBE, and

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[1] The abbreviations used are: DBCP, 1,2-dibromo-3-chloropropane; DBE, 1,2-dibromoethane; BCE, 1-bromo-2-chloroethane; DMN, dimethylnitrosamine; TIAA, thioacetamide; ETH, ethionine; DCM, dichloromethane; DBM, dibromomethane; DCE, 1,2-dichloroethane; TCE, 1,1,2-trichloroethane; TCP, 1,2,3-trichloropropane; EPI, 1,2-epoxy-3-chloropropane.

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BCE (14, 15). This loss of cytochrome P-450 appears to be a general response to intoxication with alkyl halides, but the existing data suggest that several mechanisms may be involved (14). Following treatment with CCl_4 or $CBrCl_3$, a rapid loss of cytochrome P-450 occurs during the first 4 hr accompanied by lipid peroxidation (11, 13, 16). A significant decrease in cytochrome P-450 is not observed prior to 12 hr after treatment with the other alkyl halides tested (12, 14, 15), and these compounds are not associated with a significant peroxidation of microsomal lipids. Studies on the response of other microsomal components to treatment with alkyl halides have been limited to animals treated with CCl_4 (7) or to changes occurring within the first 2 hr and their relationship to lipid peroxidation (5, 17). These studies have convincingly shown that the early changes in hepatic microsomes (within 6 hr) are associated with the extent of lipid peroxidation, and the capacity of alkyl halides to produce these effects depends on low halocarbon bond dissociation energies which are amenable to homolytic cleavage (5, 7, 16).

Recently we found that the decrease in cytochrome P-450 which followed treatment with a few alkyl halides was associated with a correlated decrease in microsomal arachidonic acid 18 hr after treatments (15). It has been shown that polyunsaturated fatty acids are required in the diet for the maintenance of drug-metabolism activity (18), particularly the level of cytochrome P-450, in the intact animal (19) and in cultured hepatocytes (20). The loss of arachidonic acid and cytochrome P-450 after alkyl halides may represent another aspect of the interrelationship between these two microsomal components. Our previous study has now been extended. Male rats have been treated with 7 additional alkyl halides (13 total) and 4 other hepatotoxins for 18 hr and the content of several microsomal components have been assayed. The results of this study demonstrate that, of the microsomal components assayed, the highest correlation among the changes were between cytochrome P-450 and arachidonic acid. This correlation was also evident when only the data from animals treated with the four non-alkyl halides were assessed.

METHODS

Treatment of animals. Male Sprague-Dawley rats (cesarean-delivered; Charles River Breeding Laboratories, Wilmington, Mass.) weighing 200–250 g were housed in steel screen-bottomed cages with food (Purina rat chow) and water provided ad libitum. The food was removed 18 hr before treatment. Before they were killed (1 or 18 hr), the animals received a single dose of one of 17 compounds. All of the alkyl halides were dissolved in mineral oil and administered by gastric intubation in a total volume of 5.0 ml/kg. DMN and TIAA were dissolved in water and also administered by gastric intubation (total volume 5.0 ml/kg). $Cd(Ac)_2$ and ETH were dissolved in water and administered i.p. in volumes of 5.0 and 20.0 ml/kg, respectively. The doses and sources of supply of the compounds were: CCl_4, 4000 mg/kg (spectro grade, Mallinckrodt, Paris, Ky.); $CBrCl_3$, 500 mg/kg (spectro grade, Eastman Kodak, Rochester, N. Y.); $CHCl_3$, 1500 mg/kg (spectro grade, Burdick and Johnson Laboratories, Inc., Muskegon, Mich.); $CHBr_3$, 1000 mg/kg (spectro grade, Eastman Kodak); DCM, 1000 mg/kg (spectro grade, Eastman Kodak); DBM, 1000 mg/kg (technical grade, Eastman Kodak); DCE, 625 mg/kg (reagent grade, Mallinckrodt); DBE, 220 mg/kg (technical grade, (Dow Chemical Company, Midland, Mich.); BCE, 250 mg/kg (technical grade, Eastman Kodak); TCE, 1080 mg/kg (technical grade, Eastman Kodak); TCP, 180 mg/kg (technical grade, Eastman Kodak); DBCP, 600 mg/kg (technical grade, Occidental Chemical Company, Lathrop, Calif.); EPI, 180 mg/kg (technical grade, Eastman Kodak); DMN, 50 mg/kg (gold label, Aldrich Chemical Company, Milwaukee, Wisc.); TIAA, 200 mg/kg (reagent grade, Fischer Scientific Company, Fair Haven, N. J.); ETH, 500 mg/kg (A grade, Calbiochem, San Diego, Calif.); and $Cd(Ac)_2$, 2 mg/kg (reagent grade, J. T. Baker Chemical Company, Philipsburg, N. J.).

Preparation of microsomes. After treatment (1 or 18 hr), the rats were anesthetized under ether, and their livers were perfused with ice-cold 0.9% NaCl solution. Liver homogenates were prepared in 0.1 M potassium phosphate (pH 7.4) and microsomes were prepared as previously described (14). The initial microsomal sediment was resuspended in 125 mM KCl and 50 mM Tris-HCl (pH 7.4), divided into fractions containing one-third and two-thirds of the microsomes and recentrifuged. The sediment in the former fraction was resuspended in 0.1 M potassium phosphate (pH 7.4), and the latter was extracted with chloroform-methanol (2:1) as described by Folch *et al.* (21).

Assays of the aqueous suspension. Protein content was determined by the method of Lowry *et al.* (22) with bovine serum albumin as standard. RNA was extracted from trichloroacetic acid precipitates of homogenates and microsomes as described by Schneider (23) and measured using the orcinol method of Ceriotti (24) with yeast RNA as standard. Microsomal suspensions diluted to 1 mg of protein per milliliter were used to quantitate cytochromes b_5 and P-450 from difference spectra (25) and to measure NADPH cytochrome *c* reductase as the rate of NADPH-dependent reduction of cytochrome *c* (26). All spectrophotometric measurements were made with an Aminco DW-2 spectrophotometer.

Assays of organic extracts. Phospholipid phosphorous was determined by the method of Bartlett (27). Diene conjugates were estimated from the absorbence of extracts at 232 nm, as described by Rechnagel and Ghoshal (16), using a Cary 118 spectrophotometer. Lipid extracts were transesterified with methanoic base reagent (3-3080; Supelco, Bellafonte, Pa.); the fatty-acid methyl esters were separated by gas chromatography (Hewlett Packard 5830 A gas chromatograph), using a Supelco SP-2330 on 100–120 chromosorb column, and detected by flame ionization as previously described (28). Standard fatty acids were obtained from Sigma Chemical Company (St. Louis, Mo.) and Supelco.

Calculations and statistical analysis. Microsomal components were considered in reference to the appropriate major component—protein, phospholipid, or total fatty acid. The values were also routinely calculated as total hepatic microsomal content relative to 100 g of body weight to assess differences arising from changes in liver weight and recovery of microsomes. Student's two-tailed

t-test was used to estimate the significance of differences between the values for treated and for control animals. When values were compared for purposes of correlation, they were expressed as the total hepatic microsomal content per 100 g of body weight, or for fatty acid as a percentage of the total detectable matter in methylated extracts. The ratios of these values to the mean control value were used with the two values from an individual treated animal as the x and y coordinates. The equations from Sokal and Rohlf (29) were used for least-squares analysis and for determination of the slope, the correlation coefficient, and its 95% confidence intervals.

RESULTS

We used relatively high doses of the 13 alkyl halides (0.5–2.0 times the reported LD_{50} values) in order to maximize the changes produced in the microsomal components. However, these doses allowed 100% survival of the rats at 18 hr, and only five treatments (DBE, TCE, EPI, DMN, and TIAA) resulted in appreciably greater loss of body weight than in control rats. In rats given TCP, ETH, and $Cd(Ac)_2$, body weight preservation was significantly greater than in control rats.

Liver-to-body weight ratios were elevated after 17 treatments, but not significantly so in animals given DCM, DBM, or TCP (data not shown). Recovery values of the major microsomal components (protein, RNA, and phospholipid) were expressed as total liver values relative to body weight, because of this diverse response of liver size to the treatments. In rats treated for 18 hr with CCl_4 or $CBrCl_3$, recovery of microsomal protein (Fig. 1a) and phospholipid (Fig. 1b) were significantly decreased. In those treated with DMN, microsomal protein and RNA (Fig. 1a), but not phospholipid (Fig. 1b), were significantly decreased. Microsomal RNA was also decreased in rats treated with BCE. Treatment with $Cd(Ac)_2$ caused an increase in microsomal protein and phospholipid. Microsomal protein and RNA were increased after treatment with DBM and $CHBr_3$, respectively (Fig. 1a).

To assess whether the perturbations in the recovery values of the microsomal components were associated with peroxidation of microsomal lipids, we treated rats with the same 17 compounds and killed them 1 hr later.[2] Lipid extracts of the microsomes were prepared and scanned against solvent in the UV range to detect diene conjugates. Only two treatments, CCl_4 and $CBrCl_3$, caused a significant increase in the diene conjugate content (Fig. 1b).

The recovery values for the microsomal proteins cytochrome P-450, cytochrome b_5, and NADPH cytochrome *c* reductase were expressed relative to total microsomal protein (Fig. 2a and b). Cytochrome P-450 levels decreased significantly after 14 of the treatments, and to a lesser extent after the other 3 (EPI, TCP, and ETH) (Fig. 2a). In contrast, NADPH cytochrome *c* reductase activity and cytochrome b_5 content decreased significantly after only four and five of the treatments, respectively. Three compounds (CCl_4, $CBrCl_3$, and DMN) consistently affected these two components, as well as microsomal protein and cytochrome P-450.

Microsomal fatty acid content was determined from peak areas of methylated extracts separated by gas chromatography and was expressed as a percentage of the material detected. Six major peaks (>3.0%) were detected, including palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), arachidonic (20:4), and docosahexenoic acids (22:6). The changes in the composition of microsomal fatty acids after the treatments showed a general pattern (Fig. 3a and b), with significant increases in the relative content of palmitic (11 treatments), oleic (9 treatments), and linoleic acids (12 treatments), and significant decreases in stearic (9 treatments) and arachidonic acids (14 treatments). The relative content of docosahexenoic acid decreased after three of the treatments, but in general showed a more variable response.

To test for correlations among the responses of the microsomal components to the treatments, we expressed the values for each treated animal as ratios to the respective mean control values. We then prepared scatter diagrams (Fig. 4) to compare the changes in arachidonic and linoleic acids to those in cytochrome P-450. These and the other diagrams prepared were linear in character and demonstrated homoscedastic patterns appropriate for assessing correlations. We found a high positive correlation ($r = 0.90$) between the decreases in cytochrome P-450 and arachidonic acid, and a high negative correlation ($r = -0.80$) between the decrease in cytochrome P-450 and the increase in linoleic acid (Fig. 4).

The relationships of the changes in cytochrome P-450, linoleic acid, and arachidonic acid to the changes in the other microsomal components were assessed in a similar fashion (Tables 1 and 2). The decreases in cytochrome P-450 demonstrated high degrees of positive correlation with the decreases in cytochrome b_5 ($r = 0.90$) and microsomal protein ($r = 0.89$), and lower degrees of correlation with the changes in NADPH cytochrome *c* reductase, phospholipid, diene conjugates, and microsomal RNA (in descending order). The decreases in arachidonic acid showed a similar pattern of correlation with changes in the other components, with relatively high degrees of correlation with the decreases in cytochrome b_5 ($r = 0.86$) and microsomal protein ($r = 0.81$). In contrast, the increase in linoleic acid showed a higher degree of correlation with the changes in NADPH cytochrome *c* reductase ($r = -0.70$) than with those in cytochrome b_5 ($r = -0.65$) or microsomal protein ($r = -0.59$).

The correlations of the changes in cytochrome P-450 with those in microsomal fatty acids are shown in Table 2. The highest degrees of correlation were with the changes in arachidonic, linoleic, and oleic acids; and there were lower degrees of correlation with the changes in palmitic, stearic, and docosahexenoic acids. A similar order of correlation was found when changes in linoleic and arachidonic acids were compared with those in the other fatty acids (Table 2).

The 17 treatments fall into three groups: (a) alkyl halides which cause measurable lipid peroxidation (CCl_4

[2] The *in vivo* detection of diene conjugates is maximal at 1–3 hr after treatments with agents known to induce lipid peroxidation (16), whereas associated alterations in protein and fatty acid contents are known to persist for longer periods. The possible induction of lipid peroxidation in this study was therefore measured in animals 1 hr after the treatments. We have also found no increase in the diene conjugate levels at 6, 12, or 18 hr after treating animals with DBCP or DMN.

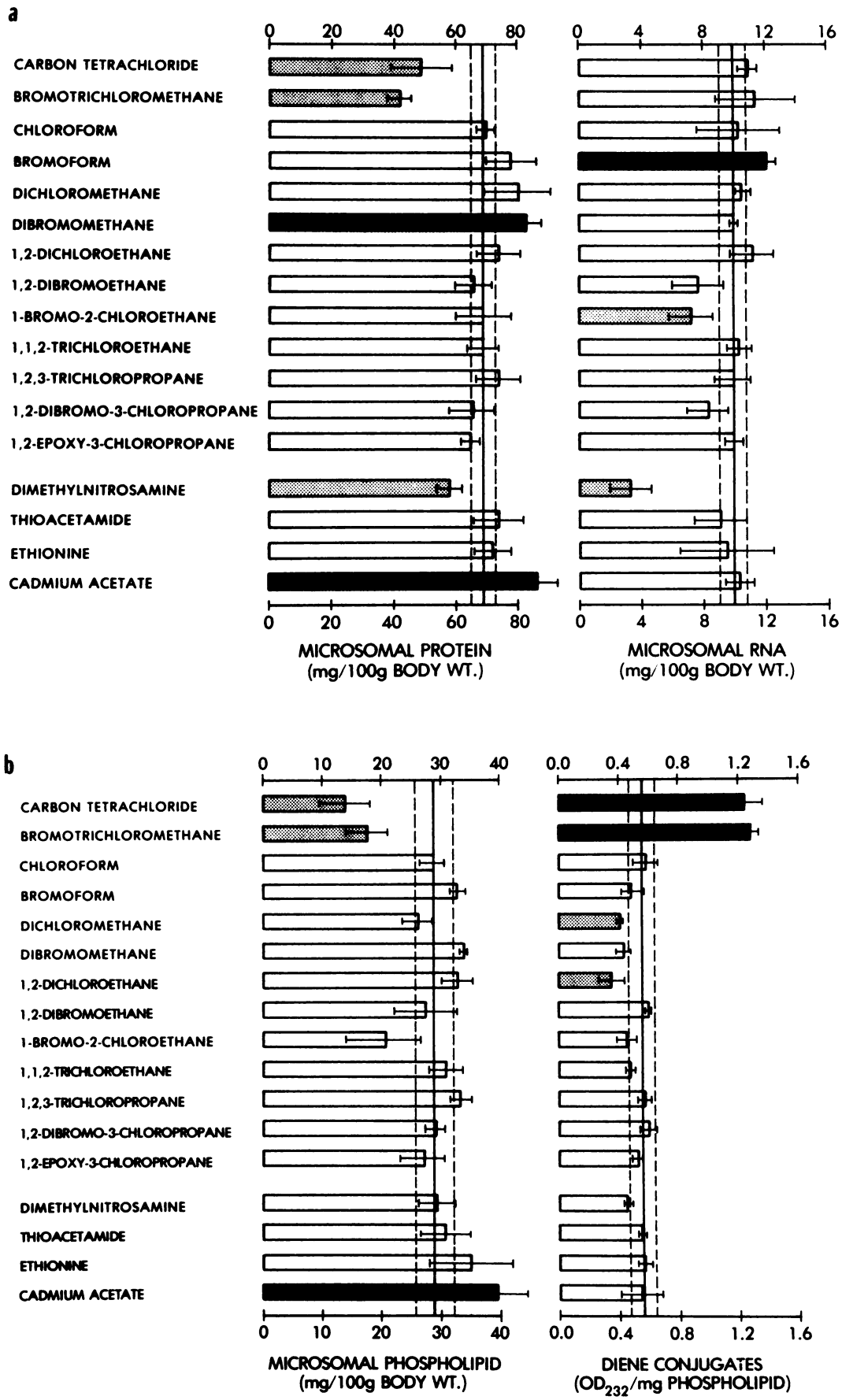

FIG. 1. *Recovery of protein, RNA, phospholipid, and diene conjugates from hepatic microsomes of rats treated with alkyl halides and other hepatotoxins*

Rats (three per treatment) received a single dose of the compounds, and microsomes were prepared as described 18 hr after treatment for (a) protein and RNA and (b) phospholipid, or 1 hr after treatment for diene conjugates.[2] —— represents the mean control ± 1 SD (- - -). *Stippled bars* show values significantly higher (*dark*) or lower (*light*) than control values ($p < 0.05$).

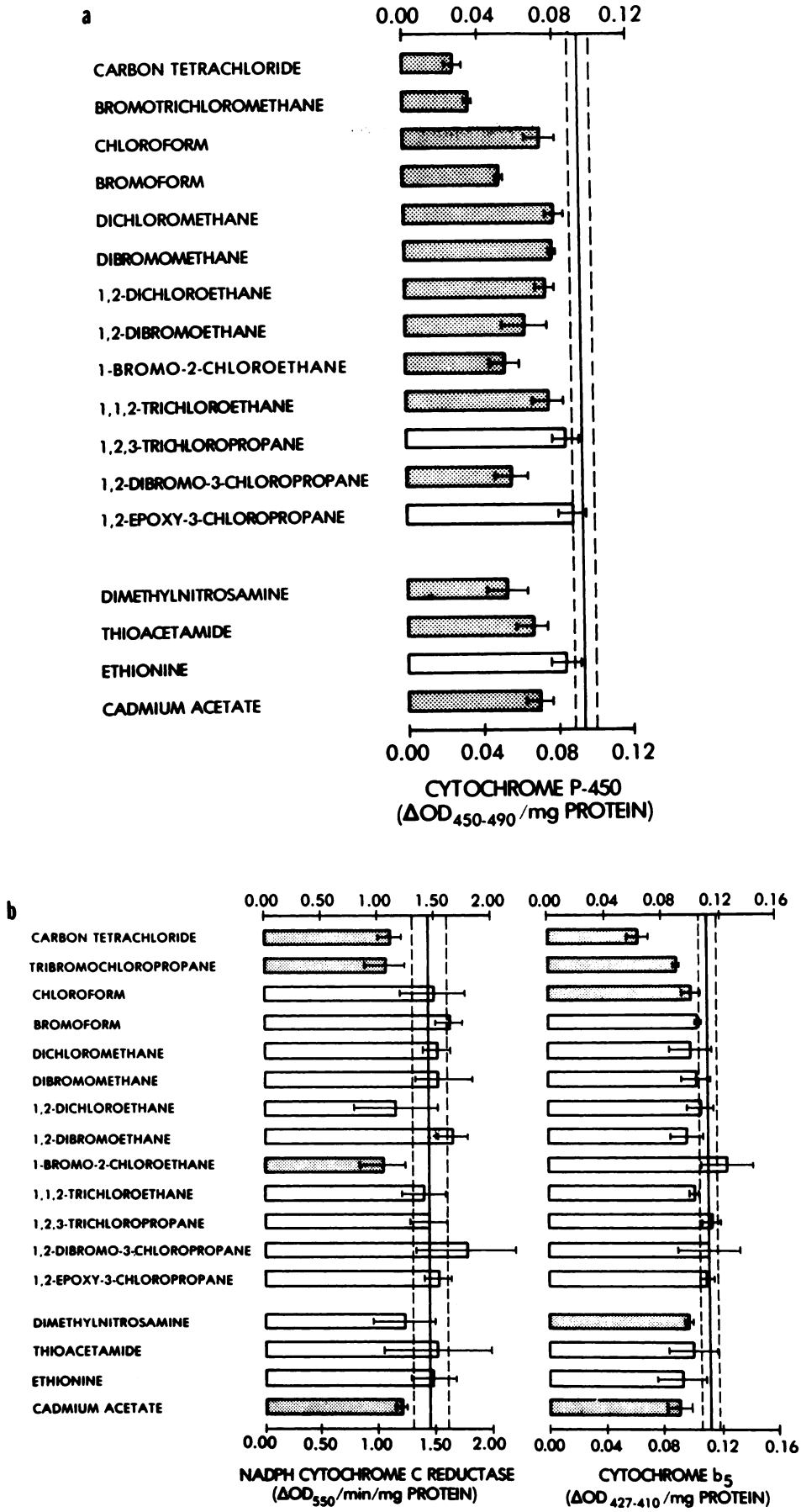

FIG. 2. *Cytochrome P-450, NADPH cytochrome c reductase, and cytochrome b_5 content of hepatic microsomes from rats treated with alkyl halides and other hepatotoxins*

Microsomes were prepared as described in Fig. 1 for the animals treated for 18 hr, and assays for (a) cytochrome P-450, (b) NADPH cytochrome *c* reductase, and cytochrome b_5 were performed as described under Methods. —— represents the mean control values ± 1 SD (- - -). *Stippled bars* show values significantly lower than control values ($p < 0.05$).

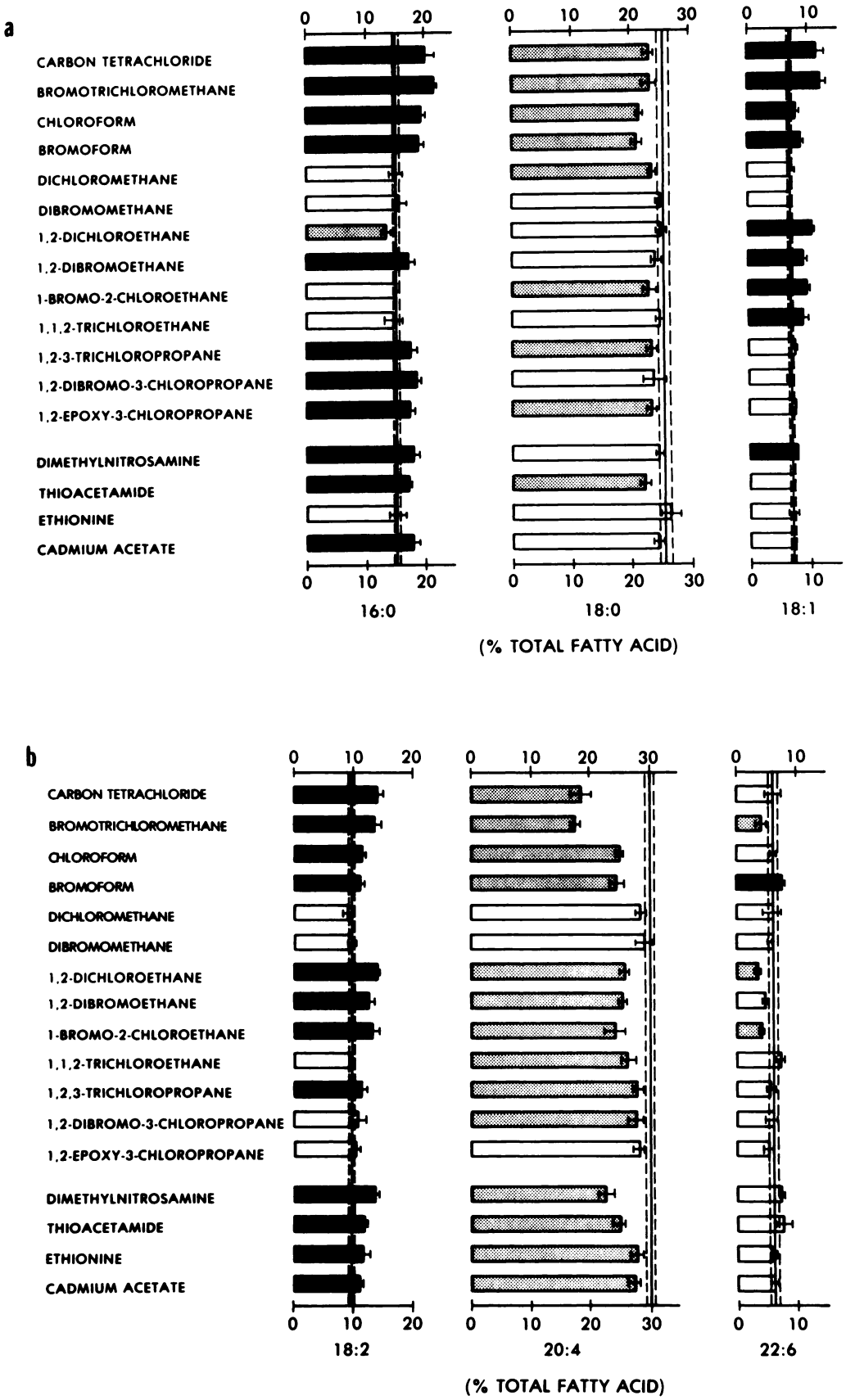

FIG. 3. *Relative content of fatty acids from lipid extracts of hepatic microsomes of rats treated with alkyl halides and other hepatotoxins*
Lipid extracts were prepared from the microsomes of animals treated for 18 hr as described in Fig. 1 and transesterified, and the fatty acids were determined by gas chromatography as described under Methods. The contents of (a) palmitic (16:0), stearic (18:0), and oleic acids (18:1), and (b) linoleic (18:2), arachidonic (20:4), and docosahexanoic acids (22:6) are expressed as percentages of total fatty acid recovered. —— represents mean control values ± 1 SD (- - -). *Stippled bars* represent values significantly higher (*dark*) or lower (*light*) than control values ($p < 0.05$).

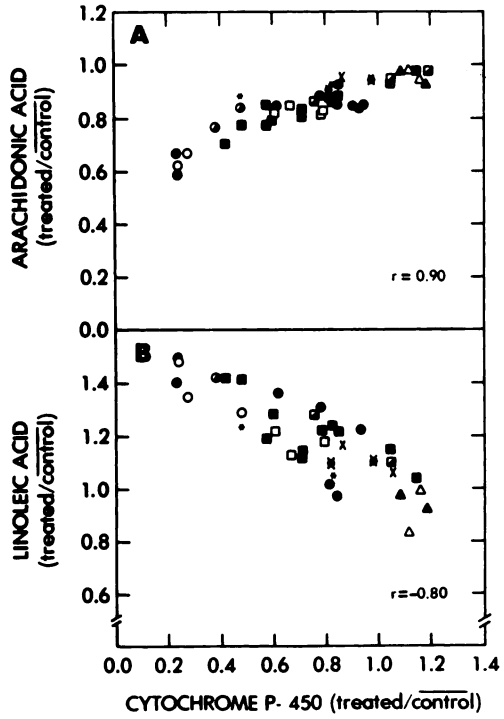

FIG. 4. *Scatter diagram of the changes in hepatic microsomal cytochrome P-450 versus the changes in (A) arachidonic acid and (B) linoleic acid*

Values from the individual treated rats (described in Figs. 2a and 3b) were expressed as a ratio to the mean control value. Hepatic microsomes were isolated from rats treated as described for Fig. 1 ($N = 51$). The corresponding treatments were as follows: carbon tetrachloride (○), bromotrichloromethane (●), chloroform (□), bromoform (■), dichloromethane (△), dibromomethane (▲), 1,2-dichloroethane (◎), 1,2-dibromoethane (◉), 1-bromo-2-chloroethane (◑), 1,1,2-trichloroethane (⊗), 1,2,3-trichloropropane (×), 1,2-dibromo-3-chloropropane (•), 1,2-epoxy-3-chloropropane (▣), dimethylnitrosamine (⊠), thioacetamide (⊡), ethionine (◪), and cadmium acetate (⊡).

and $CBrCl_3$), (b) those which do not, and (c) the four nonalkyl halides. We therefore wished to determine whether the correlations between the decrease in cytochrome P-450 and the respective decreases in arachidonic and linoleic acids would still be apparent when the data for rats treated with these three groups of chemicals were used in the calculations (Table 3). When the values for the four hepatotoxins DMN, TIAA, ETH, and $Cd(Ac)_2$ were omitted, the two correlation coefficients remained essentially the same. Consideration of these four hepatotoxins alone resulted in even higher degrees of correlation with the decreases in linoleic ($r = -0.88$) and arachidonic acids ($r = 0.97$). When the values for CCl_4 and $CBrCl_3$ were omitted, the degrees of correlation with the changes in linoleic ($r = -0.70$) and arachidonic acids ($r = 0.82$) were lower than those found when all of the compounds were considered. They were lower still ($r = -0.62$ and 0.75, respectively) when the values for CCl_4, $CBrCl_3$, and the four hepatotoxins were omitted. When the values for CCl_4 and $CBrCl_3$ were omitted from the correlations presented in Tables 1 and 2, a similar drop in the coefficient of 0.1 unit occurred in most cases (data not shown). Consideration of only the values for CCl_4 and $CBrCl_3$ resulted in coefficients ($r = -0.73$ and 0.94) that were similar to those found when all of the compounds were considered.

TABLE 1

Correlation coefficients for the comparison of changes in the content of cytochrome P-450, linoleic acid, and arachidonic acid with those in the content of other microsomal components of rats treated for 18 hr with alkyl halides and other hepatotoxins

The coefficients were determined from values plotted on scatter diagrams as shown in Fig. 4. The values in parentheses represent the 95% confidence limits of r ($n = 51$).

Component	Coefficient for comparison with		
	Cytochrome P-450	Linoleic acid	Arachidonic acid
Cytochrome P-450	—	−0.80[a] −(0.67–0.88)	0.90[a] (0.83–0.94)
Cytochrome b_5	0.90[a] (0.82–0.94)	−0.65[a] −(0.45–0.79)	0.86[a] (0.73–0.92)
NADPH cytochrome *c* reductase	0.76[a] (0.61–0.86)	−0.70[a] −(0.52–0.82)	0.73[a] (0.57–0.84)
Protein	0.89[a] (0.81–0.94)	−0.59[a] −(0.37–0.75)	0.81[a] (0.68–0.89)
RNA	0.43[a] (0.17–0.64)	−0.29 −(0.00–0.70)	0.08 (0.00–0.35)
Phospholipids	0.76[a] (0.60–0.85)	−0.51[a] −(0.01–0.53)	0.69[a] (0.51–0.82)
Diene conjugates[b]	−0.68[a] −(0.49–0.81)	0.63[a] (0.43–0.78)	−0.78[a] −(0.64–0.87)

[a] Coefficient is significantly different from zero ($p < 0.01$).

[b] Diene conjugate values for rats killed after 1 hr of treatment, which necessitated the comparison of mean values ($n = 17$).

Inclusion of the data from rats given CCl_4 and $CBrCl_3$ tends to bias the correlations of the changes in cytochrome P-450 with those in the other microsomal components. Since these compounds effected the greatest changes, omission of these data reduces the range of alterations (Table 4). This is reflected in a shift of the over-all mean changes in cytochrome P-450, linoleic acid,

TABLE 2

Correlation coefficients for the comparison of changes in the content of cytochrome P-450, linoleic acid, and arachidonic acid with those in the content of microsomal fatty acids of rats treated with alkyl halides and other hepatotoxins

Coefficients were determined from values plotted on scatter diagrams, as shown in Fig. 4. The values in parentheses represent the 95% confidence limits of r ($n = 51$).

Component	Coefficient for comparison with		
	Cytochrome P-450	Linoleic acid	Arachidonic acid
Palmitic (16:0)	−0.65[a] −(0.45–0.79)	0.48[a] (0.23–0.67)	−0.72[a] −(0.55–0.83)
Stearic (18:0)	0.46[a] (0.20–0.66)	−0.16 −(0.00–0.42)	0.36[a] (0.08–0.58)
Oleic (18:1)	−0.76[a] −(0.60–0.85)	0.78[a] (0.64–0.87)	−0.87[a] −(0.78–0.92)
Linoleic (18:2)	−0.80[a] −(0.67–0.88)	—	−0.81[a] −(0.68–0.89)
Arachidonic (20:4)	0.90[a] (0.83–0.94)	−0.81[a] −(0.68–0.89)	—
Docohexenoic (22:6)	0.01 (0.00–0.29)	−0.50[a] −(0.25–0.68)	−0.01 −(0.00–0.29)

[a] Coefficient is significantly different from zero ($p < 0.01$).

TABLE 3

Correlation coefficients for the comparison of changes in cytochrome P-450 content with those in linoleic acid and arachidonic acid content of microsomes isolated from rats treated with alkyl halides and other hepatotoxins

The coefficients were determined from values plotted on a scatter diagram, as shown in Fig. 4. Values for rats treated with CCl_4 and $CBrCl_3$, which cause lipid peroxidation, and with the four hepatotoxins DMN, TIAA, ETH, and $Cd(Ac)_2$, were omitted from the calculations or considered separately as indicated. The values in parentheses represent the 95% confidence intervals of the correlation coefficients.

Compounds considered	*N*	Coefficient for comparison with	
		Linoleic acid	Arachidonic acid
All compounds	51	−0.80[a] −(0.67–0.88)	0.90[a] (0.83–0.94)
Minus DMN, TIAA, ETH, $Cd(Ac)_2$	39	−0.82[a] −(0.67–0.90)	0.90[a] (0.69–0.89)
Minus CCl_4, $CBrCl_3$	45	−0.70[a] −(0.51–0.83)	0.82[a] (0.69–0.89)
Minus DMN, TIAA, ETH, $Cd(Ac)_2$, CCl_4, $CBrCl_3$	33	−0.62[a] −(0.34–0.80)	0.75[a] (0.54–0.87)
DMN, TIAA, ETH, $Cd(Ac)_2$	12	−0.88[a] −(0.55–0.97)	0.97[a] (0.89–0.99)
CCl_4, $CBrCl_3$	6	−0.73 −(0.00–0.99)	0.94[a] (0.16–0.99)

[a] Coefficient is significantly different from zero ($p < 0.01$).

and arachidonic acid from 0.75, 1.20, and 0.84 when all of the compounds are considered, to 0.81, 1.17, and 0.88 when CCl_4 and $CBrCl_3$ are omitted (Table 4). This skewing of the data toward the intersection of minimal relative change may be partly responsible for the loss in correlation. The small range of changes in the rats given CCl_4 and $CBrCl_3$ may preclude a useful assessment of the correlations found when these data are analyzed alone. However, the range of changes in the rats given the four hepatotoxins DMN, TIAA, ETH, or $Cd(Ac)_2$ approximates that in the entire group (Table 4) and may therefore be considered a reliable example.

DISCUSSION

It has been well established that CCl_4 intoxication results in alterations in the content and activity of numerous microsomal components. This widespread effect has been linked to the peroxidation of the microsomal polyunsaturated fatty acids, and most studies on the action of other alkyl halides have concerned their capacity to induce lipid peroxidation (7). However, it has become evident that specific components of hepatic microsomes (e.g., cytochrome P-450) are decreased after intoxication with certain alkyl halides and other hepatotoxins that do not cause a measurable peroxidation of the microsomal fatty acid (11, 14, 15, 30).

In the present study, rats received a single dose of one of thirteen short-chain (C_{1-3}) alkyl halides (x = chlorine, bromine) or four other hepatotoxins [DMN, TIAA, ETH, $Cd(Ac)_2$]. Only two of the treatments, CCl_4 and $CBrCl_3$, resulted in measurable peroxidation of microsomal lipids. These compounds also caused significant decreases in microsomal protein, phospholipid, cytochrome P-450, cytochrome b_5, NADPH cytochrome *c* reductase, stearic acid, and arachidonic acid within 18 hr after treatment. This pattern of response is consistent with previous findings concerning the capacity of alkyl halides to induce lipid peroxidation (5, 7, 17). The other alkyl halides tested have higher halocarbon bond-dissociation energies and would be less likely to be metabolized via homolytic cleavage of the halocarbon bonds, which is associated with lipid peroxidation.

Treatment with one other compound, DMN, also had a widespread effect on microsomal components. However, DMN differed from CCl_4 and $CBrCl_3$ in that it caused no peroxidation of microsomal fatty acids or loss of phospholipid, whereas a significantly lower amount of RNA was recovered from microsomes. This difference in the action of these compounds which have widespread effects on microsomes has also been documented from the difference in response of cytochrome P-450 (11) and microsomal polysomes to CCl_4 and DMN (31, 32).

Although the microsomal components responded differently to certain of the compounds, their responses to the majority showed two major similarities. There were a significant decrease in cytochrome P-450 (and to a lesser extent in cytochrome b_5) and a shift in the composition of microsomal fatty acid, primarily characterized by a decrease in arachidonic acid and an increase in linoleic acid. These three changes showed higher degrees of correlation with each other than with changes in any of the other microsomal components assayed. Furthermore, these correlations were also found when the data for the two alkyl halides that caused lipid peroxidation,

TABLE 4

Means and ranges of relative changes in cytochrome P-450, linoleic acid, and arachidonic acid, and slopes of scatter diagrams with cytochrome P-450 as the dependent variable and linoleic or arachidonic acid as the independent variable

Values for rats treated with the four hepatotoxins DMN, TIAA, ETH, and $Cd(Ac)_2$, or with CCl_4 and $CBrCl_3$, were omitted or considered separately as indicated.

Compounds considered	*N*	Cytochrome P-450		Linoleic acid			Arachidonic acid		
		Mean	Range	Mean	Range	Slope	Mean	Range	Slope
All compounds	51	0.75	0.20–1.14	1.20	0.95–1.50	−0.50	0.84	0.56–0.98	0.36
Minus DMN, TIAA, ETH, $Cd(Ac)_2$	39	0.72	0.20–1.05	1.19	0.95–1.50	−0.52	0.83	0.56–0.98	0.37
Minus CCl_4, $CBrCl_3$	45	0.81	0.38–1.14	1.17	0.95–1.44	−0.49	0.88	0.70–0.98	0.26
Minus DMN, TIAA, ETH, $Cd(Ac)_2$, CCl_4, $CBrCl_3$	33	0.81	0.38–1.05	1.14	0.95–1.44	−0.48	0.88	0.77–0.98	0.22
DMN, TIAA, ETH, $Cd(Ac)_2$	12	0.82	0.42–1.14	1.23	1.05–1.42	−0.47	0.86	0.70–0.98	0.34
CCl_4, $CBrCl_3$	6	0.24	0.20–0.28	1.45	1.31–1.50	−1.67	0.60	0.56–0.67	1.37

the remaining alkyl halides, and the four other hepatotoxins were considered separately.

It remains to be determined whether the changes in cytochrome P-450 and the composition of fatty acid are more than casually related. It is possible that they reflect similar susceptibilities to the reactive progeny of the compounds, but it is equally possible that they arise from an interrelationship between the cytochrome and fatty acid content of microsomes. We can envision two mechanisms for this interrelationship.

A decrease in arachidonic acid content is a well-known sequela to the peroxidation of microsomal fatty acids (16). It is possible that lipid peroxidation occurs after treatment with all of the alkyl halides and other hepatotoxins tested, but in all cases except CCl_4 and $CBrCl_3$ it is not detectable either by the induction of diene conjugates, as measured in the present study, or by the expiration of hydrocarbon chains (33). Indeed, Logani and Davies (34) believe that a decrease in arachidonic acid is the most sensitive indicator of peroxidation of microsomal lipids. In this instance, replacement from neutral lipid pools and the synthesis of monounsaturated fatty acids could account for the alterations in other fatty acids. The decrease in cytochrome P-450 might occur by the same, as yet unknown, mechanism by which it occurs after CCl_4 poisoning, which is intimately associated with lipid peroxidation. Should this prove to be the case, it remains to be explained why diene conjugates were not produced after treatment with $CHCl_3$, DMN, and TIAA, all of which caused considerable decrease in arachidonic acid content.

Alternatively, arachidonic acid may have decreased because of an inhibition of its synthesis from linoleic acid. Considering the strong correlation between this decrease and the increase in linoleic acid, this hypothesis is entirely consistent with our findings. Furthermore, Carreau and co-workers (35) recently found that treatment with CCl_4 inhibited the desaturase activity responsible for the synthesis of arachidonic acid. The decrease in cytochrome P-450 may follow, dependent upon the unknown mechanism by which polyunsaturated fatty acids are dietary requirements for the maintenance of its steady-state level (18–20).

As with the influence of diet on cytochrome P-450 content, alterations in microsomal lipid after treatment with alkyl halides and other xenobiotics may provide systems for studing the dependence of cytochrome P-450 on its lipid environment.

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Send reprint requests to: Dr. David E. Moody, Department of Pathology, University of California School of Medicine, San Francisco, Calif. 94143.