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MICROSOMAL EPOXIDATION OF CYCLODIENE INSECTICIDES*

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Abstract—Rabbit liver microsomes were shown to contain enzyme systems that converted heptachlor, aldrin, and isodrin into their corresponding epoxides. No other metabolites were produced. The epoxidases required $NADPH_2$ and oxygen. Epoxidation of heptachlor and aldrin was inhibited by SKF 525-A, piperonyl butoxide, parathion, and γ-BHC. Mutual inhibition between the two substrates as well as inhibition by epoxides was observed. Epoxidase activities were also found in rat liver microsomes and in insect homogenates. A model hydroxylation system (EDTA, Fe^{2+}, and ascorbate) did not simulate microsomal epoxidase. Metabolism of γ- and δ-pentachlorocyclohexene was similarly demonstrated with rabbit liver microsomes.

BIOLOGICAL epoxidation of a double bond is a rather rare reaction. It was first shown by Davidow and Radomski,[1] who found that heptachlor, a chlorinated cyclodiene insecticide (Fig. 1), was converted to heptachlor epoxide in the dog. The conversion of cyclodiene compounds to epoxides has been found also in other vertebrates,[2] in insects,[3,4] and in soil microorganisms;[5] reported epoxidation on plants[6,7] lacks experimental proof, since the epoxide could have been derived from soil microorganisms as well. No information is available on the nature of the enzymes involved in the conversion‡ although studies *in vitro* have been reported in two cases, one with insect tissues[8] and the other with cattle liver homogenates.[9] Epoxidation of steroids has been demonstrated with microorganisms,[10] with a bovine adrenal residue (5,000*g*) preparation,[11] and with liver slices.[12] Chang and Sih[13] reported the epoxidation of a steroid by a cell-free preparation from a mold and showed a close relationship between epoxidase and hydroxylase.

Epoxides have been suggested as intermediates in hydroxylation reactions.[1, 14, 15] Although liver microsomes effect hydroxylation and other types of oxidative detoxication,[16] epoxidation by such systems has never been reported. This paper describes the epoxidation of cyclodiene insecticides by liver microsomes.

MATERIALS AND METHODS

Chemicals.

Heptachlor (1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-meth-anoindene, m.p. 95°-96°) and heptachlor epoxide (1,4,5,6,7,8,8-heptachloro-2, 3-epoxy-

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‡ See addendum.

3a,4,7,7a-tetrahydro-4,7-methanoindan, m.p. 161°-163°) were supplied by Velsicol Chemical Corp. Aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene, m.p. 102°–103°); dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4,-*endo-exo*-5,8-dimethanonaphthalene, m.p. 175°–177°); isodrin (*endo-endo* isomer of aldrin, m.p. 200°–207° with partial decomposition); and endrin (*endo-endo* isomer of dieldrin, m.p. ca. 200° with decomposition) were obtained from Shell Chemical Co. γ-PCCH (γ-isomer of 1,3,4,5,6-pentachloro-1-cyclohexene, b.p. 108°–110° at 4 mm Hg) was prepared according to Nakajima *et al.*[17] δ-PCCH (m.p. 68°–69°) was supplied by Dr. H. D.

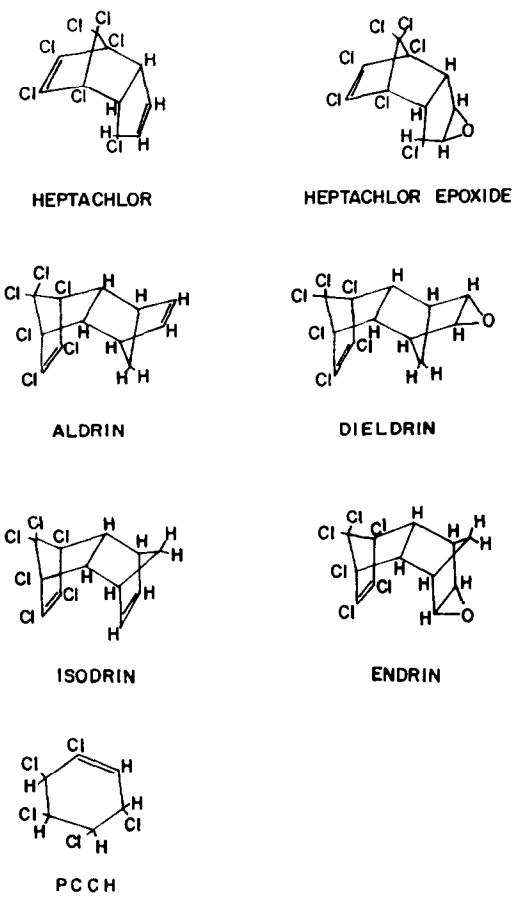

FIG. 1. Structures of substrates and products.

Orloff of Ethyl Corp. These compounds are illustrated in Fig. 1. The following chemicals were also used: SKF 525-A (β-diethylaminoethyl diphenylpropylacetate hydrochloride, from Smith Kline & French Laboratories); piperonyl butoxide (α-[2-(2-butoxyethoxy)-ethoxy]-4,5-methylenedioxy-2-propyltoluene, from Food Machinery & Chemical Corp., Fairfield Chemicals); parathion (*O,O*-diethyl *O-p*-nitrophenyl phosphorothioate, recrystallized, m.p. 6°, from Nutritional Biochemicals Corp.); γ-BHC (γ-isomer of 1,2,3,4,5,6-hexachlorocyclohexane, m.p. 112°-113°

from Hooker Chemical Corp.). All these chemicals, except SKF 525-A, were dissolved at 0·1 M in acetone containing 1% Triton X-100 (Rohm & Haas Co.), and aqueous dilutions were prepared shortly before experiments. SKF 525-A was used as an aqueous solution.

$NADPH_2$, NADP, $NADH_2$, and NAD were purchased from Sigma Chemical Co., and P–L Biochemicals, Inc.

Enzyme sources

Male albino rabbits and rats were fasted overnight, and liver microsomes were prepared by centrifuging 10% homogenates in hypertonic sucrose, according to Palade and Siekevitz.[18] Microsomal pellets were stored at about —15°, and shortly before experiments they were suspended usually in 0·25 M sucrose containing 0·01 M phosphate buffer, pH 7·4. When the volume of the suspension was made to that of the original homogenate, the suspension was designated 10% microsomes. For experiments with tissue homogenates, each tissue was homogenized in 0·25 M sucrose containing the phosphate buffer, and the homogenate was centrifuged at 600*g* for 5 min to remove large particles. Laboratory-cultured houseflies (CSMA strain), *Musca domestica* L., and American cockroaches, *Periplaneta americana* (L.), were the sources of insect enzymes.

Reaction procedures

A standard incubation mixture contained a substrate, 0·5 ml of an enzyme source and the following compounds (final concentrations indicated) to make a total volume of 2 ml: $NADPH_2$ (10^{-3} M), nicotinamide (0·01 M), KCl (0·15 M), Na_2HPO_4 (8×10^{-3} M), and KH_2PO_4 (2×10^{-3} M). The pH of the incubation mixture was 7·2 to 7·3. Incubation was carried out at room temperature (about 23°), and the reaction was stopped by addition of 2 ml of 10% trichloroacetic acid. Substrates and metabolites were then extracted with 5 or 10 ml of *n*-hexane by shaking vigorously on a wrist-action shaker for 1h. The extract was dried over anhydrous sodium sulfate for analysis.

The nonenzymatic model hydroxylation system of Udenfriend *et al.*[19] was modified to contain 0·5 ml of each of the following: 10^{-4} M substrate, 0·1 M sodium ascorbate, $2{\cdot}5 \times 10^{-2}$ M $FeSO_4$, 0·05 M EDTA, and 0·1 M sodium acetate. The reaction was started by addition of $FeSO_4$, and extraction was done in the same way as for the enzymatic system.

All incubations were done in duplicate. Since the water solubilities of the substrates were extremely low, the substrate concentrations given in the text do not refer to the true concentration of the substrates in solution, but to the overall concentrations.

Analyses

Samples in *n*-hexane were analyzed with a Jarrell-Ash model 700 gas chromatograph, equipped with an electron capture detector (100 mc tritium). *n*-Hexane extracted no materials that interfered with these analyses. At least two injections were made for each sample, and the average values were used for computations. Substrates and metabolites, except endrin, were estimated by referring to calibration curves relating concentration of the sample to peak height on the chromatogram. A 4-μ liter injection of 10^{-6} M aldrin solution served as the standard. Endrin was estimated by relating the peak area of the isomeric ketone, produced by thermal decomposition of endrin in the column,[20] to the peak height of the aldrin standard.

The following conditions were employed for gas-liquid chromatography (GLC): column, 4·4 mm (inside diameter) × 122 cm stainless steel tubing; solid support, 100/120 U.S. mesh silanized Chromosorb W; stationary phase, Dow Corning high-vacuum silicone grease (HVSG) 5%; HETP, 0·69 mm; carrier gas, prepurified nitrogen at 150 ml/min; temperatures, injector 200°, detector 195°–200°, column 200°; detector potential, 30 V. For the analysis of a composite sample containing heptachlor, heptachlor epoxide, aldrin, and dieldrin, column temperature was reduced to 180° and gas flow rate to 100 ml/min to obtain a satisfactory separation of the four compounds.

Protein nitrogen was estimated by a micro-Kjeldahl method.[21]

Identification of metabolites

For infrared spectroscopic identification, 40 ml of reaction mixture was incubated for 3 hr and extracted with *n*-hexane without addition of trichloroacetic acid. The extract was concentrated and chromatographed by thin-layer chromatography, with silica gel G as the adsorbent (Brinkmann Instruments, Inc.; prewashed with methanol) and *n*-hexane:carbon tetrachloride (1:1) as the solvent. Solutions of the substrate and its epoxide were spotted on each side of the plate. Spots of the reference compounds on the chromatogram were detected by the method of Mitchell.[22] The sample area was protected with aluminum foil. The silica gel layer corresponding to the epoxide spot was scraped off, and the adsorbed material was eluted with methanol. The solvent was evaporated, and the residue was taken up in spectroscopy-grade carbon tetrachloride for infrared analysis. Spectra were taken on a Beckman IR-5A infrared spectrophotometer by using microadapters.

For GLC identification, extracted samples were chromatographed on two or three of the following stationary phases: HVSG 5%, Apiezon N 5%, neopentylglycol succinate (NPGS) 2%, and SE-30 5% plus Epon 1001 0·5%.

RESULTS

Identification of metabolites

Rabbit and rat liver microsomes metabolized heptachlor and aldrin in the presence of $NADPH_2$. No reaction occurred when heated microsomes were used or the cofactor was omitted indicating the enzymatic nature of the reaction. GLC analyses showed single metabolite peaks with the same retention times (t_R) as those of the corresponding epoxides.

Metabolites formed by rabbit liver microsomes were recovered from thin-layer chromatograms at spots with the same R_f values as those of the epoxides. R_f Values were 0·21 for heptachlor epoxide and heptachlor metabolite, 0·10 for dieldrin and aldrin metabolite, 0·53 for heptachlor, and 0·57 for aldrin. Infrared spectra of the eluted metabolites proved that heptachlor and aldrin were converted into epoxides—i.e. heptachlor epoxide and dieldrin respectively (Fig. 2). Metabolites formed by rat liver microsomes from heptachlor and aldrin were also identified as the corresponding epoxides; the metabolites and authentic samples of the epoxides gave identical t_R values on three GLC stationary phases of different polarities; i.e. HVSG, Apiezon N, and NPGS. Rabbit microsomes also converted isodrin into its epoxide, endrin; the metabolite and endrin behaved identically on two different GLC stationary phases,

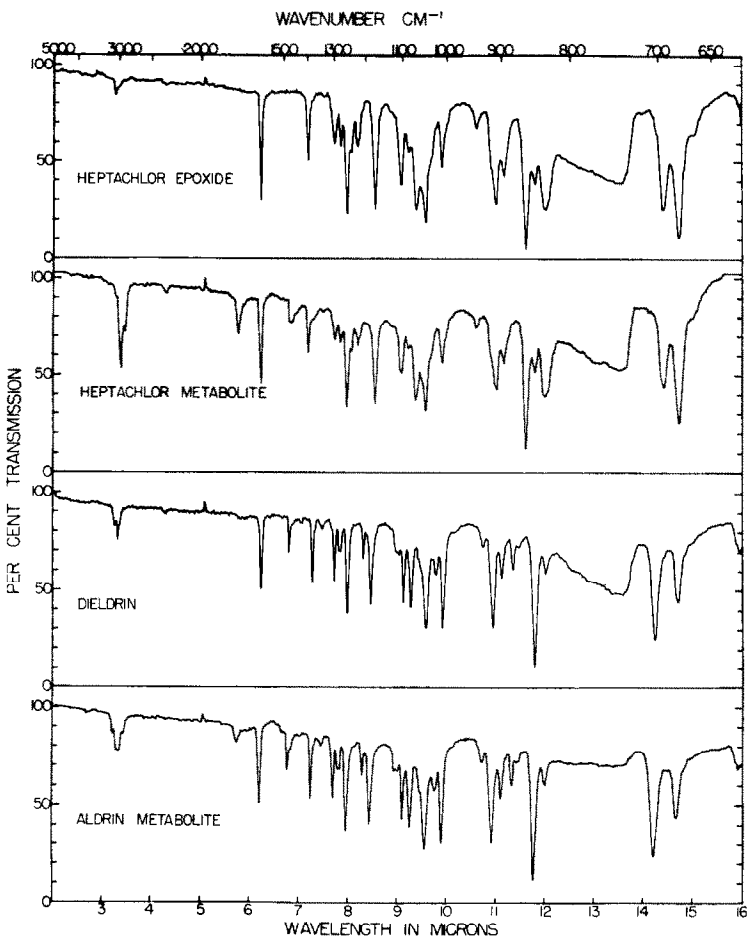

FIG. 2. Infrared spectra of epoxides and metabolites. Three-hour incubation of 10^{-6} mole heptachlor with rabbit liver microsomes (final concentration 10%) yielded $4{\cdot}50\times10^{-7}$ mole of metabolite in terms of heptachlor epoxide. Similarly, 10^{-6} mole of aldrin yielded $3{\cdot}95\times10^{-7}$ mole of metabolite in terms of dieldrin. The metabolites were isolated by thin-layer chromatography and i.r. spectra were taken. The solvent was carbon tetrachloride and the light-path was 0·05 mm. From the top down: authentic heptachlor epoxide; metabolite from heptachlor (subsequent GLC analysis showed that i.r. cell contained $1{\cdot}95\times10^{-7}$ mole of metabolite in terms of heptachlor epoxide); authentic dieldrin; metabolite from aldrin (i.r. cell contained $2{\cdot}08\times10^{-7}$ mole of metabolite in terms of dieldrin. Peaks at 1,460 cm^{-1}, 1,548 cm^{-1}, and ~3,000 cm^{-1} are due to impurities introduced during the processing of the metabolites.

HVSG and SE-30 plus Epon 1001. The t_R values for the standard compounds and metabolites are given in Table 1.

Heptachlor expoxide, dieldrin, and endrin were the only metabolites of heptachlor, aldrin, and isodrin with rabbit liver microsomes, since analyses of epoxidation systems showed that the amount of heptachlor, aldrin, or isodrin metabolized was equivalent to that of the corresponding epoxide formed. This is clearly illustrated by the time course of heptachlor epoxidation (Fig. 3). Neither heptachlor epoxide nor dieldrin was further metabolized by rabbit or rat liver microsomes in the presence or absence of cofactors.

TABLE 1. RETENTION TIMES FOR GAS-LIQUID CHROMATOGRAPHY OF CYCLODIENE COMPOUNDS, THEIR EPOXIDES AND METABOLITES

Gas-liquid chromatography (GLC) was performed by using the following stationary phases (column temperature and gas flow rate per minute as indicated): HVSG 5% (180°, 100 ml), Apiezon N 5% (200°, 150 ml), NPGS 2% (200°, 100 ml), and SE-30 5% plus Epon 1001 0·5% (200°, 150 ml).

Compound	t_R *(min)			
	HVSG	Apiezon N	NPGS	SE-30 + Epon 1001
Heptachlor	4·45	4·21	2·44	
Heptachlor epoxide and heptachlor metabolite (rat)	7·17	6·98	6·72	
Aldrin	5·65	5·61	2·52	
Dieldrin and aldrin metabolite (rat)	10·87	11·25	10·17	
Isodrin	2·83†			
Endrin and isodrin metabolite (rabbit)	5·63† (ketone) 8·23† (aldehyde)			9·00 (ketone) ‡

* Retention time.
† Temperature and gas flow rate were raised to 200° and 150 ml/min respectively.
‡ Aldehyde t_R was not determined because of diffuse appearance of the peak.

Distribution of epoxidases

Since the preceding results strongly supported the conception that the epoxidation was catalyzed by liver microsomal enzymes, the distribution of epoxidases in various rabbit tissues was examined. Ten per cent homogenates of tissues were prepared and incubated with heptachlor or aldrin in the system described in Experimental Procedure.

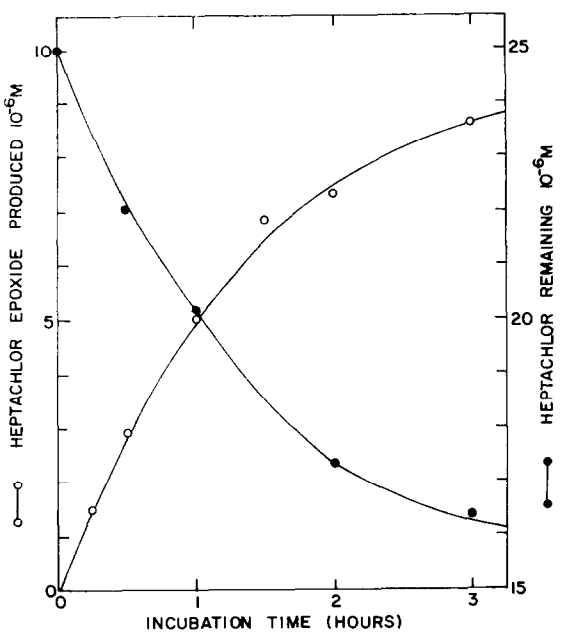

FIG. 3. Time course of heptachlor epoxidation. Rabbit liver microsomes (final concentration 1·25%) were incubated with $2·5 \times 10^{-5}$ M heptachlor. The heptachlor epoxide produced and the heptachlor remaining were determined at intervals.

The epoxidases were mainly located in the liver. Lung had a trace of activity toward heptachlor and was about 10% as active as liver toward aldrin. No epoxidase activities were detected in kidney, spleen, pancreas, heart, and brain.

Subcellular distribution of the epoxidase was checked with rabbit and rat liver homogentates, with heptachlor or aldrin as substrates (Table 2). Centrifugal fractionation was done according to Palade and Siekevitz,[18] and the heavy particulate fraction,

TABLE 2. SUBCELLULAR DISTRIBUTION OF LIVER EPOXIDASE ACTIVITIES

Ten per cent liver homogenates in hypertonic sucrose were fractioned by centrifugation into a heavy particulate fraction, microsomes, and supernatant according to Palade and Siekevitz.[18] The precipitates were resuspended in 0·88 M sucrose to the original volume. Supernatant of the rabbit preparation was further centrifuged for 1 hr at 105,000 *g* after 3·5 times dilution with distilled water. The precipitate was resuspended in 0·25 M sucrose to the original volume. Epoxidase activities were determined by incubating each fraction for 1 hr with $2{\cdot}5\times10^{-5}$ M heptachlor or aldrin.

Fraction	Protein N (mg/ml)	Epoxide production (10^{-9} mole/ml enzyme)		Specific activity (10^{-9} mole/mg protein N)	
		Heptachlor	Aldrin	Heptachlor	Aldrin
Rat					
Heavy particulate fraction	1·41	13·6	37·6	10	27
Microsomes	0·40	30·4	41·2	76	103
Supernatant	1·71	32·0	42·8	19	25
Rabbit					
Heavy particulate fraction	1·12	28·8	35·6	26	32
Microsomes	0·30	33·2	38·8	111	129
Supernatant	1·38	25·6	36·8	19	27
Supernatant centrifuged after dilution					
Precipitate	0·059	11·6	16·4	197	278
Supernatant	0·32	trace	trace	trace	trace

microsomes, and supernatant were assayed for epoxidase activity. Although microsomes had much higher specific activities than other fractions on a protein nitrogen basis, the total activities in the three fractions were about the same. Since the heavy particulate fraction contained a considerable amount of microsomes,[18] activities in this fraction were probably also due to microsomes. However, the high activities in the supernatant were not expected. Slight turbidity of the supernatant suggested that microsomes had not been sedimented completely because of the high density and viscosity of the hypertonic sucrose. Therefore, the supernatant of rabbit liver microsomes was recentrifuged after it was diluted 3·5 times to reduce the sucrose concentration to 0·25 M. Subsequent assays revealed that most activities had been sedimented, suggesting that the epoxidases were microsomal enzymes. Although the fraction sedimented from the diluted supernatant had higher specific activities than the microsomes obtained by the method of Palade and Siekevitz, the latter were used routinely for further studies.

Reported epoxidation of cyclodiene compounds in insects indicated the presence of similar insect enzymes. Fat body homogentates of female American cockroaches were incubated at a concentration of 10% for 2 hr with heptachlor ($2{\cdot}5\times10^{-5}$ M). About 17% of the substrate was epoxidized in the presence of $NADPH_2$ or $NADH_2$, but only 3% was epoxidized in the absence of cofactors. Heptachlor epoxidation was also detected with 2·5 and 5% homogenates of whole houseflies or fly abdomens.

In view of these results and the postulated relation of epoxides to the hydroxylation reaction, it seemed possible that the model hydroxylation system of Udenfriend *et al.*[19] would effect the epoxidation reaction. No epoxide, however, was detected in the model system, either in the presence or absence of ascorbate, after 1-hr incubation with any of the three substrates.

Properties of liver microsomal epoxidases

To find if the epoxidases belong to the same category as drug-oxidizing enzymes, the requirements for epoxidations *in vitro* were studied. Under anaerobic conditions only traces of epoxides were produced from heptachlor or aldrin by rabbit liver microsomes, indicating a requirement for oxygen. Table 3 shows the cofactor requirement by rabbit liver microsomes; $NADPH_2$ was the best cofactor for the epoxidation of all three substrates, heptachlor, aldrin, and isodrin. No epoxidation occurred

TABLE 3. COFACTOR REQUIREMENT OF MICROSOMAL EPOXIDATIONS

The 2-ml incubation mixture contained 0·5 ml of 10% rabbit liver microsomes, a substrate ($2·5 \times 10^{-5}$ M) and a cofactor (10^{-3} M). Other constituents are described in Experimental Procedure. The concentation of epoxide produced in 1 hr was determined with heptachlor, aldrin, or isodrin as the substrate. A part of the experiment was repeated by using once-washed microsomes.

Cofactor	Epoxide production 10^{-6} M		
	Heptachlor	Aldrin	Isodrin*
$NADPH_2$	7·04	6·58	10·1
NADP	0	0	0
$NADH_2$	1·81	3·80	4·7
NAD	trace	1·44	1·0
None	0	0	0
$NADPH_2$ with washed microsomes	5·10	5·50	
NAD with washed microsomes	0	0	0

* Gas-liquid chromatographic determination of isodrin epoxidation was not very accurate because of longer retention time of the decomposition products of endrin.

in the absence of cofactor or in the presence of NADP. NAD showed small but definite activities. When the microsomes were washed, however, NAD was no longer effective. This showed that only the reduced forms of the cofactors were effective. Perhaps NAD was partly reduced by the unwashed preparation of microsomes.

Figure 4 shows the relationship between pH and epoxidase activities. Optimum pH was about 7·1 for the epoxidation of aldrin and isodrin, and about 7·4 for heptachlor epoxidation. Since this suggested that different enzymes might be involved for different substrates, the effect of heptachlor and aldrin on each other's epoxidation was studied. Either substrate depressed the expoxidation of the other, indicating that enzymes catalyzing heptachlor epoxidation had affinity toward aldrin, and vice versa (Fig. 5). A part of the inhibitory effect on the epoxidation of one substrate was due to epoxide produced from the other substrate. An experiment, performed with a short incubation period (8 min) to minimize such by-product effects, however, showed that the inhibition due to the addition of aldrin was greater than could be explained by the dieldrin produced during the incubation (Table 4). The same was true when the substrate and inhibitor were reversed. Thus, the mutual inhibition was due partly to the other substrate and partly to the epoxide of the other substrate. Further experiments

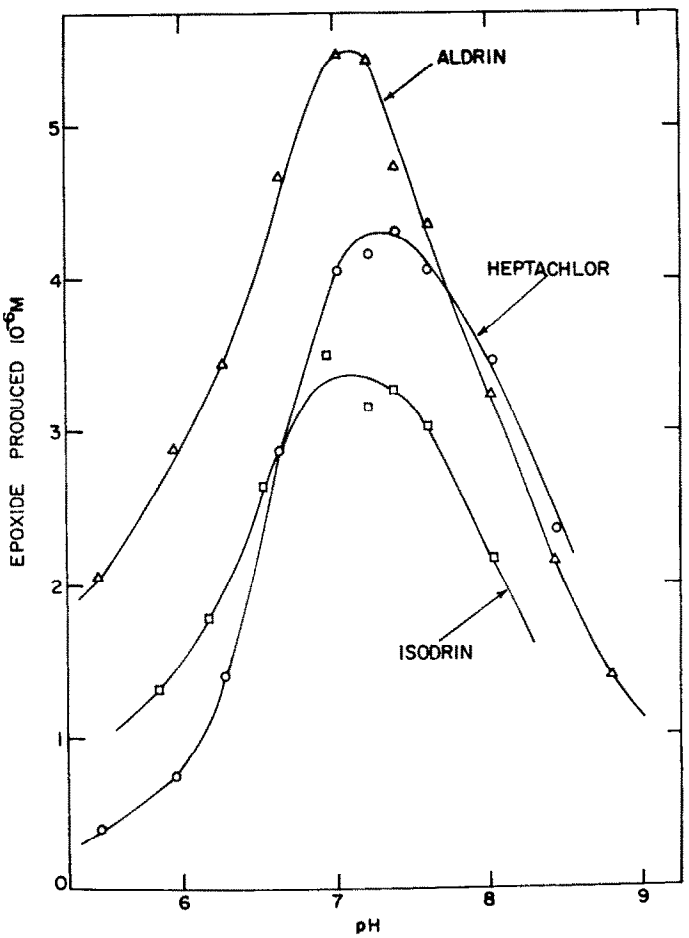

FIG. 4. Effect of pH on the epoxidation of heptachlor, aldrin, and isodrin. Rabbit liver microsomes (final concentration 1·25%) were incubated with $2{\cdot}5\times10^{-5}$ M substrate for 1 hr. Instead of phosphates, 0·5 ml of a buffer, composed of various proportions of 0·05 M borax and 0·1 M KH_2PO_4, was included in the 2-ml incubation mixture.

revealed that the epoxides produced during the incubations inhibited the epoxidation of the parent compounds also (Table 5).

Table 6 shows the inhibitory effect of SKF 525-A, piperonyl butoxide, parathion, and γ-BHC on the epoxidation of heptachlor and aldrin. SKF 525-A, a well-known inhibitor of microsomal oxidative enzymes, was particularly effective. The degree of inhibition of heptachlor epoxidation by this compound was constant in the substrate concentration range of $6{\cdot}3\times10^{-6}$ M to $2{\cdot}5\times10^{-5}$ M, indicating noncompetitive inhibition. However, this may not compare directly with similar results by others [23,24] since effective concentration of the substrates could not be estimated because of extremely low water solubilities. When a $2{\cdot}5\times10^{-5}$ M solution of heptachlor or aldrin was centrifuged at 127,000*g* for 1 hr at 20° to 25°, only traces remained in solution. Probably the rates of epoxidation were determined by the rates of dissolution of finely suspended crystalline substrates as well as by the true rates of reaction.

A brief study was made to see if compounds having an isolated double bond,

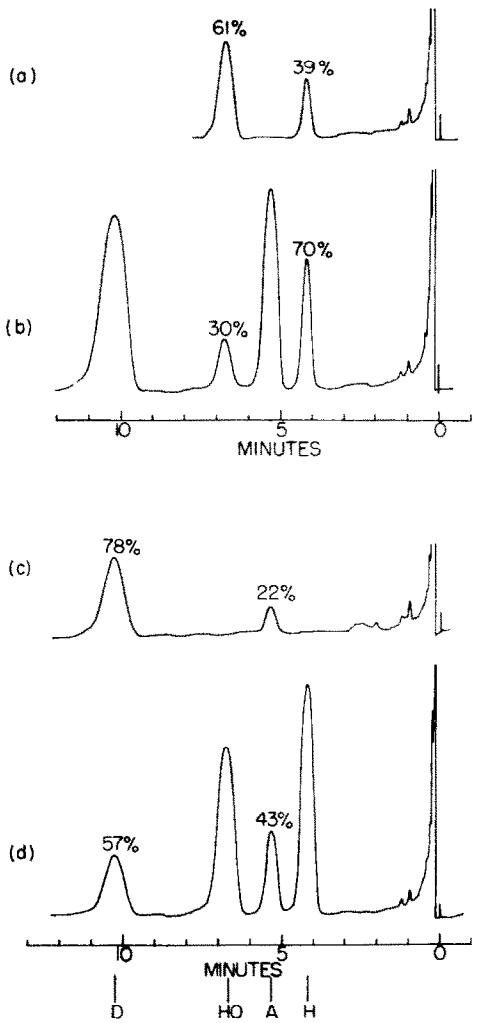

FIG. 5. Gas-liquid chromatograms showing mutual inhibition of epoxidation between heptachlor and aldrin. Rabbit liver microsomes (final concentration 2·5%) were incubated for 2 hr with (a) 5×10^{-6} M heptachlor, (b) 5×10^{-6} M heptachlor and $2{\cdot}5\times10^{-5}$ M aldrin, (c) 5×10^{-6} M aldrin, or (d) 5×10^{-6} M aldrin and $2{\cdot}5\times10^{-5}$ M heptachlor. H, HO, A, and D indicate position of the peaks for heptachlor, heptachlor epoxide, aldrin, and dieldrin respectively.

other than the cyclodiene insecticides, were also metabolized under the same conditions; γ- and δ-PCCH (5×10^{-6} M) were both readily metabolized (more than 50% in 1 hr) by 2·5% rabbit and rat liver microsomes in the presence, but not in the absence, of $NADPH_2$. Although Koransky *et al.* [25] suggested from their induction experiments that γ-BHC might be oxidized *in vivo* by liver microsomal enzymes in the rat, it was not metabolized by our microsomal preparations either in the presence of $NADPH_2$ or $NADPH_2$ plus glutathione, in contrast to the unsaturated counterpart, γ-PCCH. This result does not contradict entirely the suggestion mentioned above. Since γ-PCCH has been reported as a minor metabolite of γ-BHC in the housefly,[26] γ-BHC metabolism might involve an oxidative reaction at the γ-PCCH stage.

TABLE 4. EFFECT OF EPOXIDES IN THE MUTUAL INHIBITION OF EPOXIDATIONS

Rabbit liver microsomes (final concentration 1·25%) were incubated for 8 min, and the epoxides produced were determined. Concentrations were $2{\cdot}5 \times 10^{-6}$ M for heptachlor and aldrin and 4×10^{-7} M for heptachlor, epoxide and dieldrin.

Substrate	Inhibitor	Inhibition of epoxide production (%)	Inhibitor- or by-product-epoxide (10^{-7} M)
Heptachlor	Dieldrin	20	4·0 (dieldrin added)
Heptachlor	Aldrin	40	4·3 (dieldrin produced)
Aldrin	Heptachlor epoxide	14	4·0 (heptachlor epoxide added)
Aldrin	Heptachlor	30	3·3 (heptachlor epoxide produced)

TABLE 5. EFFECT OF EPOXIDES ON THE EPOXIDATION OF THE PARENT COMPOUNDS

Rabbit liver microsomes (final concentration 1·25%) were incubated in the standard incubation mixture containing an epoxide. The substrate remaining after 30 min was determined. The initial concentration of the substrates and epoxides was $2{\cdot}5 \times 10^{-6}$ M.

Substrate	Inhibitor	Substrate epoxidized (10^{-6} M)
Heptachlor	Heptachlor epoxide	0·73
	None	1·32
Aldrin	Dieldrin	1·04
	None	1·59

TABLE 6. EFFECT OF INHIBITORS ON MICROSOMAL EPOXIDATIONS

Rabbit liver microsomes (final concentration 2·5%) were incubated in the standard incubation mixture containing an inhibitor. The epoxide produced in 30 min was determined with heptachlor or aldrin as the substrate. The concentration of substrates and inhibitors was $2{\cdot}5 \times 10^{-5}$ M.

Inhibitor	Inhibition of epoxide production (%)	
	Heptachlor	Aldrin
SKF 525-A	82	64
Piperonyl butoxide	41	29
Parathion	21	33
γ-BHC	47	44

DISCUSSION

The demonstration of microsomal epoxidases in rabbit and rat liver adds another group of enzymes to the microsomal drug oxidation systems of the liver. Tissue distribution, requirements for reduced cofactors and oxygen *in vitro*, and subcellular distribution indicate that similar mechanisms are involved in epoxidation and in other drug oxidations, including hydroxylation. Epoxidation has been suggested as a possible intermediate step in hydroxylation reactions in animals.[1,14,15] However, rabbit microsomal preparations catalyzed only the epoxidation of cyclodiene compounds and the products stoichiometrically accounted for the substrates consumed.

No further reaction occurred with the epoxides. This result is more in line with the report by Chang and Sih[13] whose microbial cell-free preparation epoxidized androsta-4,9(11)-diene-3,17-dione, but hydroxylated the corresponding saturated steroid, in support of the hypothesis of Bloom and Shull.[10] Here, epoxidation and hydroxylation are two separate reactions dependent on the structure of the substrate, although enzyme systems for the two reactions are closely related. It might be significant that all demonstrated cases of epoxidation involve the reaction at an isolated double bond, while formation of epoxides postulated by Boyland and Sims[15] is for aromatic hydroxylation.

Close relation of the epoxidases to drug oxidation enzymes is also indicated by the inhibitor experiment. Piperonyl butoxide might be a rather general inhibitor of microsomal oxidative enzymes, although its mode of inhibition might be different from that of SKF 525-A. Sun and Johnson[27] indicated that the synergistic or antagonistic action of methylenedioxyphenyl compounds, such as piperonyl butoxide, on insecticidal actions was due to the inhibition of oxidative metabolism of various insecticides, including heptachlor and aldrin. Piperonyl butoxide also inhibited oxidation of parathion to its phosphate analog, paraoxon, by fat body microsomes of the American cockroach.[28] The inhibition by parathion might be competitive, since rabbit liver microsomes also oxidized parathion. Rubin *et al.* [29] recently reported that drugs oxidized by rat liver microsomes inhibited the microsomal oxidation of other drugs competitively, but those that were inert did not. Mutual inhibition between heptachlor and aldrin could also be due to such competition. The inhibitory effect of heptachlor epoxide, dieldrin, and γ-BHC, however, cannot be explained in such a manner since none of these was metabolized by microsomes. It might be that microsomal oxidative enzymes have affinity toward many structurally unrelated compounds whether they are metabolized or not. In this sense, the mutual inhibition does not necessarily indicate that both heptachlor and aldrin are epoxidized by the same enzyme system. There might be a group of epoxidases with different affinity toward the two substrates. This is also suggested by a small difference in pH optima for the epoxidation of the two substrates.

Boyland *et al.* [30] reported that rat liver microsomes and the model hydroxylation system of Udenfriend *et al.* [19] oxidized many aromatic compounds into the same products, while with other compounds there were marked differences in the products of the two systems. As an explanation for the differences they suggested that different mechanisms were involved in the two hydroxylation systems, epoxides being possible intermediates especially in the microsomal system. Our results also indicate a difference between the two systems. Cyclodiene compounds were epoxidized only by microsomes, and the model hydroxylation system did not simulate microsomal epoxidases.

ADDENDUM

A short communication by Wong and Terriere[31] that aldrin, isodrin, and heptachlor are converted to their respective epoxides by a rat liver microsomal system was brought to our attention after the manuscript was submitted for publication.

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