Biochemical Pharmacology, 1965, Vol. 14, pp. 263–272. Pergamon Press Ltd., Printed in Great Britain.

STUDIES OF THE POSSIBLE MECHANISMS BY WHICH CHLORDANE STIMULATES HEPATIC MICROSOMAL DRUG METABOLISM IN THE RAT*

LARRY G. HART and JAMES R. FOUTS

Department of Pharmacology, College of Medicine, State University of Iowa, Iowa City, Iowa, U.S.A.

(*Received* 28 *August* 1964; *accepted* 25 *September* 1964)

Abstract—Chlordane has been compared with phenobarbital in its stimulatory action on hepatic microsomal drug metabolism in the rat. Both agents cause increases in liver weight, microsomal protein, microsomal NADPH oxidase, and microsomal cytochrome CO-binding pigment. Both agents can effect stimulation of drug metabolism in adrenalectomized or hypophysectomized rats, and ethionine can block the enzyme stimulation by both agents in normal rats. The stimulatory actions of chlordane on microsomal drug metabolism do not add to those by phenobarbital. The actions of both chlordane and phenobarbital seems to be associated with a proliferation of smooth endoplasmic reticulum in the liver cell. Both agents appear to affect very similar mechanisms in inducing increases in enzyme activity.

MANY varieties of drugs and chemicals have been described which cause increases in the activity of the hepatic microsomal enzymes. These stimulatory effects have been variously described as "adaptive "or "induced" increases in activity of the drug-metabolizing enzymes. Stimulatory effects were first reported for 3,4-benzpyrene and other polycyclic hydrocarbons.[1–6] Subsequently. barbiturates and other drugs were shown to stimulate microsomal enzyme activity.[7–15]

More recently in our laboratory, chlordane, a chlorinated hydrocarbon insecticide, was shown to possess this stimulatory property.[16] Further work described these effects for chemically related chlorinated hydrocarbons.[17] DDT, a chlorinated insecticide not chemically related to chlordane, also was able to increase microsomal enzyme activity.[18]

Conney *et al.*[14] proposed that increased drug-metabolizing enzyme activity resulting from administration of stimulatory agents was due to increased *de novo* drug enzyme protein synthesis. However, the various agents could be divided into two groups with regard to their probable mechanisms of action: (1) the polycyclic hydrocarbons, and (2) phenobarbital and most other drugs that have this stimulatory property. Chlordane, and presumably the other related insecticides, appear to act through mechanisms more closely akin to the second group for several reasons. One is that chlordane is "nonspecific" in the variety of drug pathways that it stimulates, whereas the polycyclic hydrocarbons stimulate the metabolism of some compounds but not others.[14] Furthermore, metabolism of endogenous substrates, such as estradiol is accelerated

* This research was supported by United States Public Health Service Grants GM-06034 and CA-05648.

by chlordane,[19] but is essentially unaffected by the polycyclic hydrocarbons.[20, 21] The purpose of the work described in this paper was to elaborate on the similarities between chlordane and the nonspecific agents, with phenobarbital as the prototype, and to explore the probable mechanism(s) by which this insecticide exerts its stimulatory actions on the microsomal enzymes.

METHODS

Preparation of animals. Male Simonsen rats weighing 200–250 g were used. Chlordane, as the γ-isomer (98·91 $\pm$0·3% pure), was administered by i.p. injection in a dose of 25 mg/kg in corn oil daily for 3 days. Phenobarbital was injected i.p. in a dose of 40 mg/kg in distilled water twice daily for 3 days. In the experiments using ethionine and methionine, weanling rats weighing 60–90 g were used. DL-Ethionine and DL-methionine were dissolved by warming in 0·9% NaCl solutions. Ethionine and/or methionine in doses of 100, 150, or 200 mg/kg were injected i.p. into rats 15 min prior to an i.p. injection of 10 mg chlordane/kg. This treatment was administered once daily for 3 days. In other experiments, a fivefold larger dose of methionine, 500 mg/kg, was given concurrently with 100 mg ethionine/kg, and also equivalent doses, 200 mg/kg, of both agents were given by the s.c. route, while chlordane was given by the usual i.p. route. In all of these experiments, animals were sacrificed and their livers were excised for *in vitro* assays 16–18 hr after the third day's treatments.

For experiments on the effects of hypophysectomy or adrenalectomy, male rats, 150–200 g, were obtained, along with controls, from Hormone Assay Imports Inc., Chicago, Ill. Operated rats, bilateral adrenalectomy or hypophysectomy, were maintained on a lab chow diet supplemented with 1% NaCl and 5% glucose in the water, and were not used until at least one week after the operation.

Preparation of tissue samples. Livers were homogenized in the cold with a Potter homogenizer having a plastic pestle. Homogenates were prepared such that each gram of liver was suspended in 2 ml of cold isotonic (1·15%) KCl. For determination of the CO-binding pigment, a 10% homogenate was prepared. The supernatant fraction (9,000 *g*), containing microsomal and soluble enzymes, was prepared from the homogenate with a high-speed angle centrifuge (20 min) at 5°.

To sediment the microsomes, the supernatant was centrifuged for 60 min in a Spinco ultracentrifuge at 30,000 rpm (78,000 *g*). These microsomes were washed with KCl and resuspended with 0·1 M phosphate buffer, pH 7·4, so that 1 ml contained the microsomes from 1 *g*, 0·33 *g*, or 0·10 *g* of liver.

In vitro assays. For determinations of drug-metabolizing enzyme activity, 1 ml of liver supernatant fraction was incubated in a Dubnoff shaking incubator for 2 hr at 37° with oxygen as the gaseous phase. For the reductive pathway, the cell fractions were incubated under nitrogen for 1 hr at 37°. Final concentrations of the cofactors added were: triphosphopyridine nucleotide (NADP) ($5{\cdot}5 \times 10^{-5}$ M), glucose-6-phosphate (5×10^{-3} M), nicotinamide (2×10^{-2} M), and $MgSO_4$ (5×10^{-3} M). All concentrations are at or above optimal levels, and the final volume of all incubation mixtures was brought to 5 ml with 0·1 M phosphate buffer, pH 7·4.

The pathways studied and substrate concentrations in μmoles per 5 ml incubate were: the side chain oxidation of hexobarbital[22] (3 μmoles); the N-dealkylation of aminopyrine[23] (40 μmoles) to 4-aminoantipyrine; the oxidation of the ring sulfur of

chlorpromazine[24] (1 μmole); and the reduction of the aromatic nitro group of *p*-nitrobenzoic acid (12 μmoles) to *p*-aminobenzoic acid.[25] Disappearance of the substrate was measured to follow the metabolisms of hexobarbital and chlorpromazine. Appearance of the metabolite listed (4-aminoantipyrine, *p*-aminobenzoic acid) was determined in the other pathways studied.

The micro-Kjeldahl determination of total nitrogen[26] was used to estimate protein content of microsomes. The rate of oxidation of NADPH to NADP was determined by measuring the decrease in optical density at 340 mμ in a model DU Beckman spectrophotometer in a manner similar to that described by Gillette *et al.*[27] The cuvet used as a blank contained 2·8 ml of 0·05 M Tris buffer (pH 7·4) and 0·2 ml of the microsomal suspension equivalent to 200 mg of liver. The cuvet used for experimental samples contained 2·5 ml of Tris buffer, 0·3 ml of a NADPH solution (0·36 μmole) and 0·2 ml of the microsomal suspension.

Determination of the carbon monoxide (CO)-binding pigment of Klingenberg [28] was done after nonenzymatic reduction of the microsomal pigment with dithionite ($Na_2S_2O_4$). Stoppered reference and sample cuvets contained 3 ml of a microsomal suspension equivalent to 300 mg of liver. Nitrogen was bubbled through each cuvet for 30 sec, and reduction of the CO-binding pigment was effected with a few milligrams of solid $Na_2S_2O_4$. The contents of the experimental cell were saturated by bubbling CO through the suspension for 30 sec, and the CO-binding pigment content of the microsomes was estimated by measuring the optical density at 450 mμ in the Beckman spectrophotometer. Comparison was made against a cuvet containing microsomes treated in exactly the same way (treatment with nitrogen and dithionite) except that CO was not bubbled through.

Histological examination of liver tissue. Liver sections were routinely fixed in cold 10% neutral buffered formalin. The formalin was changed after 24 hr, and sections were stored in the cold. Tissues were embedded in paraffin, sectioned, and routinely stained with hematoxylin and eosin.

Statistical analysis of data. The statistical methods used are described by Snedecor.[29] The Student's "t" distribution was used to test the null hypothesis. The level of significance chosen for all determinations was $P \leqq 0{\cdot}05$. All values expressed in the tables are means plus or minus standard deviations. The number of animals represented by this figure is given in parentheses, unless otherwise indicated.

RESULTS

Effects of adrenalectomy or hypophysectomy on microsomal enzyme activity

These studies were done to determine whether the stimulatory effects of chlordane on drug metabolism might be mediated through the hormones of the pituitary or adrenal glands. The data in Table 1 show that removal of these glands does not prevent the stimulatory effects of chlordane on the metabolism of several drugs by the microsomal systems. These results concur with studies which showed that adrenalectomy or hypophysectory was unable to block stimulation by phenobarbital, chlorcyclizine,[15] and the polycyclic hydrocarbons.[1] It should be noted that adrenalectomy in particular depresses microsomal drug metabolism, as can be seen by comparing operated with nonoperated controls (Table 1). This has previously been reported by Remmer.[30, 31]

Effect of chlordane on liver weight and microsomal protein

Microsomes from livers of rats treated with 25 mg γ-chlordane/kg for 3 days were compared with microsomes from control livers for microsomal protein content. Microsomes were resuspended so that 1 ml of the final suspension contained the microsomes from 0·33 *g* of liver. It can be observed from Table 2 that there were significant increases in both microsomal protein and liver weight. It has been previously reported

TABLE 1. EFFECTS OF ADRENALECTOMY OR HYPOPHYSECTOMY ON STIMULATION BY CHLORDANE OF HEPATIC MICROSOMAL DRUG METABOLISM IN ADULT RATS*

Drug substrate	Chlordane	Corn oil	Chlordane	Corn oil
	Adrenalectomized†			
Hexobarbital	3·47 ± 0·39(4)‡	0·57 ± 0·17(3)	6·30 ± 0·70(3)‡	3·29 ± 0·24(3)
Aminopyrine	0·51 ± 0·03(4)‡	0·17 ± 0·03(4)	0·80 ± 0·04(3)‡	0·27 ± 0·00(3)
p-Nitrobenzoic acid	1·23 ± 0·41(4)‡	0·69 ± 0·10(4)	3·01 ± 0·30(3)‡	1·24 ± 0·09(3)
	Hypophysectomized†			
Chlorpromazine	1·27 ± 0·26(4)	0·86 ± 0·36(3)	1·65 ± 0·29(3)‡	1·14 ± 0·06(3)
Aminopyrine	0·75 ± 0·12(4)‡	0·16 ± 0·17(3)	1·47 ± 0·38(3)‡	0·39 ± 0·07(3)
p-Nitrobenzoic acid	1·55 ± 0·42(4)‡	0·59 ± 0·10(3)	2·65 ± 0·40(3)‡	0·74 ± 0·15(3)

* Values in the table indicate metabolism by supernatant fraction expressed as average μmoles drug metabolized ± standard deviation per g liver (wet wt.) in 2 hr (1 hr for *p*-nitrobenzoic acid). Numbers in parentheses represent numbers of animals per group.

† Operated animals (see Methods) and control animals were injected i.p. with γ-chlordane in corn oil, 25 mg/kg, daily for 3 days, enzyme activity being determined the following day.

‡ $P \leqq 0.05$ when compared to the respective corn-oil controls.

TABLE 2. EFFECTS OF CHLORDANE ON LIVER WEIGHT AND MICROSOMAL LIVER PROTEIN IN ADULT RATS

Pretreatment*	Total wet weight liver (g/100 g rat)	Microsomal liver protein (mg/g wet wt. liver)†	Total microsomal protein in liver (mg/100 g rat)‡
None (controls)	4·87 ± 0·30	47 ± 3	228
Chlordane	5·35 ± 0·18§	56 ± 3§	300§

* Adult male rats (260–320 g) were injected i.p. daily for 3 days with 25 mg γ-chlordane in oil/kg. Control received an equivalent volume of corn oil. Microsomes were prepared as described in the Methods section. There were 5 animals per group.

† Microsomal protein = mg nitrogen (Kjeldahl) × 6·25.

‡ Total liver weight/100 g rat × microsomal protein/g liver.

§ $P \leqslant 0.02$ as compared with control.

that phenobarbital[14] and the polycyclic hydrocarbons[6] also stimulate net synthesis of microsomal protein as well as liver growth.

Effects of chlordane on NADPH oxidase activity.

Table 3 shows that microsomes from livers of rats treated with 25 mg γ-chlordane/kg for 3 days metabolized NADPH to NADP at a markedly greater rate than did microsomes from control livers. These findings compare qualitatively with the effects of phenobarbital and chlorcyclizine treatment on NADPH oxidation in microsomes.[15]

Administration of 3-methylcholanthrene has little effect on NADPH metabolism.[20] These observations along with the present data provide further evidence that chlordane, and presumably the other chlorinated hydrocarbons, exerts its stimulatory actions by mechanisms similar to those of phenobarbital and like compounds.

TABLE 3. EFFECT OF CHLORDANE ON THE OXIDATION OF NADPH BY RAT LIVER MICROSOMES

Pretreatment*	Daily dose (mg/kg)	mμmoles NADPH metabolized/min by microsomes from 100 mg liver
Controls		17·0 ± 1·6
γ-Chlordane	25	40·9 ± 11·6†

*Adult male rats were injected i.p. with γ-chlordane, 25 mg/kg, daily for 3 days. The estimation of NADPH metabolism by liver microsomes was made by measuring the decrease in optical density at 340 mμ (see Methods). Values represent mean ± standard deviation with n = 4 (4 animals used in each group). Similar results were obtained in two additional experiments utilizing 9,000 *g* supernatant fraction as the source of enzyme.

† $P \leqq 0{\cdot}05$.

Effects of chlordane on the content of microsomal CO-binding pigment

Remmer[32] has demonstrated that repeated administration of phenobarbital to rats resulted in increases in the amount of two microsomal cytochromes, cytochrome b_5 and a second cytochrome which has been identified as a hemoprotein pigment that

TABLE 4. EFFECT OF CHLORDANE ON THE CONTENT OF CO-BINDING PIGMENT IN RAT LIVER MICROSOMES

Pretreatment*	Daily dose (mg/kg)	CO-Binding pigment in O.D. units/mg microsomal N
Control		122 ± 25(4)
γ-Chlordane	25	170 ± 40(4)†
Phenobarbital	80	341 ± 47(4)†

* Adult male rats were injected i.p. with γ-chlordane or phenobarbital for 3 days. Phenobarbital was given as two equal doses of 40 mg/kg daily. The CO-binding pigment was determined by measuring optical density at 450 mμ (see Methods). Numbers in parentheses represent number of animals per group.

† $P \leqq 0{\cdot}05$ when compared to control.

characteristically binds carbon monoxide.[28] The rise and fall of this CO-binding pigment preceded but paralleled the changes in activity of the drug-metabolizing enzymes after phenobarbital treatment.[32]

The data in Table 4 show that chlordane is able to increase significantly the amount of this pigment in rat liver microsomes. For purposes of comparison, measurements were also made after phenobarbital pretreatment.

Effects of chlordane and phenobarbital when given together as compared to the effects of either agent alone on microsomal metabolism

Gillette[33] observed that a combination of high doses of phenobarbital and 3,4-benzpyrene produced much greater increases in microsomal enzyme activity for the metabolism of acetanilide and monomethyl-4-aminoantipyrine than did these same doses of each agent when given by itself. Conversely, there were no additive effects seen on enzyme activity when 3,4-benzpyrene was given simultaneously with 3-methylcholanthrene.[33] These findings supported the view that the two hydrocarbons stimulate enzyme activity through the same mechanism, whereas phenobarbital and the hydrocarbons evoke their stimulatory effects through different mechanisms.

When optimal doses of chlordane and phenobarbital were administered concurrently for 3 days, the resultant enzyme activities for metabolism of hexobarbital and aminopyrine were no greater than the activities observed with either agent by itself

TABLE 5. STIMULATORY EFFECTS OF CHLORDANE AND PHENOBARBITAL ON HEPATIC DRUG-METABOLIZING ENZYME ACTIVITY IN THE RAT WHEN GIVEN ALONE OR IN COMBINATION

Treatment*	Dose (mg/kg)	Drug substrate† Hexobarbital	Aminopyrine
Control		4·45 ± 0·89	0·58 ± 0·16
γ-Chlordane	100	6·22 ± 0·54	2·82 ± 0·53
Phenobarbital	80	7·23 ± 1·01	2·45 ± 0·63
γ-Chlordane† plus phenobarbital	100 80	6·16 ± 0·97	2·71 ± 0·24

* Adult male rats received i.p. injections of drugs for 3 days with γ-chlordane being given as a single dose daily while the phenobarbital was divided into two equal daily doses of 40 mg/kg each.

† Values in table indicate metabolism by supernatant fraction expressed as average μmoles drug metabolized ± standard deviation per g liver (wet wt.) in 2 hr (45 min for hexobarbital). There were 3 animals per group.

(Table 5). This was felt to be further suggestive evidence that chlordane and phenobarbital were acting through similar mechanisms.

Effects of methionine on ethionine inhibition of stimulation of microsomal drug metabolism by chlordane

A previous report[16] from this laboratory showed that the stimulatory effects of chlordane on drug metabolism could be blocked by concurrent administration of ethionine. This inhibitory effect has been observed on stimulation by phenobarbital[14] and the polycyclic hydrocarbons,[1, 4] and in these cases the inhibition was overcome by simultaneous administration of the natural analog methionine. Thus, it was felt desirable to determine whether this reversal by methionine could be obtained in the present situation. In the experiments described in Table 6, administration of methionine failed to reverse the effects of ethionine.

The doses of ethionine used are not particularly toxic per se,[34] and inhibitory effects of higher doses were successfully reversed by Conney *et al.*[1, 3, 14] Histological studies of livers from the animals used in the study failed to detect any significant alterations. Even though the inhibition index of methionine with regard to ethionine is 10, a

TABLE 6. EFFECTS OF DL-ETHIONINE AND DL-METHIONINE ON STIMULATION BY CHLORDANE OF HEPATIC MICROSOMAL DRUG METABOLISM IN WEANLING RATS*

Dose of ethionine and/or methionine	Chlordane (10 mg/kg)	Chlordane + ethionine + methionine	Chlordane + ethionine	Ethionine + methionine	Ethionine	Methionine	Control
				Hexobarbital			
100	5·48 ± 0·50†	2·89 ± 0·70	2·72 ± 0·46	1·30 ± 0·44‡	1·47 ± 0·45‡	3·10 ± 1·01	2·93 ± 0·58
150	5·60 ± 0·51†	3·72 ± 0·51	3·55 ± 0·76	1·52 ± 0·68‡	1·03 ± 0·61‡	2·72 ± 0·20	2·89 ± 0·43
200	6·00 ± 0·54†	2·83 ± 0·67‡	3·41 ± 0·68	1·25 ± 0·43‡	1·99 ± 0·87‡	2·90 ± 0·19‡	4·22 ± 0·47
				Aminopyrine			
100	1·05 ± 0·27†	0·41 ± 0·11	0·35 ± 0·03	0·19 ± 0·06‡	0·16 ± 0·03‡	0·37 ± 0·21	0·36 ± 0·10
150	1·20 ± 0·51†	0·29 ± 0·16	0·46 ± 0·13‡	0·15 ± 0·03‡	0·15 ± 0·06‡	0·29 ± 0·03	0·26 ± 0·05
200	1·08 ± 0·43†	0·29 ± 0·10	0·39 ± 0·13	0·07 ± 0·03‡	0·17 ± 0·06‡	0·24 ± 0·06	0·36 ± 0·10

* Values in the table indicate metabolism by supernatant fraction expressed in average μmoles drug metabolized ± standard deviation per g liver (wet wt.) in 2 hr. Animals were treated with chlordane and/or ethionine and/or methionine for 3 days, with enzyme activity being determined the following day. Number of animals varied from 4 to 6 per group.

† $P \leqslant 0{\cdot}05$ when compared to any of the other groups in the table.

‡ $P \leqq 0{\cdot}05$ when compared with control.

fivefold larger dose of methionine was used in an experiment (500 mg/kg vs. 100 mg/kg) The inhibition index is defined as the ratio of the number of molecules of analog per molecule of metabolite required to give 50% inhibition. Reversal of ethionine inhibition was not achieved. Since the ethionine, methionine, and chlordane were all given by the same i.p. route, we felt that there might be some physical or chemical interference at the site of injection—e.g. chlordane or its vehicle might be preventing adequate absorption of methionine. Evidently this was not the case, since administration of ethionine and methionine by s.c. injection also did not result in reversal of ethionine inhibition.

It can be seen (Table 6) that administration of ethionine alone or in combination with methionine depressed drug metabolism significantly below control levels. Neubert[35] has reported significant depression with ethionine of hexobarbital and aminopyrine metabolism in male rats. However, his doses were somewhat larger and more protracted, i.e., 350 mg/kg orally for 8 days in the animals used to study hexobarbital metabolism, and 250 mg/kg orally for 5 days in the animals used to study aminopyrine metabolism. He also reported prolongation of sleeping times at larger doses.[36]

DISCUSSION

The proposal is made that the stimulatory effects of chlordane on hepatic microsomal drug metabolism are exerted through mechanisms similar to those by which phenobarbital and the other so-called nonspecific stimulatory agents exert their actions. The data presented tend to support this hypothesis.

The polycyclic hydrocarbons, including 3,4-benzpyrene and 3-methylcholanthrene, also stimulate hepatic microsomal enzyme activity. However, there are several important differences between the actions of the polycyclic hydrocarbons and chlordane. Some of these differences were cited in the introduction.

Phenobarbital is postulated to act by stimulating a net synthesis of new microsomal enzyme protein.[14] There are several lines of evidence which lend support to this postulate, and application of these same types of study to chlordane suggests a phenobarbital-like action for this compound. The similarities between chlordane and phenobarbital will now be considered.

First, the amino acid antagonist, DL-ethionine, has been shown to block the increases in microsomal enzyme activity caused by chlordane.[16] Enzyme activity in chlordane-treated rats was held at control levels by concurrent administration of ethionine. The ethionine block of enzyme stimulation by phenobarbital can be overcome with methionine, but in the present studies on chlordane, administration of methionine did not counteract the inhibition by ethionine. There is, at present, no explanation for this.

Secondly, treatment of rats with chlordane resulted in an increase in liver weight and in microsomal protein per gram liver. Such increases in liver weight and in microsomal protein do not necessarily mean there is an increased net synthesis of enzyme protein, but might be expected if such was occurring.

The drug-metabolizing enzymes were shown to be stimulated only when chlordane was administered *in vivo*.[16] Adding chlordane to microsomes *in vitro* does not stimulate drug-metabolizing enzyme activity. The same findings are true with phenobarbital.[14]

Chlordane, like phenobarbital, stimulated the enzyme system in microsomes which metabolizes NADPH to NADP. Others have shown that chlordane stimulates conversion of estradiol to polar metabolites,[19] an action also possessed by phenobarbital.[20]

Further, chlordane increased the microsomal content of the CO-binding pigment. Recent investigations[37, 38] have shown a marked increase in this pigment after phenobarbital treatment, with the time course of increases in pigment occurring prior to but parallel with increases in drug-metabolizing enzyme activity.

Adrenalectomy or hypophysectomy does not alter the ability of chlordane to stimulate microsomal enzyme activity, although these oblations do lower the "control" levels of enzyme activity from which stimulation can begin. This would suggest that the stimulatory effects of chlordane, like those of phenobarbital, are not hormonally mediated.

Finally, chlordane and phenobarbital do not appear to have any additive effects on drug metabolism when given together, as contrasted with additive effects between phenobarbital and the polycyclic hydrocarbons.[31]

All these observations provide evidence that chlordane stimulates drug-metabolizing enzyme activity through mechanisms quite similar if not identical with those by which phenobarbital and like drugs exert their effects. These findings also constitute suggestive evidence that the subcellular mechanism may be an actual increase in enzyme protein. Conclusive evidence awaits the actual solubilization, purification, and measurement of the drug-metabolizing enzyme protein.

Most of these studies are biochemical in nature and do not provide information on the morphologic alterations that may occur at the subcellular level in the hepatic cell in response to these stimulators. Using the electron microscope, Remmer and Merker[39] showed that phenobarbital caused a proliferation of smooth-surfaced endoplasmic reticulum (SER) in the hepatic parenchymal cell. Fouts[40] had previously reported that a variety of the drug-metabolizing enzymes were primarily localized in the SER or at least in the microsomes derived from this membrane system. Ortega[41] reported that chronic feeding of DDT produced a considerable increase in the amount of SER with, conversely a partial displacement of rough-surfaced reticulum. Studies in our laboratory[18] have shown stimulatory effects of DDT on drug metabolism after both acute and chronic administration, with the subsequent hypothesis that the enhancement of microsomal enzyme activity results from an increase in SER. Histologically, alterations in the hepatic cell caused by chlordane and related chlorinated insecticides are very similar to those observed with DDT.[42] Fouts and Rogers[43] in our laboratory have recently shown with the electron microscope that acute administration of chlordane (25 mg/kg for 3 days) produces a proliferation of the SER which appears to be very similar to that seen with phenobarbital.

Thus, if both biochemical and morphologic evidence is considered, it is reasonable to hypothesize that chlordane acts by causing an increase in the amount of SER in the hepatic cell with a consequent increase in the amount of the enzymes metabolizing the drugs studied.

Whether such a biochemical and morphological response to chlordane (or phenobarbital) can be considered to be a hepatotoxic effect of chlordane (or phenobarbital) or is rather, a response to the toxic actions of these chemicals, requires further study.

Acknowledgements—The authors wish to acknowledge the excellent technical assistance of Mrs. Roberta Pohl, Mrs. Diana Lewiston, and Mrs. Barbara Purdie.

REFERENCES

1. A. H. CONNEY, E. C. MILLER and J. A. MILLER, *Cancer Res.* **16**, 450 (1956).
2. A. H. CONNEY, E. C. MILLER and J. A. MILLER, *J. biol. Chem.* **228**, 753 (1957).
3. A. H. CONNEY, J. R. GILLETTE, J. K. INSCOE, E. G. TRAMS and H. S. POSNER, *Science* **130**, 1478 (1959).
4. J. W. CRAMER, J. A. MILLER and E. C. MILLER, *J. biol. Chem.* **235**, 250 (1960).
5. J. K. INSCOE and J. AXELROD, *J. Pharmacol. exp. Ther.* **129**, 128 (1960).
6. J. C. ARCOS, A. H. CONNEY and N. P. BUU-HOI, *J. biol. Chem.* **236**, 1291 (1961).
7. H. REMMER, *Naturwissenschaften* **45**, 189 (1958).
8. H. REMMER and B. ALSLEBEN, *Klin. Wschr.* **36**, 332 (1958).
9. H. REMMER, Naunyn-Schmiedeberg's, *Arch. exp. Path. Pharmak.* **235**, 279 (1959).
10. *Ibid.* **236**, 7 (1959).
11. *Ibid.* **237**, 296 (1959).
12. A. H. CONNEY and J. J. BURNS, *Nature (Lond.)* **184**, 363 (1959).
13. A. H. CONNEY and J. J. BURNS, *Ann. N.Y. Acad. Sci.* **86**, 167 (1960).
14. A. H. CONNEY, C. DAVISON, R. GASTEL and J. J. BURNS, *J. Pharmacol. exp. Ther.* **130**, 1 (1960).
15. A. H. CONNEY, I. A. MICHAELSON and J. J. BURNS, *J. Pharmacol. exp. Ther.* **132**, 202 (1961).
16. L. G. HART, R. W. SHULTICE and J. R. FOUTS, *Toxicol. Appl. Pharmacol.* **5**, 371 (1963).
17. J. R. FOUTS, *Ann. N.Y. Acad.* Sci. **104**, 875 (1963).
18. L. G. HART and J. R. FOUTS, *Proc. Soc. exp. Biol. (N.Y.)* **114** 388 (1963).
19. A. H. CONNEY, K. SCHNEIDMAN, M. JACOBSON and R. KUNTZMAN, *Ann. N.Y. Acad. Sci.* In press (1964).
20. A. H. CONNEY, in *Metabolic Factors Controlling Drug Action*, p. 250, Ed. B. B. Brodie and E. G. Erdös. Pergamon Press, London (1962).
21. A. H. CONNEY and A. KLUTCH, *J. Biol. Chem.* **238**, 1611 (1963).
22. J. R. COOPER and B. B. BRODIE, *J. Pharmacol. exp. Ther.* **114**, 409 (1955).
23. B. B. BRODIE and J. AXELROD, *J. Pharmacol. exp. Ther.* **99**, 171 (1950).
24. N. P. SALZMAN and B. B. BRODIE, *J. Pharmacol. exp. Ther.* **118**, 46 (1956).
25. J. R. FOUTS and B. B. BRODIE, *J. Pharmacol. exp. Ther.* **119**, 197 (1957).
26. J. TODD, A. SANFORM and B. WELL, *Clinical Diagnosis by Laboratory Methods*, 12th ed. Saunders, Philadelphia (1953).
27. J. R. GILLETE, B. B. BRODIE and B. N. LA DU, *J. Pharmacol. exp. Ther.* **119**, 532 (1957).
28. M. KLINGENBERG, *Arch. Biochem.* **75**, 376, 1958.
29. G. W. SNEDECOR, *Statistical Methods*, 5th ed. Iowa State College Press, Ames (1956).
30. H. REMMER, *Arch. exp. Path. Pharmak.* **233**, 184 (1958).
31. H. REMMER, *Naturwissenschaften* **45**, 522 (1958).
32. H. REMMER, *Ann. N.Y. Acad. Sci.* In press (1964).
33. J. R. GILLETTE, *Advances in Enzyme Regulation*, p. 215, Ed. G. Weber. Pergamon Press, London (1963).
34. J. M. FUJIMOTO and G. L. PLAA, *J. Pharmacol. exp. Ther.* **131**, 282 (1961).
35. D. NEUBERT, *Arch. exp. Path. Pharmak.* **232**, 235 (1958).
36. D. NEUBERT and D. MAIBAUER, *Arch. exp. Path. Pharmak.* **235**, 291 (1959).
37. R. REICHERT and H. REMMER, *Arch. exp. Path. Pharmak.* **247**, 374 (1964).
38. S. ORRENIUS and L. ERNSTER, *Biochem. biophys. Res. Commun.* **16**, 60 (1964).
39. H. REMMER and H. J. MERKER, *Klin. Wschr.* **41**, 276 (1963).
40. J. R. FOUTS, *Biochem. biophys. Res. Commun.* **6**, 373 (1961).
41. P. ORTEGA, *Fed. Proc.* **21**, 306 (1962).
42. P. ORTEGA, W. J. HAYES and W. F. DURHAM, *Arch. Path.* **64**, 614 (1957).
43. J. R. FOUTS and L. A. ROGERS, *J. Pharmacol. exp. Ther.* In press (1964).