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THE EFFECTS OF ALIPHATIC HALOGENATED HYDROCARBONS ON HEPATIC DRUG METABOLISM*†

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Abstract—Pretreatment of rats with CCl_4 prolonged the sleeping times evoked by hexobarbital. The metabolism of hexobarbital by the isolated perfused rat liver was reduced after intoxication with CCl_4 and may have been responsible, in part, for the enhanced action of the barbiturate. Poisoning with CCl_4 impaired the oxidation of hexobarbital and aminopyrine as well as the reduction of *p*-nitrobenzoic acid by liver microsomes. The activity of the drug-metabolizing enzymes declined to about 10 per cent of normal within 8 hr and remained at low levels 24 hr after the administration of CCl_4. The return of enzyme activity to normal levels started coincident with the disappearance of CCl_4 from the liver and required about 8 days for completion. In contrast to the striking toxicity of carbon tetrachloride, drug metabolism was not reduced after the administration of methylene chloride, and chloroform evoked only a moderate impairment of aminopyrine demethylation.

IT IS KNOWN that exposure of animals to certain chlorinated hydrocarbons, such as carbon tetrachloride, produces a fatty liver and hepatocellular necrosis.[1, 2] The endoplasmic reticulum is probably one of the first cellular organelles of the liver to be damaged by intoxication with CCl_4. For example, a dissolution of the endoplasmic reticulum is the only damage visible in electron micrographs of the liver taken 1–2 hr after the administration of CCl_4 to the animal.[3–5] The administration of CCl_4, moreover, causes a reduction of activity of certain enzymes derived from the endoplasmic reticulum. It has been reported, for example, that glucose 6-phosphate phosphatase activity,[6] the metabolism of aminopyrine[7] and the incorporation of amino acids into microsomal protein[8, 9] are depressed as a result of CCl_4 intoxication. Since the metabolism of a large number of drugs is catalyzed by microsomal enzymes,[10, 11] the studies reported here were undertaken to investigate the impairment of the hepatic metabolism of drugs induced by the administration of CCl_4, $CHCl_3$ and CH_2Cl_2. The effects of halogenated hydrocarbons on the oxidative and reductive metabolism of drugs were studied in the intact animal, in the isolated perfused rat liver and in hepatic microsomes.

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METHODS

Male Sprague–Dawley rats* weighing 200–350 g were used in these experiments. The animals were allowed free access to food and water at all times.

Carbon tetrachloride (2·5 ml/kg body wt.) was administered by gastric intubation. In related experiments, chloroform and methylene chloride were administered similarly in doses equimolar to that of CCl_4. In certain cases, $^{14}CCl_4$† was added to carrier CCl_4 so that each rat received $2{\cdot}1 \times 10^7$ dpm/2·5 ml CCl_4/kg body weight.

Duration of action of hexobarbital. The duration of action of hexobarbital was measured in normal rats and in rats receiving CCl_4. The rats were given hexobarbital i.p. and the time between injection of drug and the return of the righting reflex was recorded.

Metabolism of drugs in the isolated perfused liver. The metabolism of hexobarbital was studied also in the isolated perfused rat liver preparation. Details of the perfusion procedure[12] and apparatus[13] have been published previously. The perfusion medium consisted of 33 ml defibrinated rat blood, 500 i.u. heparin and sufficient Krebs–Henseleit bicarbonate buffer, pH 7·4,[14] to make a final volume of 100 ml. Hexobarbital was added to the medium in the recycling system after equilibration.

Preparation of microsomes. The rats were killed by cervical dislocation. Their livers were removed and chilled immediately on crushed ice. All subsequent manipulations were carried out at 2–4°. The livers were homogenized in a motor-driven teflon-glass homogenizer with 3 ml isotonic KCl (1·15%) per g tissue. The homogenate was centrifuged at 10,000 *g* (av.) for 30 min in an International model HR-1 centrifuge (rotor No. 856). The supernatant fraction was then centrifuged at 105,000 *g* (av.) for 1 hr in a Spinco model L preparative ultracentrifuge (rotor No. 40) The supernatant phase (soluble fraction) from the second centrifugation was removed and the microsomal pellet was then homogenized with sufficient 1·15% KCl to give a volume of 12 ml. Aliquots of this microsomal preparation were used for the assay of enzymatic activity and for the estimation of protein content.

Enzyme assays. The microsomes (1·0–1·5 ml, obtained from 250–375 mg liver) were incubated with 1·7 ml of 0·2 M potassium phosphate buffer (pH 7·4) containing glucose 6-phosphate (50 μmole) and NADP (0·5 μmole), 1·0–1·5 ml of soluble fraction (obtained from the livers of normal rats), nicotinamide (100 μmole), and semicarbazide (60 μmole). One of the following drugs was added to this mixture: aminopyrine, 5 μmole; hexobarbital, 1·9 μmole; or *p*-nitrobenzoic acid, 10 μmole. The incubation mixture was adjusted to a final volume of 6 ml with distilled water. The reaction mixtures which contained aminopyrine or hexobarbital were incubated in a Dubnoff metabolic shaker for 30 min at 37° under an atmosphere of oxygen. Those containing *p*-nitrobenzoic acid were incubated under nitrogen.

The rate of metabolism of hexobarbital was measured by estimation of the disappearance of substrate.[15] The reduction of *p*-nitrobenzoic acid was determined by measurement of the *p*-aminobenzoic acid which was formed during the incubation.[16] The rate of demethylation of aminopyrine was measured by estimation of the amount of formaldehyde formed. Formaldehyde was estimated by the Nash procedure;[17] the dye which was formed in this reaction was extracted into isoamyl alcohol and was assayed in a spectrophotometer.

* Obtained from the Holtzman Co., Madison, Wis.

† Obtained from New England Nuclear Corp.

Estimation of protein. Microsomal protein was measured by the biuret procedure.[18] Interfering materials were removed from the reaction mixture by extraction with a mixture of ether and ethanol.[19]

Measurement of $^{14}CCl_4$. The distribution of $^{14}CCl_4$ among hepatic cellular components was measured as follows: livers were homogenized with 2 ml of 0·25 M sucrose/g tissue. The soluble fraction and the microsomal fraction were prepared by differential centrifugation of the liver homogenate, as was described above. Aliquots of these preparations (3·0–6·0 ml) were pipetted into glass-stoppered centrifuge tubes containing 20 ml toluene. The tubes were shaken for 45 min and were centrifuged. Fifteen ml of the organic phase was transferred to a vial containing 2 ml phosphor (liquifluor concentrate*). The radioactivity was measured in a liquid scintillation spectrometer. When $^{14}CCl_4$ was added to liver homogenates, more than 85 per cent of the radioactivity was extracted into the toluene. The radioactivity which was extractable by toluene was assumed to be unchanged CCl_4. Since 85 per cent of the CCl_4 administered to rats is excreted unaltered into the expired air,[20] the results obtained with this method should reflect accurately the levels of CCl_4 in the liver.

RESULTS

Disposition of $^{14}CCl_4$ in liver. The concentration of $^{14}CCl_4$ in liver was at a maximum 2 hr after administration of the chlorinated hydrocarbon to the animal (Table 1);

TABLE 1. $^{14}CCl_4$ LEVELS IN RAT LIVER*

	CCl_4 (μg/g liver)					
Tissue fraction	1 hr	2 hr	3 hr	6 hr	12 hr	24 hr
Homogenate	544 ± 147	663 ± 80	484 ± 207	440 ± 82	253 ± 216	71 ± 29
Microsomes	88 ± 34	99 ± 13	97 ± 22	30 ± 18	15 ± 12	4 ± 6
Soluble fraction	129 ± 29	137 ± 24	126 ± 17	76 ± 9	32 ± 18	21 ± 16

* Rats received $^{14}CCl_4$, 2·5 ml/kg. Results are expressed as mean ± S.D. Each value is the mean of 3 experiments.

thereafter, the level of $^{14}CCl_4$ in the liver homogenate declined logarithmically, with a biological half-life of about 7 hr (Fig. 1). The endoplasmic reticulum probably was exposed to relatively high concentrations of CCl_4 shortly after the CCl_4 was administered to the animal. About 40 per cent of the radioactivity in the liver which was extracted into toluene was found in the microsomal and soluble fractions 1–3 hr after exposure of the animal to $^{14}CCl_4$ (Table 1).

Duration of action of hexobarbital. Pretreatment with CCl_4 prolonged the duration of action of hexobarbital (Table 2). Rats that received CCl_4 0·5 hr before the administration of hexobarbital (100 mg/kg) slept more than twice as long as animals not treated with CCl_4. Two hr after CCl_4 was administered, the rats were extremely sensitive to hexobarbital; it was necessary to reduce the dose of the barbiturate to an amount (50 mg/kg) which produced only transient effects in normal animals. Under these conditions, the sleeping time of the CCl_4-poisoned rats was about 15 times longer than that measured in the control animals.

* Obtained from Nuclear Chicago Corp., Des Plaines, Ill.

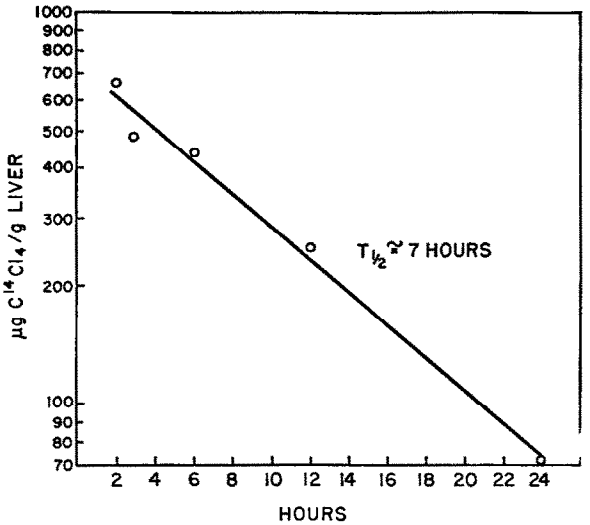

FIG. 1. Levels of $^{14}CCl_4$ in liver after the administration of 2·5 ml/kg, p.o., to rats. Each point represents the mean value obtained with 3 rats; the line was drawn according to the method of least squares.

TABLE 2. EFFECT OF CCl_4 INTOXICATION ON THE DURATION OF ACTION OF HEXOBARBITAL*

Treatment	Hexobarbital (mg/kg)	Sleeping time (min)	P
I. Experiment A			
Control	100	18 ± 3	
CCl_4, 0·5 hr	100	48 ± 19	< 0·005
CCl_4, 1·0 hr	100	44 ± 14	< 0·005
II. Experiment B			
Control	50	5 ± 6	
CCl_4, 2·0 hr	50	75 ± 20	< 0·001
CCl_4, 4·0 hr	50	83 ± 42	< 0·005

* The results are expressed as the mean values obtained with 6 rats ± S.D. The hours are the times between administration of CCl_4 (2·5 ml/kg) and injection of hexobarbital.

Metabolism of hexobarbital in the isolated perfused liver. The metabolism of hexobarbital was reduced in the perfused liver isolated from animals pretreated with CCl_4 (Table 3). When normal livers were perfused with 30 or 50 mg of hexobarbital, the amount of the drug metabolized per gram of liver was proportional to the quantity added to the system. The rate of metabolism, when 100 mg of hexobarbital was added, was probably approaching a maximum. The amount of hexobarbital metabolized by livers from CCl_4-poisoned rats was identical at all substrate concentrations; thus, a greater percentage impairment of hexobarbital metabolism was observed when larger concentrations of drug were perfused.

Effect of administration of CCl_4 on the activity of liver microsomal enzymes. Rats were pretreated with CCl_4 (2·5 ml/kg). Microsomes isolated from the liver at various

times after the administration of CCl_4 were incubated with aminopyrine, hexobarbital or *p*-nitrobenzoic acid as described under Methods. Exposure to CCl_4 rapidly reduced the rate of metabolism of these substrates (Figs. 2–4). The activity of the microsomal enzymes declined to about 10 per cent of normal within 8 hr and remained at low levels 24 hr after the CCl_4 was administered.

TABLE 3. EFFECT OF CCl_4 PRETREATMENT ON THE METABOLISM OF HEXOBARBITAL IN THE ISOLATED PERFUSED LIVER*

Treatment	Hexobarbital added (mg)	Hexobarbital metabolized (mg/g liver)	Relative activity
Experiment I			
Control	30·0	2·63	100
Experimental	30·0	1·69	64
Experiment II			
Control	50·0	4·40	100
Experimental	50·0	1·99	45
Experiment III			
Control	100·0	5·39	100
Experimental	100·0	1·63	30

* The livers were isolated from normal rats and from rats that had received CCl_4 (2·5 ml/kg) 3 hr previously. The livers were placed in the perfusion apparatus and the medium was allowed to recycle for 20 min, during which time maximal perfusion flow rates were obtained. Hexobarbital was added after this equilibration period. The quantity of hexobarbital metabolized is the difference between the amount of drug added to the system and that recovered from the liver and perfusate. Each value is the result of an individual experiment.

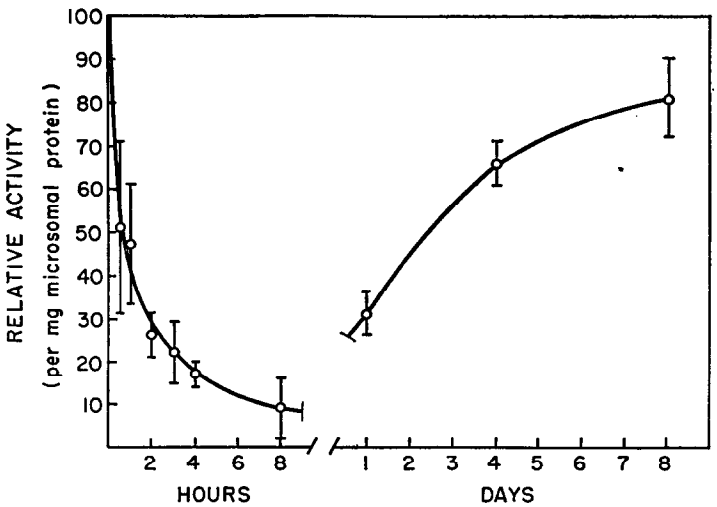

FIG. 2. Effect of CCl_4 (2·5 ml/kg, p.o.) on the demethylation of aminopyrine by liver microsomes. Each point represents the mean value obtained with 5–6 rats; vertical bars indicate ± 1 S.D.

The metabolism of both hexobarbital (Table 4) and aminopyrine (Table 5) was impaired increasingly as the dose of CCl_4 was raised. A maximum effect was obtained with a dose of 1·0 to 2·5 ml CCl_4/kg body wt.

Intoxication with CCl_4 produced an increase in the ratio of liver weight to body weight and a decrease in the concentration of microsomal protein which was dependent on the dose of CCl_4 (Table 6). The percentage increase in liver weight was equal to the percentage decrease in the concentration of microsomal protein. It may be surmised

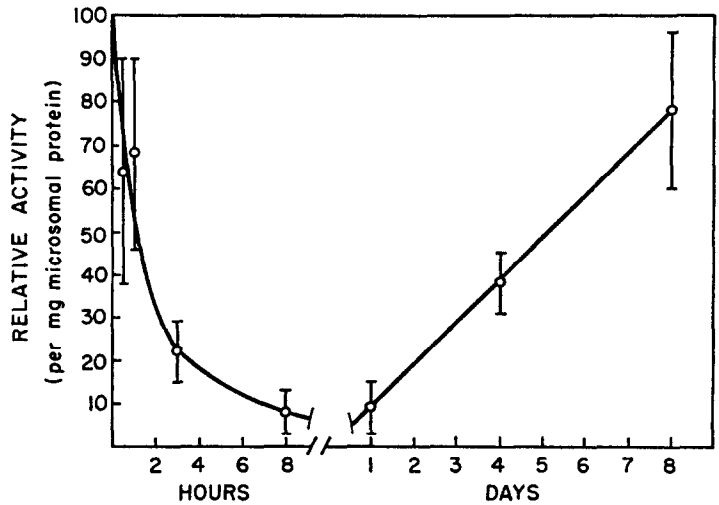

FIG. 3. Effect of CCl_4 (2·5 ml/kg, p.o.) on the metabolism of hexobarbital by liver microsomes. Each point represents the mean value obtained with 5–6 rats; vertical bars indicate ± 1 S.D.

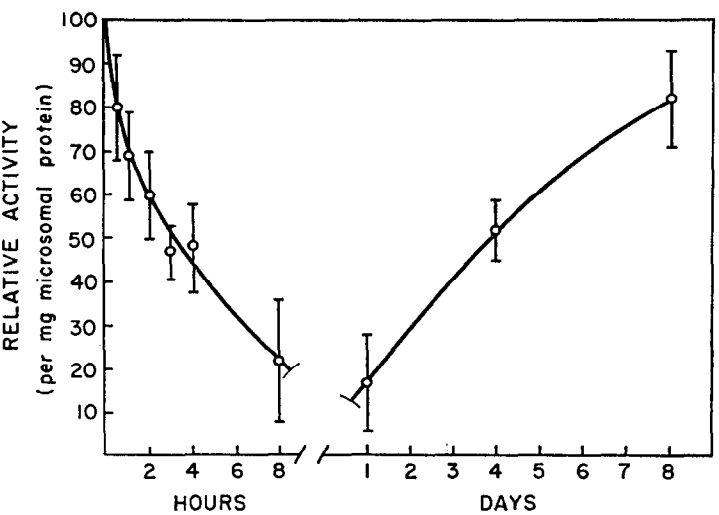

FIG. 4. Effect of CCl_4 (2·5 ml/kg, p.o.) on the reduction of *p*-nitrobenzoic acid by liver microsomes. Each point represents the mean value obtained with 5–6 rats; vertical bars indicate ± 1 S.D.

from these findings that the impairment of drug metabolism observed in CCl_4 poisoning resulted primarily from an inactivation of the microsomal enzymes rather than from a loss of enzymic protein.

Effect of chloroform and methylene chloride on drug metabolism by liver microsomes. In contrast to the striking impairment of the metabolism of both hexobarbital and

amino pyrine induced by CCl_4, the rate of metabolism of aminopyrine was 61 per cent of control values after pretreatment of the animal with equimolar doses of chloroform. Methylene chloride did not inhibit the metabolism of hexobarbital or aminopyrine (Tables 7 and 8).

TABLE 4. EFFECT OF CCl_4 ON METABOLISM OF HEXOBARBITAL BY LIVER MICROSOMES*

Treatment	Dose CCl_4 (ml/kg)	Hexobarbital metabolized (mμmoles/mg microsomal protein)	P	Relative activity
Experiment A	None (5)	94·2 ± 10·2		100
	0·10 (5)	89·2 ± 16·7	n.s.	95
	0·20 (4)	82·3 ± 9·8	n.s.	87
	0·50 (4)	41·8 ± 13·4	< 0·001	44
	1·00 (5)	7·4 ± 2·2	< 0·001	8
Experiment B	None (4)	96·0 ± 3·6		100
	1·25 (4)	27·8 ± 16·8	< 0·001	29
Experiment C	None (6)	85·0 ± 8·0		100
	2·50 (6)	8·0 ± 5·0	< 0·001	9

* Animals received CCl_4 by stomach tube 24 hr before sacrifice. The results are expressed as the mean ± standard deviation. The number of animals is in parentheses.

TABLE 5. EFFECT OF CCl_4 ON METABOLISM OF AMINOPYRINE BY LIVER MICROSOMES*

Treatment	Dose CCl_4 (ml/kg)	Formaldehyde formed (mμ moles/mg microsomal protein)	P	Relative activity
Experiment A	None (5)	55·0 ± 5·5		100
	0·10 (5)	43·0 ± 5·4	< 0·01	78
	0·20 (4)	36·5 ± 2·6	< 0·001	66
	0·50 (4)	19·0 ± 4·1	< 0·001	35
	1·00 (5)	10·6 ± 4·0	< 0·001	19
Experiment B	None (4)	78·5 ± 5·0		100
	1·25 (4)	11·2 ± 6·5	< 0·001	14
Experiment C	None (6)	98·0 ± 10·0		100
	2·50 (6)	30·0 ± 5·0	< 0·001	31

* Animals received CCl_4 by stomach tube 24 hr before sacrifice. The results are expressed as the mean ± S.D. Number of animals is in parentheses.

TABLE 6. EFFECT OF CCl_4 ON LIVER WEIGHT AND MICROSOMAL PROTEIN*

Experiment	CCl_4 (ml/kg)	Body wt. (g)	P	Liver wt-/ body wt. (mg/g)	P	Per cent increase	Microsomal protein (mg/g liver)	P	Per cent decrease
A	None (5)	211 ± 8		28·4 ± 0·8			30·7 ± 1·0		
	0·1 (5)	226 ± 15	< 0·1	30·3 ± 1·8	< 0·05	7	29·3 ± 1·5	n.s.	5
	0·2 (4)	226 ± 3	< 0·02	32·3 ± 2·3	< 0·01	14	26·6 ± 2·0	< 0·01	13
	0·5 (4)	224 ± 9	< 0·1	37·6 ± 2·4	< 0·001	32	21·0 ± 1·8	< 0·001	32
	1·0 (5)	210 ± 13	> 0·9	40·9 ± 3·0	< 0·001	44	17·0 ± 0·5	< 0·001	44
B	None (4)	347 ± 38		24·1 ± 1·5			34·1 ± 0·4		
	1.25 (4)	354 ± 14	> 0·7	34·9 ± 2·8	< 0·001	45	18·9 ± 3·2	< 0·001	44

* Animals received CCl_4 by stomach tube 24 hr before sacrifice. Number of animals is in parentheses. Results are expressed as the mean ± S.D.

Effect of antioxidants on the CCl_4-induced impairment of drug metabolism. Gallagher[21] reported that the administration of antioxidants such as α-tocopherol or *N,N*[1]-diphenyl-*p*-phenylenediamine (DPPD) to rats preceding the administration of CCl_4 protected the animals against lethal doses of the halogenated hydrocarbon. DPPD, when given in appropriate dosage, also prevented the centrilobular necrosis and diminished

TABLE 7. EFFECT OF EQUIMOLAR DOSES OF CCl_4, $HCCl_3$ AND H_2CCl_2 ON THE METABOLISM OF AMINOPYRINE BY LIVER MICROSOMES*

Pretreatment	Formaldehyde formed (mμ moles/mg microsomal protein)	P	Relative activity
None	78·5 ± 5·0		100
CCl_4 (1·25 ml/kg)	11·3 ± 6·5	< 0·001	14
$HCCl_3$ (1·05 ml/kg)	47·5 ± 6·2	< 0·001	61
H_2CCl_2 (0·85 ml/kg)	79·0 ± 9·0	n.s.	100

* Animals received the compounds by stomach tube 24 hr before sacrifice Results are expressed as the mean values obtained with 4 rats ± S.D.

TABLE 8. EFFECT OF EQUIMOLAR DOSES OF CCl_4, $HCCl_3$ AND H_2CCl_2 ON THE METABOLISM OF HEXOBARBITAL BY LIVER MICROSOMES*

Pretreatment	Hexobarbital metabolized (mμ moles/mg microsomal protein)	P	Relative activity
Control	96·0 ± 3·7		100
CCl_4 (1·25 ml/kg)	27·8 ± 16·8	< 0·001	29
$HCCl_3$ (1·05 ml/kg)	91·3 ± 7·9	n.s.	95
H_2CCl_2 (0·85 ml/kg)	94·8 ± 11·1	n.s.	99

* Animals received the compounds by stomach tube 24 hr before sacrifice. Results are expressed as the mean values obtained with 4 rats ± S.D.

the fatty infiltration of the liver. It was therefore of interest to determine whether these antioxidants could also prevent the impairment of hepatic drug metabolism seen in CCl_4 intoxication. α-Tocopherol or DPPD (100 mg) was administered i.p. to rats 40, 24 and 0 hr before the administration of CCl_4; liver microsomes were prepared 24 hr later and were incubated with aminopyrine or hexobarbital. Pretreatment with either antioxidant neither protected the drug-metabolizing microsomal enzymes nor prevented the increase in the ratio of liver weight to body weight (Table 9).

DISCUSSION

The administration of toxic doses of CCl_4 to an animal results in severe hepatic injury and eventual death of the cell.[1, 2] The biochemical mechanisms which have been postulated as responsible for the sequence of events leading to hepatocellular necrosis have been discussed in detail in several reports.[1, 22] The microsomes which are derived from the endoplasmic reticulum may be one of the earliest loci of cellular injury in the liver after administration of CCl_4. The injury to the endoplasmic reticulum precedes by many hours the damage to the mitochondria. This later mitochondrial

damage, which includes inhibition of oxidative phosphorylation, may be responsible for the cellular necrosis. The damage to the endoplasmic reticulum, which may occur even when necrosis is retarded, inhibits the activities of a number of enzymes localized in the microsomes. These activities include the metabolism of drugs and the synthesis of plasma proteins and lipoproteins. The fatty liver seen in CCl_4 poisoning may in

TABLE 9. EFFECT OF ANTIOXIDANTS ON THE CCl_4-INDUCED IMPAIRMENT OF HEPATIC DRUG-METABOLIZING ENZYME ACTIVITY*

Treatment	Hexobarbital metabolism (mμ moles)	Aminopyrine demethylation formaldehyde (mμ moles)	Liver wt./ body wt. (mg/g)
Experiment I			
(a) None (5)	72 ± 5	58 ± 3	27·9 ± 0·7
(b) α-Tocopherol (5)	61 ± 11	59 ± 7	27·8 ± 1·2
(c) CCl_4† (5)	42 ± 8	20 ± 6	42·9 ± 2·0
(d) α-Tocopherol + CCl_4† (5)	24 ± 6	15 ± 4	40·6 ± 1·8
Experiment II			
(e) None (5)	69 ± 19	39 ± 7	30·2 ± 1·0
(f) DPPD (5)	67 ± 30	39 ± 17	34·9 ± 3·0
(g) CCl_4‡ (6)	22 ± 15	8 ± 7	45·0 ± 2·8
(h) DPPD + CCl_4‡ (6)	19 ± 14	10 ± 7	45·8 ± 3·5
Statistical analysis	(P values)		
a vs. b	< 0·1	> 0·9	> 0·9
a vs. c	< 0·001	< 0·001	< 0·001
b vs. d	< 0·001	< 0·001	< 0·001
c vs. d	< 0·01	< 0·2	< 0·2
e vs. f	> 0·9	> 0·9	< 0·01
e vs. g	< 0·1	< 0·001	< 0·001
f vs. h	< 0·01	< 0·01	< 0·001
g vs. h	< 0·7	< 0·7	< 0·7

* Enzyme activities are expressed as mμ moles substrate metabolized or product formed by 1·0 mg microsomal protein in 30 min. Results are expressed as the mean values ± S.D. The number of rats is in parentheses. Rats received α-tocopherol or DPPD (100 mg, i.p.) 40, 20, and 0 hr prior to administration of CCl_4; the rats were sacrificed 24 hr after receiving CCl_4.

† 1·0 ml/kg.

‡ 1·25 ml/kg.

part be secondary to the inhibition of formation of phospholipid and protein, and therefore of the complete lipoprotein in the microsomes.[23]

In the present studies, the impairment of drug metabolism was dependent on the dose of CCl_4 administered. Equimolar doses of methylene chloride were nontoxic, and chloroform was only moderately injurious to the metabolism of aminopyrine. Recovery from the intoxication with CCl_4 was accompanied by a gradual return to normal of the metabolism of drugs by microsomes. The return to normal enzymatic activity may have started coincidentally with the disappearance of CCl_4 from the liver. In these experiments, the concentration of CCl_4 in the liver declined exponentially; 24 hr after exposure, the level of CCl_4 in the liver was about 10 per cent of that estimated at 2 hr.

The injury to the microsomes may be due to a direct action of CCl_4 on the liver and is associated with high concentrations of CCl_4 in the liver after its administration.

The concentration of CCl_4 in the liver, measured chemically, was at a peak 1·5–2·0 hr after treatment of the animal with CCl_4.[24] In our experiments, the ^{14}C extractable from the liver with toluene 2 hr after administration of $^{14}CCl_4$ was at a maximal concentration; this radioactivity probably represents unmetabolized CCl_4.[20] Fifteen per cent of the ^{14}C in the liver at this time was found in the microsomal fraction. When rats were given small doses of $^{14}CCl_4$ (or CCl_4^{36}), radioactivity which was not volatile was recovered from the liver within 2 hr from the time the rat had received an oral dose of the hepatotoxin; both of these isotopes were preferentially incorporated into the microsomal lipids.[25] It is therefore probable that the damage to the endoplasmic reticulum seen early in CCl_4 intoxication is a result of a direct physicochemical action of the chlorinated hydrocarbon on this structure. Carbon tetrachloride may attack the endoplasmic reticulum by virtue of a solvent effect on the lipoprotein structure of the organelle. In addition, it has been proposed that free radicals, which are formed during the metabolism of CCl_4, may alkylate or chlorinate the membrane structure of the endoplasmic reticulum and may also induce lipoperoxidation.[1] It has been reported by a number of workers that certain of the effects of CCl_4 on the liver (e.g. necrosis, accumulation of lipids) could be alleviated by pretreatment of the animals with antioxidants.[1] In our experiments, pretreatment of the rats with either α-tocopherol or DPPD failed to protect the rat against the destructive effects of CCl_4 on microsomal drug metabolism. Conceivably, the injuries to the various microsomal enzymes induced by CCl_4 are not uniformly distributed, or perhaps lipoperoxidation may not be involved in inactivation of all microsomal activities by CCl_4.

REFERENCES

1. R. O. RECKNAGEL, *Pharmac. Rev.* **19**, 145 (1967).
2. W. F. VON OETTINGEN (Ed.), *The Halogenated Hydrocarbons: Toxicity and Potential Dangers*, No. 414. U.S. Department of Health, Education and Welfare (1955).
3. C. OBERLING and C. ROUILLER, *Annls Anat. path.* **1**, 401 (1956).
4. M. BASSI, *Expl cell Res.* **20**, 313 (1960).
5. E. A. SMUCKLER, O. A. ISERI and E. P. BENDITT, *J. exp. Med.* **116**, 55 (1962).
6. R. O. RECKNAGEL and B. LOMBARDI, *J. biol. Chem.* **236**, 564 (1961).
7. D. NEUBERT and D. MAIBAUER, *Arch. exp. Path. Pharmak.* **235**, 291 (1959).
8. E. A. SMUCKLER, E. A. BARKER and E. P. BENDITT, *Fedn Proc.* **22**, 369 (1963).
9. E. A. SMUCKLER and E. P. BENDITT, *Science* **140**, 308 (1963).
10. B. B. BRODIE, J. R. GILLETTE and B. N. LA DU, *A. Rev. Biochem.* **27**, 427 (1958).
11. J. R. GILLETTE, *Prog. Drug Res.* **6**, 13 (1963).
12. M. HEIMBERG, I. WEINSTEIN, H. KLAUSNER and M. L. WATKINS, *Am. J. Physiol.* **202**, 353 (1962).
13. M. HEIMBERG, N. B. FIZETTE and H. KLAUSNER, *J. Am. Oil Chem. Soc.* **41**, 774 (1964).
14. H. A. KREBS and K. HENSELEIT, *Hoppe-Seyler's Z. physiol. Chem.* **210**, 33 (1932).
15. J. R. COOPER and B. B. BRODIE, *J. Pharmac. exp. Ther.* **114**, 409 (1955).
16. J. R. FOUTS and B. B. BRODIE, *J. Pharmac. exp. Ther.* **119**, 197 (1957).
17. J. COCHIN and J. AXELROD, *J. Pharmac. exp. Ther.* **125**, 105 (1959).
18. A. G. GORNALL, C. J. BARDAWILL and M. M. DAVID, *J. biol. Chem.* **177**, 751 (1949).
19. J. V. DINGELL, P. D. JOINER and L. HURWITZ, *Biochem. Pharmac.* **15**, 971 (1966).
20. B. B. PAUL and D. RUBINSTEIN, *J. Pharmac. exp. Ther.* **141**, 141 (1963).
21. C. H. GALLAGHER, *Aust. J. exp. Biol. med. Sci.* **40**, 241 (1962).
22. M. HEIMBERG, M. L. WATKINS and R. TOOKER, *J. Pharmac. exp. Ther.* **145**, 92 (1964).
23. I. WEINSTEIN, G. DISHMON and M. HEIMBERG, *Biochem. Pharmac.* **15**, 851 (1966).
24. R. O. RECKNAGEL and M. LITTERIA, *Am. J. Path.* **36**, 521 (1960).
25. E. S. REYNOLDS, *J. Pharmac. exp. Ther.* **155**, 117 (1967).