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BIOCHEMICAL CHANGES IN RAT LIVER IN RESPONSE TO TREATMENT WITH DRUGS AND OTHER AGENTS—II

EFFECTS OF HALOTHANE, DDT, OTHER CHLORINATED HYDROCARBONS, THIOACETAMIDE, DIMETHYLNITROSAMINE AND ETHIONINE

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Abstract—In this second of a series of three papers relating patterns of response of rat liver enzymes to the administration of various chemical agents, the effects of DDT, halothane, DMN, ethionine, thioacetamide and various chlorinated hydrocarbons are compared.

The established toxins showed marked similarities, characterised principally by reductions in microsomal metabolism. DDT, a compound traditionally considered to be hepatotoxic, gave a pattern of response quite unlike that due to the toxins, as also did halothane. The DDT effect was similar to that produced by the barbiturates.

IN THIS second paper, the results of the administration of various halogenated compounds and hepatotoxins on the liver parameters indicated in the first paper[1] are reported. In a previous report,[2] the results of treatment with the well-established hepatotoxins, CCl_4 and thioacetamide, were found to be markedly different from those due to DDT, CPIB, I.C.I. 53,072 and barbiturates. We have, therefore, in the present group of experiments, examined a larger number of toxic compounds and results are reported following treatment with CCl_4, chloroform, 1,1,2-trichloroethane (112-TCE), thioacetamide, dimethylnitrosamine (DMN) and ethionine. For comparison, a chlorinated hydrocarbon reputed not to produce liver toxicity,[3] viz. 1,1,1-trichloroethane (111-TCE), has also been examined.

In addition, similar studies have been made with Fluothane* (1) halothane; 1,1,1-trifluoro-2-bromo-2-chloroethane) and DDT (2,2-*bis* (p-chlorophenyl)trichloroethane).

METHODS

The methods used are given in the first of this series of papers.[1] Details of the doses used and the duration of dosing are given in Table 1.

RESULTS

The effects of the various treatments on the whole body growth are shown in Table 2. DDT and 111-TCE had no effect on growth, whereas 112-TCE, chloroform, CCl_4

* I.C.I. Trade Mark.

(at 2000 mg/kg), DMN and ethionine virtually abolished growth. Halothane, CCl_4 (at 200 mg/kg) and thiocetamide produced marginal reductions in growth.

Liver weight changes, protein concentrations and enzyme activities are given in Tables 3, 4 and 5–8 respectively.

TABLE 1. DETAILS OF EXPERIMENTS PERFORMED

Expt. no.	Compounds investigated	Dose given*
8	DDT	0·10% w/w in diet for 14 days.
	Control	Powdered diet.
9	1,1,2-Trichloroethane†	180 mg/kg (0·125 ml/kg) p.o. for 7 days in liquid paraffin.
	1,1,1-Trichloroethane	1650 mg/kg (1·25 ml/kg) p.o. for 7 days in liquid paraffin.
10	Control	5 ml liquid paraffin/kg p.o. for 7 days.
	Halothane (CF_3-CHBrCl)	1870 mg/kg (1·0 ml/kg) p.o. for 14 days in liquid paraffin.
	$CHCl_3$†	750 mg/kg (0·5 ml/kg) p.o. for 14 days in liquid paraffin.
	Control	5 ml liquid paraffin/kg p.o. for 14 days.
11	CCl_4	200 mg/kg (0·125 ml/kg) p.o. for 13 days in liquid paraffin.
	Control	5 ml liquid paraffin/kg p.o. for 13 days.
12	CCl_4	2000 mg/kg (1·25 ml/kg) p.o. for 13 days in liquid paraffin.
	Control	5 ml liquid paraffin/kg p.o. for 13 days.
13	Thioacetamide	0·025% w/w in diet for 13 days.
	Control	Powdered diet.
14	Dimethylnitrosamine	10 mg/kg p.o. for 7 days.
	Ethionine (*dl*)	200 mg/kg p.o. for 7 days.
	Control	5 ml water/kg p.o. for 7 days.

* Powdered diet given *ad libitum* for at least 7 days before dosing commenced.

† 1,1,2-TCE was lethal to our rats after 5–7 doses at double this dose (i.e. 360 mg/kg, 0·25 ml/kg); $CHCl_3$ was lethal to our rats after 3–4 doses at 2½ times this dose (i.e. 1875 mg/kg, 1·25 ml/kg).

TABLE 2. BODY WEIGHT CHANGES*

Expt. no.	Compound	Mean body wt. (g)				Ratio terminal to initial body wt. (%)	
		Initial		Terminal			
		Treated	Control	Treated	Control	Treated	Control
8	DDT	121	124	200	210	165	169
9	112-TCE	122	122	132	163	108	134
	111-TCE	123		158		128·5	
10	Halothane	115	117	171	187	149	160
	$CHCl_3$	120		133		111	
11	CCl_4	160	157	201	211	126	135
12	CCl_4	121	125	132	203	109	163
13	Thioacetamide	121	126	179	202	148	160
14	DMN	124	121	120	157	97	130
	Ethionine	119		120		101	

* Dosing schedules given in Table 1.

Hepatotoxic agents

CCl_4 at both dose levels gave rise to a 35–38 per cent increase in the relative liver weight but chloroform induced a smaller rise of 8 per cent. Thioacetamide, in contrast to a previous observation,[2] also caused liver enlargement. From literature evidence this effect of thioacetamide is to be expected.[5,23] Both DMN and ethionine produced

a decrease of approximately 15 per cent in liver weight, whereas 112-TCE had no effect.

Five of the six toxins examined reduced the microsomal protein concentration, 112-TCE being the only exception. Chloroform, CCl_4 (at the higher dose), DMN and ethionine also reduced cell-sap protein concentrations significantly, although the depression of microsomal levels was more pronounced in all cases.

TABLE 3. LIVER WEIGHT CHANGES

Expt. No.	Compound	Liver wt.: Body wt. ratio					
		Mean (g/100 g)	± S.E.M.	(N)	CV (%)	Per cent control gp.	P*
8	DDT	6·51	0·14	(5)	4·7	129	§
	Control	5·05	0·15	(4)	5·9	100	
9	112-TCE	4·51	0·12	(5)	6·0	96	n.s.
	111-TCE	4·96	0·16	(5)	7·3	105·5	n.s.
	Control	4·70	0·16	(5)	7·7	100	
10	Halothane	5·50	0·21	(5)	8·4	119	‡
	$CHCl_3$	4·98	0·16	(5)	7·2	108	†
	Control	4·61	0·10	(5)	5·0	100	
11	CCl_4	6·26	0·22	(5)	7·7	138	§
	Control	4·53	0·09	(5)	4·2	100	
12	CCl_4	6·56	0·25	(4)	7·7	135	§
	Control	4·87	0·11	(5)	5·2	100	
13	Thioacetamide	5·55	0·15	(5)	6·0	120	§
	Control	4·61	0·07	(5)	3·4	100	
14	DMN	3·92	0·21	(4)	10·5	83·5	‡
	Ethionine	4·06	0·14	(5)	7·9	87	‡
	Control	4·69	0·07	(5)	3·4	100	

* P—Treated group compared with control group by Student's *t* test
n.s.—$P > 0{\cdot}10$, not significant.
† $P < 0{\cdot}10$.
‡ $P \leqslant 0{\cdot}01$.
§ $P \leqslant 0{\cdot}001$.

All six toxins reduced the activity of microsomal AP-demethylase (Table 5); DMN and particularly ethionine virtually abolished this function of the $NADPH_2$-electron transport chain, but $NADPH_2$-cyt. *c* reductase activity was less profoundly affected, ethionine and 112-TCE having no observable effect.

$NADH_2$-cyt. *c* reductase activity (Table 6) was reduced by 112-TCE, CCl_4, thioacetamide and DMN but not by either ethionine or chloroform. G6Pase activity was also reduced by the majority of these toxic compounds, 112-TCE being the only exception; LDH and GDH activities were generally reduced, although the degree of reduction varied considerably. Ethionine elicited a rise in GDH activity in experiment 14 but the significance of this observation is doubtful.

The most striking effect of all 6 compounds was the large increase in G6PDH activity, associated with a marginal reduction of PGDH activity. The G6PDH activity was at least doubled and in the case of CCl_4 and thioacetamide was increased 3 to 4-fold.

111-TCE

The response of the liver to this agent was generally unremarkable, the most significant changes being the increases in both microsomal and cell-sap protein concentrations.

TABLE 4. LIVER MICROSOMAL AND CELL-SAP PROTEIN CONCENTRATIONS

Expt. No.	Compound	Microsomal protein concn (mg/equiv. g fresh liver)						Cell-sap protein concn (mg/equiv. g fresh liver)					
		Mean	± S.E.M.	(N)	CV (%)	Per cent control gp.	P*	Mean	± S.E.M.	(N)	CV (%)	Per cent control gp.	P*
8	DDT	13·0	‖			186		75·5	‖			107·5	
	Control	7·0	‖			100		70.3	‖			100	
9	112-TCE	9·08	0·41	(4)	9·0	98	n.s.	60·0	2·35	(5)	9·1	98	n.s.
	111-TCE	11·72	0·43	(4)	7·2	127	§	66·6	1·34	(5)	4·5	109	++
	Control	9·25	0·22	(5)	5·3	100		61·3	0·80	(5)	2·9	100	
10	Halothane	12·66	0·40	(5)	7·0	107	n.s.	65·3	1·75	(5)	6·0	92·5	†
	$CHCl_3$	10·02	0·50	(4)	10·0	85	‡	64·4	1·48	(4)	4·6	91·5	†
	Control	11·82	0·24	(5)	4·6	100		70·5	1·24	(5)	2·9	100	
11	CCl_4	7·6	‖			78·5		66·0	‖			95	
	Control	9·7	‖			100		69·7	‖			100	
12	CCl_4	10·1	‖			90		58·1	‖			66·5	
	Control	11·2	‖			100		87·5	‖			100	
13	Thioacetamide	9·4	‖			79		77·0	‖			104	
	Control	11·9	‖			100		74·2	‖			100	
14	DMN	8·08	0·31	(5)	8·5	74	§	61·3	1·49	(4)	4·8	87·5	++++
	Ethionine	9·12	0·39	(5)	9·6	83·5	‡	63·5	0·89	(5)	3·1	90·5	++++
	Control	10·94	0·30	(5)	6·1	100		70·1	1·20	(5)	3·8	100	

* See footnote to Table 3 for levels of significance.

‖ Concentrations assayed on pooled sample from all animals in group.

TABLE 5. LIVER ENZYME ACTIVITIES: $NADPH_2$-CYTOCHROME *c* REDUCTASE AND AMINOPYRINE DEMETHYLASE

Expt. No.	Compound	$NADPH_2$-cyt.*c* reductase (μmoles cyt.*c* reduced g/ min)						Aminopyrine demethylase (mμmoles HCHO formed/g/min)					
		Mean	± S.E.M.	(N)	CV (%)	Per cent control gp.	P*	Mean	± S.E.M.	(N)	CV (%)	Per cent control gp.	P*
8	DDT	2·85	0·20	(4)	14·3	361	§	271	13	(5)	10·3	228	§
	Control	0·79	0·02	(3)	3·4	100		119	4	(3)	5·7	100	
9	112-TCE	1·05	0·07	(4)	13·3	93	n.s.	168	9	(5)	11·7	86·5	†
	111-TCE	1·42	0·11	(5)	17·6	126	†	209	16	(4)	15·0	108	n.s.
	Control	1·13	0·04	(5)	8·0	100		194	7	(4)	6·8	100	
10	Halothane	2·92	0·13	(5)	10·3	193	§	204	22	(4)	21·6	92	n.s.
	$CHCl_3$	1·15	0·12	(4)	20·9	76	†	115	11	(5)	21·6	52	§
	Control	1·51	0·09	(5)	13·9	100		222	13	(5)	13·4	100	
11	CCl_4	0·59	0·05	(5)	19·9	51	§	89	14	(5)	35·2	38	§
	Control	1·16	0·07	(5)	12·6	100		236	10	(4)	8·3	100	
12	CCl_4	0·84	0·04	(4)	8·7	60	‡	59	6	(4)	19·0	25	§
	Control	1·40	0·12	(5)	19·1	100		236	12	(5)	11·1	100	
13	Thioacetamide	1·14	0·07	(4)	12·5	76·5	‡	96	5	(4)	11·2	43·5	§
	Control	1·49	0·06	(4)	7·5	100		220	18	(5)	18·0	100	
14	DMN	0·60	0·04	(5)	13·3	35	§	39	9	(4)	43·1	17	§
	Ethionine	1·85	0·16	(5)	19·5	109	n.s.	9	5	(5)	‖	4	§
	Control	1·70	0·12	(5)	15·9	100		229	13	(5)	12·7	100	

* See footnote to Table 3 for levels of significance.

‖ Three of the 5 observations gave zero activity.

TABLE 6. LIVER ENZYME ACTIVITIES: $NADH_2$-CYTOCHROME *c* REDUCTASE AND GLUCOSE-6 PHOSPHATASE

Expt. No.	Compound	$NADH_2$ cyt.*c* reductase (μmoles cyt.*c* reduced/g/min)						Glucose-6 phosphatase (μmoles inorg. PO_4 liberated/g/hr)					
		Mean	± S.E.M.	(N)	CV (%)	Per cent control gp.	P*	Mean	± S.E.M.	(N)	CV (%)	Per cent control gp.	P*
8	DDT	6·25	0·30	(4)	9·5	107	n.s.	572	30	(5)	11·7	68·5	‡
	Control	5·85	0·46	(4)	15·8	100		837	76	(4)	18·0	100	
9	112-TCE	4·98	0·56	(5)	25·1	67·5	‡	901	37	(5)	9·2	104·5	n.s.
	111-TCE	7·26	0·34	(5)	10·6	98·5	n.s.	857	32	(5)	8·2	99·5	n.s.
	Control	7·38	0·24	(4)	6·4	100		862	49	(5)	12·6	100	
10	Halothane	9·22	0·67	(4)	14·4	106	n.s.	929	29	(4)	6·3	128·5	‡‡
	$CHCl_3$	10·81	1·11	(5)	22·8	125	n.s.	592	41	(4)	13·7	82	‡‡
	Control	8·68	0·38	(4)	8·7	100		722	34	(4)	9·4	100	
11	CCl_4	6·09	0·49	(4)	16·2	74·5	†	289	27	(4)	18·3	38·5	§§
	Control	8·15	0·55	(4)	13·5	100		754	50	(4)	13·2	100	
12	CCl_4	5·58	0·30	(4)	10·5	75	‡	295	27	(4)	18·6	38·5	§§
	Control	7·43	0·31	(4)	8·2	100		764	24	(5)	6·9	100	
13	Thioacetamide	6·41	0·33	(5)	11·4	75	‡	506	19	(5)	8·3	56·5	§§
	Control	8·54	0·48	(4)	11·3	100		900	20	(4)	4·4	100	
14	DMN	3·75	0·12	(5)	6·9	42·5	§	342	18	(5)	11·9	40·5	§§§
	Ethionine	8·08	0·84	(4)	20·8	91·5	n.s.	643	14	(4)	4·3	76	§§§
	Control	8·83	0·29	(4)	6·6	100		848	25	(5)	6·5	100	

* See footnote to Table 3 for levels of significance.

TABLE 7. LIVER ENZYME ACTIVITIES: LACTATE AND GLUTAMATE DEHYDROGENASES

Expt. No.	Compound	Lactate dehydrogenase (units/g)‖						Glutamate dehydrogenase (units/g)‖					
		Mean	± S.E.M.	(N)	CV (%)	Per cent control gp.	P*	Mean	±S.E.M.	(N)	CV (%)	Per cent control gp.	P*
8	DDT	254	15	(5)	13·1	57	§	2·55	0·35	(4)	27·7	53	†
	Control	443	24	(4)	10·7	100		4·80	0·41	(3)	14·8	100	
9	112-TCE	343	9	(5)	5·9	86·5	†	9·98	0·44	(4)	8·7	115	n.s.
	111-TCE	351	13	(4)	7·2	88·5	n.s.	10·39	0·44	(4)	8·5	119	†
	Control	397	21	(5)	12·0	100		8·70	0·72	(4)	16·6	100	
10	Halothane	648	22	(5)	7·4	160	§	8·86	0·39	(5)	9·9	70	§
	$CHCl_3$	235	17	(5)	16·0	58	§	11·63	0·70	(4)	12·0	92	n.s.
	Control	405	16	(5)	8·9	100		12·63	0·65	(4)	10·3	100	
11	CCl_4	309	18	(5)	13·0	89	n.s.	11·41	1·04	(5)	20·4	70	‡
	Control	347	17	(4)	9·8	100		16·37	0·45	(4)	5·5	100	
12	CCl_4	277	17	(4)	12·2	72	§	8·15	0·56	(4)	13·8	59	‡
	Control	385	12	(5)	6·8	100		13·85	1·04	(5)	16·8	100	
13	Thioacetamide	337	8	(5)	5·3	83	§	16·01	0·91	(5)	12·6	105	n.s.
	Control	406	9	(5)	4·9	100		15·22	1·21	(4)	15·8	100	
14	DMN	295	9	(5)	6·5	81	§	2·72	0·24	(5)	19·5	38	§
	Ethionine	321	12	(5)	8·4	88	†	9·37	0·70	(4)	15·1	131	†
	Control	364	10	(4)	5·5	100		7·17	0·55	(4)	15·2	100	

* See footnote to Table 3 for levels of significance.
‖ 1 unit = change of absorbance of 0·001/min.

TABLE 8. LIVER ENZYME ACTIVITIES: GLUCOSE-6 PHOSPHATE AND 6-PHOSPHOGLUCONATE DEHYDROGENASES

Expt. No.	Compound	G6PDH (units/g)‖ Mean	± S.E.M.	(N)	CV (%)	Per cent control gp.	P*	PGDH (units/g)‖ Mean	±S.E.M.	(N)	CV (%)	Per cent control gp.	P*
8	DDT	6·05	0·67	(4)	22·0	134·5	n.s.	13·07	0·69	(5)	11·8	130	‡
	Control	4·50	0·45	(3)	17·3	100		10·04	0·57	(4)	11·3	100	
9	112-TCE	7·70	0·56	(5)	16·4	195	§	9·79	0·62	(5)	14·1	78	†
	111-TCE	4·48	0·36	(4)	16·1	114	n.s.	11·16	0·42	(5)	8·3	89	n.s.
	Control	3·94	0·29	(5)	16·3	100		12·56	0·83	(4)	13·2	100	
10	Halothane¶	9·42	1·48	(5)	35·0	198	†	10·83	0·37	(5)	7·7	88·5	†
	$CHCl_3$	9·35	0·71	(4)	15·3	196	§	11·22	0·48	(5)	9·6	91·5	n.s.
	Control	4·76	0·35	(4)	14·5	100		12·28	0·34	(4)	5·5	100	
11	CCl_4	11·28	1·23	(5)	24·6	264	‡	9·14	0·19	(5)	4·6	85	†
	Control	4·26	0·51	(4)	24·0	100		10·78	0·55	(4)	10·1	100	
12	CCl_4	10·09	0·71	(4)	14·0	350	§	7·44	0·56	(4)	15·1	62·5	§
	Control	2·88	0·44	(4)	30·9	100		11·90	0·56	(5)	10·5	100	
13	Thioacetamide	14·26	1·91	(5)	30·0	407	§	11·39	0·38	(5)	7·4	95·5	n.s.
	Control	3·50	0·54	(5)	34·4	100		11·93	0·59	(5)	11·0	100	
14	DMN	8·55	0·40	(5)	10·5	230	§§	7·47	0·13	(5)	3·8	77·5	§§
	Ethionine	8·22	0·30	(4)	7·2	221	§§	8·21	0·30	(5)	8·2	85·5	++
	Control	3·72	0·69	(4)	36·8	100		9·61	0·18	(4)	3·5	100	

* See footnote to Table 3 for levels of significance.

‖ 1 Unit = change of absorbance of 0·001/min.

¶ See text.

DDT

The response of the liver to this agent was well-defined; in particular, highly significant increases in relative liver weight, microsomal protein concentration, $NADPH_2$-cyt. *c* reductase, AP-demethylase and PGDH activities were observed. G6Pase, LDH, and GDH activities were significantly reduced whereas cell-sap protein concentration and $NADH_2$-cyt. *c* reductase activity were unaffected. The effect on G6PDH activity was anomalous when compared with previous results; the insignificant increase found here was in contrast to the reduced activity previously found in this and other laboratories.[2,4]

Halothane

Halothane produced liver enlargement, but had no effect on microsomal protein concentration. Cell-sap protein concentration was apparently reduced by some 8 per cent compared with controls. Like diphenylhydantoin,[1] halothane had a differential effect on the components of microsomal $NADPH_2$-electron transport, $NADPH_2$-cyt. *c* reductase activity being doubled whereas AP-demethylase was not affected.

$NADH_2$-cyt. *c* reductase activity was not changed, but G6Pase in this experiment was significantly increased in marked contrast to the effect of the toxic agents. Halothane was the only compound examined apart from the CPIB-type compounds[1] to produce an elevated level of LDH activity. Both GDH and PGDH activities were reduced, but G6PDH activity was doubled.

DISCUSSION

The biochemical results in this paper are in general agreement with previous observations from this and other laboratories; CCl_4, chloroform and thioacetamide have been shown to increase the relative liver weight;[2,5,17,23] CCl_4 inhibits microsomal protein synthesis,[18] an effect also seen after DMN,[19] ethionine[12] and thioacetamide;[20] DDT has been found to raise both liver weight[4,14,15] and microsomal protein concentration and to stimulate microsomal protein synthesis.[21] These effects on protein synthesis agree with the levels of protein observed in these experiments. CCl_4 reduces both microsomal drug metabolism (e.g. ref. 22) and G6Pase activity[2,23] and raised G6PDH activity;[2,23] thioacetamide has been shown to reduce the metabolic conversion of pentobarbitone *in vivo*,[24] to reduce G6Pase activity,[2,23] and to increase G6PDH activity,[2] although Feuer *et al.*[23] were unable to demonstrate this change; DMN and ethionine have similar effects on these parameters.[23] In contrast, DDT stimulates microsomal, oxidative drug metabolism,[21,25,26] produces a marginal reduction in G6Pase[2] and reduces G6PDH activity,[2,4] although the latter effect does not agree with the result found in the present experiment. The results of treatment with CCl_4, thioacetamide and DDT on rat liver LDH, GDH and PGDH activities confirmed previously reported results.[2] The results of halothane treatment of rats, however, did not conform with those reported by Kunz *et al.*[27] in mice, but Remmer (see ref. 36) found halothane to have no stimulatory effect on rat liver microsomal drug metabolism, a result confirmed by the present findings.

The results in this paper can be grouped into three distinct patterns of response, designated VI–VIII, as shown in Table 9.

CCl_4 is probably the most investigated liver toxin and an extensive review of this agent by Recknagel has recently appeared.[7] The main morphological features of CCl_4

TABLE 9. PATTERNS OF RESPONSE OF VARIOUS LIVER PARAMETERS TO TREATMENT OF RATS WITH VARIOUS AGENTS

Pattern Code	Agents showing this pattern	Effect on liver parameters*										
		RLW	Mic. prot. concn	Cell-sap prot. concn	$NADPH_2$-cyt. *c* reduct.	AP-demeth.	$NADH_2$ cyt. *c* reduct.	G6PDH	PGDH	G6Pase	LDH	GDH
VI	CCl_4	I	D	(D)	D	D	D	I	D	D	D	D
	Chloroform§	I										
	112-TCE†	n.c.										
	Thioacetamide‖	I										
	DMN	D										
	Ethionine‡	D										
VII	DDT	I	I	n.c.	I	I	n.c.	(I)	I	D	D	D
VIII	Halothane	I	n.c.	(D)	I	n.c.	n.c.	I	(D)	(I)	I	D

* I = increase of concentration or activity; D = decrease of concentration or activity; n.c. = no change of concentration or activity; values in parentheses are considered of doubtful significance.

† 112-TCE had less pronounced effects on several parameters than the other agents in this group.

‡ Ethionine had no effect on $NADPH_2$-cyt. *c* reductase or $NADH_2$-cyt. *c* reductase and increased GDH activity.

§ $CHCl_3$ had no effect on $NADH_2$-cyt. *c* reductase or GDH activities.

‖ Thioacetamide had no effect on GDH or PGDH activities.

hepatotoxicity are centrilobular necrosis and fatty infiltration (e.g. ref. 8). Thioacetamide produces centrilobular necrosis[5] but only minor changes in the fat content of the liver.[9] DMN gives rise to centrilobular necrosis and fatty infiltration[10,11] and is also an hepatic carcinogen.[11] Ethionine in contrast does not give rise to a centrilobular necrosis but does produce a fatty liver (e.g. ref. 12). In this study, the toxic group of compounds (pattern VI) showed some minor differences in the way various parameters reacted (see footnotes to Table 9), but despite these differences there was a marked degree of similarity between all six agents, characterised by the following changes:

(i) marked reductions of both G6Pase and AP-demethylase activities
(ii) marked increase of G6PDH activity
(iii) significant reductions of $NADH_2$- and $NADPH_2$-cyt. *c* reductases, microsomal and cell-sap protein levels and also of extra-microsomal oxidoreductase activities.

Liver enlargement was not associated with the pattern of response observed. Klaassen and Plaa[3] compared the degree of hepatic dysfunction induced in mice by a series of halogenated hydrocarbons and showed that chloroform and CCl_4 produced moderate to severe damage, 112-TCE produced moderate damage and 111-TCE produced only mild dysfunction even at near-lethal doses. The biochemical changes correlated well with this assessment in that 112-TCE showed less significant changes than those induced by either CCl_4 or chloroform; 111-TCE was without significant effect on those parameters most notably affected by the toxic analogues, viz: AP-demethylase, G6Pase and G6PDH activities. The lower dose of CCl_4 used in experiment 11 (200 mg/kg) showed effects equal to those from the higher dose used in experiment 12 (2000 mg/kg) on the microsomal enzymes and also G6PDH activity, but the higher dose elicited greater changes in extra-microsomal PGDH, GDH and LDH activities and also in cell-sap protein concentration.

DDT is traditionally considered to be hepatotoxic since it produces liver enlargement and some fatty infiltration in the rat.[13,14] Centrilobular necrosis is rarely observed, however, and then only as a terminal event at high doses.[13] Ortega[15,16] could find no evidence of necrosis nor any clinical evidence of toxicity in rats fed a diet containing as much as 1000 ppm (the dose used in the present study) for up to 18 months, and on the basis of electron-microscopical evidence (to be discussed later[6]) has suggested that the effect of DDT on the liver should be re-evaluated.[16] The present studies have shown that the DDT-pattern of response (pattern VII) was completely different from that due to the toxic compounds particularly in respect of microsomal drug metabolism and microsomal protein concentration. This pattern of response was identical to that of the barbiturates found in the first paper of this series,[1] although an equivocal response of G6PDH was observed after DDT.

Considerable controversy has arisen in recent years on whether halothane produces liver damage in man, but the extensive retrospective survey carried out by the National Research Council in the U.S.A.[31] effectively refuted this implication. Studies in experimental animals[32–34] have also indicated that halothane is not hepatotoxic. Rees and Zuckermann[35] in experiments using human, embryonic liver tissue-cultures were unable to find any abnormalities due to halothane, whereas chloroform induced necrosis and fatty infiltration analogous to its effects in rat liver. In the present experiments, where halothane was given orally, i.e. more compound would be expected to

reach the liver more rapidly than after inhalation, the changes induced in the liver (pattern VIII) were quite distinct from those produced by the established toxic agents. There was, however, some resemblance to the CPIB-type response (pattern IV[1]), particularly in that liver enlargement was associated with equivocal effects on drug metabolism, and also with marked elevations of both G6PDH and LDH activities. Schimassek *et al.*[28] have suggested from experiments in mice that halothane exerts its principal effect on the liver by changes in mitochondrial metabolism, a postulate also advanced for the action of CPIB,[29,30] but other evidence suggests that both agents may have a primary effect on the endoplasmic reticulum (see the third paper in this series[6]).

It is clear from the results presented here that DDT and halothane induce a very different pattern of response in rat liver to that produced by established toxic agents. All the agents examined, however, induced changes in microsomal metabolism and in extra-microsomal oxidoreductase activities, an effect which seems to be common to a wide variety of agents foreign to the animal body.[1,2,6]

The wider implications of these results are discussed in the third paper of this series.[6]

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