Biochemical Pharmacology, Vol. 18, pp. 459–473. Pergamon Press. 1969. Printed in Great Britain

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

EFFECTS OF CENTRALLY ACTING DRUGS

D. S. PLATT and B. L. COCKRILL

Research Department, I.C.I. Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire

(*Received* 29 *July* 1968; *accepted* 30 *September* 1968)

Abstract—In this third paper, results are presented on the effects on various rat liver parameters of chronic administration of several agents with activity in the central nervous system. Three patterns of response were found with the nine agents examined. Two of the groups were virtually identical except that the glutethimide group induced liver enlargement whereas the chlorpromazine-group did not. Both groups showed marked elevation of microsomal drug metabolism. Eight of the nine agents showed elevation of microsomal $NADH_2$-cyt. *c* reductase activity.

An overall discussion of the results in this and the two precedings papers is presented which suggests that toxicity and liver enlargement can be separated by biochemical means and that drug-induced liver enlargement is not necessarily a toxic response of the liver but can be a functional response. Evidence for the primary involvement of the endoplasmic reticulum is presented and an hypothesis is advanced in an attempt to simplify and rationalise the interpretation of the effects of foreign agents on the liver.

MANY drugs exhibiting activity in the central nervous system have been shown to stimulate microsomal oxidative metabolism in rat liver following their administration to the intact animal.[1–3, 7] Liver enlargement, however, was not always associated with this change and it was of interest therefore to examine the effects of several such drugs on the range of parameters detailed in the first of this series of papers,[4] to further clarify the relationship between hepatomegaly and stimulation of drug metabolism.

The results of this study are given in this third paper together with a general discussion of the wider implications of all the results presented in this and the two preceding papers.[4, 5]

METHODS

The methods employed are given in detail in the first of this series of papers.[4]

Details of the compounds investigated, the doses used and the duration of dosing are given in Table 1.

I.C.I. 49,455, an analgesic in rats, has recently been investigated in man for analgesic activity and has the following chemical structure.

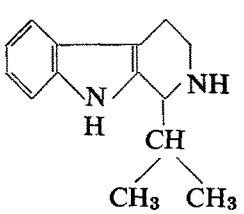

TABLE 1. DETAILS OF EXPERIMENTS PERFORMED

Expt. no.	Compounds investigated	Dose given*
15	Glutethimide	100 mg/kg p.o. for 14 days.
	Control	5 ml suspending fluid/kg p.o. for 14 days.
16	Methaqualone	100 mg/kg p.o. for 14 days.
	Methylpentynol carbamate	200 mg/kg p.o. for 14 days.
	Methylpentynol	150 mg/kg p.o. for 14 days.
	Mephenesin	200 mg/kg p.o. for 14 days.
	Control	5 ml suspending fluid/kg p.o. for 14 days.
17	Methaqualone	30 mg/kg p.o. for 14 days.
	Meprobamate	150 mg/kg p.o. for 14 days.
	Control	5 ml suspending fluid /kg p.o. for 14 days.
18	Chlorpromazine	0·10% w/w in diet for 14 days.
	Control	Powdered diet.
19	I.C.I. 49455	100 mg/kg p.o. for 13 days.
	Control	5 ml water/kg p.o. for 13 days.
20	*dl*-Amphetamine	25 mg/kg p.o. for 14 days.
	Control	5 ml water/kg p.o. for 14 days.

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

Methylpentynol-carbamate is designated methylpentynol-C in the tables for convenience.

RESULTS

The body weight changes during the period of treatment are given in Table 2. Only chlorpromazine (at a dose equivalent to about 100 mg/kg/day) caused a reduction in growth rate.

TABLE 2. BODY WEIGHT CHANGES*

Expt. no.	Compound	Mean Body wt. (g)				Ration terminal to initial body wt. (%).	
		Initial		Terminal			
		Treated	Control	Treated	Control	Treated	Control
15	Glutethimide	123	125	185	185	150	148
16	Methaqualone	85	86	182	194	214	225
	Methylpentynol-C	87		178		205	
	Methylpentynol	87		197		226	
	Mephenesin	83		187		225	
17	Methaqualone	119	122	193	191	162	157
	Meprobamate	120		190		158	
18	Chlorpromazine	126	124	170	210	135	169
19	I.C.I. 49455	166	163	211	226	127	139
20	Amphetamine	93	116	174	194	187	167

* Dosing schedules given in Table 1.

Relative liver weights are shown in Table 3. Three of the nine agents examined produced liver enlargement, *viz*; methaqualone (at both dose levels), methylpentynol-carbamate and glutethimide. The enlargement, however, was not as pronounced as with some of the agents reported in the first two papers,[4, 5] and amounted to an increase of approximately 10 per cent compared with controls. These same three

TABLE 3. LIVER WEIGHT CHANGES

Expt. No.	Compound	Liver wt. Body wt. ratio					
		Mean (g/100g)	± S.E.M.	(N)	CV (%)	% control gp.	P*
15	Glutethimide	5·03	0·08	(5)	3·4	112·5	‡
	Control	4·47	0·13	(5)	6·3	100	
16	Methaqualone	5·51	0·18	(5)	7·4	114	‡
	Methylpentynol-C	5·17	0·07	(5)	3·2	107	†
	Methylpentynol	4·97	0·18	(5)	8·0	103	n.s.
	Mephenesin	4·61	0·07	(5)	3·5	95·5	n.s.
	Control	4·84	0·10	(6)	5·1	100	
17	Methaqualone	5·03	0·13	(4)	5·2	109	†
	Meprobamate	4·79	0·07	(4)	2·7	104	n.s.
	Control	4·61	0·09	(5)	4·3	100	
18	Chlorpromazine	4·75	0·13	(5)	6·2	94	n.s.
	Control	5·05	0·15	(4)	5·9	100	
19	I.C.I. 49455	4·59	0·03	(4)	1·4	102	n.s.
	Control	4·49	0·08	(5)	4·2	100	
20	Amphetamine	4·23	0·16	(5)	8·5	97·5	n.s.
	Control	4·34	0·11	(5)	5·8	100	

* P—treated group compared with control group by Student's *t* test; n.s.—P>0·10, not significant
† P<0·10.
‡ P≤0·01.
§ P≤0·001.

compounds and also chlorpromazine and I.C.I. 49,455 induced an increase in microsomal protein concentration (Table 4). Methylpentynol and mephenesin caused a reduction of doubtful significance in microsomal protein concentration. The effects on cell-sap protein concentration were more equivocal, although glutethimide, chlorpromazine and probably I.C.I. 49,455 induced marginal increases.

$NADPH_2$-cyt. *c* reductase and AP-demethylase activities (Table 5) were both increased after administration of either glutethimide, methaqualone (at both dose levels), methylpentynol carbamate, meprobamate or chlorpromazine, i.e. five of the nine agents examined. Methylpentynol and mephenesin raised the reductase activity to a small extent but at these doses had no observable effect on demethylase activity; I.C.I. 49,455 had the opposite effect in this experiment. Amphetamine was the only compound to reduce $NADPH_2$-cyt.*c* reductase activity but a parallel effect on aminopyrine demethylase activity was not observed.

The results of the $NADH_2$-cyt.*c* reductase and G6Pase assays are given in Table 6. An unexpected finding was that all of these compounds, with the exception of mephenesin, produced an elevation of $NADH_2$-cyt. *c* reductase activity. The increased activity was particularly noticeable after chlorpromazine and methaqualone (at 100 mg/kg). In the other groups of compounds examined,[4, 5] propranolol was the only compound to induce significant elevations of this enzyme activity. The effects of these agents on G6Pase were unremarkable, although methaqualone at the higher dose of 100 mg/kg gave a significant reduction in activity.

The activities of LDH and GDH are shown in Table 7. Methaqualone at a dose of 100 mg/kg reduced both activities but had no effect at 30 mg/kg. Methylpentynol carbamate reduced LDH, glutethimide reduced GDH but the effects of the other agents were unremarkable.

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*
15	Glutethimide	14·89	0·64	(5)	9·5	124	‡	90·2	1·27	(5)	3·1	109	‡
	Control	12·00	0·33	(5)	6·2	100		82·6	1·76	(5)	4·8	100	
16	Methaqualone	13·41	0·51	(5)	8·5	141·5	§	70·1	1·90	(5)	6·0	97	n.s.
	Methylpentynol-C	11·24	0·38	(4)	6·7	118·5	‡	70·7	1.43	(3)	3·5	97·5	n.s.
	Methylpentynol	8·22	0·26	(4)	6·2	86·5	†	65·5	2·17	(5)	7·4	90·5	†
	Mephenesin	8·02	0·40	(4)	9·9	84·5	†	75·8	0·27	(3)	0·6	104·5	†
	Control	9·49	0·28	(5)	6·6	100		72·4	1·01	(5)	3·1	100	
17	Methaqualone	9·40	0·40	(4)	8·5	104·5	n.s.	77·8	2·13	(4)	5·5	107	†
	Meprobamate	9·53	0·49	(3)	8·9	106	n.s.	70·6	1·54	(4)	4·3	97	n.s.
	Control	9·00	0·21	(5)	5·2	100		72·6	0·87	(5)	2·7	100	
18	Chlorpromazine	11·6‖				166		83·5‖				118·5	
	Control	7·0‖				100		70·3‖				100	
19	I.C.I.49455	11·0‖				113·5		75·2‖				112	
	Control	9·7‖				100		67·0‖				100	
20	Amphetamine	13·28	0·55	(5)	9·2	106	n.s.	76·4	3·08	(5)	9·0	104	n.s.
	Control	12·50	0·39	(5)	7·0	100		73·5	1·65	(5)	5·0	100	

* See footnote to Table 3 for levels of significance.

‖ Concentrations assayed on pooled sample from all animals in the 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*
15	Glutethimide	1·61	0·15	(4)	19·3	159·5	‡	409	20	(5)	11·1	180	§
	Control	1·01	0·01	(4)	4·0	100		227	12	(4)	10·4	100	
16	Methaqualone	2·69	0·18	(4)	13·3	234	§§§	402	32	(4)	15·7	180	§
	Methylpentynol-C	2·22	0·18	(5)	18·2	193	§§§	319	12	(5)	8·3	143	‡‡
	Mephenesin	1·41	0·01	(4)	1·7	122·5	‡‡	258	11	(4)	8·5	116	n.s.
	Methylpentynol	1·34	0·08	(5)	13·7	116·5	†	202	21	(4)	21·2	90·5	n.s.
	Control	1·15	0·05	(6)	10·6	100		223	18	(6)	19·7	100	
17	Methaqualone	1·59	0·13	(3)	14·5	130·5	†	212	15	(4)	14·2	155	‡‡
	Meprobamate	1·43	0·06	(4)	8·4	117	†	189	14	(4)	14·5	138	††
	Control	1·22	0·07	(5)	13·1	100		137	10	(4)	14·5	100	
18	Chlorpromazine	1·61	0·17	(5)	23·4	204	‡	350	20	(5)	12·9	294	§
	Control	0·79	0·02	(3)	3·4	100		119	4	(3)	5·7	100	
19	I.C.I. 49455	1·39	0·13	(5)	21·1	103	n.s.	247	10	(4)	7·8	131	‡
	Control	1·35	0·08	(5)	12·5	100		189	12	(4)	13·1	100	
20	Amphetamine	1·15	0·09	(5)	18·3	70	‡	206	4	(4)	3·5	90·5	n.s.
	Control	1·65	0·10	(5)	12·1	100		228	10	(5)	10·0	100	

* See footnote to Table 3 for levels of significance.

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

Expt. no.	Control	$NADH_2$—cyt.*c.* reductase (μmoles. cyt.*c.* reducted/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*
15	*Glutethimide*	9·93	0·59	(5)	13·3	134·5	‡	814	19	(5)	5·3	92	n.s.
	Control	7·39	0·39	(4)	10·6	100		886	35	(5)	8·8	100	
16	Methaqualone	15·46	0·91	(5)	13·1	244	§	780	9	(4)	2·2	77	‡‡†
	Methylpentynol-C	8·43	0·47	(4)	11·2	133	‡	886	16	(4)	3·5	87	†
	Methylpentynol	7·89	0·34	(4)	8·6	124·5	‡	952	62	(5)	14·5	94	n.s.
	Mephenesin	6·14	0·44	(5)	16·0	97	n.s.	1032	75	(4)	14·5	102	n.s.
	Control	6·33	0·23	(5)	8·2	100		1016	45	(5)	10·0	100	
17	Methaqualone	8·79	0·54	(4)	12·3	136·5	‡	927	29	(4)	6·3	107	n.s.
	Meprobamate	7·65	0·44	(4)	11·4	119	†	892	21	(4)	4·7	103	n.s.
	Control	6·44	0·42	(4)	13·2	100		865	42	(5)	11·0	100	
18	Chlorpromazine	10·81	0·40	(5)	8·3	185	§	866	35	(4)	8·0	103·5	n.s.
	Control	5·85	0·46	(4)	15·8	100		837	76	(4)	18·0	100	
19	I.C.I. 49455	15·27	1·08	(4)	14·1	157·5	‡	724	36	(5)	11·1	84	†
	Control	9·69	0·57	(5)	13·2	100		864	31	(4)	7·1	100	
20	Amphetamine	8·83	0·39	(4)	8·7	130	‡	819	38	(4)	9·2	101	n.s.
	Control	6·79	0·18	(4)	5·3	100		811	22	(5)	6·0	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*
15	Glutethimide	340	26	(5)	17·3	94	n.s.	5·57	0·25	(5)	9·9	71	§
	Control	362	11	(5)	6·8	100		7·88	0·19	(4)	4·8	100	
16	Methaqualone	217	9	(5)	9·5	65	§	9·63	0·18	(5)	4·1	83·5	†
	Methylpentynol-C	246	11	(5)	10·3	73·5	‡	11·11	0·21	(5)	4·2	96·5	n.s.
	Methylpentynol	307	19	(5)	14·0	91·5	n.s.	11·23	0·30	(5)	6·0	97·5	n.s.
	Mephenesin	346	22	(5)	14·3	103	n.s.	12·81	0·40	(4)	6·2	111	n.s.
	Control	335	19	(6)	13·7	100		11·51	0·59	(5)	11·5	100	
17	Methaqualone	399	41	(4)	20·4	92	n.s.	10·63	0·61	(4)	11·5	117·5	n.s.
	Meprobamate	458	39	(4)	17·0	105·5	n.s.	9·58	0·78	(4)	16·4	106	n.s.
	Control	433	27	(5)	14·1	100		9·05	0·65	(4)	14·4	100	
18	Chlorpromazine	416	23	(4)	11·1	94	n.s.	5·39	0·37	(4)	13·8	112	n.s.
	Control	443	24	(4)	10·7	100		4·80	0·41	(3)	14·8	100	
19	I.C.I. 49455	337	13	(5)	8·5	93·5	n.s.	17·69	1·17	(5)	14·7	108	n.s.
	Control	361	23	(4)	12·8	100		16·35	1·20	(4)	14·7	100	
20	Amphetamine	504	24	(5)	10·7	100	n.s.	13·36	0·67	(5)	11·3	94	n.s.
	Control	504	18	(5)	8·0	100		14·21	0·22	(5)	3·5	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*
15	Glutethimide	5·88	0·42	(4)	11·9	183·5	§	10·66	0·39	(5)	8·1	127·5	‡
	Control	3·20	0·31	(5)	21·6	100		8·36	0·30	(5)	7·1	100	
	Methaqualone¶	16·45	1·64	(3)	17·3	272	§	17·04	1·30	(4)	15·2	138·5	‡
		5·48	0·58	(2)	14·8	91	n.s.						
16	Methylpentynol-C¶	11·76	1·13	(3)	16·6	195	‡	11·95	0·62	(4)	10·3	97	n.s.
		6·30	0·48	(2)	10·7	104	n.s.						
	Methylpentynol	6·69	0·62	(5)	20·6	111	n.s.	12·09	0·29	(4)	4·9	98	n.s.
	Mephenesin	6·73	0·21	(4)	6·2	111·5	†	11·60	0·61	(5)	11·7	94·5	n.s.
	Control	6·04	0·18	(4)	6·0	100		12·30	0·23	(5)	4·2	100	
17	Methaqualone	4·69	0·19	(4)	8·1	129·5	†	12·69	1·01	(4)	15·9	114	n.s.
	Meprobamate	4·45	0·57	(4)	25·9	123	n.s.	11·32	0·32	(4)	5·7	102	n.s.
	Control	3·62	0·39	(5)	24·3	100		11·10	0·43	(5)	8·6	100	
18	Chlorpromazine	9·40	1·27	(4)	27·1	209	†	13·96	0·91	(4)	13·1	139	‡
	Control	4·50	0·45	(3)	17·3	100		10·04	0·57	(4)	11·3	100	
19	I.C.I. 49455	15·53	1·24	(4)	15·9	234	§	14·38	0·73	(5)	11·4	136·5	‡
	Control	6·63	0·44	(4)	13·3	100		10·51	0·54	(4)	10·2	100	
20	Amphetamine	6·94	0·68	(4)	19·5	174	‡	10·56	0·38	(4)	7·1	113	†
	Control	3·99	0·40	(4)	20·1	100		9·35	0·26	(5)	6·3	100	

* See footnote to Table 3 for levels of significance.
‖ 1 Unit = change of absorbance of 0·001/min.
¶ See ref. 4 (results section) for discussion.

The activities of G6PDH and PGDH are given in Table 8. Seven of the nine drugs examined produced elevations of G6PDH, the increase being particularly marked after treatment with glutethimide, methaqualone (at 100 mg/kg), methylpentynol carbamate, chlorpromazine, I.C.I. 49,455, and amphetamine. Smaller parallel increases in PGDH activity were seen with these same compounds. The differential response of G6PDH to treatment with methaqualone (100 mg/kg) and methylpentynol carbamate was discussed in the first paper of this series.[4]

DISCUSSION

The results presented in this paper demonstrated that a wide range of centrally-acting drugs are able to stimulate microsomal drug metabolism confirming previous results.[2, 3, 6–8] Amphetamine has been shown to have no stimulatory effect,[2, 3, 6] whereas glutethimide,[2, 3, 6, 7] meprobamate[2, 3, 8] and chlorpromazine[2, 3, 6] all stimulate microsomal oxidative metabolism. Methylpentynol carbamate was reported to have no effect on pentobarbitone sleeping time in rats[3] although it stimulated meprobamate metabolism[2]; our results showed a clear stimulation of aminopyrine metabolism together with an elevated $NADPH_2$- cyt. *c* reductase activity. Neither mephenesin nor methylpentynol are reported[2, 3, 6] to induce drug metabolism, in good agreement with the present findings.

Despite, therefore, a wide variation in chemical structure, these nine agents with activity on the central nervous system, had remarkably similar effects on the pattern of response of various liver parameters. The most notable and the most unexpected change was the increase of $NADH_2$-cyt.*c* reductase activity. Three patterns of response (designated IX–XI) are shown empirically in Table 9, derived from seven of the nine agents investigated. The remaining two compounds, methylpentynol and mephenesin gave more equivocal responses at the doses employed but the trends observed indicated that these compounds could be compared with the chlorpromazine type effect, pattern X.

The only difference between the glutethimide and chlorpromazine type patterns of response was the failure of the latter group (pattern X) to produce liver enlargement; the effects on extra-microsomal LDH and GDH activities were also less marked. The glutethimide-type response (pattern IX) was virtually identical to the barbiturate-type response (pattern I, ref. 4) and the DDT-type response (pattern VII, ref. 5), with the exception that liver enlargement was not so pronounced and $NADH_2$-cyt. *c* reductase activity was increased. It was clear from these results that the compounds exhibiting patterns of response IX and X had pronounced effects on the microsomal fraction of the liver.

Amphetamine (pattern XI) had similar effects to the other centrally-acting compounds on $NADH_2$-cyt. *c* reductase, G6PDH and PGDH activities but differed markedly in its lack of effect on microsomal drug metabolism; $NADPH_2$-cyt. *c* reductase activity was in fact reduced in this experiment.

GENERAL DISCUSSION

In this and the two preceding papers,[4, 5] the effects of thirty agents on rat liver weight, protein concentrations and enzyme actitivities have been reported and briefly discussed. In all, eleven patterns of response, summarised in Table 10 have been elucidated, although the distinction between some of the patterns was often confined to the

TABLE 9. PATTERNS OF RESPONSE IN THE LIVERS OF RATS TREATED WITH CENTRALLY-ACTING DRUGS

Pattern code	Compounds showing this pattern	Effects on liver parameters*										
		RLW	Mic. prot. conc.	Cell-sap prot. conc.	$NADPH_2$ -cyt.*c* reduct	AP-demeth.	$NADH_2$ -cyt.*c* reduct.	G6PDH	PGDH	G6Pase	LDH	GDH
IX	Glutethimide Methaqualone Methylpentynol-C	I	I	nc	I	I	I	I	I	D†	D†	D
X	Chlorpromazine I.C.I. 49455 Meprobamate‡	nc	I	nc	I	I	I	I	I	D†	nc	nc
XI	Amphetamine	nc	nc	nc	D	nc	I	I	I	nc	nc	nc

* I—increase of concentration or activity;
D—decrease of concentration or activity;
nc—no change of concentration or activity.

† Values of doubtful significance.

‡ meprobamate had less significant effect than either chlorpromazine or I.C.I. 49455 but the trends were essentially identical.

TABLE 10. SUMMARY OF PATTERNS OF RESPONSE OF LIVER PARAMETERS

Pattern Code	Reference	Effect on liver parameter*										
		RLW	Mic. prot. conc.	Cell-sap. prot. conc.	$NADPH_2$-cyt.*c* reduct.	AP-demeth.	$NADH_2$-cyt.*c* reduct.	G6PDH	PGDH	G6Pase	LDH	GDH
I and VII	4 5	I	I	nc	I	I	nc	I	I	D	D	D
IX	This paper	I	I	nc	I	I	I	I	I	D†	D†	D
X	This paper	nc	I	nc	I	I	I	I	I	D†	nc	nc
III	4	nc	I†	nc	I	I	nc	nc	nc	nc	D	D
II	4	nc	—	—	I	nc	nc	nc	nc	D	D	—
IV	4	I	I†	I	I	I†	nc	I	nc	nc	I	D
VIII	5	I	nc	D†	I	nc	nc	I	D†	I†	I	D
V	4	D	—	—	nc	D	I	D	nc	nc	D	—
XI	This paper	nc	nc	nc	D	nc	I	I	I	nc	nc	nc
VI	5	I or D	D	D†	D	D	D	I	D	D	D	D

* See footnote to Table 9 for explanation of symbols.

response of one particular parameter. Some of the compounds tested (indomethacin, I.C.I. 45,763, I.C.I. 50,172, 1,1,1-trichloroethane, methylpentynol and mephenesin) had little or no observable effect on the liver.

The aim of these investigations as set out in the first of this series of papers[4] was firstly to gain some insight into the extent to which the liver can react to the presence of foreign chemicals and, secondly to attempt to separate hepatomegaly from hepatotoxicity. As an extension to the latter, an attempt was made to define hepatotoxicity more clearly in biochemical terms.

It was indicated previously[11] that drug-induced liver enlargement may not necessarily be a toxic response. The extra data presented in these papers has shown that those compounds inducing liver enlargement (patterns I, IV, VII, VIII and IX), in direct contrast to the established toxins (pattern VI), tended to show stimulation of microsomal enzymes, increases in protein concentration, less profound reductions in oxidoreductase enzymes and less marked stimulation of G6PDH activity. The only apparent similarity was the tendency for G6Pase activity to be reduced in both groups but the fall in activity (expressed as concentration per unit weight of whole liver) in the liver enlarging group was less marked than in the toxic group of agents and is probably explained by a dilution effect due to the overall liver enlargement and the raised concentration of microsomal protein. There is little evidence that frank pathological lesions are caused even at near-lethal doses of many of the compounds inducing hepatomegaly, and Golberg[12] has advanced the hypothesis that in such cases, the enlargement should be considered more as an adaptive, functional response of the liver to an increased work load. Ortega[9] has emphasised the similarities in electronmicrographs prepared from livers of rats treated with a range of hepatotoxins, the principal observations being early proliferation of the smooth endoplasmic reticulum (SER), alterations and reductions in the granular lamellae and general depletion of glycogen. Although hypertrophy of the SER is commonly found following administration of foreign chemicals,[10] differentiation can be made between the toxic and nontoxic agents under the electron-microscope.[9] Ortega's investigations with DDT[9] revealed a marked similarity between DDT, phenobarbitone and an hydantoin derivative, all of which showed SER hypertrophy but without either pronounced rupture of the granular endoplasmic membranes or ribosomal detachment. In addition, glycogen depletion was incomplete and fatty accumulation only moderate. The biochemical observations in our work,[4,5] therefore, correlate well with the electronmicroscopical evidence, suggesting that DDT is not hepatotoxic to the rat.

The enlargement observed following CPIB (and I.C.I. 53,072) and halothane—administration to rats requires further comment. Unlike the barbiturates or DDT, neither CPIB, I.C.I. 53,072 nor halothane had a pronounced effect on endoplasmic-reticular enzymes in the present experiments. There was, however, no similarity with the effects of the established toxins. Hess *et al.*[15] and Schimassek *et al.*[14] have suggested that CPIB and halothane respectively exert their effects on mitochondrial metabolism, but there is evidence to show that, in parallel with many other agents, both CPIB and halothane have effects on the endoplasmic reticulum, e.g. CPIB rapidly stimulates microsomal protein synthesis[13] and induces an early proliferation of the SER;[15,24] halothane has a similar effect on dog liver SER[16] and exhibits spectral changes *in vitro* consistent with binding to components of the microsomal $NADPH_2$-electron transport chain.[21] There is no evidence to contradict the hypothesis that

CPIB acts indirectly on the liver by displacement of thyroxine and other endogenous factors from serum protein-bound sites,[13] and it would be surprising if halothane, by analogy with CCl_4 and chloroform, did not penetrate the endoplasmic reticulum. It was clear, however, from the evidence presented here that neither compound is hepatotoxic in the rat.

The accumulated evidence[4,5,9–11,23] supports Golberg's hypothesis that drug-induced liver enlargement can represent a functional response of the liver and suggests that without critical biochemical and electron-microscopical examination of the liver, it would be unreasonable to classify liver enlargement *per se* as a toxic manifestation.

The overall similarity observed between the effects on the biochemical parameters of the various toxins (pattern VI), despite the variable pathological end-points (see ref. 5 for details), was striking and was in good agreement with the similar ultrastructural changes.[9,10] Meldolesi[10] reviewed the incidence of SER-hypertrophy in response to many foreign chemicals and demonstrated that the majority of hepatotoxins elicit this effect. In addition, the biochemical evidence presented by Feuer *et al.*[23] and ourselves[5,11] on a wide range of toxins points to the conclusion that most hepatotoxic agents exert their effects as a result of a primary involvement with the endoplasmic reticulum. Secondary direct effects at some extra-microsomal location cannot, however, be ruled out but profound disturbances in the metabolic balance of the endoplasmic reticulum would be expected to result in equally deleterious effects in extra-microsomal metabolism, e.g. to the oxidoreductases involved in the redox equilibrium of the cell.

The major implication, therefore, from the present studies and those of others[9,10,12,23] is an involvement of the endoplasmic reticulum as a primary site of action of many agents foreign to the liver. As a consequence of this, either a toxic or a non-toxic effect results. In association with toxicity, there occurs inhibition of microsomal protein synthesis and marked reduction of certain microsomal enzymes. Liver enlargement, however, is not invariably found in hepatotoxic cases. Liver enlargement is frequently associated with hypertrophy of the SER, induction of microsomal protein synthesis, induction of microsomal $NADPH_2$-electron transport and drug metabolising activity, and induction of G6PDH activity, although again there is not always a parallel between these individual effects. Meldolesi[10] postulates that SER-hypertrophy is always produced by the same mechanism and that all substances able to elicit the membrane proliferation are metabolised by microsomal enzymes and thus might act as potential inducers of microsomal enzyme activity. He further suggests that parallel changes in the SER and microsomal enzyme activity can only be observed if the agent or its metabolite do not inhibit microsomal protein synthesis. Despite such an inhibition, however, the cell still retains its capacity for SER-hypertrophy. Both toxic and non-toxic compounds fit into this conception, the toxic agents or their metabolites only differing from the non-toxic compounds in their capacity to inhibit microsomal protein synthesis; both types, however, promote hypertrophy of the SER. There is evidence in the literature to show that many of the well-established liver toxins have to undergo metabolic conversion before becoming toxic, e.g. CCl_4,[18] thioacetamide,[19] DMN[20] and others.[10] The close juxtaposition of the toxic entity formed and the endoplasmic reticular membranes may in part account for the marked reductions in activity of enzymes associated with this fraction of the liver cell. An explanation

for the lack of halothane toxicity may be offered by such an interpretation, i.e. that halothane is not metabolised to a toxic metabolite, possibly due to the stabilising influence of the C—F bonds (see ref. 22).

The present investigations have indicated variable reactions to the introduction of foreign agents into the endoplasmic reticular environment but certain interesting interrelationships have been observed, such as the identical patterns of response with different chemical types (the barbiturates, the I.C.I. compounds, 45,337 and 51,426 and DDT), the apparent stimulation of $NADH_2$-electron transport by many unrelated centrally-acting drugs and the identical responses to structurally-related compounds (e.g. CPIB and I.C.I. 53,072). Furthermore the stimulus to liver enlargement was apparently separate from the stimulus to increase microsomal protein synthesis, (compare patterns III, VIII and X—Table 10), and also from the stimulus to induce drug metabolising enzymes, (patterns II, III and X). The latter was apparently separate from the induction of microsomal protein synthesis (patterns IV and VIII) and there were differences also in the induction of microsomal protein synthesis *per se*, e.g. the protein laid down by such agents as phenobarbitone and DDT was retained within the microsomal fraction whereas that from CPIB and I.C.I. 53,072 was diverted to the cell-sap, implying synthesis of different enzyme proteins. Some agents such as propranolol and I.C.I. 45,763, although metabolised by the microsomal enzyme system, had no inducing effect, whereas others such as barbitone and phenobarbitone which are largely unmetabolised had a profound inducing effect. Elevated G6PDH activity was often correlated with liver enlargement (patterns I, IV, VII and VIII) but not exclusively so (patterns VI, X and XI). Similarly the relationship between induced drug-metabolising enzyme activity and induction of G6PDH was not absolute, as shown particularly by the toxic agents (pattern VI). The evidence now available (this paper, and refs. 4, 5, 10, 12, 23), however, suggests a close correlation between G6PDH activity and hypertrophy of the SER. The presence of the foreign chemical in the endoplasmic reticulum appears to elicit a response in this system, the function of which is to facilitate removal of the unwanted material. This is achieved usually by metabolic conversion,[17] which requires involvement of the $NADPH_2$-electron transport chain. It is possible, therefore, that an increased demand for $NADPH_2$ could be associated with stimulation of G6PDH activity, since this enzyme represents a major source of cellular $NADPH_2$.

The evidence presented, therefore, leads us to postulate that there may be receptors in the endoplasmic reticulum sensitive to the physicochemical properties of the chemical agent introduced, since chemically-unrelated compounds can only be related by such properties. Even when compounds have some structural similarity, they may possess different physicochemical properties; a good example in these investigations is the variation observed with the adrenergic β-blockers, an effect clearly related to their widely different chloroform: water partition ratios (propranolol, 34·5; I.C.I. 45,763, 4·95; I.C.I. 50,172, 0·0135). It is not apparent, however, at this stage what physicochemical properties are required to elicit the various reactions in the liver cell discussed above, nor is it apparent how many such receptors may exist. Unless, however, one envisages an infinite capacity of the liver cell to react specifically to every particular chemical, it would seem rational to suppose that there are relatively few such receptors. The binding of the compound to one or more of these sites would then give rise to the various combinations of effects observed.

In summary, therefore, now that sufficient evidence exists to allow differentiation between hepatomegaly and hepatotoxicity, it is not enough to rely on standard histological evidence in the interpretation of the effects of foreign agents on the liver. Biochemical investigations such as those used here, preferably supported by electron-microscopical evidence, allow a clearer definition of the effects of a foreign chemical.

Acknowledgement—The skilled technical assistance of Mrs. J. Siddall is most gratefully acknowledged.

REFERENCES

1. A. H. Conney, *Proc. 2nd Int. Pharmac. Meet. Prague* **4**, 277 (1963).
2. R. Kato and P. Vassanelli, *Biochem. Pharmac.* **11**, 779 (1962).
3. R. Kato and E. Chiesari, *Br. J. Pharmac.* **18**, 29 (1962).
4. D. S. Platt and B. L. Cockrill, *Biochem. Pharmac.* **18**, 429 (1969).
5. D. S. Platt and B. L. Cockrill, *Biochem. Pharmac.* **18**, 445 (1969).
6. R. Kato, E. Chiesara and P. Vassanelli, *Biochem. Pharmac.* **13**, 69 (1964).
7. H. Remmer, in *Ciba Foundation Symposium: Enzymes and Drug Action* (Eds. J. L. Mongar and A. V. S. de Reuck), p. 276. Churchill, London (1962).
8. J. A. Smith, W. J. Waddell and T. C. Butler, *Life Sci.* **3**, 486 (1963).
9. P. Ortega, *Lab. Invest.* **15**, 657 (1966).
10. J. Meldolesi, *Biochem. Pharmac.* **16**, 125 (1967).
11. D. S. Platt and B. L. Cockrill, *Biochem. Pharmac.* **16**, 2257 (1967).
12. L. Golberg, *Proc. Eur. Soc. Study Drug Toxicity* **7**, 171 (1966).
13. D. S. Platt and J. M. Thorp, *Biochem. Pharmac.* **15**, 915 (1966).
14. H. Schimassek, W. Kunz and D. Gallwitz, *Biochem. Pharmac.* **15**, 1957 (1966).
15. R. Hess, W. Stäubli and W. Riess, *Nature, Lond.* **208**, 856 (1965).
16. A. H. Giesecke, Jr., J. R. Clark, V. A. Stembridge, F. T. Callus and M. T. Jenkins, Anesthesiology, **27**, 214 (1966).
17. B. B. Brodie, J. R. Gillette and B. N. La Du, Ann. Rev. Biochem. **27**, 427 (1958).
18. A. A. Seawright and A. E. M. McLean, *Biochem. J.* **105**, 1055 (1967).
19. O. Nygaard, L. Eldjarn and K. F. Nakken, *Cancer Res.* **14**, 625 (1954).
20. J. A. J. Brouwers and P. Emmelot, *Exp. Cell Res.* **19**, 467 (1960).
21. A. E. M. McLean, *Biochem. Pharmac.* **16**, 2030 (1967).
22. T. F. Slater, *Nature Lond.*, **209**, 36 (1966).
23. G. Feuer, L. Golberg and J. R. le Pelley, *Food. Cosmet. Toxicol.* **3**, 235 (1965).
24. G. E. Paget, *J. Atheroscler. Res.* **3**, 729 (1963).