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

EFFECTS OF ANTICONVULSANT, ANTI-INFLAMMATORY, HYPOCHOLESTEROLAEMIC AND ADRENERGIC β-BLOCKING AGENTS

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Abstract—Results are presented on the effects of 1–2 week administration of several therapeutically-active agents on various rat liver parameters. The data form part of a larger study presented in a series of three papers.

At least 5 distinct patterns of response have been observed in rat liver following administration of anti-convulsant, anti-inflammatory, hypocholesterolaemic and adrenergic β-receptor blocking agents. Agents of analogous chemical structure tended to show similar patterns of response but in the anticonvulsant group, agents with different chemical structures have also been shown to exert identical effects in the liver. Liver enlargement with different underlying patterns of response of liver enzyme measurements, stimulation of microsomal drug metabolism with or without liver enlargement and differential effects on two components of the microsomal $NADPH_2$-electron transport chain have all been observed.

A PREVIOUS paper from these laboratories reported the results of an investigation into the possible relationship of drug-induced liver enlargement and hepatotoxicity;[1] it was concluded that (a) liver enlargement may not necessarily reflect a toxic response of the liver to drug treatment, and (b) that "functional" enlargement may be associated with changes in the dehydrogenase levels in the liver cell and in microsomal metabolism. Well-established liver toxins such as carbon tetrachloride and thioacetamide also exerted their primary effects on the microsomal fraction of the liver cell.

We have pursued these observations further by examining the effects of a wide range of agents on the activities of liver enzymes, particularly those associated with microsomal metabolism and the oxidation and reduction of endogenous substrates.

A considerable mass of data has been accumulated to date and to facilitate the presentation, the investigations have been divided into three separate papers, this and the two subsequent papers in this series.[2,3] To avoid repetition, details of the parameters measured and the methods used are given in this paper, and a general discussion of all the results is given in the third paper of the series.[3]

In this first paper, the results of treatment with various anticonvulsant, anti-inflammatory, hypocholesterolaemic and adrenergic β-receptor antagonist agents are reported; the majority are well-established therapeutic agents in man.

METHODS

Experimental animals

Male rats of a specific pathogen-free Wistar-derived Alderley Park Strain were used throughout. The rats weighed 120–170 g at the start of each experiment and carefully matched controls in terms of body weight were included in each experiment.

Dosing procedures

The rats were maintained on a standard powdered diet (Powder 'O' of Scottish Agricultural Industries) given *ad libitum*. The compounds to be tested were either incorporated in the diet or dosed orally by tube as solutions or dispersions. Control animals were treated in an exactly similar way in each experiment so that variations due to handling techniques could be discounted.

Diet feeding *ad libitum* or oral dosing (once daily at 9.00–9.30 a.m.) was continued for 7–14 days. In all cases, dosing was terminated 24 hr before sacrifice.

The doses of the compounds used in these experiments were usually close to the maximal tolerated dose in rats, i.e. were in considerable excess of therapeutically-effective doses.

The compounds examined in this paper together with details of the doses used are listed in Table 1.

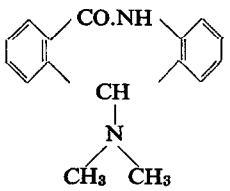
TABLE 1. DETAILS OF EXPERIMENTS PERFORMED

Expt. No.	Compounds Investigated	Dose given*
1a	Phenobarbitone	0·20% w/w in diet for 14 days
	Control	Powdered diet
1b	Phenobarbitone	200 mg/kg p.o. for 7 days
	Control	5 ml water/kg p.o. for 7 days
2	Barbitone	200 mg/kg p.o. for 7 days
	I.C.I. 45337	500 mg/kg p.o. for 7 days
	Diphenylhydantoin	200 mg/kg p.o. for 7 days
	Propranolol	150 mg/kg p.o. for 7 days
	Control	5 ml saline/kg p.o. for 7 days
3	I.C.I. 51426	250 mg/kg p.o. for 14 days
	Control	5 ml suspending fluid/kg p.o. for 14 days
4	CPIB	0·25% w/w in diet for 14 days
	I.C.I. 53072	0·025% w/w in diet for 14 days
	Control	Powdered diet
5	Indomethacin	3 mg/kg p.o. for 7 days
	Control	5 ml suspending fluid/kg p.o. for 7 days
6	Phenylbutazone	0·15% w/w in diet for 14 days
	Control	Powdered diet
7	I.C.I. 45763	100 mg/kg p.o. for 7 days
	I.C.I. 50172	100 mg/kg p.o. for 7 days
	Control	5 ml saline/kg p.o. for 7 days

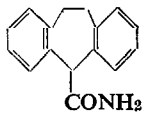
* Powdered diet given *ad libitum* for at least 7 days before dosing commenced.

The structures of the unfamiliar I.C.I. compounds are as follows:

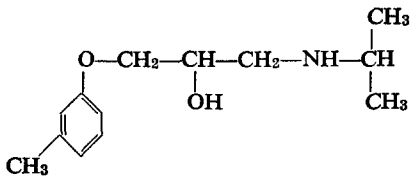
I.C.I. 45,337



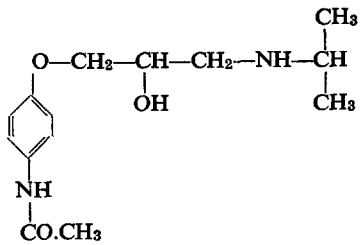
I.C.I. 51,426



I.C.I. 45,763



I.C.I. 50,172



The compounds are classified pharmacologically as follows:

Anticonvulsants: phenobarbitone, barbitone, diphenylhydantoin, I.C.I. 45,337 and I.C.I. 51,426 (identical with Ayerst compound AY-8682)

Anti-inflammatories: phenylbutazone, indomethacin

Hypocholesterolaemics: Atromid-S* (ethyl α-(4-chlorophenoxy)-α-methyl propionate, CPIB), I.C.I. 53,072 (structure in ref. 1)

Adrenergic β-blockers: Inderal* (propranolol), I.C.I. 45,763 and I.C.I. 50,172.

With the exception of I.C.I. 53,072, all these compounds have been administered to man.

Parameters measured

The liver enzymes assayed are shown in Table 2. In addition to these parameters, liver weights were recorded and protein concentrations of the microsomal and cell-sap fractions were also assayed.

To avoid any confusion in interpretation, some definition of three of these enzymes is given.

(*a*) *Microsomal NADPH$_2$-cytochrome* c *reductase.* The activity measured refers to the activity of the flavoprotein-enzyme in the microsomal NADPH$_2$-electron transport chain, which is capable of using exogenous cytochrome *c* as the electron-acceptor from NADPH$_2$ (see ref. 4).

(*b*) *Microsomal aminopyrine* N-*demethylase.* The *N*-demethylation of a variety of drug substrates is one of a group of well-established reactions of the NADPH$_2$-electron transport chain (e.g. see ref. 5). As a measure of the activity of this electron-transport chain with respect to drug metabolism, the *N*-demethylation of aminopyrine has been chosen as a model for comparison with the NADPH$_2$-cytochrome *c* reductase activity, the object being to determine whether differential effects can be

* Atromid-S and Inderal are I.C.I. Trade Marks.

TABLE 2. ENZYME ACTIVITIES MEASURED

Trivial name	Systematic name	E.C. code	Abbreviations used in these papers	Reference to assay method	Homogenate used in assay
Glucose-6-phosphate dehydrogenase	D-glucose-6-phosphate: NADP oxidoreductase	1.1.1.49	G6PDH	7	15000 *g* KCl
6-Phosphogluconate dehydrogenase	6-phospho-D-gluconate: NADP oxidoreductase (decarboxylating)	1.1.1.44	PGDH	7	15000 *g* KCl
Lactate dehydrogenase	L-lactate: NAD oxidoreductase	1.1.1.27	LDH	8	15000 *g* KCl
Glutamate dehydrogenase	L-glutamate: NAD oxidoreductase (deaminating)	1.4.1.2	GDH	9	600 *g* sucrose
$NADH_2$-cytochrome *c* reductase	$NADH_2$-cytochrome *c* oxidoreductase	1.6.99.3	As trivial name	11	15000 *g* KCl
$NADPH_2$-cytochrome *c* reductase	$NADPH_2$-cytochrome *c* oxidoreductase	1.6.99.1	As trivial name	13	15000 *g* KCl
Aminopyrine *N*-demethylase	—	—	AP-demethylase	12	15000 *g* KCl
Glucose-6 phosphatase	D-glucose-6-phosphate phosphohydrolase	3.1.3.9	G6Pase	10	600 *g* sucrose

exerted by drugs on these two components of what is in effect an integrated enzyme system.

(*c*) *Microsomal $NADH_2$-cytochrome* c *reductase.* The activity measured refers to the activity of the enzyme system which is able to utilise exogenous cytochrome *c* as an acceptor at the stage subsequent to cytochrome b_5 (see ref. 4) in the $NADH_2$-electron transport chain. Exogenous cytochrome *c* is unable to accept electrons from the flavoprotein in this system) i.e. unlike the $NADPH_2$-system.

Assay procedures

Rat livers were collected and processed as described in detail previously.[1]

The methods of enzyme determination were either direct applications of established methods or minor modifications of the methods indicated in Table 2; in all cases, the control homogenates were processed in an identical manner to the 'treated' homogenates.

Dehydrogenase activities were assayed spectrophotometrically by following the rate of reduction of the respective pyridine-nucleotide coenzyme (either NADP for G6PDH and PGDH, or NAD for LDH and GDH) at 340 mμ at 25° using a Unicam SP800 dual-beam recording spectrophotometer. PGDH activity was determined at pH 7·6.[7] $NADPH_2$-and $NADH_2$-cytochrome *c* reductases were similarly assayed by following the rate of reduction of cytochrome *c* at 550 mμ at 25°.

The protein concentrations of microsomal and cell-sap fractions were measured by the method of Lowry *et al.*[6] after centrifugation of either pooled or individual 15,000 *g* KCl homogenates at 105,000 *g* for 60 min in a Spinco Model L ultracentrifuge.

Expression of activities

The dehydrogenase and G6Pase activities were derived and expressed as given previously.[1] The activities of the pyridine-nucleotide: cytochrome *c* reductases were derived from the initial linear sections of the spectrophotometric recordings and expressed as μmoles cytochrome *c* reduced/g fresh liver/min. Aminopyrine demethylase activity was expressed as mμmol formaldehyde formed/g fresh liver/min, and protein concentrations were expressed as mg per equivalent g fresh liver.

The results were statistically analysed as in a previous report.[1]

RESULTS

The effects of the various treatments on the growth of the rats are shown in Table 3. Propranolol, at a dose many times in excess of that therapeutically-effective in rats, was the only agent in this group to cause a marginal reduction in whole body growth.

Liver weight changes, protein concentrations and enzyme activities are given in Tables 4, 5 and 6–9 respectively.

Anticonvulsant agents

Phenobarbitone, barbitone, I.C.I. 45,337 and I.C.I. 51,426 all gave rise to a qualitatively similar pattern of response in rat liver. Hepatomegaly was associated with increased activities of $NADPH_2$-cyt. *c* reductase, AP-demethylase, G6PDH and PGDH, and decreased activities of LDH, GDH and G6Pase. $NADH_2$-cyt. *c* reductase activity was not affected by any of these 4 agents.

TABLE 3. BODY WEIGHT CHANGES*

Expt. No.	Compound	Mean body wt. (g)				Ratio terminal to initial body wt. (%)	
		Initial		Terminal			
		Treated	control	Treated	control	Treated	control
1a	Phenobarbitone	123	120	204	206	166	172
1b	Phenobarbitone	169	172	197	213	117	124
2	Barbitone	122	120	171	157	140	131
	I.C.I. 45337	116		152		131	
	Diphenylhydantoin	127		159		125	
	Propranolol	115		136		118	
3	I.C.I. 51426	134	138	208	202	155	146
4	CPIB	125	127	212	225	170	177
	I.C.I. 53072	126		218		173	
5	Indomethacin	119	118	160	161	135	137
6	Phenylbutazone	119	124	195	210	164	169
7	I.C.I. 45763	126	125	168	169	133·5	135
	I.C.I. 50172	125		164		131	

* Dosing schedules given in Table 1.

TABLE 4. LIVER WEIGHT CHANGES IN RESPONSE TO TREATMENT WITH THE VARIOUS COMPOUNDS

Expt. No.	Compound	Liver wt.: Body wt. ratio				Per cent control group	P*
		Mean (g/100 g)	± S.E.M.	(N)	CV (%)		
1a	Phenobarbitone	5·90	0·14	(5)	5·5	114	‡
	Control	5·17	0·15	(5)	6·3	100	
1b	Phenobarbitone	6·31	0·09	(5)	3·1	126	§
	Control	5·00	0·11	(5)	4·8	100	
2	Barbitone	5·50	0·18	(4)	6·6	108·5	†
	I.C.I. 45337	6·61	0·16	(4)	4·9	130	‡
	Diphenylhydantoin	5·29	0·14	(3)	4·6	104	n.s.
	Propranolol	4·68	0·06	(5)	2·7	92·5	‡
	Control	5·07	0·07	(4)	2·8	100	
3	I.C.I. 51426	5·86	0·09	(5)	3·4	129·5	§
	Control	4·53	0·06	(5)	3·0	100	
4	CPIB	6·83	0·18	(5)	6·0	131	§
	I.C.I. 53072	8·36	0·14	(5)	3·7	161	§
	Control	5·20	0·09	(5)	3·8	100	
5	Indomethacin	4·87	0·16	(5)	7·3	105	n.s.
	Control	4·65	0·19	(5)	9·1	100	
6	Phenylbutazone	5·34	0·10	(5)	4·2	106	n.s.
	Control	5·05	0·15	(4)	5·9	100	
7	I.C.I. 45763	4·55	0·10	(5)	5·0	99	n.s.
	I.C.I. 50172	4·58	0·09	(5)	4·1	100	n.s.
	Control	4·60	0·08	(4)	3·5	100	

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

Microsomal and cell-sap protein concentrations (Table 5) were determined on samples of pooled homogenates from the individual rats and significance levels, therefore, could not be determined. It was clear, however, that phenobarbitone and I.C.I. 51,426 gave rise to elevated concentrations of protein in the microsomal fraction,

TABLE 5. 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)		
		Treated	Control	Treated as per cent control	Treated	Control	Treated as per cent control
1a	Phenobarbitone	14·0	9·7	144	75·5	71·6	105
1b	Phenobarbitone	12·9	9·3	139	75·2	76·7	98
2	Barbitone I.C.I. 45337 Diphenylhydantoin Propranolol	experiments not performed			experiment not performed		
3	I.C.I. 51426†	13·3	10·2	130	65·9	68·9	95·5
4	CPIB	11·8	10·1	117	83·5	58·0	144
	I.C.I. 53072	11·3		112	77·7		134
5	Indomethacin	9·5	7·8	122	65·2	66·5	98
6	Phenylbutazone	7·9	7·0	113	68·7	70·3	98
7	I.C.I. 45763	10·2	9·3	110	76·4	67·9	112·5
	I.C.I. 50172	8·8		94·5	84·5		124·5

* Protein concentrations determined on aliquots of pooled homogenates (see Methods Section).
† See text for further results.

and, by analogy, barbitone and I.C.I. 45,337 would be expected to show the same effect. The homogenates from the I.C.I. 51,426 treated group were further examined by treating each rat sample individually, with the following results:—

	Treated			Control			P
	Mean (mg/g)	± S.E.M.	(N)	Mean (mg/g)	± S.E.M.	(N)	
Microsomal protein	15·39	0·49	(5)	9·98	0·42	(5)	< 0·001
Cell-sap protein	74·0	1·7	(5)	74·7	2·8	(5)	n.s.

These determinations of the protein concentrations in individual rat liver preparations confirmed the results obtained from pooled homogenates.

It was of considerable interest that an identical pattern of response in rat liver was seen after treatment with these different chemical types, the I.C.I. compounds being related basic chemicals quite unlike the acidic barbiturates.

In contrast to this "barbiturate-type" pattern of response diphenylhydantoin gave rise to a qualitatively dissimilar pattern in that liver enlargement (Table 4) and stimulation of AP-demethylase (Table 6) were not observed. There was a similarity, however, in the elevated $NADPH_2$-cyt. *c* reductase activity, in the decreased G6Pase and LDH activities and the lack of a significant effect on $NADH_2$-cyt. *c* reductase.

The diphenylhydantoin pattern of response was of most interest in the differential effect observed on the separate stages of microsomal $NADPH_2$-electron transport,

TABLE 6. LIVER ENZYME ACTIVITIES: $NADPH_2$-CYTOCHROME-*c* REDUCTASE AND AMINOPYRINE DEMETHYLASE

Expt. No.	Compound	NADPH₂-Cytochrome *c* reductase (μmoles cyt. *c* reduced/g/min)						Aminopyrine demethylase (mμ moles 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*
1a	Phenobarbitone	3·22	0·25	(5)	17·5	238	§	547	28	(5)	11·4	242	§
	Control	1·35	0·11	(5)	17·4	100		226	8	(5)	8·2	100	
1b	Phenobarbitone	3·04	0·34	(5)	25·1	264	§	568	30	(5)	11·6	280	§
	Control	1·15	0·08	(5)	15·0	100		203	15	(5)	16·5	100	
2	Barbitone	2·11	0·11	(4)	10·0	358	§§	380	15	(4)	8·0	312	§§
	I.C.I. 45337	1·76	0·11	(3)	11·1	298	§§	289	31	(4)	21·7	237	§§
	Diphenylhydantoin	1·63	0·29	(3)	30·8	276	‡	127	6	(3)	8·7	104	n.s.
	Propranolol	0·72	0·10	(4)	28·2	122	n.s.	81	9	(3)	20·0	66·5	†
	Control	0·59	0·06	(4)	19·5	100		122	8	(4)	12·8	100	
3	I.C.I. 51426	3·83	0·21	(5)	12·0	190	§	255	18	(4)	14·4	161	‡
	Control	2·02	0·21	(5)	23·4	100		159	10	(4)	12·0	100	
4	CPIB	1·94	0·15	(5)	17·1	149	‡	338	40	(4)	23·8	136	†
	I.C.I. 53072	1·73	0·07	(5)	9·4	133	‡	271	20	(5)	16·5	109	n.s.
	Control	1·30	0·06	(5)	9·4	100		248	11	(4)	9·2	100	
5	Indomethacin	1·04	0·06	(5)	12·9	96·5	n.s.	187	13	(5)	15·5	94·5	n.s.
	Control	1·08	0·12	(5)	25·2	100		198	19	(5)	21·2	100	
6	Phenylbutazone	1·34	0·09	(5)	14·2	170	‡	232	12	(5)	11·7	195	§
	Control	0·79	0·02	(3)	3·4	100		119	4	(3)	5·7	100	
7	I.C.I. 45763	1·13	0·05	(5)	9·5	113	n.s.	212	10	(5)	10·4	117	†
	I.C.I. 50172	0·97	0·06	(5)	14·3	97	n.s.	181	11	(5)	13·2	100	n.s.
	Control	1·00	0·05	(5)	11·2	100		181	5	(5)	4·9	100	

* See footnote to Table 4 for levels of significance.

viz:—stimulated flavoprotein enzyme activity with no observable effect on the terminal enzyme responsible for drug substrate oxidation.

Anti-inflammatory agents

Neither indomethacin nor phenylbutazone produced a significant effect on relative liver weight (Table 4), although both produced an apparent increase in microsomal protein concentration (Table 5). Indomethacin produced an unremarkable response in the liver with no significant effect on any of the enzymes measured, except a 15 per cent fall in G6Pase activity, which in isolation was considered to be of doubtful relevance.

Phenylbutazone gave rise to significant reductions in both LDH and GDH (Table 8), but had no effect on pentose-phosphate shunt activity (Table 9), G6Pase or $NADH_2$ cytochrome *c* reductase activities (Table 7). There was, however, marked stimulation of microsomal drug metabolism (Table 6), the levels of both $NADPH_2$-cyt. *c* reductase and AP-demethylase being approximately doubled.

Hypocholesterolaemic Agents

Both CPIB and I.C.I. 53,072 produced liver enlargement which was associated in these investigations not only with marginally increased concentrations of microsomal protein but also with a much greater increase of cell-sap protein concentration. Both agents caused an elevation of LDH activity, and also moderate increases in $NADPH_2$-cyt. *c* reductase activity. Only CPIB, however, had an observable effect on AP-demethylase activity, the level being increased by 36 per cent compared with controls.

Both agents stimulated G6PDH activity, but neither agent had any effect on PGDH or G6Pase activities. In a previous experiment,[17] CPIB caused a reduction of $NADH_2$-cyt. *c* reductase activity in rat liver, but in this investigation this same agent had no effect. I.C.I. 53,072, however, depressed the activity of this enzyme by some 25 per cent.

Qualitatively, however, there was a closely-similar pattern of response to these structurally-related compounds.

Adrenergic β-blocking agents

Three I.C.I. compounds in this relatively new pharmacological category were compared. Propranolol and I.C.I. 45,763 behave similarly in that they have both β-blocking and anti-arrhythmic properties *in vivo*, whereas I.C.I. 50,172 possesses only β-blocking activity.

No unequivocal pattern of response related to all three compounds could be observed even at the high doses used, the effects of both I.C.I. 50,172 and I.C.I. 45,763 being largely unremarkable. Propranolol, however, showed a significant decrease in relative liver weight (probably associated with retarded body growth—Table 3) and an apparent inhibition of AP-demethylase activity (Table 6). This was not accompanied by a reduction in G6Pase activity. Propranolol was the only compound in this group to increase $NADH_2$-cyt. *c* reductase activity. This agent also reduced both LDH and G6PDH activities, but had no effect on PGDH activity.

TABLE 7. LIVER ENZYME ACTIVITIES: $NADH_2$-CYTOCHROME *c* REDUCTASE AND GLUCOSE-6 PHOSPHATASE

Expt. No.	Compound	$NADH_2$-Cytochrome *c* reductase (μmoles cyt. *c.* reduced/g/min)						Glucose-6 phosphatase (μmoles inorganic '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*
1a	Phenobarbitone	11·69	0·63	(4)	10·7	107·5	n.s.	558	41	(5)	16·5	63·5	§
	Control	10·88	1·20	(5)	24·7	100		880	29	(4)	7·4	100	
1b	Phenobarbitone	9·96	0·59	(5)	13·2	107·5	n.s.	601	60	(5)	22·2	75	†
	Control	9·27	0·34	(5)	8·5	100		804	33	(5)	9·3	100	
2	Barbitone	5·53	0·24	(4)	8·7	102·5	n.s.	606	24	(4)	8·0	70	††††
	I.C.I. 45,337	5·58	0·46	(3)	14·4	103	n.s.	646	29	(4)	9·0	75	††††
	Diphenylhydantoin	6·85	1·12	(3)	28·2	127	n.s.	715	22	(3)	5·2	83	††
	Propranolol	8·60	0·51	(5)	13·2	159	‡	695	79	(4)	22·7	80·5	n.s.
	Control	5·40	0·45	(4)	16·6	100		863	44	(4)	10·2	100	
3	I.C.I. 51,126	9·96	0·40	(5)	9·0	100·5	n.s.	712	16	(4)	4·6	78	‡‡
	Control	9·90	0·41	(5)	9·3	100		914	64	(4)	7·0	100	
4	CPIB	10·15	0·58	(4)	11·4	102·5	n.s.	831	54	(5)	14·6	104	n.s.
	I.C.I. 53,072	7·39	0·36	(4)	9·8	75	†	764	55	(5)	16·1	95·5	n.s.
	Control	9·89	0·97	(5)	21·8	100		800	30	(5)	8·5	100	
5	Indomethacin	6·90	0·38	(5)	12·2	101·5	n.s.	790	10	(5)	2·9	84·5	‡
	Control	6·80	0·40	(5)	13·1	100		937	32	(4)	6·9	100	
6	Phenylbutazone	6·94	0·71	(5)	22·8	118·5	n.s.	672	26	(4)	9·9	80·5	n.s.
	Control	5·85	0·46	(4)	15·8	100		837	76	(4)	18·0	100	
7	I.C.I. 45,763	8·72	0·72	(5)	18·3	112	n.s.	905	46	(4)	10·2	100	n.s.
	I.C.I. 50,172	7·73	0·26	(5)	7·5	99·5	n.s.	924	55	(4)	12·0	102	n.s.
	Control	7·77	0·59	(4)	15·1	100		908	39	(5)	9·5	100	

* See footnote to Table 4 for levels of significance.

TABLE 8. LIVER ENZYME ACTIVITIES: LACTATE DEHYDROGENASE AND GLUTAMATE DEHYDROGENASE

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§
1a	Phenobarbitone	264	13	(5)	10·9	75·5	‡	2·98	0·23	(4)	15·7	53	‡
	Control	350	14	(5)	8·6	100		5·61	0·48	(5)	19.0	100	
1b	Phenobarbitone	269	5	(5)	4·0	77	§	2·87	0·20	(5)	15·9	60·5	§
	Control	349	12	(5)	7·6	100		4·74	0·12	(5)	5·5	100	
2	Barbitone	193	7	(4)	6·8	57·5	§	assay not performed—see text					
	I.C.I. 45,337	198	6	(4)	5·7	59	‡						
	Diphenylhydantoin	264	12	(3)	8·1	78·5	†						
	Propranolol	218	13	(4)	10·0	64·5	‡						
	Control	337	24	(4)	14·1	100							
3	I.C.I. 51.426	239	9	(5)	7·9	72	§	3·23	0·35	(5)	24·4	45·5	§
	Control	332	3	(5)	2·2	100		7·07	0·23	(4)	6·5	100	
4	CPIB	682	18	(5)	6·0	152	§§	assay not performed see text					
	I.C.I. 53,072	857	64	(4)	15·0	191	§§						
	Control	448	17	(5)	8·3	100							
5	Indomethacin	373	22	(5)	13·3	105	n.s.	3·88	0·17	(4)	9·0	105·5	n.s.
	Control	355	11	(5)	7·0	100		3·67	0·44	(4)	24·1	100	
6	Phenylbutazone	334	20	(5)	11·1	75·5	‡	3·77	0·07	(4)	3·5	78·5	†
	Control	443	24	(4)	10·7	100		4·80	0·41	(3)	14·8	100	
7	I.C.I. 45,763	447	13	(5)	6·4	105	n.s.	5·77	0·37	(4)	15·6	75·5	†
	I.C.I. 50,172	432	19	(5)	9·9	101·5	n.s.	5·52	0·12	(4)	4·3	87·5	†
	Control	425	25	(5)	13·0	100		6·32	0·33	(4)	10·5	100	

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

§ See footnote to Table 4 for levels of significance.

TABLE 9. LIVER ENZYME ACTIVITIES: GLUCOSE-6 PHOSPHATE AND 6-PHOSPHOGLUCONATE DEHYDROGENASES

Expt. No.	Compound	G6PDH* (units/g)						PGDH* (units/g)					
		Mean	± S.E.M.	(N)	CV (%)	Per cent control gp.	P§	Mean	± S.E.M.	(N)	CV (%)	Per cent control gp.	P§
1a	Phenobarbitone¶	6·29	0·99	(4)	31·3	97·5	n.s.	9·04	0·35	(4)	7·7	130	†
	Control	6·45	0·42	(4)	13·2	100		6·95	0·60	(5)	19·2	100	
	Phenobarbitone	20·61	1·89	(5)	20·5	264	§	9·92	0·31	(5)	7·1	131	‡
	Control	7·81	0·66	(4)	17·0	100		7·56	0·51	(5)	15·2	100	
2	Barbitone¶	6·71	1·71	(2)	36·0	118·5	n.s.	11·26	0·06	(2)	0·7	100	n.s.
		12·51	2·27	(2)	25·7	221	§	17·52	0·29	(2)	2·3	156	‡‡‡
	I.C.I. 45,337	8·46	0·07	(3)	1·5	149	‡	15·16	0·71	(4)	9·4	135	‡‡‡
	Diphenylhydantoin	5·19	0·72	(3)	24·0	91·5	n.s.	12·71	1·05	(3)	14·3	113·5	n.s.
	Propranolol	3·84	0·35	(3)	16·0	68	†	12·12	1·09	(3)	15·5	108	n.s.
	Control	5·66	0·46	(4)	16·3	100		11·21	0·59	(4)	10·6	100	
3	I.C.I. 51,426	5·72	0·27	(4)	9·2	114·5	n.s.	18·08	1·40	(4)	15·5	134	‡
	Control	5·00	0·47	(4)	18·6	100		13·50	0·29	(5)	4·8	100	
4	CPIB¶	21·42	4·35	(2)	28·7	286	‡	10·53	0·40	(5)	8·5	93·5	n.s.
		6·92	0·68	(3)	17·0	92	n.s.						
	I.C.I. 53,072	21·83	2·77	(4)	25·3	290	‡	11·96	0·34	(4)	5·7	106	n.s.
	Control	7·52	0·89	(4)	23·6	100		11·27	0·36	(5)	7·1	100	
5	Indomethacin	4·57	0·46	(4)	20·0	89	n.s.	9·06	0·31	(5)	7·6	93·5	n.s.
	Control	5·15	0·42	(5)	18·3	100		9·71	0·37	(5)	8·6	100	
6	Phenylbutazone	4·71	0·54	(3)	19·7	105	n.s.	10·76	0·39	(4)	7·3	107	n.s.
	Control	4·50	0·45	(3)	17·3	100		10·04	0·57	(4)	11·3	100	
7	I.C.I. 45,763	4·14	1·04	(4)	50·5	117	n.s.	8·29	0·16	(5)	4·3	103·5	n.s.
	I.C.I. 50,172	3·69	0·32	(4)	17·5	104	n.s.	8·27	0·42	(5)	11·3	103	n.s.
	Control	3·55	0·23	(5)	14·4	100		8·00	0·26	(5)	7·4	100	

* 1 unit = change of absorbance of 0·001/min.

§ See footnote to Table 4 for levels of significance.

¶ See text.

G6PDH Activity and PGDH Activity

The activities given in Table 9 require further comment, in particular the data in experiments 2 and 4. In the barbitone-treated rats in experiment 2, two of the group of four rats showed an elevated G6PDH activity (221per cent of control) and PGDH activity (156 per cent of control). In the CPIB group (experiment 4), 2 of the five animals showed elevated G6PDH activity (286 per cent of control), the remaining animals showing levels insignificantly different from controls. A similar differential effect on G6PDH activity was noted after treatment with methaqualone and methylpentynol carbamate in the third paper in this series.[3] In a previous paper,[1] CPIB under similar conditions to the present experiment had no effect on G6PDH activity although even earlier studies[17] had shown unequivocally that CPIB elevated G6PDH activity in rat liver.

The reason for these discrepancies is not fully understood, but it may be related to differing degrees of response of individual rats to the treatment either in the extent to which G6PDH (and PGDH) activity is raised or to the rate at which an elevated level reverts to normal. Kunz *et al.*[18] have demonstrated a rapid elevation of G6PDH activity in mouse liver during the early phase of phenobarbitone administration, followed, however, by a return to normal levels on continued administration. It is possible, therefore, that the effects on G6PDH recorded in these experiments represent transient changes but we have evidence that elevated G6PDH levels can be maintained for at least three months during treatment with several agents.[19]

DISCUSSION

The observed results on some of the individual liver parameters were in general agreement with those reported by this and other laboratories:

(a) *Phenobarbitone* causes liver enlargement (e.g. ref. 22) and stimulates microsomal protein synthesis,[20] the protein synthesised being retained within the microsomes.[20,21] The barbiturates represent classical examples of non-specific inducers of microsomal oxidative drug metabolism (e.g. refs. 23, 24). Bresnick and Yang[29] have previously reported a similar effect of phenobarbitone and barbitone on G6PDH and PGDH activities. It is worth noting that when these investigators measured PGDH activity at its pH optimum of 9·0, instead of pH 7·6 as done here, they found virtually equal increases of both G6PDH and PGDH activities. Our estimates, therefore, of the increase of PGDH activity were probably low.

(b) *Diphenylhydantoin* has an equivocal effect on oxidative drug metabolism.[14,15]

(c) *Phenylbutazone* has a similar effect to the barbiturates on oxidative drug metabolism (e.g. ref. 24). Silvestrini *et al.*[25] have demonstrated liver enlargement in mice after treatment with a 0·3% diet of phenylbutazone, but not after a 0·1% diet. We have been unable to find reference to a similar hepatomegalic effect of this agent in rats.

(d) Previous results of treatment with *CPIB and I.C.I. 53,072* are in good agreement with present findings[1,16,17] Azarnoff *et al.*[26] have reported that CPIB does not stimulate its own metabolism, but we have been able to demonstrate marginal elevations of the activities of both $NADPH_2$-cyt. *c* reductase and AP-demethylase in the liver microsomes.

The changes in the various liver parameters are recorded empirically in Table 10, which shows 5 distinct patterns of response (designated I–V) to nine of the twelve

TABLE 10. PATTERNS OF RESPONSE OF VARIOUS LIVER PARAMETERS TO TREATMENT OF RATS WITH VARIOUS THERAPEUTIC AGENTS

Pattern Code	Agents showing this pattern	Effect on liver parameters*										
		RLW§	Mic. Protein concn	Cell-sap Protein concn	$NADPH_2$-cyt. *c* reduct.	AP-demeth.	$NADH_2$-cyt. *c* reduct.	G6PDH	PGDH	G6Pase	LDH	GDH
I	Phenobarbitone Barbitone I.C.I. 45,337 I.C.I. 51,426	I	I	n.c.	I	I	n.c.	I	I	D	D	D
II	Diphenylhydantoin	n.c.	†	†	I	n.c.	n.c.	n.c.	n.c.	D	D	†
III	Phenylbutazone	n.c.	I‡	n.c.	I	I	n.c.	n.c.	n.c.	n.c.	D	D
IV	CPIB I.C.I. 53,072	I	I‡	I	I	I‡	n.c.	I	n.c.	n.c.	I	D¶
V	Propranolol	D	†	†	n.c.	D	I	D	n.c.	n.c.	D	†

* I = increase of concentration or activity,
D = decrease of concentration or activity,
n.c. = no change of concentration or activity;
† = assay not performed,
‡ = values of doubtful significance.
§ RLW = relative liver weight.
¶ data from previous work (ref. 1).

agents examined. Three of the agents (indomethacin, I.C.I. 45,763 and I.C.I. 50,172) showed equivocal effects.

Certain points emerged from these five patterns of response; (a) Liver enlargement was associated with a different underlying pattern of change of liver parameters (cf. patterns I and IV) confirming the results found previously; (b) compounds of different chemical structure induced identical changes in the liver, cf. the barbiturates, I.C.I. 45,337 and I.C.I. 51,426 in pattern I, (c) compounds with certain structural similarities, e.g. propranolol, I.C.I. 45,763, and I.C.I. 50,172 did not necessarily give rise to identical responses in the liver, cf. pattern V with the lack of effect of I.C.I. 45,763 and I.C.I. 50,172, (d) compounds with similar pharmacological activity did not always induce similar changes in the liver, cf. patterns I and II, and also pattern III with the lack of effect of indomethacin, (e) liver enlargement was not always associated with marked stimulation of all the parameters of microsomal $NADPH_2$-electron transport, and vice-versa cf. patterns I and IV, and pattern III, and (f) stimulation of $NADPH_2$-cyt. *c* reductase was not paralleled in all instances by a similar increase in AP-demethylase, e.g. pattern II and also the effects of polycyclic hydrocarbons such as 3,4-benzpyrene (e.g. ref. 24).

The phenylbutazone and diphenylhydantoin patterns of response (patterns II and III), apart from the lack of effect on liver weight and some other parameters in these investigations, revealed a similar overall trend to the barbiturate-type response (pattern I), suggesting that these agents have a primary effect in the microsomal fraction.

The liver enlargement induced by CPIB and I.C.I. 53,072 showed a different underlying pattern of response of liver parameters when compared with the "barbiturate-type" response. The effect on microsomal metabolism in particular was not so pronounced. It was shown previously[16] that CPIB induced an early stimulation of microsomal protein synthesis, but the present results showed that in contrast to phenobarbitone, the protein synthesised was largely accumulated in the cell-sap fraction. CPIB, at these doses is extensively bound to serum proteins and as a result is thought not to enter the liver cells. The effects in the liver, therefore, are considered to be indirect, and due to the displacement of thyroxine and other endogenous factors from serum protein bound sites.[16,27,28] Phenobarbitone, in contrast to CPIB, is known to be rapidly localised in liver microsomes[23] and the effects exerted on the liver are thought to be direct.

The wider implications of the results reported here are discussed further in the third paper.[3]

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