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LIVER ENLARGEMENT AND HEPATOXICITY: AN INVESTIGATION INTO THE EFFECTS OF SEVERAL AGENTS ON RAT LIVER ENZYME ACTIVITIES

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Abstract—Rat liver enzyme activities have been examined following treatment of the rat with various liver enlarging or hepatotoxic agents. The agents examined were CPIB, I.C.I. 53072, phenobarbitone, barbitone, DDT, carbon tetrachloride and thioacetamide. Characteristic patterns of response were established enabling differentiation between various liver enlarging agents to be made. The problem of the detection of hepatotoxicity by measurement of various parameters is discussed with the conclusions that hepatomegaly may not necessarily imply hepatoxicity, nor can "functional" liver enlargement be ascribed to a single factor.

THE TENDENCY towards an association of drug-induced liver enlargement with liver toxicity has led not only to a growing awareness of this problem but also to an increasing number of attempts to rationalise the observations made. Golberg's review[1] is an excellent recent appraisal of the subject. This particular problem had arisen in our laboratories in connection with the hypocholesterolaemic drug, Atromid-S* (ethyl α-(4-chlorphenoxy)-α-methylpropionate, clofibrate, CPIB) and several of our studies aimed principally at elucidating its mode of action have already been reported.[2, 3]

The investigations were expanded in order to examine more fully the effects of not only CPIB but also several other agents known to produce either hepatomegaly or hepatotoxicity or both. It was hoped that, by measuring various parameters in the liver, it might be possible to determine (a) if hepatomegaly of the so-called "functional" type[1] was the result of some common change irrespective of the chemical type initiating the enlargement or (b) if it was possible to predict whether a compound would give rise to liver damage on chronic treatment.

The results of investigations with carbon tetrachloride, thioacetamide, DDT, CPIB, I.C.I. 53072 (an analogue of CPIB producing similar hepatomegalic and hypolipidaemic effects)†, phenobarbitone and barbitone are given in this paper. Investigations were made after 14 days of treatment with these agents by which time liver enlargement had reached a maximum and a new dynamic equilibrium had been established (see refs. 2 and 3 for discussion of this point). It was assumed that, at such a time, the state of the liver would reflect the nature of the response to be expected after chronic treatment with these agents.

* I.C.I. Trade Mark.

† This compound was made initially by CIBA Laboratories and is quoted in U.K. Patent No. 1002667—see Table 1 for formula.

The parameters examined were gross liver weight, liver protein concentration, glutamate pyruvate transaminase, glucose-6 phosphatase, phosphohexose isomerase, glucose-6 phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, lactate dehydrogenase and glutamate dehydrogenase. Four of these seven enzymes are associated with the metabolism of glucose-6 phosphate and three with pyruvate metabolism, substrates occupying pivotal positions in intermediary metabolism.[4] The four oxidoreductase enzymes are affected by the oxidation-reduction state of the cell. In addition, two of the seven (glutamate pyruvate transaminase and glutamate dehydrogenase) are of mitochondrial origin, one (glucose-6 phosphatase) of microsomal origin and the remaining four are of cytoplasmic origin enabling some localisation of the effects to be made.

METHODS

Experimental animals

Male rats of a specific pathogen-free Wistar-derived Alderley Park strain were used throughout. Body weights at the beginning of each experiment were in the range 130–170 g, and carefully matched controls in terms of body weight were included in each experiment.

Dosing procedures

The rats were maintained on a powdered diet (Powder "O" of Scottish Agricultural Industries, supplemented with 25 mg vitamin E/kg). The compounds were either incorporated in the diet or dosed orally by tube, as solutions or dispersions. In either case, control animals were treated in exactly the same way such that any variations arising from handling techniques could be accounted for. Diet feeding *ad libitum* or oral dosing (once daily at 9.00–9.30 a.m.) was continued for 14 days. The medicated diet was not removed before termination of the experiment, and where oral dosing was employed the last dose was given about 30 min before killing the animals. Details of the compounds examined, the dose and route of administration are given in Table 1.

Assay procedures

At the end of each experiment, the rats were killed by stunning with a blow to the head and exsanguinating from a severed throat. The livers were rapidly removed to an ice-cooled container, the weight of the whole liver recorded and samples of liver homogenised at 0° with a Potter–Elvehjem Perspex-Fluon homogeniser (loose-fitting—clearance approx. 0·5 mm) driven by an air motor.

A standardised homogenising procedure was followed in all cases to give 10% homogenates in either 0·25 M sucrose or 0·15 M KCl (containing 0·8% v/v. 0·02 M $KHCO_3$ at pH 7·0). The sucrose homogenates were centrifuged at 600 *g* for 5 min and the KCl homogenates at 15,000 *g* for 15 min in an MSE-17 refrigerated centrifuge at 0°. The respective supernatant fractions were subdivided into several portions to avoid excessive freezing and thawing and stored at −20° until assayed. Significant changes in the enzyme activities measured were not seen in preparations stored in this way for up to three weeks at −20°, providing freezing and thawing was not carried out more than once on each aliquot of homogenate.

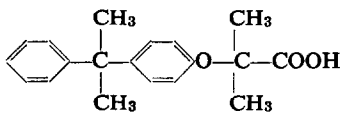
The methods of enzyme determination employed were either direct applications of established methods or minor modifications of the methods indicated and in all

TABLE 1. DETAILS OF EXPERIMENTS PERFORMED

Expt. no.	Compounds investigated	Dose* given
1	CPIB	0·25% w/w in diet
	I.C.I. 53072†	0·025% w/w in diet
	(Control)	(Powdered diet)
2	Phenobarbitone	0·25% w/w in diet
	Barbitone	0·25% w/w in diet
	(Control)	(Powdered diet)
3	Phenobarbitone	200 mg/kg p.o. once daily
	Barbitone	200 mg/kg p.o. once daily
	(Control)	(5 ml water/kg p.o. once daily
4	DDT	0·10 %w/w in diet
	Thioacetamide	0·05 %w/w in diet
	(Control)	(Powdered diet)
5	CCl_4	5 ml/kg p.o. of a 1:4 dilution in liquid paraffin once daily (i.e. = 1·25 ml CCl_4/kg)
	(Control)	(5 ml liquid paraffin/kg p.o. once daily)

* N.B. All animals were given powdered control diet *ad libitum* for at least 7 days before starting the experiment Dosing was carried out for 14 days.

† I.C.I. 53072:—



cases, the control homogenates were processed in an identical manner allowing direct comparisons with 'treated' samples to be made.

The enzymes assayed, together with relevant references, are given in Table 2. The dehydrogenase enzymes were assayed spectrophotometrically by following the rate of reduction of the respective pyridine nucleotide (either NADP for G6PDH and PGDH or NAD for LDH and GDH) at 340 mμ at 25° using a Spectronic 505 dual-beam recording spectrophotometer or at 22° using a Hilger–Gilford single-beam instrument. Both homogenate and coenzyme were included in the control cuvettes, which differed from the test cuvettes only in the absence of the specific substrate. Phosphogluconate dehydrogenase (PGDH) was assayed at pH 7·6.[5]

The protein concentration in the 600 *g* and 15,000 *g* supernatants was determined by the method of Lowry *et al.*[11]

Expression of activities

The dehydrogenase activities were derived from the initial linear sections of the spectrophotometric recordings and are expressed as units/mg fresh liver, 1 unit being equal to a change in extinction at 340 mμ of 0·001/min. G6Pase and GPT activities are expressed as m-mole inorganic phosphate or pyruvate liberated/g fresh liver/hr respectively, and PHI activity as μmole fructose-6 phosphate formed/g fresh liver/min. No attempt has been made in the individual experiments to correct the dehydrogenase activities to a constant temperature of incubation since the control samples were processed in exactly the same way in any one experiment.

TABLE 2. ENZYME ACTIVITIES DETERMINED

Trivial name	Systematic name	E.C. code no.	Abbreviation used in this paper	Reference for assay method	Homogenate used in assay
Glucose 6-phosphate dehydrogenase	D-glucose 6-phosphate:NADP oxidoreductase	1.1.1.49	G6PDH	5	15,000 g KCl
6-Phosphogluconate dehydrogenase	6-phospho-D-gluconate:NADP oxidoreductase (decarboxylating)	1.1.1.44	PGDH	5	15,000 g KCl
Lactate dehydrogenase	L-lactate:NAD oxidoreductase	1.1.1.27	LDH	6	15,000 g KCl
Glutamate dehydrogenase	L-glutamate:NAD oxidoreductase (deaminating)	1.4.1.2	GDH	7	600 g sucrose
Glutamate-pyruvate transaminase	L-alanine:2-oxoglutarate aminotransferase	2.6.1.2	GPT	8	600 g sucrose
Glucose-6 phosphatase	D-glucose-6-phosphate phosphohydrolase	3.1.3.9	G6Pase	9	600 g sucrose
Phosphohexoseisomerase	D-glucose-6-phosphate ketol isomerase	5.3.1.9	PHI	10	15,000 g KCl

Statistical treatment

Occasionally, within any one investigation, large scatters were observed due presumably to variable responses of the liver to the compound under test. This was confined to one or at most two observations within the group of 5 examined. Some selection, therefore, was made of the results analysed to keep the CV† below 30 per cent. It was felt that this was valid since the essential object of the experiments was to compare the pattern of response of the liver to the various agents. The divergent values excluded in this way did not markedly influence the overall result and where the group scatter was in any way equivocal, no attempt was made to exclude the observations involved. The treated values were compared to the equivalent control values by Student's *t* test.

RESULTS

The seven compounds investigated were divided into 5 separate experiments, each with its own control group. The details of the experiments are given in Table 1.

The extents of growth during the 14 day periods of dosing are given in Table 3. At the doses administered, CPIB, I.C.I. 53072, phenobarbitone, barbitone and DDT

TABLE 3. BODY WEIGHT CHANGES*

Expt. no.	Compound	Mean body wt.		Terminal body wt.
		Initial (g)	Terminal (g)	Initial body wt. (%)
1	CPIB	163	226	139
	53072	160	213	133
	Control	171	250	146
2	Phenobarbitone	171	235	137
	Barbitone	163	216	132·5
	Control	173	256	148
3	Phenobarbitone	153	206	134
	Barbitone	164	228	139
	Control	167	237	142
4	DDT	160	228	143
	Thioacetamide	161	150	93
	Control	158	236	149
5	CCl_4	142	152	107
	Control	131	212	162

* Dosing routines given in Table 1.

exerted a slight retardation of growth but this was accounted for by an effect in the first day or so when the presence of drug in the diet caused a slight reduction in food intake. The rate of growth subsequently followed the control group. When phenobarbitone and barbitone were given orally, a similar lag was observed. Thioacetamide and CCl_4 at the doses given completely prevented an increase in body weight. When thioacetamide was administered orally at 50 mg/kg for several days, a similar cessation of growth was observed.

The liver weight observations are given in Table 4. A marked similarity in the extent of enlargement was seen after the administration of CPIB, I.C.I. 53072, phenobarbitone, DDT and CCl_4. Barbitone had a significant effect when given in the diet,

† Coefficient of variation (CV) is expressed as a percentage of the S.D. divided by the group mean.

but not when administered orally (expt. 3). (Subsequent experiments have confirmed, however, that barbitone does cause hepatomegaly in our rats, but, dose for dose, not to the same extent as phenobarbitone.) Thioacetamide abolished body growth and liver growth since it had no observable effect on the relative liver weight.

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

Expt. no.	Compounds	Liver wt.:Body wt. ratio					
		Mean (g/100g)	± S.E.M.	(N)	CV (%)	% Control group	P†
1	CPIB	6·87	0·21	(5)	6·8	137·5	<0·001
	53072	7·10	0·19	(5)	6·0	142·5	<0·001
	Control	4·98	0·09	(5)	4·0	100	—
2	Phenobarbitone	6·77	0·20	(5)	6·7	134	<0·001
	Barbitone	5·91	0·12	(5)	4·4	117	<0·001
	Control	5·06	0·06	(5)	3·4	100	—
3	Phenobarbitone	6·63	0·26	(4)	8·0	141·5	<0·001
	Barbitone	5·03	0·23	(5)	10·1	107·0	N.S.
	Control	4·69	0·07	(9)	4·3	100	—
4	DDT	6·51	0·19	(5)	6·4	135	<0·001
	Thioacetamide	4·89	0·13	(5)	6·0	101·5	N.S.
	Control	4·82	0·20	(5)	9·3	100	—
5	CCl_4	6·82	0·59	(3)	14·9	147	0·005
	Control	4·64	0·09	(5)	4·3	100	—

* Dosing routines given in Table 1.
† Treated vs. control group, analysed by Student's *t* test.
N.S. not significant, $P > 0·10$.

The protein concentrations in the supernatants used for enzyme assay are given in Table 5. It was seen that CPIB, I.C.I. 53072, phenobarbitone, barbitone and possibly DDT increased the protein concentration, the effect usually being most significant in the microsomal-cytoplasmic fraction (15,000 g KCl supernatant). Thioacetamide and CCl_4 caused a slight fall in concentration.

The enzyme activities are given in Tables 6–10. The effects of CPIB and I.C.I. 53072 are shown in Table 6. A close similarity in the pattern of response was seen in all the enzyme activities measured, except G6PDH where CPIB had no observable effect in this experiment. It has been shown previously, however, that the activity of this enzyme is raised following CPIB treatment.[2] In contrast to previous observations with CPIB,[2] GDH activity was reduced in this experiment. Not only the pattern of response but also the extent of change of each enzyme was very similar following treatment with CPIB and I.C.I. 53072 at the stated doses and it is of considerable interest, therefore, that at these same doses, these compounds produced very similar reductions in plasma cholesterol. This evidence indicates an essentially similar mechanism of action on the liver enzymes and plasma cholesterol. The mode of action of CPIB in the light of presently available evidence has already been discussed at some length.[2, 3]

Tables 7 and 8 show the results of treatment with phenobarbitone and barbitone either in the diet or by oral dosing. The results of either method of treatment were in good agreement (except PGDH after barbitone in the diet—further experiments have confirmed, however, that the increase observed after oral dosing in expt. 4 is the

TABLE 5. LIVER HOMOGENATE PROTEIN CONCENTRATIONS : EFFECT OF THE VARIOUS COMPOUNDS TESTED*

Expt. no.	Compound	15,000 g KCl supernatant						600 g sucrose supernatant					
		Mean†	±S.E.M.	(N)	CV (%)	% Control group	P‡	Mean†	±S.E.M.	(N)	CV (%)	% Control group	P‡
1	CP1B	10·40	0·31	(5)	6·7	113	0·02	15·80	0·34	(4)	4·2	103·5	N.S.
	53072	10·50	0·08	(5)	1·7	113·5	0·005	17·24	0·37	(5)	4·8	113·0	<0·05
	Control	9·24	0·26	(5)	6·4	100		15·28	0·60	(5)	8·8	100	
2	Phenobarbitone	10·79	0·42	(4)	7·8	111	<0·05	14·70	0·44	(5)	6·7	97	N.S.
	Barbitone	10·63	0·17	(5)	3·6	109·5	0·01	14·70	0·44	(5)	6·6	97	N.S.
	Control	9·72	0·20	(5)	4·6	100		15·20	0·50	(5)	7·4	100	
3	Phenobarbitone	10·85	0·23	(4)	4·2	114·5	<0·001	14·15	0·13	(4)	6·0	107·5	<0·05
	Barbitone	10·42	0·21	(5)	4·6	110	<0·01	13·90	0·06	(4)	0·9	105·5	N.S.
	Control	9·47	0·17	(9)	5·5	100		13·15	0·26	(9)	5·8	100	
4	DDT	9·33	0·26	(4)	5·5	100	N.S.	16·46	0·26	(5)	3·5	110	<0·01
	Thioacetamide	8·85	0·27	(5)	6·9	95	N.S.	13·58	0·48	(5)	7·9	91	0·07
	Control	9·31	0·31	(5)	7·5	100		14·91	0·37	(5)	5·5	100	
5	CCl_4	8·11	0·36	(4)	8·9	93	N.S.	11·02	0·51	(4)	9·3	90·5	N.S.
	Control	8·75	0·32	(5)	8·2	100		12·19	0·52	(5)	9·5	100	

* Dosing routines given in Table 1.
† Protein concn. as g/100 g Equiv. fresh liver wt.
‡ See Table 4.

typical response to barbitone). The effects of these barbiturates on the dehydrogenase enzymes were identical qualitatively, differing only in the degree of response. The activities of the intermediary metabolic enzymes, PHI, GPT and G6Pase, responded in an equivocal manner and it is doubtful whether any real significance can be attached to these results.

TABLE 6. EXPT. NO. 1. EFFECTS OF CPIB AND I.C.I. 53072 IN DIET ON RAT LIVER ENZYME ACTIVITIES

Enzyme	Compound	Treated activity*				Control activity*				Treated as % control group	P†
		Mean	±S.E.M.	(N)	CV (%)	Mean	±S.E.M.	(N)	CV (%)		
G6PDH	CPIB	5·03	0·74	(3)	25·2	4·60	0·32	(5)	15·6	109·5	N.S.
	53072	8·58	0·96	(4)	22·4					187	0·005
PGDH	CPIB	5·64	0·42	(5)	16·5	6·70	0·52	(5)	17·2	84	N.S.
	53072	5·62	0·24	(5)	9·7					84	N.S.
GDH	CPIB	3·32	0·22	(5)	14·2	5·26	0·54	(4)	20·5	63	<0·01
	53072	3·24	0·28	(4)	17·4					61·5	<0·02
LDH	CPIB	769	40	(5)	11·6	583	39	(5)	14·9	132	0·01
	53072	830	24	(5)	6 4					142·5	<0·001
PHI	CPIB	116	5	(5)	9·6	166	5	(5)	6·8	70	<0·001
	53072	125	3	(5)	5·2					75·5	<0·001
GPT	CPIB	3·27	0·12	(5)	8·0	3·57	0·08	(5)	4·8	91	0·07
	53072	3·27	0·10	(5)	6·6					91·5	0·05
G6Pase	CPIB	0·95	0·06	(5)	14·0	1·14	0·06	(5)	10·9	83·5	0.05
	53072	0·87	0·02	(5)	4·5					76·5	<0·001

* See Methods section for units.
† See Table 4.

The effects of DDT and thioacetamide are shown in Table 9. The contrast was striking, particularly in the activities of G6PDH, PGDH, LDH, GPT and G6Pase. Thioacetamide decreased the activities of PGDH, GDH, GPT and G6Pase, had no effect on LDH and PHI and markedly increased G6PDH activity. There was a pronounced similarity between these results and those obtained after CCl_4 treatment (Table 10) and it is significant that both agents are known to produce a centrilobular necrosis at these dose levels. DDT was the only compound in this investigation to produce a significant fall in G6PDH activity. Thioacetamide differed from CCl_4, however, in its failure to produce liver enlargement.

DISCUSSION

Although there were points of similarity between the different agents in their effects on some of the liver enzymes, the patterns of response differed to a varying extent.

The results presented in Tables 6–10 indicated that the enzymes generally showing the most pronounced changes in activity were in the oxidoreductase group rather than in the intermediary metabolic enzyme group. This is shown diagrammatically

TABLE 7. EXPT. NO. 2. EFFECTS OF PHENOBARBITONE AND BARBITONE ADMINISTERED IN THE DIET ON RAT LIVER ENZYME ACTIVITIES

Enzyme	Compound	Treated activity*				Control activity*				Treated as % control group	P†
		Mean	±S.E.M.	(N)	CV (%)	Mean	±S.E.M.	(N)	CV (%)		
G6PDH	P-Barb.	11·1	0·40	(3)	6·3	6·9	0·82	(4)	23·6	161	0·01
	Barb.	10·1	1·18	(4)	23·4					146·5	0·05
PGDH	P-Barb.	10·6	0 47	(3)	7 6	8 0	0 58	(4)	14 6	132 5	0 02
	Barb.	7·6	0 41	(4)	10 8					95	N.S.
GDH	P-Barb.	3·43	0·42	(5)	27·5	5·53	0·35	(5)	14·2	62	<0·01
	Barb.	4·50	0·30	(5)	15·0					81·5	0·05
LDH	P-Barb.	268	14	(5)	11·9	489	16	(4)	6·3	55	<0·001
	Barb.	255	12	(4)	9·2					52	<0·001
PHI	P-Barb.	231	4	(5)	4·2	194	9	(5)	9·9	119	<0·01
	Barb.	184	8	(5)	9·8					95	N.S.
GPT	P-Barb.	3·17	0·13	(5)	9·1	3·33	0·12	(4)	6·9	95	N.S.
	Barb.	2·67	0·19	(4)	14·3					80	0·02
G6Pase	P-Barb.	0·70	0·02	(5)	6·3	1·04	0·04	(5)	7·9	67·5	<0·001
	Barb.	0·94	0·03	(5)	7·9					91	N.S.

* See Methods section for units.
† See Table 4.

TABLE 8. EXPT. NO. 3. EFFECTS OF PHENOBARBITONE AND BARBITONE ADMINISTERED ORALLY ON RAT LIVER ENZYME ACTIVITIES

Enzyme	Compound	Treated activity*				Control activity*				Treated as % control group	P†
		Mean	±S.E.M.	(N)	CV (%)	Mean	±S.E.M.	(N)	CV (%)		
G6PDH	P-Barb.	8·89	0·84	(3)	16·4	4·45	0·36	(7)	21·4	200	<0·001
	Barb.	6·99	1·13	(4)	32·4					157	<0·05
PGDH	P-Barb.	7·38	0·49	(4)	13·4	5·45	0·16	(8)	8·1	135	<0·001
	Barb.	6·84	0·45	(5)	14·6					125·5	0·005
GDH	P-Barb.	3·13	0·34	(4)	21·6	6·20	0·40	(9)	19·5	50·5	<0·001
	Barb.	4·47	0·18	(4)	8·1					72	0·02
LDH	P-Barb.	223	6	(4)	5·4	363	12	(9)	10·1	61·5	<0·001
	Barb.	276	14	(5)	10·9					76	<0·001

* See Methods section for units.
† See Table 4.

TABLE 9. EXPT. NO. 4. EFFECTS OF DDT AND THIOACETAMIDE ADMINISTERED IN THE DIET ON RAT LIVER ENZYME ACTIVITIES

Enzyme	Compound	Treated activity*				Control activity*				Treated as % control group	P†
		Mean	±S.E.M.	(N)	CV (%)	Mean	±S.E.M.	(N)	CV (%)		
G6PDH	DDT	3·64	0·79	(4)	21·6	5·54	0·34	(5)	13·8	66	0·05
	Thioacetamide	7·97	0·57	(4)	14·2					144	<0·01
PGDH	DDT	6·99	0·17	(4)	4·9	6·50	0·51	(5)	17·5	107·5	N.S.
	Thioacetamide	3·88	0·20	(4)	10·3					59·5	0·005
GDH	DDT	3·29	0·34	(4)	20·6	6·65	0·16	(5)	5·3	49·5	<0·001
	Thioacetamide	4·81	0·37	(5)	17·2					72·5	<0·005
LDH	DDT	280	13	(5)	10·6	411	16	(4)	7·7	68	<0·001
	Thioacetamide	372	14	(5)	8·6					90·5	N.S.
PHI	DDT	248	10	(5)	8·9	217	10	(5)	9·9	114	0·05
	Thioacetamide	206	7	(5)	7·9					95	N.S.
GPT	DDT	2·59	0·20	(5)	16·9	2·59	0·24	(5)	20·8	100	N.S.
	Thioacetamide	1·60	0·08	(5)	10·9					62	<0·01
G6Pase	DDT	0·71	0·04	(5)	10·9	0·85	0·04	(5)	11·4	83·5	<0·05
	Thioacetamide	0·50	0·03	(5)	13·3					59·5	<0·001

* See Methods section for units.
† See Table 4.

TABLE 10. EXPT. NO. 5. EFFECT OF CCl_4 GIVEN ORALLY ON RAT LIVER ENZYME ACTIVITIES

Enzyme	Treated activity*				Control activity*				Treated as % control group	P†
	Mean	±S.E.M.	(N)	CV (%)	Mean	±S.E.M.	(N)	CV (%)		
G6PDH	7·89	0·61	(4)	15·5	3·72	0·42	(5)	25·2	212	<0·001
PGDH	2·95	0·12	(4)	7·8	4·73	0·29	(5)	13·5	62·5	0·001
GDH	2·97	0·50	(3)	29·0	6·16	0·17	(5)	5·6	48	<0·001
LDH	198	11	(4)	10·9	305	6	(5)	4·4	65	<0·001
PHI	171	3	(4)	3·8	201	4	(5)	4·3	85	<0·001
GPT	1·31	0·07	(4)	11·1	2·76	0·19	(5)	15·4	47·5	<0·001
G6Pase	0·43	0·01	(4)	2·2	1·07	0·03	(5)	6·2	40	<0·001

* See Methods section for units.
† See Table 4.

in Fig. 1, from which it can be seen that the changes in activities of the four enzymes of the oxidoreductase class fall into four distinct groups;

(a) after CPIB and I.C.I. 53072 administration, characterised principally by the elevated LDH activity;

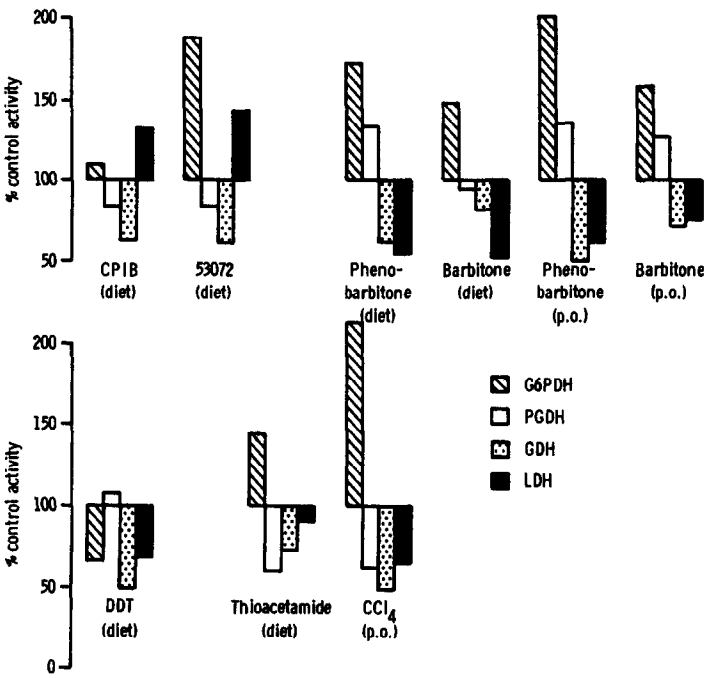


FIG. 1. Pattern of response of liver dehydrogenase enzymes to various compounds.

(b) after barbiturate administration, characterised by the increased activities of G6PDH and PGDH and the decreased activities of GDH and LDH.

(c) after DDT administration, characterized principally by the reduced G6PDH activity, and,

(d) after CCl_4 and thioacetamide administration, characterized by the increased G6PDH activity and the decreased activities of the other three enzymes.

CCl_4 and thioacetamide also differed from the other agents in their effects in reducing GPT and G6Pase activities.

The observed results are in general agreement with those reported from this and other laboratories, e.g. the changes after CPIB treatment agree with those reported;[2] DDT has been shown to reduce G6PDH,[13, 14] to increase liver weight,[13–16] and to increase the concentration of microsomal protein;[17] CCl_4 has been shown to reduce microsomal protein synthesis,[18] G6Pase activity,[12, 22] and to raise G6PDH activity;[22] thioacetamide has been shown to depress protein synthesis,[20] to reduce G6Pase,[22] and in contrast to the results reported in this paper, to have no observable effect on liver G6PDH activity;[22] the barbiturate results agree well with those of Kunz *et al.* in mice.[23]

The principal objective of this investigation was to try to determine whether heptatomegaly is necessarily associated with hepatotoxicity. A toxic response as seen anatomically is a change from the normal, but, is a change from the normal necessarily an indication of toxicity? From Fig. 1 and the other results presented in this paper, it was clear that liver enlargement *per se* was not always associated with the same changes in the biochemical parameters of the liver cell. It cannot be claimed from these results that liver enlargement is not a toxic response, nor can it be claimed that hepatomegaly always implies hepatotoxicity. Much work, therefore, remains to be done to elucidate fully the underlying causes of drug-induced hepatomegaly and it may not be valid at present to classify liver enlargement *per se* as a toxic response.

Based on the observations that a wide range of known hepatotoxins gave rise to marked reductions in liver microsomal G6Pase and drug-metabolising activities and that over liver-enlarging agents such as phenobarbitone did not,[22, 29] Golberg[1] has suggested that certain drug-induced hepatomegalies should be considered as "functional" responses of the liver. Feuer *et al.*[22] failed to demonstrate increased G6PDH activity following the administration to rats of non-hepatotoxic agents, in contrast to the observations in rats given in this paper and to the observations of Kunz *et al.*[23] in mice after phenobarbitone and halothane treatment. The marked similarity, however, in the observed patterns of response to CCl_4 and thioacetamide (characterized by a general reduction in most of the enzyme activities measured) in this study suggests that these changes might be associated with the necrogenic activity of these agents (see ref. 28 for a survey of the hepatotoxicity of thioacetamide). The pattern of response produced by these established toxins was in marked contrast to the patterns observed after administration of the other 5 agents, but it is not possible at this stage to be sure that all hepatotoxins will elicit a similar pattern of response in the liver enzymes as CCl_4 and thioacetamide. Thioacetamide did not give rise to liver enlargement in this investigation, in contrast to the effect of CCl_4. The CCl_4-induced liver enlargement was probably the result of fat accumulation.

Kunz *et al.*[23] investigated the effects of phenobarbitone and halothane on mouse liver enzymes and made essentially the same observations as those reported here, i.e. that the liver enzymes showing the most pronounced changes in response to drug treatment were associated with the redox-state of the liver cell. They have stressed the central importance of the NADP-$NADPH_2$ system in the co-ordination of many of

the major biosynthetic pathways of the cell, and have demonstrated a time-course correlation between the effects of phenobarbitone and halothane on the NADP: $NADPH_2$ equilibrium, on the hexosemonophosphate-shunt pathway (G6PDH activity) and on the liver growth period.

It is possible, therefore, that changes in the redox-state of the cell may be related to drug-induced "functional" enlargement of the liver. The distinct patterns of response observed after either CPIB and I.C.I. 53072, or the barbiturates or DDT had one factor in common, the marked changes in the activities of the oxidoreductases of the liver cell. It was not possible, however, to decide from the observed results (a) whether the initial mechanism producing the liver enlargement and the associated enzymes changes was the same for all the groups examined, (b) whether specific mechanisms peculiar to one group of compounds were involved, (c) whether the enzyme changes preceded or simply reflected the liver enlargement. Some differences in the mechanism involved, either initially or secondarily, must occur to give the different patterns of response observed after 14 days of treatment.

The proposed mode of action of CPIB (and by analogy I.C.I. 53072) is a displacement of physiological amounts of serum protein-bound thyroxine and other factors into the liver cells, leading to the observed effects within the liver cells. This has been discussed at some length previously.[2, 3] Golberg[1] has also raised the question of hormonal involvement as a contributory factor to drug-induced liver enlargement.

The barbiturates are known to be rapidly taken up by the liver microsomal drug-metabolising system[24] and this is thought to be closely associated with an increased microsomal protein synthesis and proliferation of the endoplasmic reticulum. DDT has been observed to stimulate microsomal drug-metabolising activity[17, 26, 27] and to cause an increase in the concentration of protein in the microsomal fraction.[17, 25] In this study, CPIB and I.C.I. 53072 increased the microsomal-supernatant protein concentration (Table 5) as also did the barbiturates, and CPIB has been shown previously[3] to stimulate protein synthesis in the microsomal fraction. These 5 agents, therefore, have an effect on microsomal protein synthesis and protein concentration, which is in direct contrast to the effect of CCl_4. CCl_4 inhibits microsomal protein synthesis.[18, 21] DDT has been considered a liver toxic agent[13, 15, 19] giving rise to some fatty infiltration as well as liver enlargement.[15, 16, 19] In this study, however, the effects on the liver enzymes were in marked contrast to those seen after CCl_4 or thioacetamide, particularly G6Pase and G6PDH activities.

In summary, therefore, the results given in this paper, when considered along with the results of other workers, indicate (a) that the prediction of hepatotoxicity remains a difficult problem, (b) that liver enlargement may not necessarily be a toxic effect, (c) that the measurement of a single parameter can give rise to misleading conclusions, (d) that by measurement of several parameters, it is possible to differentiate between various classes of compounds despite the grossly similar effects on total liver weight, (e) that so-called "functional" enlargement of the liver may be associated with changes in the redox balance of the liver cell and changes in microsomal metabolism and (f) that hepatotoxicity of the CCl_4-thioacetamide type is associated with marked reductions in enzyme activity, particularly in the microsomal fraction.

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REFERENCES

1. L. GOLBERG, *Proc. Eur. Soc. Study Drug Toxicity*, **7**, 171 (1966).
2. D. S. PLATT and B. L. COCKRILL, *Biochem. Pharmac.* **15**, 927 (1966).
3. D. S. PLATT and J. M. THORP, *Biochem. Pharmac.* **15**, 915 (1966).
4. C. A. NICHOL, in *Inflammation and Diseases of Connective Tissue*, Hahnemann Symposium (Eds. L. C. MILLS and J. H. MOYER), p. 372. Saunders, London (1961).
5. G. E. GLOCK and P. MCLEAN, *Biochem. J.* **55**, 400 (1953).
6. E. AMADOR, L. E. DORFMAN and W. WACKER, *Clin. Chem.* **9**, 391 (1963).
7. J. E. SNOKE, *J. biol. Chem.* **223**, 271 (1956).
8. J. KING, *J. med. Lab. Technol.* **17**, 1 (1960).
9. M. A. SWANSON, in *Methods in Enzymology* (Eds. S. P. COLOWICK and N. O. KAPLAN), vol. 2, p. 541. Academic Press, London (1955).
10. G. E. GLOCK, P. MCLEAN and J. K. WHITEHEAD, *Biochem. J.* **63**, 520 (1956).
11. O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. biol. Chem.* **193**, 265 (1951).
12. R. O. RECKNAGEL and B. LOMBARDI, *J. biol. Chem.* **236**, 564 (1961).
13. I. J. TINSLEY, *Nature, Lond.* **202**, 113 (1964).
14. I. J. TINSLEY, *Biochem. Pharmac.* **14**, 847 (1965).
15. E. P. LAUG, A. A. NELSON, O. G. FITZHUGH and F. M. KUNZE, *J. Pharmac. exp. Ther.* **98**, 268 (1950).
16. P. ORTEGA, *Fedn Proc.* **21** 306 (1962).
17. A. MORELLO, *Can. J. Biochem.* **43**, 1289 (1965).
18. E. A. SMUCKLER and E. P. BENDITT, *Biochemistry, N.Y.* **4**, 671 (1965).
19. O. G. FITZHUGH and A. A. NELSON, *J. Pharmac. exp. Ther.* **89**, 18 (1947).
20. P. D. CLEVELAND and E. A. SMUCKLER, *Proc. Soc. exp. biol. Med.* **120**, 808 (1965).
21. A. A. SEAWRIGHT and A. E. M. MCLEAN, *Biochem. J.* **100**, 11P (1966).
22. G. FEUER, L. GOLBERG and J. R. LE PELLEY, *Food Cosmet. Toxic.* **3**, 235 (1965).
23. W. KUNZ, G. SCHAUDE, H. SCHIMASSEK, W. SCHMID and M. SIESS, *Proc. Eur. Soc. Study Drug Toxicity* **7**, 138 (1966).
24. L. ERNSTER and S. ORRENIUS, *Fedn Proc.* **24**, 1190 (1965).
25. D. S. PLATT and B. L. COCKRILL, unpublished observations.
26. L. G. HART and J. R. FOUTS, *Proc. Soc. exp. biol. Med.* **114**, 388 (1963).
27. F. K. KINOSHITA, J. P. FRAWLEY and K. P. DUBOIS, *Toxic. appl. Pharmac.* **9**, 505 (1966).
28. Z. HRUBAN, W. GRADMAN, A. SLESERS and M. LUBRAN, *Lab. Invest.* **15**, 1748 (1966).
29. G. FEUER, L. GOLBERG and J. R. LE PELLEY, *Food Cosmet. Toxic.* **3**, 251 (1965).