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A COMPARISON OF MOUSE AND RAT LIVER ENZYMES AND THEIR RESPONSE TO TREATMENT WITH VARIOUS COMPOUNDS

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Abstract—The responses of various mouse and rat liver parameters to administration of several pharmacologically-active agents are compared. Similar, but not identical, changes occurred in the livers of both species after the administration of chlorpromazine, clofibrate, carbon-tetrachloride and DDT. Greater differences in the responses were observed after administration of phenobarbitone or griseofulvin.

THE PROBLEM of species differences is the most important single problem in the evaluation of the safety of a drug for man. A comparison of the species differences in liver enzymes between rats and mice and the different species reactions to foreign substance is of some value in the elucidation of this problem. In this paper the data from the two species are compared. The biochemical changes in rat liver after treatment with various agents in these laboratories have been already reported.[1–5]

The metabolism and toxicity of many compounds is known to be species and strain dependent.[6–8] Male and female animals of the same strain often metabolise foreign compounds at different rates.[6,9] Other factors (e.g. dosage age,[10,11] diet,[12–14] hormonal status,[13] microbial status,[15] stress and the presence of other foreign chemicals[6,8]) are known to affect the metabolism of foreign compounds and some of the other enzymes studied.

METHODS

The methods used in these experiments have been described.[1–3]

Experimental animals

Rats of a specific pathogen-free Wistar derived Alderley Park strain were used throughout. The rats weighed between 100–170 g at the start of each experiment. The mice were a specific pathogen-free Alderley Park strain and weighed between 30 and 40 g at the start of each experiment. The rats and mice were maintained on a standard powdered or cube diet given *ad lib*. Each group of five animals was housed together in suspended galvanised all mesh cages. The open mesh floor ensured that urine and faeces did not collect on the floor of the cages. The ambient temperature was maintained at 72° ± 5°. Light cycle with 12 hr fluorescent light was maintained.

Dosing procedures

The animals were dosed daily (between 9:30 and 11:30 a.m.) for 13–14 days and sacrificed 24 hr after the last dose. Rats were dosed with phenobarbitone (0·20%,

w/w in diet) chlorpromazine (0·10%, w/w in diet), clofibrate (0·25%, w/w in diet) carbon tetrachloride (200 mg/kg p.o.), DDT (0·10% w/w in diet) and griseofulvin (500 mg/kg p.o.). Mice were dosed with phenobarbitone (100 mg/kg p.o.), chlorpromazine (50 mg/kg p.o.) clofibrate (500 mg/kg p.o.), carbon tetrachloride (25 mg/kg p.o.), DDT (100 mg/kg p.o.) and griseofulvin (2%, w/w in diet). Matched groups of control animals dosed with vehicle alone were included in each experiment.

The doses of the compounds used in these experiments were usually close to the maximum tolerated in rats or mice. This inevitably leads to some large differences in the actual doses administered to rats and mice.

Assay procedures

The livers were collected and processed as reported previously.[1,2] Aminopyrine *N*-demethylase, $NADH_2$- and $NADPH_2$-cytochrome c reductases, 6-phosphogluconate dehydrogenase, lactate dehydrogenase and glucose-6-phosphatase activities were assayed as described.[1–3] In the assay of glucose 6-phosphate dehydrogenase activity an excess of 6-phosphogluconate dehydrogenase[16] was added to increase the sensitivity of the method.

RESULTS

A comparison of the effects of various drug treatments on the liver parameters in rat and mouse

The control levels of the liver enzymes in mice and rats are shown in Table 1. The effect of the various drug treatments on the males of both species is shown in Tables 2 and 3. These results are expressed as percentage of the values in control animals.

TABLE 1. COMPARISON OF CONTROL MALE RAT AND MOUSE LIVER ENZYME ACTIVITIES OF RELATIVE LIVER WEIGHTS

Parameter measured*	I.U.B. code	Rats ± S.E.M.	Mice ± S.E.M.
R.L.W.†	—	4.9 ± 0·12	6·07 ± 0·27
AP‡	—	188 ± 23	117 ± 18
$NADPH_2$†	1.6.99.1	1·04 ± 0·11	0·37 ± 0·16
$NADH_2$‡	1.6.99.3	7·52 ± 1·05	11·17 ± 0·62
G6PDH‡	1.1.1.49	4·81 ± 0·83	1·6 ± 0·70
PGDH§	1.1.1.44	10·65 ± 1·05	3·88 ± 0·12
LDH§	1.1.1.27	394 ± 22	93 ± 18
G6Pase	3.1.3.9.	788 ± 37	1150 ± 283

The results refer to male animals aged about 8 weeks.

* Abbreviations and expression of activities: R.L.W., relative liver weight (liver weight/body weight × 100); AP, aminopyrine *N*-demethylase (mμmoles H.CHO formed/g/min); $NADPH_2$, $NADPH_2$-cytochrome c reductase (μmoles cyt. c reduced/g/min); $NADH_2$, $NADH_2$-cytochrome c reductase (μmoles cyt. c reduced/g/min); G6PDH, glucose-6-phosphate dehydrogenase (change in absorbance of 0·001/min/g); PGDH, 6 phosphogluconate dehydrogenase (change in absorbance of 0·001/min/g); LDH, lactate dehydrogenase (change in absorbance of 0·001/min/g); G6Pase, glucose-6-phosphatase (μmoles inorg. PO_4 liberated/g/hr).

† $P \leqslant 0{\cdot}01$
‡ $P \leqslant 0{\cdot}1$
§ $P \leqslant 0{\cdot}001$
} Significance of differences between species.

TABLE 2. CHANGES IN RELATIVE LIVER WEIGHT AND HEPATIC ENZYME ACTIVITIES FOLLOWING TREATMENT OF RATS WITH VARIOUS AGENTS (EXPRESSED AS PERCENTAGE OF VALUES IN CONTROL ANIMALS)

Compound	R.L.W.	AP	$NADPH_2$	$NADH_2$	G6PDH	PGDH	LDH	G6Pase
Phenobarbitone	114*	242†	238†	108	98	130‡	76*	64†
Chlorpromazine	94	294†	204*	185†	209‡	139*	94	104
Clofibrate	131†	136‡	149*	103	286* 92	94	152†	104
CCl_4	138‡	38†	51†	75‡	264*	85‡	89	39†
DDT	129†	228†	361†	107	135	130*	57†	69*
Griseofulvin	106‡	103	128‡	127	60	97	85*	90‡

Abbreviations: see Table 1. Levels of significance: P = treated group compared with control groups

* $P \leqslant 0{\cdot}01$.
† $P \leqslant 0{\cdot}001$.
‡ $P \leqslant 0{\cdot}1$.

TABLE 3. CHANGES IN RELATIVE LIVER WEIGHT AND HEPATIC ENZYME ACTIVITIES FOLLOWING TREATMENT OF MICE WITH VARIOUS AGENTS (EXPRESSED AS PERCENTAGE OF VALUES IN CONTROL ANIMALS)

Compound	R.L.W.	AP	$NADPH_2$	$NADH_2$	G6PDH	PGDH	LDH	G6Pase
Phenobarbitone	154†	296†	135	115	120	106	72*	111
Chlorpromazine	125†	263†	170‡	118	83	100	70*	102
Clofibrate	129*	151‡	198*	125	107	84	94	103
CCl_4	109‡	63	76	103	106	112	95	83
DDT	136*	290*	565†	170‡	112	98	80‡	103
Griseofulvin	151†	214†	200‡	79	75	135‡	77*	98

Abbreviations: see Table 1. Levels of significance: see Table 2.

Phenobarbitone. The dose per kilogram given to the rats was about twice that given to the mice. The elevation of $NADPH_2$-cytochrome c reductase and PGDH activities was less marked in the mouse liver than in rat liver although the increase in relative liver weight was greater in mouse than in rat. G6P'ase activity was reduced in rat liver, but not in mouse liver.

Chlorpromazine. Aminopyrine *N*-demethylase, $NADH_2$- and $NADPH_2$-cytochrome c reductases, G6PDH and PGDH activities were increased in rats without an increase in relative liver weight.

In mice, chlorpromazine increased the relative liver weight and aminopyrine *N*-demethylase, $NADH_2$- and $NADPH_2$-cytochrome c reductases activities but decreased G6PDH and LDH activities. The body weight of these mice did not increase during the 14-day dosing period.

Clofibrate (*ethyl* p-*chlorophenoxyisobutyrate*). In both species the relative liver weight, aminopyrine *N*-demethylase, and the $NADPH_2$-cytochrome c reductase activities were increased. LDH activity was stimulated in rat but not in mouse liver.

Carbon tetrachloride. The dose per kilogram given to rats was eight times that given to mice. The increase in relative liver weight, and the decrease in aminopyrine *N*-demethylase, $NADPH_2$-cytochrome c reductase and G6P'ase activities were correspondingly smaller in mouse than rat. In rats but not mice, the G6PDH activity was increased but $NADH_2$-cytochrome c reductase and LDH activities were reduced.

DDT (1,1,1,-*trichloro*-2,2- *di*-(4-*chlorophenyl*) *ethane*). Both species responded similarly to DDT, the only differences were that in mouse liver $NADH_2$-cytochrome c reductase activity was increased and no reduction in the G6P'ase activity occurred.

Griseofulvin. The dose per kilogram given to the mice was approximately four times that given to the rats. The increase in relative liver weight was correspondingly much greater in mouse than rat. Aminopyrine *N*-demethylase activity was unchanged in rat liver but was greatly increased in mouse liver. In mice the changes in the activity of $NADPH_2$-cytochrome c reductase and LDH were more marked than in rats. PGDH activity was increased in mouse but not in rat liver. G6P'ase activity was unchanged in mouse liver but was slightly reduced in rat liver.

DISCUSSION

A large number of compounds has been shown to stimulate drug metabolizing enzymes in small animals (mainly rats, mice and rabbits). In man only a few compounds have been shown to increase drug metabolism, these include the barbiturates, glutethimide, meprobamate, phenylbutazone, and griseofulvin.[11]

Phenobarbitone illustrates an interesting species difference. In rats the relative liver weight increased to 114 per cent of control and $NADPH_2$-cytochrome c reductase activity was increased to 238 per cent of control. In mice although the relative liver weight increase was much greater (154 per cent of control) $NADPH_2$-cytochrome c reductase activity was unchanged. The G6P'ase activity in rats was reduced in agreement with previous results[2,17,18] but in mice G6P'ase activity was slightly increased by phenobarbitone.

Another species difference was noted after treatment with griseofulvin. The aminopyrine *N*-demethylase activity was increased by griseofulvin in mice but not in rats. This correlates with the observation that griseofulvin decreased the hexobarbital sleeping time in the mouse.[19]

Mouse and rat liver respond in a similar but not identical way to treatment with foreign compounds. This may be related to the different activities of the enzymes measured in untreated animals. The activities of G6PDH, 6PGDH, LDH, $NADPH_2$-cytochrome c reductase, aminopyrine *N*-demethylase are greater in male rat than in male mouse liver. Relative liver weight, and $NAPH_2$-cytochrome c reductase activity are greater in male mouse liver. Differences in these hepatic enzyme activities are maintained from 2–5 months of age but in both species $NADH_2$-cytochrome c reductase activity increases whilst relative liver weight decreases with age.[17,20] These species differences although characteristic of the strains of mice and rats used in this work may not be typical of all strains of mice and rats.

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