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

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**Abstract**—Results are presented on the effects of administration of several pharmacologically active agents on various rat liver parameters. The compounds examined fall into three groups. (1) Compounds which stimulated an increase in enzymes of the microsomal $NADPH_2$-electron transport chain that was accompanied by an increase in relative liver weight. (2) Compounds which stimulated an increase in enzymes of the microsomal $NADPH_2$-electron transport chain without an increase in liver weight. (3) Ethanol. The patterns of response induced in the liver by these compounds were frequently not easily classified. The weak "hyperfunctional" responses induced by compounds in groups 1 and 2 were clearly differentiated from those given by ethanol and other hepatotoxins.

PREVIOUS studies[1–4] in these laboratories have demonstrated that the rat liver can exhibit a wide variety of responses to foreign compounds. These effects were monitored by following the response of several parameters of liver function, particularly those concerned with the metabolism of foreign compounds. The factors involved in determining the response of the liver to foreign compounds are poorly understood. Some attempts have been made to classify the "type" of response elicited by various agents. For example differentiation has been made between "toxic" and "hyperfunctional" responses with and without liver enlargement. However, the significance of many of these changes is obscure. The response of the same parameters of rat liver function to a further series of compounds with a wide variety of pharmacological actions and chemical types is now reported. The majority are well established therapeutic agents in man.

## METHODS

The methods employed, the parameters measured and the assay procedures used were as described by Platt and Cockrill.[2]

### *Experimental animals*

Male rats of a specific pathogen-free Wistar-derived Alderley Park strain were used. The rats weighed 95–150 g at the start of each experiment and carefully matched controls in terms of body weight were included in each experiment. Each group contained five rats.

### *Dosi ng procedures*

The rats were maintained on a standard cube diet* given *ad lib*. The animals were dosed orally by tube as solutions or dispersions. Control animals were similarly dosed

* Supplied by Oakes (Millers Ltd.) Congleton, Cheshire.

with vehicle alone. Dosing was continued for 14 days and terminated 24 hr before sacrifice.

The doses of the compounds used were as follows: chlorcyclizine, diphenhydramine and orphenadrine (50 mg/kg), carisoprodol (200 mg/kg), nikethamide, methyprylone and aminopyrine (250 mg/kg), tolbutamide (400 mg/kg), sulphadimidine and griseofulvin (500 mg/kg) and ethanol (4 g/kg). 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 the therapeutically-effective doses.

The compounds are classified pharmacologically as follows:

| | |
|---|---|
| anti-histamine: | chlorcyclizine, diphenhydramine |
| muscle relaxants: | orphenadrine, carisoprodol |
| sulphonamides: | sulphadimidine, tolbutamide |
| anti-fungal: | griseofulvin |
| sedatives: | ethanol, methyprylone |
| antipyretic: | aminopyrine |
| CNS stimulant: | nikethamide. |

All these compounds have been administered to man.

*Parameters measured and assay procedures*

Rat livers were collected and homogenised as previously described.[1] Protein concentrations of microsomal and soluble fractions were measured by the method of Lowry *et al.*[5] after centrifugation of the 15,000 *g* KCl supernatant at 105,000 *g* for 60 min in a Spinco model L ultracentrifuge.

Aminopyrine *N*-demethylase was assayed as described by Ernster and Orrenius.[6] $NADH_2$- and $NADPH_2$-cytochrome c reductases ($NADH_2$- and $NADPH_2$-cytochrome c oxidoreductases; I.U.B. codes 1.6.99.3 and 1.6.99.1) were assayed following the rate of reduction of cytochrome c at 550 nm at 25°.[7,8] Glucose-6-phosphatase (D-glucose-6-phosphate phosphohydrolase I.U.B. code 3.13.9) was assayed as described by Swanson.[9] The dehydrogenase activities were assayed by following the rate of reduction of the appropriate pyridine-nucleotide co-enzyme at 340 nm at 25°.[10,11] [NADP for glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: NADP oxidoreductase I.U.B. code 1.1.1.49), and 6-phosphogluconate dehydrogenase (6-phospho-D-gluconate: NADP oxidoreductase I.U.B. code 1.1.1.44) and NAD for lactate dehydrogenase (L-lactate: NAD oxidoreductase I.U.B. code 1.1.1.27).]

## RESULTS

Ethanol was the only agent to cause a reduction in whole body growth. Liver weight changes, protein concentrations and enzyme activities are given in Table 1.

The compounds examined fall into three groups (see Table 1).

(1) Compounds which stimulated an increase in aminopyrine *N*-demethylase and/or $NADPH_2$-cytochrome c reductase that was accompanied by an increase in relative liver weight (R.L.W.).

(2) Compounds which stimulated an increase in aminopyrine *N*-demethylase and/or $NADPH_2$-cytochrome c reductase without an increase in liver weight.

(3) Ethanol.

TABLE 1. CHANGES IN LIVER PROTEIN CONCENTRATIONS AND ENZYME ACTIVITIES FOLLOWING TREATMENT OF RATS WITH VARIOUS AGENTS (EXPRESSED AS % OF VALUES IN CONTROL ANIMALS)

| Pattern code | Agents showing this pattern | R.L.W. | Mic | Sol | AP | $NADPH_2$ | $NADH_2$ | G6PDH | PGDH | LDH | G6Pase |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | Chlorcyclizine | 112* | 148† | 101 | 228† | 240* | 87 | 56 | 124* | 67‡ | 78† |
| | Orphenadrine | 112‡ | 127‡ | 98 | 203† | 137 | 90 | 100 | 116‡ | 77 | 89 |
| | Nikethamide | 120‡ | 113 | 92 | 222* | 195† | 114 | 124 | 101 | 87 | 100 |
| | Methyprylone | 118* | 121 | 108 | 221* | 180† | 125 | 200† | 109 | 84‡ | 95 |
| | Aminopyrine | 115† | 175† | 115‡ | 117 | 172 | 125 | 91 | 109 | 85* | 105 |
| | Griseofulvin | 106‡ | 99 | 104 | 103 | 128‡ | 127 | 60 | 97 | 73* | 90‡ |
| II | Diphenhydramine | 100 | 124‡ | 104 | 194* | 114 | 105 | 89 | 106 | 80 | 95 |
| | Carisoprodol | 103 | 108 | 99 | 131 | 137‡ | 100 | 87 | 92 | 100 | 97 |
| | Tolbutamide | 109 | 112 | 97 | 159‡ | 126‡ | 127 | 102 | 113 | 69† | 77* |
| | Sulphadimidine | 110 | 117 | 100 | 108 | 153† | 213† | 182‡ | 88 | 70† | 53† |
| III | Ethanol | 101 | 88 | 124† | 63* | 83 | 68 | 146† | 77‡ | 118† | 112 |

*Abbreviations and expression of results.* R.L.W.: relative liver weight (liver wt./body wt. × 100). Mic: microsomal protein concentration (mg/equiv. g fresh liver). Sol.: soluble protein concentration (mg/equiv. g fresh liver). AP: aminopyrine *N*-demethylase (mμmoles H.CHO formed/g/min). $NADPH_2$: $NADPH_2$-cytochrome c reductase (μ moles 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).

‡ $P < 0{\cdot}1$
* $P < 0{\cdot}01$
† $P < 0{\cdot}001$
} significance of difference from control.

*Group* 1

The most marked changes in this group were induced by chlorcyclizine, nikethamide and methyprylone, all of which stimulated aminopyrine *N*-demethylase and $NADPH_2$-cytochrome c reductase as well as affecting some of the other parameters in an apparently random manner. The changes induced by orphenadrine were restricted to an increase in R.L.W. and aminopyrine *N*-demethylase accompanied by an increase in microsomal protein concentration. Aminopyrine increased R.L.W. and both microsomal and soluble protein concentrations but the increase in aminopyrine *N*-demethylase activity was not significant. Griseofulvin increased R.L.W., $NADPH_2$-cytochrome c reductase but had no effect on the *N*-demethylase activity.

In this group glucose-6-phosphate dehydrogenase activity was increased only by methyprylone and glucose-6-phosphatase was decreased by chlorcyclizine and griseofulvin. Chlorcyclizine, aminopyrine and orphenadrine increased microsomal protein concentration. Lactate dehydrogenase was reduced by four compounds in this group.

*Group* 2

The sulphonamides, tolbutamide and sulphadimidine, induced similar changes characterised by an increase in $NADPH_2$- or $NADH_2$-cytochrome c reductase activity and a reduction of glucose-6-phosphatase and lactate dehydrogenase activities. Aminopyrine *N*-demethylase was stimulated by tolbutamide and glucose-6-phosphate dehydrogenase was stimulated by sulphadimidine.

Diphenhydramine had no effects other than stimulating aminopyrine *N*-demethylase and increasing microsomal protein concentration. Carisoprodol stimulated $NADPH_2$-cytochrome c reductase.

*Group* 3

Ethanol increased soluble protein concentration, glucose-6-phosphate dehydrogenase and lactate dehydrogenase activities but decreased aminopyrine *N*-demethylase and 6-phosphogluconate dehydrogenase activities.

## DISCUSSION

In this paper the effects of eleven agents on rat liver weight, protein concentrations and enzyme activities have been reported. The alterations in these parameters generally were less marked with these compounds than with those reported previously.[2–4] Moreover, the patterns of response did not correspond precisely to any of the eleven patterns previously observed. The responses given by the compounds in Group I resembled the "phenobarbitone response", (Pattern I, Table 10, ref. 2), but not all the features of a typical phenobarbitone response were observed. The compounds in Group I illustrate a graded response of the liver similar to that induced by increasing doses of phenobarbitone,[2,15] (see Table 1). A minimal response was generated by griseofulvin and aminopyrine, larger responses were given by orphenadrine, methyprylone and chlorcyclizine. Chlorcyclizine,[12,13] orphenadrine,[14] nikethamide,[15,16] methyprylone[17] and aminopyrine[14] have all been shown to stimulate hepatic drug metabolising enzymes, in agreement with our present results. Griseofulvin caused an increase in relative liver weight in rats and mice but a decrease in hexobarbital sleeping time was noted only in mice.[18] We found no increase in aminopyrine *N*-demethylase

activity after griseofulvin treatment. Within this group and group II the responses of soluble protein concentration, $NADH_2$-cytochrome c reductase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, glucose-6-phosphatase and lactate dehydrogenase, did not follow any discernible pattern.

The response elicited by the compounds in group II was comparable to that previously observed with some compounds acting on the C.N.S. (Pattern X, Table IX, ref. 4), but the effects were less pronounced. There was an increase of one or more of the enzymes involved in the $NADPH_2$ microsomal electron-transport chain but there was no corresponding increase in relative liver weight. The pattern of change in the liver produced by these compounds was most clearly seen with tolbutamide. Sulphadimidine, carisoprodol and diphenhydramine produced less marked changes. Increases in hepatic drug metabolising enzyme activity by treatment with tolbutamide[16,17] carisoprodol[19,20] or diphenhydramine[21] treatment have been reported previously.

In contrast to the other agents ethanol has been shown to cause hepatic injury in the rat typified by fatty deposition in the centrilobular cells;[22,23] vacuolation of the rough-surfaced endoplasmic reticulum occurred with some ribosomal detachment but alterations to the smooth surfaced endoplasmic reticulum were minimal.[24] However, these changes were much less severe than those caused by much smaller doses of direct liver toxins (e.g. $CCl_4$) which are typified by disruption of the rough-surfaced endoplasmic reticulum followed by proliferation of the smooth-surface endoplasmic reticulum.[25,26] The level of cytochrome P-450 was unaffected by ethanol in contrast to marked reduction caused by $CCl_4$ treatment.[27]

Alterations in enzyme levels in the liver following ethanol treatment were also much less generalised than those induced by other liver toxins[3] (e.g. carbon tetrachloride, chloroform, thioacetamide, dimethyl nitrosamine and ethionine). Only the decreases in aminopyrine *N*-demethylase and 6-phosphogluconate dehydrogenase activities coupled to a stimulation of glucose-6-phosphate dehydrogenase were typical of the "hepatotoxic"[3] response. There was no change in glucose-6-phosphatase, $NADPH_2$- and NADH-cytochrome c reductase activities; enzymes lowered by treatment with other hepatotoxins.

Recently Rubin *et al.*[28] demonstrated that ethanol administered to Sprague–Dawley rats caused a marked stimulation of hepatic drug metabolism (aniline and pentobarbital hydroxylase) and that ethanol inhibited these oxidations *in vitro*. Lieber and De Carli[29] showed that ethanol was oxidised by the microsomal drug metabolizing system and that pretreatment with ethanol increased the rate of oxidation.

The patterns of response induced in the liver by treatment of rats with foreign compounds frequently are not clear cut and often do not permit precise classification of the compound. The weak "hyperfunctional" responses induced by all the compounds in Groups I and II were readily differentiated from that given by ethanol and other hepatotoxins. The biological significance of drug-induced changes in the liver remains obscure.

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