Table 1 shows that the suramin-treated rats did not differ significantly in the weight of the liver relative to that of body weight from those treated with normal saline. Twenty-four hr after phenobarbital treatment there was an increase of about 20 per cent in liver weight relative to body weight, but additional treatment of these animals with suramin did not alter this increase. The *N*-demethylating activity and the level of cytochromes, P-450 and b_5, per unit of microsomal protein appeared to be unaffected by suramin treatment compared to those of control animals. Single-dose treatment with suramin, however, resulted in a substantial inhibition of the enzyme and cytochromes being induced by phenobarbital. The duration of action of hexobarbitone (Table 2) shows that the suramin-treated animals metabolized hexobarbital in the same time as the control ones. Phenobarbital treated rats, however, metabolized hexobarbital about four times more rapidly than either suramin treated or control animals while the metabolic rate was increased by a factor of 3 when phenobarbital-treated animals were given suramin.

The present studies have thus shown that the administration of phenobarbital to rats increased the activity of *N*-demethylase and cytochromes, P-450 and b_5, to about 200 per cent of control animals; these are in agreement with the observation of others[11] who showed a similar increase in the activity of a number of hepatic microsomal enzymes. The phenobarbital-induced increases in the enzyme system was inhibited by the administration of suramin at a dose of 20 mg/kg body wt. As suramin did not alter the microsomal enzyme system from the control values, the inhibition of the system being induced by phenobarbital is unlikely to be due to the inhibition of protein synthesis or of the active sites of the enzymes. The possibility of the rate of membrane absorption of the phenobarbital being impaired because of the membrane receptors being blocked by the suramin is also excluded by the observation that phenobarbital treatment increased the amount of lipid phosphorus and this was not altered by suramin (unpublished observations in our laboratory). However, it is possible that the inhibition of the phenobarbital-induced increases in the enzyme system by suramin could be due to interference with the newly synthesized enzyme protein. Nevertheless, the present investigation is only a preliminary report based on a single-dose treatment. To elucidate the mechanism of suramin action in presence of phenobarbital, it is necessary to investigate the chronic effects of suramin, which are now under current investigation in our laboratories.

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REFERENCES

1. G. MACLEAN, *Ann. Trop. Med. Parasitol.* **23**, 337 (1929).
2. J. K. T. CHERRY, *East Af. Med. J.* **37**, 550 (1960).
3. B. W. TOWN, E. D. WILLS, E. J. WILSON and A. WORMALL, *Biochem. J.* **47**, 149 (1950).
4. J. WILLIAMSON, *Exp. Parasitol.* **12**, 273 (1962).
5. J. C. BOURSNELL, W. G. DANGERFIELD and A. WORMALL, *Biochem. J.* **33**, 81 (1930).
6. S. ORRENIUS and L. ERNSTER, *Biochem. biophys. Res. Commun.* **16**, 60 (1964).
7. N. E. SLADEK and G. J. MANNERING, *Biochem. biophys. Res. Commun.* **24**, 668 (1966).
8. J. B. SCHENKMAN, I. FREY, H. REMMER and R. W. ESTABROOK, *Molec. Pharmac.* **3**, 516 (1967).
9. J. L. HOLTZMAN, T. E. GRAM, P. L. GIGON and J. R. GILLETTE, *Biochem. J.* **110**, 407 (1968).
10. O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDAL, *J. biol Chem.* **193**, 265 (1951).
11. T. K. BASU, J. W. T. DICKERSON and D. V. PARKE, *Biochem. J.* **124**, 19 (1971).

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Inhibition by ethylmorphine and pentobarbitone *in vitro* of the metabolism of [ureyl-^{14}C]tolbutamide by hepatic microsomal preparations from sexually-immature male and female rats treated with phenobarbitone

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IN A previous paper,[1] it was shown that phenobarbitone treatment of adult male and female rats *in vivo* increased the degree of inhibition by pentobarbitone and ethylmorphine *in vitro* of the hepatic

microsomal metabolish of [ureyl-^{14}C]tolbutamide relative to control animals. Microsomal preparations from female animals seemed generally less susceptible to the inhibitors than those from male animals. This communication describes similar inhibition experiments performed with sexually immature rats in the expectation that such sex differences would be less marked, or even not present.

Methods. The treatment of the animals and the preparations of hepatic microsomal fractions were performed essentially as described in the earlier paper,[1] except that the rats (Wistar strain, from Scientific Products Farm Ltd., Manston Research Centre, Margate, Kent) weighed 40–50 g at the time of the first injection and weighed a maximum of 75 g when killed. The assay of the microsomal metabolism of [ureyl-^{14}C]tolbutamide (gift of Farbwerke Hoechst A/G., Frankfurt/Main, Germany) *in vitro* differed only in that all incubation mixtures contained [ureyl-^{14}C]tolbutamide (1·0 μCi/μmole) at a final concentration of 0·4 mM. The ethyl acetate used to extract the hydroxy [ureyl-^{14}C]tolbutamide was evaporated to dryness after direct transfer to counting vials, and radioactivity measured as described in the previous paper.

Results and discussion. Phenobarbitone-treament of both male and female immature rats caused statistically significant ($P < 0·001$) threefold increases in the rate of metabolism of [ureyl-^{14}C]tolbutamide by the animals' hepatic microsomes *in vitro*. The rates for male animals [sham-injected, 0·34 ± 0·02 (7)nmole hydroxy[ureyl-^{14}C]tolbutamide formed/minute/milligram microsomal protein; phenobarbitone-treated, 1·17 ± 0·08 (7)] were not statistically different from those found using female animals [(sham-injected 0·37 ± 0·02 (7); phenobarbitone-treated, 1·15 ± 0·08 (7)]. This is in contrast to the earlier findings that the rates of metabolism associated with microsomal preparations from adult male rats were higher (1½-times) than those associated with preparations from adult female rats, although the previous study used the kinetic parameter V_{max} for comparative purposes and not the rate of metabolism at a fixed concentration of [ureyl-^{14}C]tolbutamide. Values for adult rats comparable with those described above for immature animals, i.e. rates of metabolism *in vitro* with 0·4 mM [ureyl-^{14}C]tolbutamide, are 0·46 ± 0·02 (8) (male, sham-injected), 0·29 ± 0·04 (7) (female, sham-injected), 1·01 ± 0·06 (5) (male, phenobarbitone-treated), and 0·74 ± 0·06 (8) (female, phenobarbitone-treated), all highly significantly ($P < 0·01$ or 0·001) different from each other, and obviously paralleling the kinetic data. The degree of stimulation of the microsomal metabolism by phenobarbitone-administration *in vivo* to immature animals (3–2½-times the control values) is higher than the 2–2½-fold increase found previously for adult males and females, and may support the contention that the microsomal mixed-function oxidases are more markedly increased in activity in young animals than in older ones following phenobarbitone-treatment.[2–4].

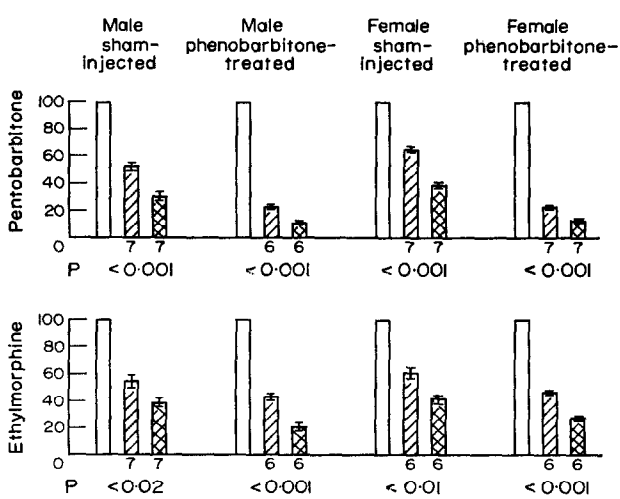

FIG. 1. Effect *in vitro* of pentobarbitone or ethylmorphine on the metabolism of [ureyl-^{14}C]tolbutamide by hepatic microsomes from sexually immature male or female rats sham-injected or treated with phenobarbitone *in vivo*. □, No inhibitor *in vitro*, expressed as 100 per cent. ▨, Inhibitor concentration *in vitro* 0·5 mM. ▩, Inhibitor concentration *in vitro* 1·5 mM. The height of each vertical bar represents the rate of metabolism expressed as a percentage relative to the corresponding no-inhibitor value and calculated as a mean. The solid vertical lines at the top of each bar represent twice the S.E.M. P values were derived by Student's *t*-test from the data for 0·5 and 1·5 mM inhibitor concentrations. The numbers under the vertical bars represent the number of animals used.

Figure 1 summarizes the differences associated with phenobarbitone-treatment *in vivo* on the effect *in vitro* of 0·5 or 1·5 mM pentobarbitone or ethylmorphine on the rate of [ureyl-^{14}C]tolbutamide metabolism. There is a marked increase in the sensitivity to pentobarbitone of the hepatic microsomal preparations from male and female animals, but the increase in sensitivity to ethylmorphine is not so clear cut, as also reported earlier for adult rats.[1] The metabolism of [ureyl-^{14}C]tolbutamide by microsomes prepared from the livers of immature female rats is less sensitive to pentobarbitone and (to a lesser extent) ethylmorphine *in vitro* at either concentration than the metabolism by hepatic microsomes from immature male rats, but this is not nearly as marked as for adults.[1]

TABLE 1. EFFECT OF PHENOBARBITONE-TREATMENT OF SEXUALLY-IMMATURE MALE AND FEMALE RATS ON THE RATIO OF THE RATES OF METABOLISM OF [UREYL-^{14}C]TOLBUTAMIDE BY THEIR HEPATIC MICROSOMES IN THE PRESENCE OF 0·5 AND 1·5 mM PENTOBARBITONE OR ETHYLMORPHINE

	Male		Female	
Inhibitor	Sham-injected	Phenobarbitone treated	Sham-injected	Phenobarbitone treated
Pentobarbitone	1·80 ± 0·19 (7)	2·00 ± 0·14 (6)	1·72 ± 0·10 (7)	2·02 ± 0·07 (6)
P	N.S.		<0·05	
Ethylmorphine	1·43 ± 0·05 (7)	2·20 ± 0·35 (6)	1·48 ± 0·11 (6)	1·78 ± 0·06 (7)
P	<0·05		<0·05	

Values are calculated as:

$$\frac{\text{Rate of formation of hydroxy [ureyl-}^{14}\text{C]tolbutamide in the presence of 0·5 mM inhibitor}}{\text{Corresponding rate of metabolism in the presence of 1·5 mM inhibitor}}$$

and expressed as mean ± S.E.M. Figures in backets indicate the number of animals used.

Differences between pairs of values ("Control" vs "phenobarbitone-treated") for each inhibitor have been calculated using Student's *t*-test. N.S. = not significantly different.

Table 1 compares the ratios of the rates of metabolism of [ureyl-^{14}C]tolbutamide at the two different concentrations of each inhibitor, and it is clear that in general phenobarbitone increases the degree of inhibition at the higher concentrations of inhibitors, but that there is no obvious sex difference. Figure 1 confirms the (anticipated) finding that the hepatic microsomal metabolism of [ureyl-^{14}C]-tolbutamide *in vitro* would be inhibited by pentobarbitone and ethylmorphine, but with only a marginal sex difference with the more immature rats used. In fact, the only statistically significant differences are those associated with 0·5 mM pentobarbitone (male and female sham-injected, $P < 0·02$) and 1·5 mM ethylmorphine (male and female phenobarbitone-treated, $P < 0·05$). Marked sex-differences in the degree of inhibition have been found with sexually mature rats,[1] and from the extra evidence described here, it is reasonable to suppose that male sex hormones bound to microsomes are causing the effect by a mutual interaction with the inhibitors, which are themselves substrates. It is still unclear why microsomes from adult females should be "sensitized" by phenobarbitone treatment *in vivo* compared with those from adult males, when tolbutamide as well as being a substrate will probably also be itself inhibiting the (unmeasured) metabolisms of the pentobarbitone and ethylmorphine.

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REFERENCES

1. F. J. DARBY, *Biochem. Pharmac.* **21**, 1649 (1972).
2. A. H. CONNEY, *Pharmac. Rev.* **19**, 317 (1967).
3. R. KATO and A. TAKANAKA, *J. Biochem. Jap.* **63**, 406 (1968).
4. W. KLINGER, T. KURCH, A. NEUGEBAUER, F.-K. SPLINTER and H. ANKERMANN, *Acta biol. Med. Ger.* **21**, 257 (1968).

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Hepatotoxicity of CS_2 in rats: relation to postexposure liver weight and pre-exposure cytochrome P-450 level

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MICROSOME mixed function oxidase activity and cytochrome P-450 levels are depressed in animals given CS_2.[1,2] Such animals are less sensitive to carbon tetrachloride liver injury.[3,4] In animals pretreated with phenobarbitone, CS_2 causes a greater fractional decrease in both drug metabolizing enzymes and cytochrome P-450 levels.[1] In contrast to this biochemical effect, CS_2 does not induce liver lesions after a single toxic oral dose[5] but does so after phenobarbitone pre-treatment[5,6] and this effect is aggravated by short-term starvation.[6] In the present work we examined the relation between histological damage and the level of cytochrome P-450 at the time of CS_2 exposure. Different liver cytochrome P-450 levels were achieved by feeding or starving rats given an identical phenobarbitone dose but varying the time before exposure to CS_2.

Albino rats of Porton–Wistar strain (200–220 g) were given two doses of phenobarbitone sodium (BDH) i.p., the first 80 mg/kg and the second 50 mg/kg 6 hr later. Control animals were given saline. Animals were killed decapitation 24 or 48 hr after the first injection and liver cytochrome P-450 estimated using the procedures described by Bond and De Matteis,[1] or were subjected to a 4 hr inhalation exposure to 2·0 mg/l. CS_2 in an inhalation chamber described earlier.[7] Animals were divided into six groups and treated as shown in Table 1. All animals were males except in Group F. Food was withdrawn at the start of exposure but for some animals food was withdrawn at either at the time of the first phenobarbitone injection or 24 hr later. Exposed animals were killed 18 hr after the end of exposure, livers were removed, weighed and fixed in formol alcohol or formol saline. Paraffin sections were prepared and stained with Harris' haematoxylin and eosin. Sections were scored as follows: indistinguishable from normal, 0; few hydropic cells in the region of central veins, 1+; approximately half of the lobules were changed to hydropic cells, 2+.

Table 1 shows that phenobarbitone pretreatment significantly increased the cytochrome P-450 level in every group, while starvation of the phenobarbitone pretreated animals caused a further significant increase. In control rats 1 day fasting resulted only in a slight change in cytochrome P-450 levels but after 2 days starvation P-450 increased significantly (Group E saline-treated controls) and was even higher than in phenobarbitone-treated fed rats (Group A) ($t = 3{\cdot}19$; $P < 0{\cdot}05$). However, these animals which were starved for 2 days without phenobarbitone treatment (Group E) did not develop histological change on exposure to CS_2.

In phenobarbitone-treated rats the lowest cytochrome levels were found in the female 24 hr starved group (Group F) or in the male fed group (Group A) and the highest in the male 48 hr starved group (Group E). The female group had no liver damage, only 4 of the 8 fed male had slight liver damage, while the last group developed the most extensive hydropic degeneration. However, within these two extremes, no correlation was found between the pre-exposure cytochrome P-450 levels and post-exposure liver damage. Thus only one animal in Group C which had approximately the same cytochrome level as in Group B developed a slight liver lesion. In group D with significantly higher cytochrome P-450 concentrations than in Group B ($t = 4{\cdot}31$ $P < 0{\cdot}0125$) the extent of liver damage was less pronounced.

Liver weight after exposure increased in starved animals in the same order as liver damage, that is F < C < D < B < E. The correlation between liver weights and liver damage can be seen in Table 2.