Conclusion. The present data and previous findings[3–6,8] are consistent with the assumption (1) that C_{10} is transported into renal cells by a carrier mechanism capable of transporting quaternary ammonium compounds (for instance carbamoylcholine); and (2) that C_{10} once inside the cell is strongly bound to some intracellular structure.

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Effect of suramin (Bayer 205) on hepatic microsomal cytochromes P-450, b_5, and demethylation activity in rats

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SURAMIN is commonly used for protecting an individual against trypanosomiosis (sleeping sickness) because of its powerful trypanocidal action.[1] It is the symmetrical 3″-urea of the sodium salt of 1-(3-benzamido-4-methylbenzamido)-napthalene-4,6,8-trisulphonic acid which was first synthesized in 1922.[2] The precise mode of action of suramin on trypanosomes is not yet clear. It is possible that the suramin combines with the plasma protein, which may then gradually be released in the blood stream and pass into trypanosomes in amounts sufficient to inactivate essential enzymes of the trypanosomes.[3] However, a prolonged latent period of contact of suramin and trypanosomes before the trypanocidal effect is manifested, has been reported by the earlier workers.[4] In addition to plasma protein, various body tissues in animals, such as kidneys, spleen, liver, heart, brain and adrenals have also been found to bind suramin for a considerable period after administration.[5] It seems probable that the long retention of suramin in the plasma and tissues without being metabolized[5] accounts for the very satisfactory prophylactic value of this drug as far as sleeping sickness is concerned. However, a knowledge which would be useful is whether suramin retention in the body can impair detoxication mechanisms. If suramin retention decreases the efficiency of detoxication mechanisms, then it may be supposed that toxic manifestations might occur in suramin treated persons.

The present work was undertaken to investigate the effect of suramin on the NADPH-dependent oxidative demethylation of various drugs, e.g. ethylmorphine and the concentrations of cytochrome b_5 and cytochrome P-450 which are probably involved in liver microsomal drug-hydroxylating reactions.[6] This study is merited because suramin is retained in the liver, where it might interact with the drug-metabolizing enzymes.

Weanling Sprague–Dawley rats were used throughout and maintained on a stock pellet diet (Diet 41B) and water *ad lib.* The rats were divided into four groups: group A was given 0·95% saline and served as controls, groups B, C and D were treated with suramin (20 mg/kg), phenobarbital sodium (100 mg/kg) and suramin plus phenobarbital, respectively 24 hr before the animals were

killed. Suramin and phenobarbital sodium were dissolved in 0·95% saline, and both the drugs as well as the saline in control animals were given intraperitoneally.

The rats were killed by cervical fracture and livers were quickly removed, weighed and homogenized in ice-cold 1·15% (w/v) KCl using a glass homogenizer and a loose-fitting Teflon pestle, to give a final concentration of liver of 25% (w/v). Homogenates were centrifuged at 10,000 *g* for 20 min and the supernatant fractions centrifuged at 100,000 *g* for 1 hr to sediment the microsomal fraction. The microsomes were then washed and suspended in isotonic KCl (1 ml of the microsomal suspension is equivalent to 250 mg liver). Cytochrome P-450,[7] cytochrome b_5[8] and the activity of ethylmorphine demethylase[9] were determined in the microsomal preparation. Microsomal protein was determined by the method of Lowry.[10]

Further groups of animals were treated as described for A, B, C and D. After 24 hr these animals were given a dose of hexobarbitone (100 mg/kg) and the duration of action of the barbiturate was determined. The duration of action of hexobarbitone was regarded as the time which elapsed between the disappearance and the reappearance of the righting reflex, after intraperitoneal injection of hexobarbitone.

TABLE 1. EFFECT OF SURAMIN ON HEPATIC MICROSOMAL CYTOCHROMES AND THE DEMETHYLATION OF ETHYLMORPHINE

Treatment	Liver wt (% body wt)	*N*-demethylase (μmole HCHO formed/hr/mg microsomal protein)	Cytochrome P-450 (nmole/mg microsomal protein)	Cytochrome b_5 (nmole/mg microsomal protein)
Control	4·5 ± 0·08	0·24 ± 0·04	0·75 ± 0·08	0·35 ± 0·04
Suramin	4·8 ± 0·08	0·25 ± 0·03	0·79 ± 0·06	0·35 ± 0·05
Phenobarbital	*5·5 ± 0·10	*0·44 ± 0·06	*1·76 ± 0·10	*0·72 ± 0·08
Suramin + phenobarbital	5·4 ± 0·15	0·31 ± 0·04	†1·10 ± 0·09	†0·51 ± 0·06

Rats were treated with suramin (20 mg/kg), phenobarbital (100 mg/kg) and suramin plus phenobarbital 24 hr before the animals were killed. Each result is the mean for six animals with ± S.E.

* Difference between control of suramin and phenobarbital, statistically significant ($P < 0·01$).

† Differences between phenobarbital and suramin + phenobarbital, statistically significant ($P < 0·05$).

TABLE 2. EFFECT OF SURAMIN ON THE DURATION OF ACTION OF HEXOBARBITAL

Treatment	Sleeping time (min)
Control	69 ± 6·5
Suramin	71 ± 8·5
Phenobarbital	*16 ± 2·6
Suramin + phenobarbital	†24 ± 2·0

Rats were treated with suramin (20 mg/kg), phenobarbital (100 mg/kg) and suramin + phenobarbital 24 hr before the animals were given hexobarbital (100 mg/kg) intraperitoneally. Each result is the mean for six animals with ± S.E.

* Difference between control of suramin and phenobarbital, statistically significant ($P < 0·001$).

† Difference between phenobarbital and suramin + phenobarbital, statistically significant ($P < 0·05$).

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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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