TABLE 1. CHANGES IN LACTIC AND PYRUVIC ACIDS AND IN INORGANIC PHOSPHATE OF BLOOD AFTER ADMINISTRATION OF COMPOUND I (100 mg/kg)

	0 (hr)	2 (hr)	*T* between means
Lactic acid	17·35 ± 2·32 (12)	49·00 ± 8·79 (12)	3·4*
Pyruvic acid	2·19 ± 0·1 (9)	2·96 ± 0·26 (9)	2·7
Pi	4·39 ± 0·3 (9)	6·06 ± 0·26 (8)	4·2*

Figures in parentheses indicate number of observations.
* Significant at 5 per cent level.
Values expressed in milligram per cent indicate mean ± S.E. of the mean.

lactic acid and liver and muscle glycogen compares directly with that of guanidine derivatives. Therefore, Compound I may act in a similar manner to guanidine derivatives as speculated by Hollunger.[7]

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Effect of intracellular accumulation of inert carbon particles on the cytochromes P-450 and b_5 levels of rat liver microsomes

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HISTOLOGICAL and ultrastructural studies have shown that carbon particles injected into the blood stream are quickly resorbed by the reticuloendothelial cells, particularly by the Kuppfer cells of the liver.[1–4] Biochemical effects of this intracellular accumulation of carbon in the whole liver are not well known. In this communication we report the results of a systematic study, showing a decrease in the microsomal cytochrome levels.

Carbon particles suspension was obtained as described by Stenger *et al*:[4] Gunther Wagner "Pelikan" china ink (type C 11/1431a) was centrifuged (8000 *g* for 15 min) to precipitate larger carbon particles. The supernatant was diluted eight times with saline. Charles River male rats (C.D. strain) weighing about 230 g were injected intravenously (0·4 ml) under light ether anaesthesia, either with carbon particles (43 mg/kg) or with saline. Animals were injected on each of two successive days and sacrificed on the third day after 12 hr of fasting. The liver microsomes were isolated by ultracentrifugation of the postmitochondrial supernatant at 105,000 *g* for 60 min in 0·25 M sucrose. Protein was measured by the method of Lowry *et al*.[5] The amounts of P-450 and cytochrome b_5 were determined by the method of OMURA and SATO[6] on a Perkin–Elmer double beam spectrophotometer model 402 equipped with a Hewlett–Packard XY recorder which amplified three times the spectrophotometer signal.

Microsomal ethylmorphine demethylase activity was determined according to Hildebrandt and Estabrook[7] by measuring the formaldehyde formed by the colorimetric method of Nash.[8] Glucose-6-phosphatase activity was determined in whole liver homogenate (1% wt/vol in 0·25 M sucrose) and in microsomes by the method of Ricketts.[9] Total iron in the liver was evaluated by the following procedure: the organs were carefully perfused with heparinized isotonic saline in order to wash out haemoglobin and were then mineralized in sulfonitric mixture. After neutralization, an iron *o*-phenantroline complex was formed which was measured spectrophotometrically.

Results are given in Table 1. There was a slight increase of the ratio liver weight/body weight, which is an indication of the increase in the number of reticuloendothelial cells.[4] The microsomal protein however was not significantly altered. Glucose-6-phosphatase activity was not altered, either in the whole homogenate or in the microsomes. This indicates that carbon particles do not affect the synthesis of this enzyme and that their presence in the liver tissue does not change the centrifugation properties of the liver particles.

There was however a considerable decrease in the levels of cytochromes b_5 and P-450, the latter being confirmed by a decrease in demethylase activity.

TABLE 1.

	Control saline (0·25 ml i.v)	Treated carbon particles (43 mg/kg i. v)	Variation (%)	P*
Number of rats	12	11	—	—
Weight of rats (g)	229 ± 14	213 ± 7	− 7	N.S.
Weight of livers (g)	7·8 ± 0·6	8·8 ± 1	+ 13	N.S.
Ratio $\frac{\text{liver wt}}{\text{rat wt}} \times 100$	3·45 ± 0·47	4·18 ± 0·16	+ 21	*
Microsomal protein mg/g of liver	8·65 ± 1·64	7·25 ± 0·60	−15	N.S.
Glucose-6-phosphatase				
Whole liver homogenate†	28·8 ± 3·6	26·7 ± 1·9	−7	N.S.
Microsomes‡	3·05 ± 0·15	2·8 ± 0·20	−8	N.S.
Cytochrome b_5				
nmoles/mg protein	0·64 ± 0·13	0·36 ± 0·12	−44	*
nmoles/g of liver	5·5 ± 1·5	2·6 ± 0·7	−53	*
Cytochrome P-450				
nmoles/mg protein	0·84 ± 0·12	0·38 ± 0·11	−55	***
nmoles/g of liver	7·2 ± 0·9	2·7 ± 0·2	−63	***
Ethylmorphine demethylase§				
nmoles FA/min/mg protein	6·5 ± 1·0	2·3 ± 0·5	−64	***
nmoles FA/min/g of liver	56 ± 10	16·7 ± 5	−70	***
Total liver iron¶				
μg/g of liver	51·0 ± 8·5	57·7 ± 20·1	+13	N.S.

* 0·05 > P > 0·01, ** 0·01 > P > 0·001, *** P > 0·001, N.S.—not significant.

† μmoles P/min/g of liver.

‡ μmoles P/min/mg protein.

§ FA: formaldehyde.

¶ Eight rats in each group.

Since total microsomal protein and glucose-6-phosphatase activity were not significantly changed, the cytochrome synthesis was probably inhibited. The liver cytochromes are principally located in the hepatocytes in which the endoplasmic reticulum is much more developed than in the Kuppfer cells.[10] On the other hand, the majority of the carbon particles accumulate in the latter cells.[4] Thus the decrease in microsomal cytochromes is an indirect effect showing that very close biochemical relations exist between the two principal types of liver cells. The cause of the inhibition of cytochrome synthesis could be a lack of iron in the liver. We have found no variation in the total liver iron, but this does not exclude a perturbation in the exchange of this metal between the Kuppfer cells and the hepatocytes.

Loading of the liver reticuloendothelial cells with carbon particles is therefore a non-toxic method useful for the study of microsomal cytochrome turnover and function. It supplements the information obtained from the induction of cytochromes by phenobarbital or methylcholantrene. Using this method we have shown that a decrease in the level of P-450 could explain the protective effect of carbon particle injection against carbon tetrachloride injury,[4,11] since the hepatotoxicity of this compound is thought to be due to its activation as a free radical by cytochrome P-450.[12]

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Steric hindrance to hydroxyl-group assisted acyl group migration in 2′(3′)-*O*-acylribonucleosides: a reassessment and correction

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WE REPORTED earlier[1] on aminoacylribonucleosides, prepared as potential antitumour agents and derived from the tumour inhibitor, 1-aminocyclopentanecarboxylic acid. It was suggested that migration of the aminoacyl group between the 2′- and 3′-positions in compounds (I) and (II) (and their adenosine analogues) might be inhibited by steric factors. More recent studies, however, have led us to revise this view.

When 3′,5′-di-*O*-benzyluridine[2] was fused with 1-(benzyloxycarbonylamino)cyclopentanecarboxylic anhydride (reaction in solvents, e.g. pyridine, dimethylformamide, was slower and more incomplete)