to be based on the possible disturbance of this equilibrium [10]. An efflux of Hg can be caused by the addition of SH-compounds to the extracellular fluid, as was shown by White and Rothstein [1], Giblin [2] and in this paper. The amount of Hg released would be expected to depend on the extracellular SH-concentration and the affinity of the added chelating agent for MMC. As shown in Fig. 3(a), only DMSA is able to remove all the MMC from human erythrocytes, the affinity of the other compounds apparently being smaller. Some of the MMC extracted by all the chelating agents tested is lost again gradually from the extracellular fluid. However, as Fig. 2 suggests, the underlying mechanisms may be different:

PA and NAPA. At low concentrations 5–12 per cent of the MMC was mobilized from the erythrocytes, but almost none of this was lost again during the observation period indicating that the complex between the mercurial and these substances, once formed, is quite stable. The loss of MMC from the extracellular fluid at higher PA or NAPA concentrations points to a damage of the cell wall which then permits diffusion of the complex into the cell. This is corroborated by the finding that haemolysis occurs soon after the addition of the highest dose of PA or NAPA, respectively.

DMSA. At low doses only part of the mobilized MMC is lost gradually. The reason may be that there are two binding sites on the DMSA-molecule of differing affinity for MMC.

DMPS. The loss of MMC from the extracellular fluid after mobilization by this chelating agent, at all concentrations, seems to reflect two superimposed effects: first, a dissociation of the mercurial from DMPS and back diffusion into the cell, and secondly, as shown by the study with the ^{14}C-labelled DMPS, an associaton of the whole complex with the erythrocyte. The order of effectiveness of the chelating agents for the removal of MMC from human and rat erythrocytes is the same. Recent experiments [15] have shown that in rats this is also true for the mobilization of MMC *in vivo*. We believe, therefore, that the *in vitro* system described in this paper could possibly serve as a test system to compare the effectiveness of chelating agents for the decorporation of MMC from the human body.

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Phagocytic activity of the reticulo-endothelial system in the rat and rates of *in vivo* excretion of metabolites of carbaryl and *in vitro* microsomal metabolism

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The role of the reticuloendothelial system (RES) in the toxicity and metabolism of xenobiotics is not well known. However, it has been shown that depending on the functional activity of the macrophages of the RES the toxicity of benzylpenicillin can be modified, RES inhibition increasing it and RES activation decreasing it [1]. In addition the RES is involved in the metabolism of sulfonamides [2]. For our part, we have previously observed that inhibition of the RES increases the duration of the anticholinesterase toxic effect of a carbamate pesticide, carbaryl [3], widely used as an insecticide in agriculture and veterinary medicine. Moreover, the administration of colloidal carbon decreases both the phagocytic activity of the RES and the blood clearance of carbaryl, whereas the administration of glyceryl trioleate increases phagocytic activity as well as the clearance of carbaryl [4].

From these data we wondered that if, parallel to the activation and inhibition of the RES, there were no perturbations in the metabolism of carbaryl. We measured the biliary, urinary and pulmonary excretions of ^{14}C-labelled metabolites *in vivo* after administration of [^{14}C]carbaryl in rats with the RES inhibited by colloidal carbon [5, 6] or activated by glyceryl trioleate [7], compared to control animals. In addition we evaluated in animals having undergone the same treatment, the *in vitro* *N*-demethylation of carbaryl and three other mixed function oxidase activities since it is known that hepatic drug-metabolizing enzymes metabolise, for the most part, this pesticide [8, 9].

Materials and Methods. [^{14}C]carbaryl (1-naphthyl *N*-[^{14}C]methylcarbamate) was prepared by reaction of freshly distilled naphthyl chloroformate with [^{14}C]methylamine, HCl (41.2 mCi/mmole) (CEA, Saclay, France) (10) and

Table 1. Effects of intravenous pretreatment of colloidal carbon and glyceryl trioleate on body weight and various hepatic parameters of rats*

	Control	Colloidal carbon	Glyceryl trioleate
Body weight (g)	216.50 ± 2.85	214.81 ± 2.07	211.50 ± 2.19
Liver weight (g/100 g body wt)	3.085 ± 0.037	3.864 ± 0.076†	4.012 ± 0.061†
Hepatic proteins (mg. in liver/100 g body wt)	556.90 ± 7.15	684.29 ± 13.00†	717.40 ± 12.43†
Hepatic proteins (mg/g dry wt liver)	441.32 ± 2.46	438.51 ± 1.91	442.27 ± 2.39
Liver microsomal proteins (mg/100 g body wt)	64.12 ± 1.20	64.89 ± 2.15	78.90 ± 1.81†
Liver microsomal RNA (mg/100 g body wt)	9.72 ± 0.17	13.06 ± 0.29†	11.24 ± 0.24†

* Each value is the mean ± S.E.M. for 16 rats
† Significantly different $P < 0.001$ from control animals.

was kept in a benzene solution; radiochemical purity was > 99 per cent. Unlabelled carbaryl was kindly supplied by Pépro, Lyon (France); after recrystallisation in ethanol it had a melting point of 142° and was chromatographically pure (determination by thin-layer chromatography on silica gel in hexane–diethylether: 1/1). The colloidal carbon suspension (C 11/1431 a, Pelikan), came from Günther–Wagner, Hanover, W. Germany. Glyceryl trioleate, obtained from Prolabo, Paris (France) was of analytical grade; the suspension was prepared shortly before injection by adding 4 g of glyceryl trioleate to 16 ml of an isotonic glucose solution (glucose 5.4 g/100 ml; Tween 20, 2.8 mg/100 ml) and homogenized in a MSE homogenizer for 2 min. Nicotinamide–adenine dinucleotide phosphate, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, reduced nicotinaminde–adenine dinucleotide and nicotinamide were purchased from Calbiochem. Other chemicals used were of analytical grade. Male OFA Sprague–Dawley rats weighing 239.3 ± 2.5 g were obtained from Iffa-Credo, Saint-Germain-sur-l'Arbresle (France). A balanced complete standard feed (UAR, Villemoisson-sur-Orge, France) and water were freely available. Eighteen hours before each experimentation the animals required for the study (numbers indicated below) were divided into 3 groups: one group received an intravenous dose of colloidal carbon (10 mg/100 g body weight), RES inhibitory; another received an intravenous administration of a glyceryl trioleate suspension (7.5 mg/100 g body weight), RES activatory. The control animals received, under the same conditions, an equivalent volume of an isotonic solution (0.1 ml/100 g). The pretreated rats were deprived of food. The evaluation of the phagocytic activity of the RES in 8 rats of each group was effected by the colloidal carbon blood clearance technique [11]. The value of the phagocytic index K was calculated according to a previously described method [3]. 16 rats of each group were decapitated and the liver immediately removed and weighed; the water content of the liver was measured by overnight drying of an aliquot in an oven at 100°. Proteins were measured [12] by means of another aliquot; protein standard was carried out with fraction V human albumin serum (Nutritional Biochemicals Corp.). In order to evaluate the activities of hepatic microsomal drug-metabolizing enzymes the rat liver was homogenized in 4 vol. (w/v) of ice cold 0.1 M phosphate buffer, pH 7.4, using a motor-driven Potter homogenizer with a close-fitting Teflon pestle. Liver homogenates were centrifuged at 4° at 9000 g for 15 min and the supernatant was then centrifuged at 4° at 105,000 g for one hour (MSE ultra centrifuge). The pellet was resuspended in the cold isolation medium to give a protein concentration of approximately 8 mg/ml and was used for the following assays: carbaryl N-demethylase by evaluation of formaldehyde formed [13] aniline aromatic hydroxylase [14], p-nitroanisole o-demethylase [14] and pyramidon N-demethylase [15]. The cytochrome P-450 content was evaluated using the extinction coefficient of 91 cm^{-1} mM^{-1} [16].

Protein concentration was determined by the same method and standard as above. RNA content was evaluated [17] using sheep's liver RNA (Choay, France) as the RNA reference. The reaction vessel contained 6 ml of the medium of incubation consisting of 0.75 μmoles of NADP, 50 μmoles of glucose-6-phosphate, 0.5 IU of glucose-6-phosphate dehydrogenase, 25 μmoles of Cl_2Mg, 100 μmoles of nicotinamide, 5 μmoles of NADH and 290 μmoles of potassium phosphate buffer, pH 7.4. Substrate quantities were: 3 μmoles for p-nitroanisole, 5 μmoles for aniline, pyramidon and carbaryl. Carbaryl which is insoluble in distilled water was dissolved in acetone (5 μmoles/50 μl) and a blank with acetone but without carbaryl was included in the dosage; in this particular case 60 μmoles of semicarbazide was added to the medium of incubation. The quantity of microsomal proteins present in the 6 ml incubation medium was approximately 20 mg. Incubation was carried out for 30 min by means of shaking at 150 oscillations/min in a Gallenkhamp apparatus at 37°, air being used as the gaseous phase. To evaluate the ^{14}C-labelled metabolites of [^{14}C]carbaryl in the bile 8 animals of each group had a cannula inserted in the common bile duct under light ether anaesthesia. Alterations in bile flow due to hypothermia [18] were prevented by maintaining body temperatures at 37° by means of a heat lamp. The rats received by intravenous administrations an aqueous suspension of carbaryl in an isotonic glucose solution (glucose 5.4 mg/100 ml; Tween 20, 2.8 mg/100 ml); after dilution with unlabelled carbaryl to a specific activity of 2.16 μCi/mg, an administration of 15 mg/kg (4 mg/ml) of carbaryl via the dorsal vein of the penis, was made. The LD_{50} of carbaryl was, under our experimental conditions, of the order of 50 mg/kg body weight. After administration of [^{14}C]carbaryl, the bile volume for each collection period was measured gravimetrically. The total concentrations of metabolites–^{14}C of carbaryl in the bile (99.8% of radioactivity non extracted by hexane) were determined by adding 50 μl of bile collected at appropriate time intervals to 10 ml of a toluene–triton X-100 mixture, Scintix, supplied by Isotec, Versailles, France. Radioactivity was measured by an Intertechnique liquid scintillation counter. Sample counting efficiency was determined by an automatic external standardization. In order to evaluate the ^{14}C-labelled metabolites of [^{14}C]carbaryl in the urine, 8 animals of each group for each time period considered were used. Thirty min, 1 hr and 2 hr after the carbaryl administration, the bladders were removed after binding and the urine retrieved; the bladders were subsequently rinsed and the urine volume adjusted to 5 ml with distilled water. The total concentration of ^{14}C-metabolites of carbaryl (99.9% of radioactivity non extracted by hexane) was determined by adding 50 μl of diluted urine to 10 ml of Scintix; radioactivity was evaluated as indicated previously. To evaluate the [^{14}C]carbon dioxide in the expired air 8 animals of each group received an intravenous administration of [^{14}C]carbaryl and were placed in metabolism cages designed

Table 2. Effects of intravenous pretreatment of colloidal carbon and glyceryl trioleate on the *in vitro* activities of drug-metabolizing enzymes and on the cytochrome P-450 content of rat liver microsomes

	Control	Colloidal carbon	Glyceryl trioleate
Carbaryl *N*-demethylase*	11.97 ± 1.38	8.05 ± 0.82‡	7.05 ± 1.03§
Aniline hydroxylase*	25.11 ± 1.34	12.96 ± 0.94‖	15.27 ± 0.75‖
P-Nitroanisole *O*-demethylase*	24.57 ± 0.97	16.61 ± 0.74‖	14.01 ± 0.41‖
Pyramidon *N*-demethylase*	19.83 ± 1.16	14.16 ± 1.00§	13.64 ± 0.56‖
Cytochrome P-450†	0.679 ± 0.028	0.426 ± 0.040‖	0.533 ± 0.024‖

* Each value is the mean of the microsomal enzyme activity ± S.E.M. for 8 rats. Results are expressed in nmoles of metabolite formed/mg of microsomal proteins/30 min. of incubation.

† Each value is the mean of cytochrome P-450 content ± S.E.M. for 16 rats. The cytochrome P-450 content is expressed in nmoles/mg of microsomal proteins.

‡ Significantly different $P < 0.02$ from control animals.

§ Significantly different $P < 0.01$ from control animals.

‖ Significantly different $P < 0.001$ from control animals.

for the collection of CO_2. [^{14}C]carbon dioxide was trapped in 20 ml of a mixture of 4.4 ml of ethanol, 6.6 ml of phenylethylamine, 9 ml of toluene, (PPO: 2,5-diphenyloxazole, 0.5 g/100 ml; POPOP: 1,4-di-[2-(5-phenyloxazolyl)] benzene, 0.05 g/100 ml). Radioactivity was evaluated as previously. The results of treated and control animals were compared using Student's *t*-test.

Results and discussion. Eighteen hours after intravenous administration of colloidal carbon the phagocytic activity of the RES was inhibited by 70 per cent ($K = -0.0057 \pm 0.0020$) compared to the control group ($K = -0.0187 \pm 0.0019$) ($P < 0.001$). Following intravenous administration of glyceryl trioleate the phagocytic activity of the RES was activated by 160 per cent ($K = -0.0502 \pm 0.0052$) compared to the control group ($P < 0.001$). The results given in Table 1 show that the weight of the animals treated with colloidal carbon or trioleine was not different to that of the control animals after being deprived of food for 18 hr. Conversely, liver weight per 100 g body weight was significantly increased in the two pretreated groups of rats. Given the variations in liver weight, we have expressed the hepatic protein level, firstly, in relation to the liver weight per 100 g body weight and, secondly, in relation to the dry weight of the liver. Thus colloidal carbon and glyceryl trioleate had no effect on the hepatic protein level, but the protein mass of the liver per 100 g body weight significantly increased compared to the control animals. In the animals pretreated with colloidal carbon the microsomal protein level was stable per one gramme of liver per 100 g body weight, but after glyceryl trioleate treatment the microsomal protein level increased. There was an increase in the microsomal RNA level whatever the pretreatment. The results given in Table 2 show that colloidal carbon and glyceryl trioleate significantly decreased the activities of the microsomal drug-metabolizing enzymes of the liver for the 4 substrates tested; there was correspondingly a decrease in the cytochrome P-450 level. The results obtained with colloidal carbon cross-check those of Leterrier *et al.* [19]. Comparable results to those involving glyceryl trioleate pretreatment were obtained after treating rats with endotoxines [20], also known as RES activators [21]. Figure 1 shows the effects of pretreatment with colloidal carbon and glyceryl trioleate on the biliary and urinary excretions and expired CO_2 of ^{14}C-labelled metabolites of carbaryl. With carbon pretreatment, ^{14}C-labelled metabolites decreased in the bile, increased in the urine and remained unchanged in the air expired; with glyceryl trioleate, carbaryl metabolites were unchanged in the bile, increased in the urine and increased in the air expired. A

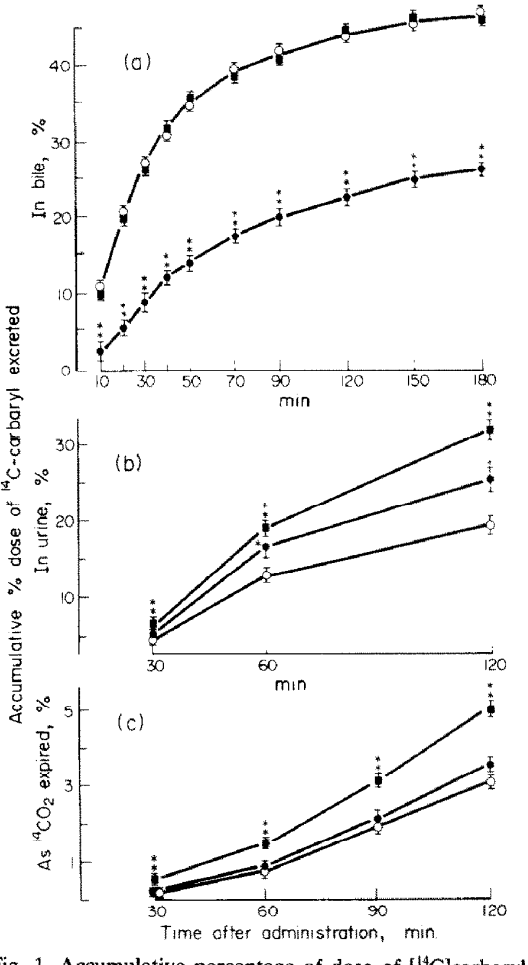

Fig. 1. Accumulative percentage of dose of [^{14}C]carbaryl excreted in (a) bile (b) urine and (c) air expired following intravenous administration of [^{14}C]carbaryl (15 mg/kg; 2.6 μCi/mg) in control rats (○——○) and in rats pretreated with colloidal carbon (●——●) or glyceryl trioleate (■——■). Each point represents the mean ± S.E.M. for 8 rats; one asterisk(*) ($0.01 < P < 0.05$) and two asterisks (**) ($P < 0.01$) indicate values significantly different from the control animals.

decreased biliary excretion and an inhibition of microsomal metabolism after colloidal carbon administration show that the hepatic function is important in determining the disposition of carbaryl. It is believed to be particularly metabolised by the hepatic microsomes [8, 9] and the metabolites formed *in vitro* with the liver microsomes–$NADPH_2$ system suggest that this enzymatic system metabolises carbaryl in the same way as it detoxicates other drugs [22]. The proof of microsome participation is also given by SKF 525A, an inhibitor of microsomal monooxygenases, which increases the lethality of carbaryl whereas inductors of these enzymes such as pentobarbital and DDT decrease the toxicity of carbaryl in the mouse [23]. Moreover, the liver damage produced by colloidal carbon could also explain the decrease of the rate of the previously observed disappearance of carbaryl from the blood.* However following glyceryl trioleate administration, the carbaryl excretion of metabolites was unchanged in the bile compared to the controls despite a decrease in microsomal metabolizing capacity. In addition, the increases in excreted ^{14}C-metabolites obtained in the urine after treatment by colloidal carbon or in the urine and the CO_2 after glyceryl trioleate administration remain unexplained, and seem rather to be in contradiction with the diminished hepatic metabolism; it can hardly be related to RES activity since in the urine, for example, an increase in metabolites was observed following RES inhibition as well as RES activiation. This could suggest that the pretreatment used performed actions in addition to those it carries out on the hepatic microsomes and hepatic RES. Indeed, it is known that there is accumulation and metabolism of carbaryl in organs other than the liver, in particular in the spleen, the lungs and the bone marrow [24]. Several studies have also demonstrated the ability of the pulmonary tissue [25, 26] and whole lungs [27] to metabolize significant quantities of carbaryl in several of the metabolites reported in whole animals and in liver microsomes studies. In addition, and in identical experimental conditions to those used in this study, we have demonstrated an increase in the fixation of [^{14}C]carbaryl in the lungs of animals pretreated with glyceryl trioleate*.

To sum up, it appears that the colloidal carbon which inhibits RES activity and the liver microsomal metabolism, equally decreases the excretion capacity of ^{14}C-labelled metabolites of the carbaryl by the liver and increases urinary excretion. Glyceryl trioleate, a pretreatment which stimulated RES activity, decreased the liver microsomal metabolism while the hepatic excretion of ^{14}C-labelled metabolites remained unchanged and showed an increase in the urine and air expired. The results, in contradiction with the diminished hepatic metabolism, could suggest that the pretreatment used performed actions in addition to those it normally carries out on the hepatic RES and hepatic microsomes; the extrahepatic metabolism of carbaryl, which needs further study, could be increased.

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