incubating rat brain and liver homogenates and blood with GHB-^{14}C (sodium salt) in the presence of malonic acid (2×10^{-2} M), added to block the Krebs cycle at the succinate level. Succinic acid was isolated from possibly interfering substances by passing an 80% ethanolic extract of the tissue over a Dowex-2-formate column, and eluting with 6 N formic acid. The eluate was then passed over a Dowex-50 column to remove interfering cations. Gas chromatography was used to separate and estimate succinic acid after methylation with diazomethane. By means of an effluent splitter, about 95% of the succinate peak was trapped in a vial containing an ethanolic PPO–POPOP* scintilllation-counting mixture and counted in a Packard Tri-Carb liquid scintillation spectrophotometer. One to two per cent of the ^{14}C-isotope of GHB was found in the succinic acid from brain and up to 6% in liver; no isotope could be detected in the blood succinate. In view of the negative findings of Walkenstein *et al.*[3] with regard to labelling of succinate by GHB-^{14}C *in vivo*, we feel that the small percentage of isotope found in brain succinate in our experiments was the result of random labeling of succinate by CO_2 fixation, which could have been overlooked by a less sensitive method.

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* Supplied by Pilot Chemicals Inc., Watertown, Mass., under the trade name "Liquifluor".

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A note on the relative toxic activities of tetrachloromethane and trichloro-fluoro-methane on the rat

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THE administration of carbon tetrachloride to rats (and to many other species) produces central necrosis and fatty degeneration in the liver (see Cameron and Karunaratne,[1] and Van Oettingen,[2] for early references). Carbon tetrachloride administration also results in many early disturbances in metabolic processes: for example, in protein synthesis (Smuckler, Iseri and Benditt,[3]); in nucleotide levels (Thielmann, Schulze, Kramer and Frunder,[4]; Slater, Sawyer and Sträuli,[5]); in serum enzyme levels (Rees and Sinha,[6]; Rees, Sinha and Spector, [7]); and in detoxication mechanisms (Neubert and Maibauer,[8]; Rees, private communication).

The metabolic disturbances which result in the accumulation of fat and in the histological appearanc of necrosis appear to proceed along largely independent pathways of development (See for example, Rees,[9]). Necrosis can be virtually prevented by various treatments (for example, adrenalectomy: Recknagel, Stadler, and Litteria,[10]; administration of certain drugs such as the phenothiazines: Rees, Sinha and Spector,[7]), whereas fat accumulation is little affected by such procedures. Since these 'protective treatments' also suppress several of the metabolic alterations usually observed after dosing with carbon tetrachloride (for example, nucleotide changes, Slater, Sawyer and Sträuli[5]; serum enzyme changes, Rees, Sinha and Spector[7]), it would seem not unreasonable to suppose that such changes have a role, however indirect, in the developing lesions which culminate in necrosis.

Many hypotheses have appeared concerning the toxicity of carbon tetrachloride. In most, the assumption has been implicit that the physical properties of carbon tetrachloride are the key to its hepatotoxicity. Thus, since carbon tetrachloride is a powerful lipid solvent, the view has grown that it produces damage to the lipid-rich membranes of the cell by a direct solvent-like attack resulitng in disruption of the ordered arangement of the membrane components.

This note is an attempt to investigate the above assumption that hepatotoxicity of CCl_4 is dependent upon its physical properties by considering the effect of a closely similar lipophilic solvent, trichlorofluoromethane, on two of the systems which are disturbed by CCl_4. Since both disturbances respond to 'protective treatments' (e.g. administration of 'Phenergan') they are taken to be connected with the development of necrosis.

The rats used were albino females, body wt. approx. 130 g CCl_4 and CCl_3F were administered by stomach tube with the rats under light ether anaesthesia. CCl_4 and CCl_3F were given as 1 : 3 and 1 : 1 mixtures respectively with liquid paraffin at dose levels of 0·5 ml mixture/100 g body wt. Blood was obtained by severing the carotid artery under ether anaesthesia and serum β-glucuronidase was assayed as described by Slater, Greenbaum and Wang.[11] Liver nicotinamide-adenine dinucleotides were estimated by the method of Slater, Sawyer and Sträuli[12] on liver samples after killing the rats by cervical dislocation. Liver samples for histology were fixed in formol-saline and stained with hemotoxylin-eosin.

Table 1 shows serum β-glucuronidase levels at various times after dosing with either CCl_4 or CCl_3F. It can be seen that although CCl_4 produces a rapid increase CCl_3F is inactive in this respect even

TABLE 1. SERUM β-GLUCURONIDASE ACTIVITY AFTER THE ADMINISTRATION OF CARBON TETRACHLORIDE OR TRICHLORO-FLUORO-METHANE TO RATS. THE RESULTS ARE EXPRESSED IN TERMS OF AN ARBITRARY UNIT: EXTINCTION CHANGE/HR; INCUBATION/ML SERUM. IN THIS AND IN TABLE 2, MEAN VALUES ARE GIVEN $\pm$ S.E.M. AND THE NUMBERS OF RATS USED ARE IN PARENTHESES. THE RESULTS FOR CARBON TETRACHLORIDE ADMINISTRATION HAVE PREVIOUSLY BEEN REPORTED[11] AND ARE GIVEN HERE FOR COMPARATIVE PURPOSES

	Time after dosing (hr)		
	0	3	24
Control	·16 ± 03(5)		
CCl_4		3·23 ± ·65(10)	0·29 ± ·10(6)
CCl_3F		0·17 ± ·02(6)	0·08 ± ·02(6)

at a dose twice that of CCl_4. There is no appreciable deviation from normal values even 24 hr after CCl_3F (Table 1). An increase in serum β-glucuronidase has often been used as an index of liver injury following dosing with CCl_4 (for example, see Bangham, Rees and Shotlander,[13]; Judah, Ahmed and McLean,[14]). Since the administration of 'Phenergan' concomitantly with CCl_4 stops such rise in serum activity it would appear connected with the onset of necrosis. Judged by this criterion, CCl_3F does not cause significant hepatic injury.

Table 2 gives the results obtained for the liver levels of NADP and $NADPH_2$ measured 1 hr after dosing. It can be seen that CCl_4 produces a considerable fall in the $NADPH_2$ level and that this drop is prevented by a "protective treatment": administration of the phenothiazine drug "Phenergan". CCl_3F does not produce any significant change in liver $NADPH_2$ levels.

Histological examination of liver sections obtained 3 hr and 24 hr after dosing with CCl_3F revealed an absence of necrosis.

Thus, CCl_3F does not produce two disturbances which follow CCl_4 administration (serum enzyme leakage, and decreased liver $NADPH_2$) and which, by virtue of their response to "Phenergan" are presumably connected with the onset of necrosis. It might be thought that this lack of toxicity results from a failure of CCl_3F to reach the liver. However, it has previously been reported (Lester and Greenberg,[15]) that rats exposed to an atmosphere of 10% v/v CCl_3F for 30 min did not develop liver necrosis; it would appear unlikely that no CCl_3F reaches the liver under such conditions.

CCl_3F possesses physical properties closely similar to CCl_4 particularly in so far as solubilities and surface tension are concerned (Table 3). However, the introduction of a fluorine atom for chlorine

can be expected to alter the chemical properties of the resulting CCl_3F in favour of an increased stability. As a general rule, the band dissociation energy is higher for a C—F than for a C—Cl band (121 ± 4 k cals./mole in CF_4 and 68 ± 3 k cals./mole in CCl_4 respectively, Cottrell[16]). Further, the fluorine atom in CCl_3F can be expected to increase the bond dissociation energies of the carbon-chlorine bonds (This is illustrated by the finding that the bond dissociation energy of the carbon-chlorine bond in CF_3Cl is 83 ± 3 k. cals compared to the value of 68 ± 3 in CCl_4 itself, Cottrell[16]). Since CCl_4 has been shown to undergo limited transformation *in vivo* (Butler,[17]; Paul and Rubenstein,[18]) it could be expected that such a process, or processes, would be less likely with CCl_3F.

TABLE 2. EFFECT OF CARBON TETRACHLORIDE OR OF TRICHLORO-FLUORO-METHANE ON THE LEVELS OF NADP AND $NADPH_2$ IN RAT LIVER. ESTIMATIONS WERE PERFORMED 1 HR AFTER DOSING; THE RESULTS ARE EXPRESSED AS μg. NUCLEOTIDE/WHOLE LIVER CORRECTED TO A 100 g BODY WT. BASIS.*

Treatment	NADP	$NADPH_2$
Untreated	$144 \pm 10(10)$	$1041 \pm 33(14)$
LP†	$107 \pm 6(10)$‡	$1050 \pm 20(9)$
CCl_4 + LP	$128 \pm 8(14)$	$734 \pm 28(15)$
CCl_4 + LP + Phenergan	$87 \pm 6(6)$‡	$1039 \pm 63(6)$‡
CCl_3F + LP	$163 \pm 18(6)$	$1020 \pm 79(6)$

* See Slater, Sawer and Sträuli,[5]. For further details see text.
† LP = Liquid paraffin.
‡ Values taken for comparison from Slater, Sawyer and Sträuli[5].

TABLE 3. PHYSICAL PROPERTIES OF CARBON TETRACHLORIDE AND OF TRICHLORO-FLUORO-METHANE*.

Density (g/cm³)	1·59	1·49
Melting point (°C)	−22·8	−111
Boiling point (°C)	76·8	24
Surface tension at 25°C (dynes/cm)	26·6	19
Viscosity (centipoises)	0·9 at 25°C	0·40 at 30°C
Solubility in water	0·08% at 20°C	0·011% at 25°C
in ethanol	soluble	soluble
ether, benzene	soluble	soluble

* Values for the former are taken from the Handbook of Chemistry and Physics, CHEMICAL Publishing Co., 40th Ed. 1958–59 and from the International Critical Tables. Values for tri-chloro-fluoro-methane are taken from a pamphlet "Data on Arcton Refrigerants" issued by Imperial Chemical Industries, Ltd.

In conclusion, it appears that a substance (CCl_3F) having physical properties closely similar to CCl_4 has none of its necrogenic activity. The most likely explanation at present is that CCl_4 has to undergo some biochemical metabolism before the powerful hepatotoxicity can appear. Such a transformation appears insignificant in the case of CCl_3F.

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Effect of aminoazo dyes on rat serum paraphenylene diamine oxidase activity

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HEPATOCARCINOGENIC substances including aminoazo dyes caused a temporary decrease in the activity of rat serum para-phenylenediamine oxidase (a copper-containing enzyme probably identical with caeruloplasmin) and in the level of serum copper when they were injected as solutions or suspensions in arachis oil intraperitoneally into male albino rats.[1] Woodhouse[2] confirmed this effect with the carcinogenic aminoazo dye 4-dimethylaminoazo-benzene (DAB).

Attention is now drawn to a similar suppressive effect which has been observed subsequently with the supposedly non-carcinogenic dye, 2-methyl-4-dimethylaminoazobenzene (2-MeDAB).

EXPERIMENTAL

Serum was obtained by tail-bleeding male albino rats (body weight ~ 200 g) 24 hr before and 24, 48, 72 and 120 hr after intraperitoneal injection of 16·5 mg of 2-MeDAB (or the molar equivalent of other azo dyes) in 0·6 ml of arachis oil per 100 g body weight. Control rats received arachis oil only. Sera were stored at −15° until required for para-phenylenediamine oxidase (PPDO) determination which was carried out according to the method of Ravin.[3]

RESULTS

As shown in the table, arachis oil injection was followed by a rise in serum PPDO which was sustained for at least 5 days after injection. The same effect was observed after injection of the non-carcinogenic dye 2-methoxy-4-dimethylaminoazobenzene (2-MeODAB) in arachis oil.

On the other hand, the strong carcinogen 3′-methyl-4-dimethylaminoazobenzene (3′-MeDAB) caused a pronounced drop in serum PPDO during the first 3 days post injection after which the enzyme activity began to increase gradually. With the carcinogen DAB of intermediate activity serum PPDO reached a minimum value at 48 hr after injection. Practically the same course of events was observed with the strong carcinogen 4′-fluoro-4-dimethylaminoazobenzene (4′-FDAB).