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THE EFFECT OF AN EQUIMOLAR MIXTURE OF CARBON TETRACHLORIDE AND CARBON DISULPHIDE ON THE LIVER OF THE RAT

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Abstract—The inclusion of an equimolar mixture of CS_2 in an oral dose of about 4 LD_{50}'s of CCl_4 (5 mmoles/kg) to phenobarbitone pretreated, fasted 100 g male rats reduced the amount of liver injury due to the CCl_4 and prevented deaths occurring. At 24 hr after dosing with the CS_2 + CCl_4 mixture, the morphological changes and chemical composition of the liver more closely resembled those due to CS_2 alone rather than those due to CCl_4. Subsequently the liver lesions due to the mixture became those of a typical but non fatal CCl_4 toxicity. Elevation of conjugated dienes in the hepatic microsomal lipids occurred in the CS_2 + CCl_4 group but not in the CS_2 group, and were less than in the rats given CCl_4 alone.

The inclusion of CS_2 with $^{14}CCl_4$ dosed orally to rats caused an initial increase followed by a reduction in the uptake of $^{14}CCl_4$ into the liver within 15 min of dosing, reduction in the amount of bound radioactivity in the liver from 50 per cent in the $^{14}CCl_4$ controls to 30 per cent and a decrease in the respiratory excretion over 24 hr of $^{14}CO_2$ derived from the $^{14}CCl_4$ of about 65 per cent, findings which indicate reduced metabolism in the animal of the CCl_4. In addition, the CS_2 + CCl_4 mixture caused a 50 per cent loss of cytochrome P450 from liver microsomes within 5–10 min of dosing, while CS_2 and CCl_4 alone, respectively, caused no change during this period.

Since the toxic effect of CCl_4 on the liver is directly related to its metabolism in the organ, the present findings suggest that the protection caused by the inclusion of CS_2 in the mixture is due to reduction of hepatic microsomal oxidative metabolism of the CCl_4 through an enhanced loss of cytochrome P450.

A theoretical basis is thus proposed for the beneficial effects on the liver of sheep of using a CS_2 + CCl_4 mixture rather than CCl_4 alone as a drug treatment in the field.

Carbon tetrachloride (CCl_4) is very effective in the treatment of liver fluke (*Fasciola hepatica*) infestation in sheep [14], but its use has been restricted because of its unpredictable toxicity even when given in small therapeutic doses [25].

Recent studies of the mechanism of CCl_4 toxicity in sheep indicated that significant reduction in the toxicity of the drug for the animals occurred without loss of its effectiveness against the liver fluke when carbon disulphide (CS_2) was administered simultaneously [22].

CS_2 was used in sheep in combination with CCl_4 following the report by Bond and De Matteis [1] that a single oral dose of CS_2 in the rat caused a depression of the hepatic microsomal mixed function oxidases (HMFO) through the loss of microsomal cytochrome P450 without causing hepatocellular necrosis nor other significant untoward effects in the animal. CCl_4 requires metabolism by the HMFO for toxicity [13] and the susceptibility to the drug in both the rat and sheep appeared directly related to the level of these enzymes at the time of dosing [13, 18, 19, 5, 20, 21].

In the present experiments simultaneous oral dosing with CS_2 was also shown to protect the rat against a lethal dose of CCl_4 and the mechanism of the effect was investigated in this species.

MATERIALS AND METHODS

Animals

Young male rats (90–110 g body wt) of a highly inbred Wistar strain were supplied by the Central Animal Breeding House of the University of Queensland. They were kept in wire cages and fed a proprietary cubed diet and given tap water *ad lib.*

On two consecutive days prior to each experiment the rats were given an intraperitoneal injection of 80 mg/kg sodium phenobarbitone dissolved in normal saline in order to enhance the HMFO. All animals were fasted for 24 hr prior to the start of each experiment and for 24 hr thereafter. The LD_{50} for oral CCl_4 dosing in male rats prepared in this manner was determined by the method of Weil [27].

CS_2 and CCl_4 (analytical grade) were dissolved in arachis oil so that each solution contained 0.5 mmole/ml. A mixture of CS_2 and CCl_4 in arachis oil was prepared containing 0.5 mmole of each compound per ml. Each dose of CS_2, CCl_4 or the CS_2 + CCl_4 mixture, respectively, was given by gavage under light ether anaesthesia using a 2.5 mm plastic nasal catheter. Control rats were dosed with 1 ml of arachis oil. $^{14}CCl_4$ was supplied by The Radiochemical Centre, Amersham, England.

Animals were killed by exsanguination from the

jugular vein and carotid artery while under light ether anaesthesia, except that in one experiment the rats were killed rapidly by cervical dislocation at fixed times after dosing with the hepatotoxic agents.

Immediately after death the animals were weighed and their livers rapidly removed and weighed. Thin slices were fixed in formal alcohol for subsequent histopathological examination. Other samples of liver were collected at various times as described for particular chemical and other determinations.

Chemical determinations on livers

In order to measure water content, approximately 1 g of liver was weighed accurately and placed for 24 hr in a hot air oven at 105°. The water content was calculated from the change in weight of the slices before and after heating.

Measurements of sodium, potassium and calcium ions were carried out on the dried residue of liver following water determination by the method of Henckmann and Parsons [7] modified by Judah *et al.* [8].

Triglyceride determinations were carried out using fresh liver by the method of Folch *et al.* [4]. For measurement of conjugated dienes, 5 g samples of liver from freshly killed animals were homogenized in ice cold 0.3 M sucrose containing 0.003 M EDTA. Microsomes were prepared from the homogenate and the lipid extract prepared from them according to Klassen and Plaa [9]. This lipid solution in chloroform was scanned against a reference blank between 210 and 310 nm in a Pye Unican SP1800 Spectrophotometer using 1 cm wide glass cuvettes. The absorption peak for the conjugated dienes was found to be at 243 nm, as shown by Sell and Reynolds [24].

For cytochrome P450 determination, 3 g pieces of liver were homogenized in 9 vol. of ice cold 1.15% (w/v) KCl. This was then centrifuged at 9000 g_{av} for 20 min and the resultant supernatant spun at 100,000 g_{av} for 1 hr. The microsome pellet was resuspended and washed once in 1.15% (w/v) KCl and resuspended in 0.1 M phosphate buffer at pH 7.4. The cytochrome P450 was determined in microsomal suspensions according to Omura and Sato [15]. Microsomal protein was measured by the method of Lowry *et al.* [10].

Collection of expired CS_2, $^{14}CCl_4$ *and* $^{14}CO_2$ *after intragastric administration of* CS_2 *and* $^{14}CCl_4$

Immediately after administration of the respective agents the rats were placed individually in a tubular chamber and the expired air drawn through a series of traps [6]. The procedure for the measurement of CS_2 was carried out in accordance with that described by De Matteis and Seawright [2], and that for $^{14}CCl_4$ and $^{14}CO_2$ as set out by Seawright and McLean [18]. The specific activity of the $^{14}CCl_4$ used in this experiment was 3.6 μCi/mmole.

All samples were counted in a Packard Tricarb Liquid Scintillation Spectrometer.

Measurement of total and bound radioactivity in the liver after dosing with $^{14}CCl_4$

For determination of total radioactivity in the liver after dosing with $^{14}CCl_4$, approximately 100 mg of wet liver was weighed in a scintillator vial. One milliliter Soluene-100 (Packard Co.) was added to the vial which was then capped and left overnight at 40°. The solution was decolorized by the addition of 0.2 ml isopropanol and 0.2 ml 100% v/v hydrogen peroxide. After 30 min, 9 ml of scintillator fluid [toluene containing 0.53 per cent of 2,5 diphenoxyoxazole and 0.04 per cent 1,4-bis-(4-methyl-5-phenyloxazol-2yl) benzene] was added to the vial and the radioactivity counted.

In order to determine the amount of CCl_4 present, 1 g of liver was weighed and placed in 10 ml ice cold toluene and stored at 4° for 3 days with occasional shaking [12]. A 1 ml aliquot of the toluene was then added to 9 ml scintillator and counted. It was assumed that the radioactivity so extracted represented unchanged CCl_4. This was not verified by an alternative assay.

For measurement of bound radioactivity a 20 per cent w/v homogenate of liver in ice cold water was prepared. One milliliter of the homogenate was placed in a scintillator vial and dried at 80° overnight. The residue was dissolved in 1 ml Soluene-100, decolorized as before and 9 ml scintillator added for counting [17]. It was assumed that the radioactivity lost through vaporization was CCl_4 and that present was due to chemically reactive CCl_4 metabolites bound to tissue macromolecules.

The sum of the radioactivity extractable with toluene and that remaining in the dried residue after heating overnight was found to be the same as that measured directly in an equivalent amount of fresh wet liver solubilized in Soluene-100, and was used as the measurement of total radioactivity in the organ after $^{14}CCl_4$ dosing.

RESULTS

The oral LD_{50} of the CCl_4 in the phenobarbitone presented rats was 1.2 mmole/kg with 95 per cent confidence limits of 0.9–1.5 mmole/kg body wt.

The progressive mean changes in body weight over 4 days in each of four groups of 8 rats given 5 mmoles/kg CS_2, 5 mmoles/kg CCl_4, 5 mmoles/kg CS_2 + 5 mmoles/kg CCl_4 and arachis oil alone, respectively, are shown in Fig. 1. Rats given CS_2 alone appeared unaffected and responded similarly to the arachis oil controls. Those given CCl_4 alone became very ill, did not resume eating when food was offered and all died within 36–48 hr of dosing. The rats given the CS_2 + CCl_4 mixture all survived. Although they had appetite when food was restored to them 24 hr after dosing, their weight gain up to 96 hr was much less than in the CS_2 and control groups.

In a duplicate experiment 2 rats from each group were killed at each successive 24 hr period after dosing for pathological examinations of their livers. The livers of the control rats remained consistently normal. Those animals given CS_2 alone were pale and swollen at 24 and 48 hr but were indistinguishable from the controls by 72 and 96 hr after dosing. Histologically there was marked foamy vacuolation of the centrolobular hepatocytes at 24 and 48 hr. The cytoplasm of affected hepatocytes was less vacuolated by 72 hr and virtually normal by 96 hr.

The livers of the rats given CCl_4 alone were mark-

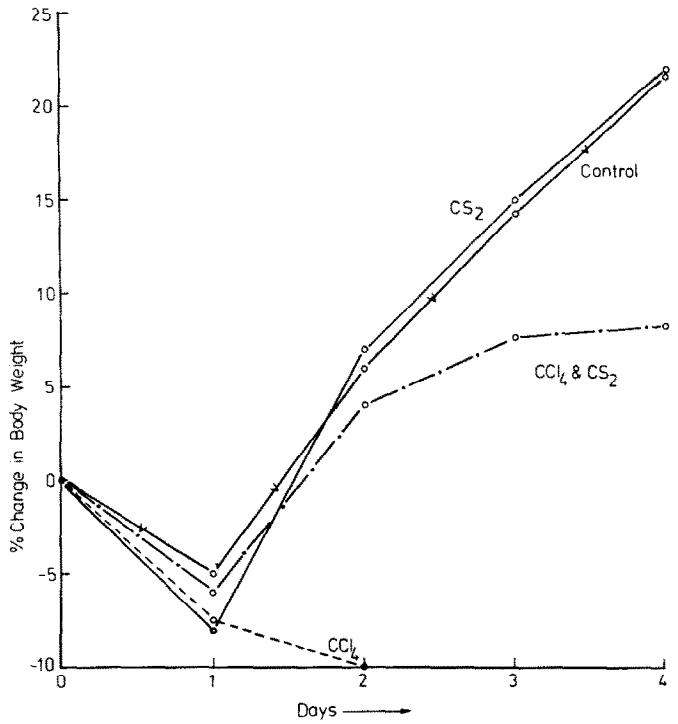

Fig. 1. The effect of oral dosing 5 mmoles/kg CS_2, 5 mmoles/kg CCl_4, 5 mmoles/kg CS_2 + 5 mmoles/kg CCl_4 and 1 ml arachis oil on the progressive mean body wt changes in groups of 4 phenobarbitone pretreated, fasted 100 g male rats. Food was restored on day 1 after 48 hr of fasting. Rats given CCl_4 alone did not resume eating and died within 48 hr of dosing.

edly swollen, pale with reddish mottling and of firm consistency at 24 and 48 hr. Histologically all hepatocytes contained multiple small vacuoles due to fatty infiltration, pyknotic nuclei and markedly eosinophilic cytoplasm typical of coagulative necrosis.

The livers of the rats given the CS_2 + CCl_4 mixture grossly resembled those from rats given the CS_2 alone. They remained pale and swollen for 72 hr after dosing and their surface still retained a mottled, granulated appearance at 96 hr. The histological appearance of the livers at 24 hr shared features of both the CS_2 and CCl_4 lesions in that in the centrolobular zone, most hepatocytes had a pink, granular, finely vacuolated cytoplasm, while in the associated periportal zone most of the cells were more extensively vacuolated and the cytoplasm was less eosinophilic. By 48 hr the parenchymal cells of the centrolobular zone were clearly necrotic and fragmenting, while in the periportal zone the cytoplasmic vacuolation had disappeared and mitotic figures were plentiful in the surviving hepatocytes. At 72 hr the necrotic centrolobular zone was extensively infiltrated by macrophages and by 96 hr the central veins were surrounded by plentiful new connective tissue still containing scattered macrophages and calcified hepatocyte debris. Cell proliferation for replacement of lost hepatocytes was active in the periportal parenchyma.

In a further experiment 5 groups, each of 3 rats, were dosed with 5 mmoles/kg of CCl_4. Each group was then given a dose of 5 mmoles/kg of CS_2 at 0, 2, 4, 6 or 8 min later, respectively. All 12 rats given CS_2 after the CCl_4 dose died within 60 hr with liver lesions similar to those seen in the CCl_4 dosed animals above.

Comparative chemical features of the liver lesions produced by CCl_4, CS_2 *and* CCl_4 + CS_2 *in combination*

(1) *Water and cations.* Sixteen rats were divided into 4 groups of 4 and given 5 mmoles/kg CCl_4, 5 mmoles/kg CS_2, a mixture of 5 mmoles/kg of CCl_4 and 5 mmoles/kg CS_2 and arachis oil alone, respectively. All animals were killed 24 hr after dosing and water, sodium, potassium and calcium ions measured on duplicate 1 g samples of the fresh tissue.

This experiment was repeated twice, giving a total of 12 sets of duplicate observations of each parameter which are set out in Table 1. From the table it is seen that all treatments except the oil control caused an increase in liver water. In the CCl_4 dosed group, there were increases in the concentrations of calcium and sodium ions and a decrease in that of potassium ions. In the livers of the CS_2 and CS_2 + CCl_4 treated groups there were slight increases in both the total levels of sodium and potassium ions, but their respective concentrations in the liver water were similar to that of the oil dosed controls.

(2) *Conjugated dienes in hepatic microsomes.* Two groups of 16 rats were dosed with 5 mmoles/kg CCl_4 and 5 mmoles/kg CCl_4 + 5 mmoles/kg CS_2 while 2 additional groups of 8 animals were dosed with 5 mmoles/kg CS_2 and 1 ml arachis oil, respectively. Two animals in each of the former groups and one

Table 1. Liver water and cation concentrations in phenobarbitone-induced, fasted 100 g male rats 24 hr after oral dosing with 5 mmoles/kg CCl_4, 5 mmoles/kg CS_2, 5 mmoles/kg CCl_4 + 5 mmoles/kg CS_2 in combination and arachis oil, respectively

	Water		Ca^{2+}	Na^+		K^+	
Treatment*	mg/total liver/ 100 g body wt	g/kg dry wt	mEq/kg dry wt	mEq/kg dry wt	mEq/kg liver water	mEq/kg dry wt	mEq/kg liver water
Control	3115 ± 302†	2257 ± 36	6.0 ± 1.2	89 ± 14	40‡	314 ± 40	139‡
CS_2	5340 ± 725	2571 ± 78	5.7 ± 1.8	96 ± 13	38	342 ± 13	133
CCl_4 + CS_2	4537 ± 150	2649 ± 55	9.5 ± 4.6	118 ± 24	44	335 ± 57	126
CCl_4	4551 ± 285	2267 ± 82	36.8 ± 8.8	243 ± 38	107	151 ± 44	67

* The CS_2, CCl_4 and CCl_4 + CS_2 in combination were dosed dissolved in 1 ml arachis oil. Control rats were dosed with 1 ml arachis oil alone.

† All figures represent the means ± S.D. of 12 determinations.

‡ The figures in this column are derived from the other data contained in the table.

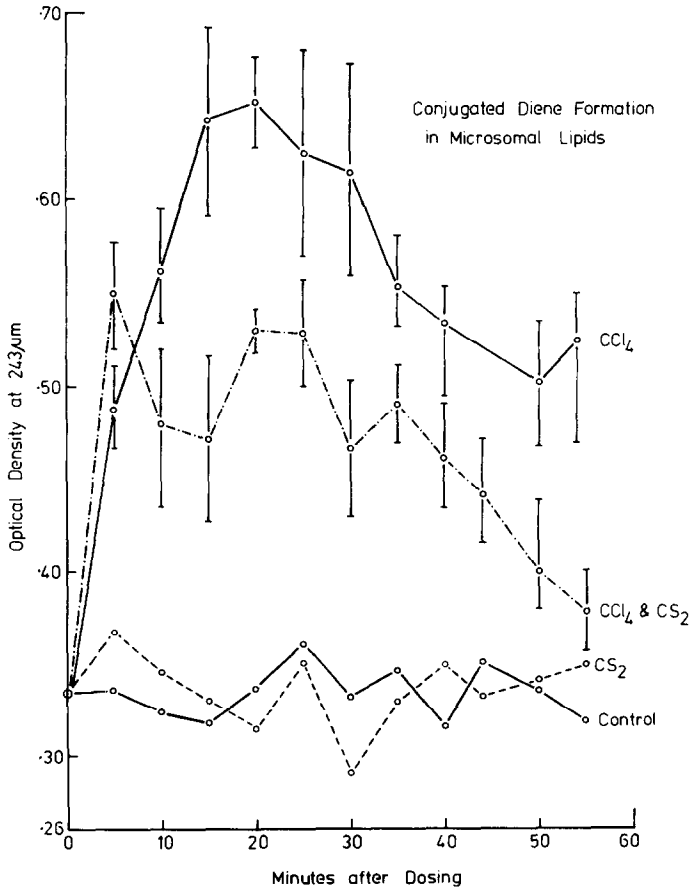

Fig. 2. The effect of the CS_2, CCl_4, CS_2 + CCl_4 and control treatments on the formation of conjugated dienes in the hepatic microsomal lipids. The lines on the CCl_4 and CS_2 + CCl_4 graphs represent the range of the readings for the two rats on each time point.

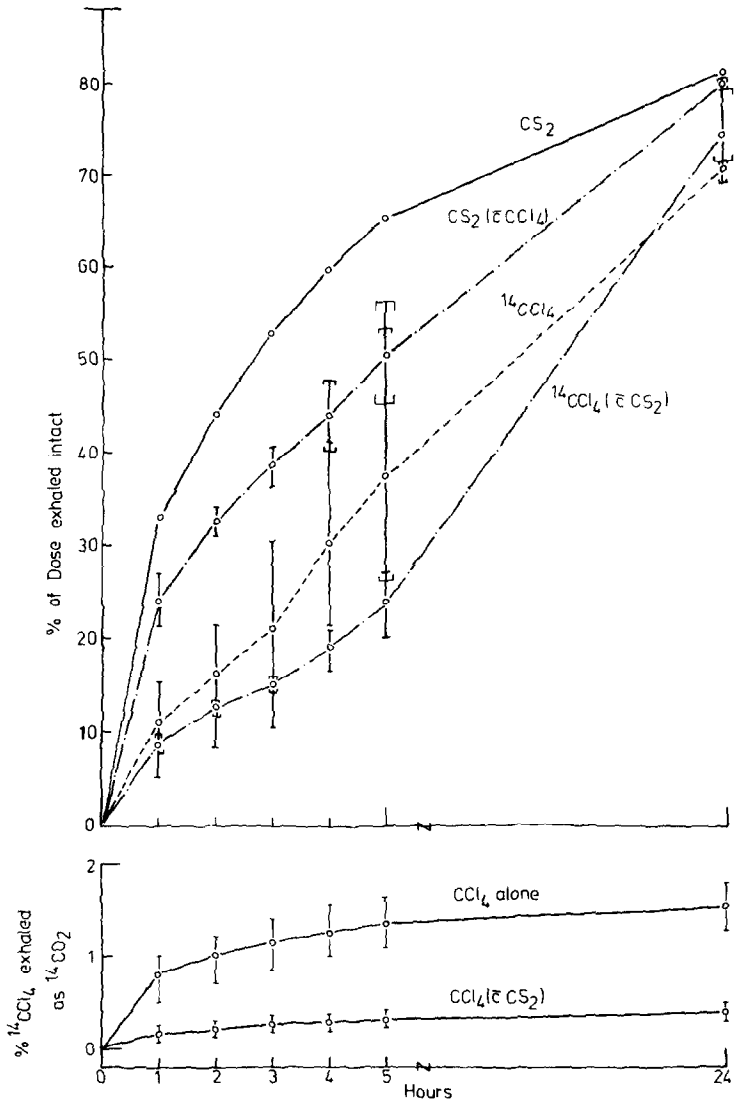

Fig. 3. The effect of simultaneous dosing to rats of equimolar amounts of CS_2 and $^{14}CCl_4$ (5 mmoles/kg) on the total respiratory excretion of the $^{14}CO_2$ derived from the latter compound through HMFO metabolism. The progressive respiratory excretions of CS_2 and $^{14}CCl_4$, each dosed alone and in combination over the same period are also shown. Each point represents the mean + range of readings from 2 animals. The range bars for the CS_2 control rats are not included.

from each of the latter groups were killed at 5 min intervals after dosing for the first 30 min and then at 45 min and 60 min for measurement of microsomal conjugated dienes. The results are shown in Fig. 2 and indicated that treatment with CS_2 alone or arachis oil was not followed by formation of conjugated dienes in the microsomal lipids. Inclusion of CS_2 in the CCl_4 dose did not prevent formation of dienes by the CCl_4 component, but except for the initial samples in which there was an increase, did cause the level of them generally to be reduced.

Effects of CS_2 *on the metabolism of* CCl_4 in vivo

(1) *Metabolism of* $^{14}CCl_4$ to $^{14}CO_2$. Two rats were each dosed with 5 mmoles/kg $^{14}CCl_4$ and 5 mmoles/kg $^{14}CCl_4$ + 5 mmoles/kg CS_2 and the respiratory excretion of unchanged $^{14}CCl_4$ and $^{14}CO_2$, respectively, determined over the next 24 hr. A further two rats were each dosed with 5 mmoles/kg CCl_4 + 5 mmoles/kg CS_2 and 5 mmoles/kg CS_2, respectively, and the respiratory excretion of the unchanged CS_2 measured over the same period after dosing. The progressive cumulative excretion of these respective compounds is shown in Fig. 3. It can be seen that the presence of an equimolar amount of CS_2 in the $^{14}CCl_4$ dose substantially reduced the total amount of $^{14}CO_2$ excreted in the breath over 24 hr by about 65 per cent. In addition, both CCl_4 and CS_2 delayed the respiratory excretion of the other, respectively.

(2) *Binding in the liver of radioactivity due to* $^{14}CCl_4$. Twenty rats were dosed with 5 mmoles/kg

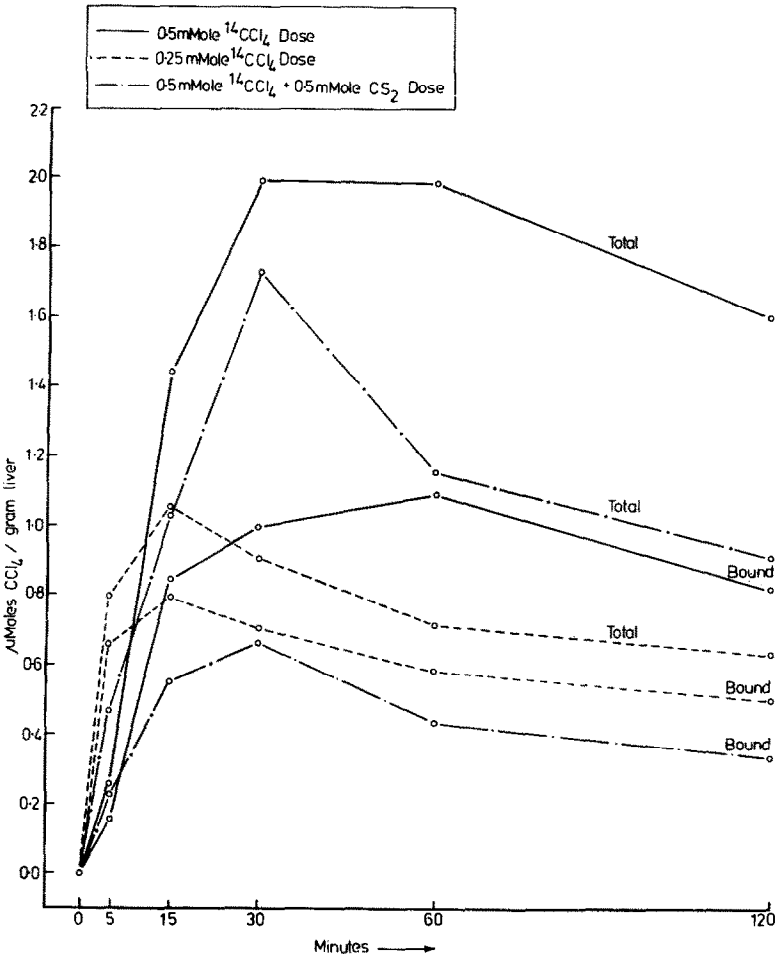

Fig. 4. The progressive concentrations of total and bound $^{14}CCl_4$ in the liver of 100 g male rats with time after dosing. Each time point represents the average determination for 2 animals.

$^{14}CCl_4$ and a further 20 dosed with 5 mmoles/kg $^{14}CCl_4$ + 5 mmoles/kg CS_2. In addition, 10 rats were dosed with 2.5 mmoles/kg $^{14}CCl_4$ of twice the specific activity of the former groups, such that the same amount of radioactivity was dosed to each animal. Two further animals were dosed with 1 ml arachis oil to serve as background controls. Of the first three groups, 2 animals in each were killed at 5, 15, 30, 60 and 120 min after dosing for determination of total and bound radioactivity in the liver, respectively. The results are set out in Fig. 4.

After 5 min of dosing there was a greater uptake of $^{14}CCl_4$ in the pair of rats given the CS_2 + $^{14}CCl_4$ mixture than with animals given 5 mmoles/kg $^{14}CCl_4$ alone. By 15 min and subsequently, however, the total hepatic radioactivity was higher in the latter group, suggestive of a biphasic effect due to the presence of the CS_2. At the lower dose level of 2.5 mmoles/kg $^{14}CCl_4$, the rate of uptake of the compound was higher and peak levels of radioactivity occurred at 15 min rather than 30 min as for the 2 groups dosed with 5 mmoles/kg CCl_4.

The proportion of the radioactivity bound in the livers of rats given 5 mmoles/kg $^{14}CCl_4$ alone remained at about 50 per cent, while for the CS_2 + $^{14}CCl_4$ mixture it varied from 30 per cent at 30 min to 40 per cent at 120 min after dosing. At the lower dose of 2.5 mmoles/kg $^{14}CCl_4$, on the other hand, bound radioactivity was consistently about 80 per cent of the total, indicating a higher proportion of the CCl_4 metabolized at the lower dose level of the compound.

The time course of cytochrome P450 *loss from the liver after dosing with* CCl_4, CS_2 *and* CCl_4 + CS_2 *in combination*

Groups of 15 rats were dosed with 5 mmoles/kg CCl_4, 5 mmoles/kg CS_2 and 5 mmoles/kg CCl_4 + 5 mmoles/kg CS_2, respectively, and killed in groups of three at 0, 5, 10 and 15 min thereafter for determination of hepatic microsomal cytochrome P450. The levels of the cytochrome for each group, expressed as nmoles/mg microsomal protein with increasing time after the respective treatment, are set out in Fig. 5. There was no decrease in microsomal protein levels in the livers in any group by 15 min after administration of the respective treatments. The data indicated that when given alone, CCl_4 caused destruction of cytochrome P450 slightly more rapidly than the CS_2 by 15 min, but when both compounds

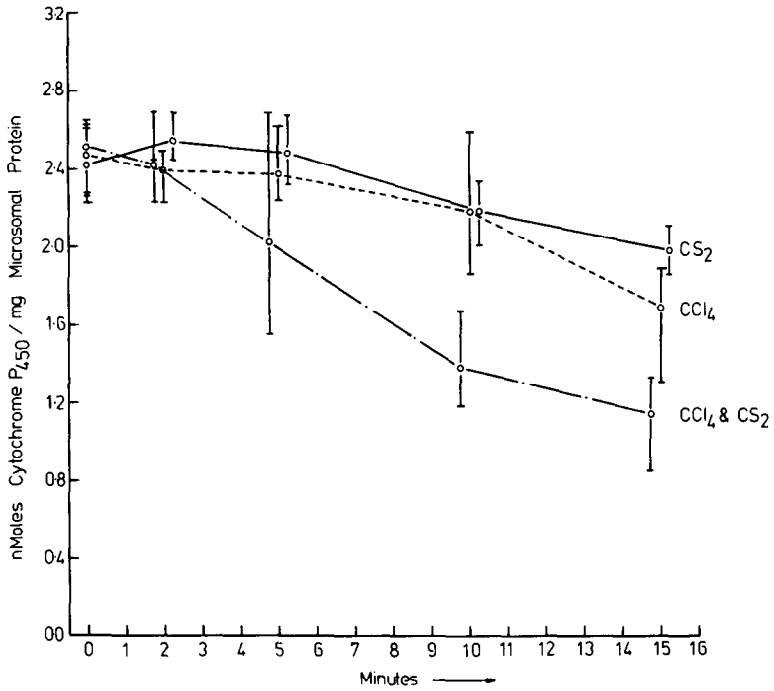

Fig. 5. The effect of CS_2, CCl_4 and the CS_2 + CCl_4 mixture on the microsomal cytochrome P450 concentrations in the livers of rats with time after dosing. Each point with bars represents the mean of 3 rats with the range.

were given together in equivalent amounts the rate of loss of the cytochrome from the microsomes was much greater than with the CCl_4 alone.

DISCUSSION

Those sheep which are excessively susceptible to CCl_4 have been shown also to have high HMFO at the time of dosing, probably due to intake of natural inducers [21]. Phenobarbitone pretreatment enhances the HMFO in sheep and likewise markedly increases the susceptibility of the animals to CCl_4 [19, 20]. Rats so pretreated have similarly increased HMFO and susceptibility to CCl_4 [5, 13] and were thus considered to be an appropriate model for the present study.

From the practical view points of safety, convenience and expense involved in dosing large numbers of sheep in the field, the preferred method of administration of CCl_4 is by means of a single dose by the oral route, and the inclusion of CS_2 in this drench has already been found to be effective in preventing liver damage caused by the former compound [22]. For this reason inclusion of the CS_2 with the CCl_4 in a single oral dose was considered to be the most relevant mode of administration of these agents in the present study in the rat.

As found in the sheep studies above [22], inclusion of an appropriate amount of CS_2 in the CCl_4 dose protected rats against a fatal dose of the latter compound (approximately 4 LD_{50}'s in the case of the rats). Administration of the CS_2 as soon as 2 min after the CCl_4 was entirely without such protective effect and underlines the need for simultaneous dosing of the compounds if a single administration of the drug treatment is to be preferred under practical conditions of use.

The pathological studies of the livers in the present experiment indicated that there was far less damage produced in the rats given the CS_2 + CCl_4 mixture than in those given the CCl_4 alone. While CS_2 on its own caused a benign centrolobolar hydropic degeneration [11], CCl_4 alone caused a marked, extensive and fatal coagulative necrosis of the liver cells. The livers of the rats given the mixture showed features of both lesions, mainly resembling the CS_2 type at 24 hr and then a mild CCl_4 type of injury thereafter. The content of water and concentration of cations in the livers of the CS_2 and CCl_4 mixture group also resembled those of the CS_2 group, rather than those of the CCl_4 group at 24 hr after dosing [23], the only time point at which these parameters were studied.

Although CS_2 alone caused no formation of conjugated dienes in the hepatic microsomal lipids, enhanced conjugated diene formation did occur in both the CS_2 + CCl_4 and CCl_4 groups. Except initially when the level was transiently higher, the conjugated dienes remained consistently lower in the CS_2 + CCl_4 group than in the CCl_4 group, a further indication of a lesser degree of CCl_4 type liver injury in the mixture group than in the CCl_4 dosed animals.

The conversion *in vivo* of $^{14}CCl_4$ to $^{14}CO_2$, the latter determined in the expired air, has been used in the rat as a measure of the HMFO of CCl_4 [18]. The effect of combining CS_2 with the $^{14}CCl_4$ in the present experiment was the reduction of the respiratory excretion of $^{14}CO_2$ in 24 hr by about 65 per cent, indicating that there was a significant decrease in the metabolism of $^{14}CCl_4$ in the liver of these animals.

Although initially greater, there was generally a lower uptake of $^{14}CCl_4$ into the livers of the CS_2 + CCl_4 rats than those given $^{14}CCl_4$ alone. However, there was a lower proportion of the radioactivity bound in the CS_2 + CCl_4 mixture group than in both the 2.5 and 5 mmoles/kg CCl_4 groups, indicating further that hepatic $^{14}CCl_4$ metabolism in the former group was reduced as compared with the latter two groups.

The $^{14}CCl_4$ uptake data, at least at the 5 mmoles/kg dose level of the compound (Fig. 4), together with the initially higher level of the conjugated dienes in the microsomal lipids in the CS_2 + CCl_4 rat livers as compared with the CCl_4 alone group (Fig. 2), suggested that the action of the CS_2 on the uptake and subsequent action of CCl_4 in the liver is biphasic. In each experiment, however, there were only 2 rats on each time point. This effect is of particular interest and requires further investigation using larger numbers of animals.

Since after oral dosing CS_2 appeared much sooner in the expired air than CCl_4 (Fig. 3), it was highly probable that when both compounds were administered together, the former could be expected to be absorbed and reach the liver in effective amounts well before the latter. CS_2 itself produces damage to the hepatic endoplasmic reticulum through its own metabolism by this organelle, above all causing loss of cytochrome P450 [1, 2]. The present results indicate, however, that when the compounds are dosed separately under the same conditions, neither causes significant loss of cytochrome P450 from the microsomes within the first 10 min. When both compounds are given together in the same doses there is a marked loss of the cytochrome in some livers by 5 min and in all by 10–15 min. This is not necessarily surprising since the CS_2 + CCl_4 mixture represents for the animals' total hepatic endoplasmic reticulum a potential doubling of its exposure to the hepatotoxic agents, as compared with the situation where each is administered alone.

CCl_4 is believed to cause its toxic and lethal effect to the liver cell through microsomal metabolism to a toxic intermediate free radical which initiates a cascade of peroxidative decomposition of lipids throughout the cell leading to typical coagulative necrosis [16, 26]. CS_2 is considered to cause its injurious effect in the liver cell through a similar microsomal metabolism which leads to the formation of active atomic sulphur which binds covalently to the microsomes [2]. The latter injury is confined to the endoplasmic reticulum and is characterized mainly by loss of cytochrome P450, decrease in mixed function oxidative activity and a transient, benign accumulation of water in distended cysternae [3]. When the two compounds are given together, the CS_2 rapidly gains access to and damages a substantial proportion of the appropriate microsomal enzyme sites, thereby preventing much of the toxic metabolism of the CCl_4. Initially at least, this would tend to confine the injury to the endoplasmic reticulum itself, and at the same time restrict its expansion to more vital organelles and structures which could result in the death of the cell.

In order, therefore, for CS_2 to be employed with CCl_4 in protecting the liver against the lethal effects of the latter, it should be given simultaneously with the CCl_4 and in sufficient amount to allow it to compete successfully for the available microsomal metabolism.

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