of 3-methylcholanthrene on liver steatosis and triglyceride secretion, suggesting that the initial steps of CCl₄ hepatotoxicity are not associated with the interference by the polycyclic hydrocarbon; 3-methylcholanthrene would act on only those metabolites responsible for hepatic necrosis. To obtain a better understanding of the lack of effect by 3-methylcholanthrene on fatty liver and triglyceride secretion, we studied the polyribosomal disassociation induced by CCl₄ in rats pretreated with 3-methylcholanthrene. Even in this case no protection was found.

To evaluate the role of drug-metabolizing enzymes on hepatotoxicity, we studied the effects of phenobarbital and 3-methylcholanthrene on the liver damage produced by white phosphorus. As already shown by Pani et al. (1972), phenobarbital does not alter phosphorus hepatotoxicity, and as far as the lethality is concerned neither does 3-methylcholanthrene. As this hepatotoxic agent is probably not metabolized by the drug-metabolizing enzyme system, it is quite likely that the role of the drug-metabolizing enzyme system in drug toxicity concerns just those drugs that are primarily metabolized within its system. An extension of these studies to other hepatotoxins may clarify this point. Moreover, we may then better understand the role played by smooth membranes in drug metabolism and detoxification related with hepatic necrosis. In fact, the phenobarbital and 3-methylcholanthrene models are suitable ones, as the first enhances the toxicity of many drugs, whereas the latter seems to act by means of some protective mechanism.

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flame-ionization detector and the other one going to the electron-capture detector. Each
detector was connected with a separate recorder.

With polar columns suitable for fatty acid analysis, the electron-capture detector did
not give any response; clearly the chlorinated compounds occurring in liver microsomal
lipids of CCl₄-treated rats are so changed in their properties with respect to normal fatty
acids that they are not eluted by these columns. In fact, when we used columns with a
less-polar liquid phase (OV-101-SE-30-OV225, 28:11:11, w/w/w) we did obtain
responses with the electron-capture detector. Fig. 1 shows the results obtained for fatty
acids of phospholipids of rat liver microsomal fraction 1 h after the administration of
CCl₄ (0.25 ml/100 g body wt.). The response of the electron-capture detector shows
two major peaks (really groups of unresolved peaks). These peaks are eluted long after
the elution of the major fatty acids (shown by the response of the flame-ionization de-
tector). When the sensitivity of the response of the flame-ionization detector is strongly
enhanced, after the elution of the major fatty acids, a number of peaks appear in the
response of the flame-ionization detector. However, none of these peaks coincides with
the peaks occurring in the response of the electron-capture detector. Further, these
peaks also occur in the response of the flame-ionization detector with the controls, with
which the response of the electron-capture detector does not show any peak. Clearly
these peaks do not represent the compounds revealed by the electron-capture detector. It
is therefore likely that the compounds revealed by the electron-capture detector occur in
such a small amount that they are not detectable in the response of the flame-ionization
detector.

Similar results were obtained 5 min, 15 min and 4 h after CCl₄ poisoning. The same
peaks also occur in the response of the electron-capture detector with fatty acid methyl
esters of total lipids of liver microsomal fraction at the same stages after poisoning.

In an effort to identify the nature of the peaks detected with the electron-capture
detector in liver microsomal lipids of CCl₄-treated rats, we tested by use of the electron-
capture detector fatty acid methyl esters (g.l.c. standards) previously subjected to a
reaction with the free radicals deriving from CCl₄, according to the experimental

![Fig. 1. Responses of electron-capture detector (a) and flame-ionization detector (b) for fatty acid methyl esters of rat liver microsomal phospholipids 1 h after CCl₄ administration](image)

The column temperature was 210°C. Peaks 1, 2 and 3 in (b) are the responses for methyl
esters of C₁₆, C₁₈ and C₂₀ fatty acids respectively.
procedure described by Gordis (1969). According to Gordis (1969), methyl oleate thus treated behaves like the compounds formed by the action in vivo of CCl₄ on the fatty acids of liver phospholipids. The chromatograms obtained with the use of the electron-capture detector for methyl oleate and methyl linoleate treated with CCl₄ showed two groups of unresolved peaks. From a comparison of a chromatogram of fatty acid methyl esters of liver microsomal phospholipids from CCl₄-treated rats with these chromatograms, it seems likely that some peaks of the former chromatogram are derived from linoleate and others from oleate (the comparison is based on the retention times). The response of the electron-capture detector for methyl arachidonate subjected to the reaction with the free radicals deriving from CCl₄ showed a number of peaks that are eluted after those obtained for oleate and linoleate, as well as those obtained for fatty acids of liver microsomal phospholipids from CCl₄-treated rats. As expected, no response of the electron-capture detector was obtained for saturated fatty acids such as methyl palmitate after the same treatment with CCl₄.

The above results may mean that the response of the electron-capture detector for fatty acids of liver microsomal lipids of CCl₄-treated rats is due to compounds formed by an addition reaction of CCl₄ free radicals with fatty acids such as oleic acid and linoleic acid.

However, during the present work it was found that fatty acids of liver microsomal lipids peroxidized in vitro also give a response with the electron-capture detector. Such a response is somewhat similar to that found for fatty acids of liver microsomal lipids of CCl₄-treated rats. Fig. 2 shows the result of an experiment carried out with liver microsomal fraction incubated with ascorbic acid. Lipid peroxidation was assessed by the production of malondialdehyde and by the occurrence of conjugated dienes. The response of the electron-capture detector shows two major peaks, which are eluted after the elution of the major fatty acids (shown by the response of the flame-ionization detector). The second major peak shows a retention time that falls within the second peak

![Fig. 2. Responses of electron-capture detector (a) and flame-ionization detector (b) for fatty acid methyl esters of rat liver microsomal lipids peroxidized in vitro](image)

Liver microsomal fraction (250 mg equiv.) was incubated with potassium phosphate buffer, pH 7.4, in the presence of 0.1 mM-ascorbic acid at 37°C for 120 min. The column temperature for the analysis was 210°C.
found in the response of the electron-capture detector for fatty acids of microsomal lipids from CCl₄-treated rats (the latter peak is clearly a group of unresolved peaks).

Since serum very-low-density lipoproteins derive directly from the hepatic cell and since the phospholipid fraction of such lipoproteins is believed to derive from the phospholipids of the endoplasmic reticulum, we investigated whether alterations like those observed in liver microsomal phospholipids could also be detected in the phospholipid fraction of very-low-density lipoproteins (d<1.006) after CCl₄ poisoning. Therefore fatty acid methyl esters of phospholipids of very-low-density lipoproteins from rats given CCl₄ 4 h earlier were analysed by use of the electron-capture detector. The response of the electron-capture detector showed one small peak only. This peak apparently corresponds to the second peak found in the response of the electron-capture detector for fatty acids of liver microsomal phospholipids of CCl₄-treated rats. However, the first peak of the latter response is absent.

It was deduced that the phospholipids of very-low-density lipoproteins are also damaged by CCl₄. However, the difference between the response of the electron-capture detector for phospholipids of very-low-density lipoproteins and that for phospholipids of liver microsomal fraction may mean that some of the CCl₄-damaged molecular structures in the membranes of the endoplasmic reticulum are not available for the assembly of lipoproteins in CCl₄-treated rats.

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The Effect of Zinc Deficiency and Food Restriction on Hepatic Zinc Proteins in the Rat

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As a part of a programme of work to investigate the nature of competitive metabolic interactions between zinc and copper we have been examining the nature of the metalloprotein components of the liver cytosol. Relevant to these studies are the observations by Webb (1972) on the existence of a low-molecular-weight zinc-containing protein in the livers of female but not of male rats. Synthesis of this protein can be induced by injections of a zinc salt. The protein is similar in some respects to metallothionein (Kägi & Vallee, 1960, 1961), a cadmium- and zinc-binding protein whose synthesis can be induced by cadmium (Shaikh & Luck, 1970). Proteins having similar properties, but binding both zinc and copper, occur in sheep, pig, calf and human liver, where they may bind more than 50% of the total zinc and more than 20% of the total copper, although they are absent from zinc-deficient animals (I. Brenner, unpublished work). A study has now been made of the changes in the binding of zinc and copper in rat liver during the onset of zinc deficiency, after restriction of food intake and after the injection of ZnSO₄.

Male rats were used in all these studies. In most experiments the animals initially weighed about 150 g. The semi-synthetic basal diet described by Williams & Mills (1970) was used throughout and this was either supplemented with ZnSO₄ (40 p.p.m. of Zn) or was offered in unsupplemented form (<1 p.p.m. of Zn).

Pooled livers from three or four rats were homogenized in 10 mM-Tris–acetate buffer, pH 8.2, at 1°C and centrifuged at 100000 g for 1 h, and the supernatants were fractionated on Sephadex G-75 with the same buffer. Fig. 1 illustrates a typical elution pattern of soluble proteins containing zinc and copper. Three fractions were obtained, these

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