TABLE 1. EFFECT OF EMETINE AND CYCLOHEXIMIDE ON THE INCORPORATION OF ^{14}C-AMINO ACIDS BY DIFFERENT SUBCELLULAR FRACTIONS WHEN CELL-FREE EXTRACT OF RAT LIVER WAS INCUBATED WITH ^{14}C-ALGAL PROTEIN HYDROLYSATE

System	Incorporation: cpm/mg protein		
	Mitochondria	Microsome	Soluble supernatant
Complete	2113 ± 153	2749 ± 169	672 ± 73
Complete + emetine (10^{-5} M)	842 ± 69	761 ± 70	195 ± 82
Complete + emetine (10^{-4} M)	450 ± 81	236 ± 58	84 ± 70
Complete + cycloheximide (50 μg/ml)	1948 ± 158	718 ± 43	254 ± 86
Complete + cycloheximide (75 μg/ml)	1863 ± 168	301 ± 32	131 ± 95

Results are expressed as cpm/mg protein and the averages of five experiments with ±S.D.

were incubated in presence of ^{14}C-amino acids. Protein in soluble supernatant may include some proteins released after their formation on the microsomes. Protein synthesized in mitochondria is not so easily released.[9]

The data given in Table 1, also indicate that emetine and cycloheximide inhibit microsomal protein synthesis in rat liver, but unlike cycloheximide emetine, strongly inhibits mitochondrial synthesis when cell-free extract of rat liver was incubated with ^{14}C-amino acids. It has previously been reported that cycloheximide inhibits protein synthesis in the cytoribosome-cell sap, but has no effect on protein synthesis by isolated mitochondria.[3] Our findings are consistent with those previously reported.[3] Hence, it may be concluded that emetine is more potent inhibitor for protein synthesis than cycloheximide in living system.

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Damage effect of chronic intoxication by CCl_4 on structural organization of liver microsomes and cytochromes $(b)_5$ and P-450

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IT IS now widely known that carbon tetrachloride is metabolized in the liver and can lead to centrolobular necrosis, accumulation of neutral lipids[1] and decreased activity of microsomal mixed-function oxidases;[2,3] which are involved in the processes of drug biotransformation. However, other investigators have succeeded in proving that lipoperoxidation of microsomal lipids is an important factor,

the "vector" of CCl_4-hepatotoxicity.[1,4] Also the peroxidative destruction of microsomal membranes is responsible for the observed electron microscopic data and biochemical disturbances.[5] In particular, it has been shown that the impairment of liver enzymes is accompanied by a decrease of microsomal cytochrome P-450.[2,3]

At this time very little is known of the drug-metabolizing capacity of microsomal enzymes of the CCl_4-induced cirrhotic liver. Hartman *et al.*,[6] have reported the possible role of lipoperoxidation in the development of chronic hepatitis and subsequent liver cirrhosis. The purpose of the present investigation is to analyse the impairment of the structural component of microsomal membranes, damage to the cytochrome P-450 content and aminopyrine *N*-demethylation activity, during long-term exposure to CCl_4.

Male Wistar rats, of 150–200 g, initial body weight, were used. Inhalations of CCl_4 by the method of Rabinovici and Weiner[7] were conducted for 4 hr, twice a week. The animals were sacrificed at 3 and 6 weeks; 2 days after the last inhalation. A histological examination of the liver was carried out for hepatic fibrosis, necrosis amount, fatty degeneration and cytoplasmic changes. The liver microsomes were isolated by ultracentrifugation of postmitochondrial supernatant at 105,000 *g* for 60 min, in a medium containing 0·35 M sucrose, 0·025 M KCl, 0·01 M $MgCl_2$, 0·05 M Tris–HCl (pH 7·5). The amount of P-450, P-420 and cytochrome b_5 was determined by the method of Omura and Sato,[8] on a Hitachi two-wavelength, double beam, difference recording spectrophotometer, model 356.

TABLE 1. AMOUNT OF MICROSOMAL CYTOCHROMES AND AMINOPYRINE DEMETHYLASE ACTIVITY DURING LIVER CIRRHOSIS DEVELOPMENT*

	Control groups (30)†	%	CCl_4-treated 3 weeks (24)	%	CCl_4-treated 6 weeks (27)	%
b_5 ΔA(424–409 nm) nM/mg protein	0·95 ± 0·16	100	0·65 ± 0·05	68·4	0·81 ± 0·08	85·2
P-450 ΔA(450–490 nm) nM/mg protein	1·0 ± 0·18	100	0·20 ± 0·07	20·0	0·47 ± 0·08	47·0
P-420 ΔA(420-490 nm) nM/mg protein	not detected		0·23 ± 0·06		0·042 ± 0·01	
HCHO (nM formed min/mg protein)	3·76 ± 0·22	100	1·13 ± 0·16	30·0	2·57 ± 0·20	68·5

* The livers from four rats were combined for each microsomal preparation. The results are expressed as averages ±S.D. of the results of 5–8 preparations.

† Rats were divided into three groups. The number of each group are given in parentheses.

When aminopyrine (4-dimethyl-amino-2,3-dimethyl-1-phenyl-5-pyrazolone) metabolism was determined, mixtures were incubated for 10 min at 37°. Estimates of the amount of formaldehyde formed during *N*-demethylation of aminopyrine were determined by the method of Nash.[9] Fluorescence efficiency of 8-Anilino-1-naphthalene sulphonate (ANS) in cuvette, after adding microsomal suspension, was measured by the recording spectrofluorimeter. The basic incubation medium consisted of: 125 mM KCl, 20 mM Tris–HCl, pH 7·4. Protein was determined by the method of Lowry.

The main experimental data are given in Table 1. It can be seen from this table that the amount of microsomal cytochromes decreased considerably during the development of liver cirrhosis. Thus, after 6 weeks (cirrhotic changes in liver) it had been found that the P-450 content was 47 per cent of the control level. After 3 weeks (morphologically-hepatitis pattern) the quantity of this hemoprotein was only 20 per cent of controls, which is approximately the same as during acute CCl_4 intoxication when after 24 hr the content of P-450 dropped by 86 per cent.[3] It is very important that changes in *N*-demethylase activity of microsomal mixed-function oxidases closely corresponds to P-450 content changes. The decrease in amount of P-450 suggests that the changes in the structural organization of microsomes have occurred, because mixed-function oxidases are firmly bound to the microsomal membrane and their activities are very sensitive to alteration in the microsomal structure.[10] Even more indicative is the appearance in the 3-week-CCl_4 samples of P-420, whose very

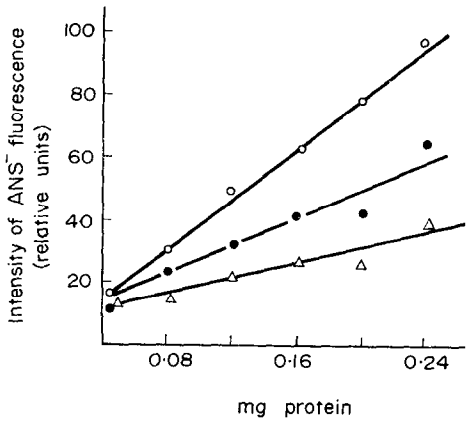

FIG. 1. Intensity of ANS^- fluorescence in a medium containing control (○——○) and experimental microsomes of rats having received CCl_4 over a period of 3 (△——△) and 6 (●——●) weeks. Incubation mixture: 125 mM KCl + 20 mM Tris-HCl, pH 7·4. Protein in experimental and control cuvettes is the same. Excitation wavelength, 360 nm, emission wavelength 470 nm. Cuvette volume, 3 ml, ANS^-, 10 μM.

existence is considered to be one of the proofs of alteration in the phospholipid component in the membranes of endoplasmic reticulum.[11]

From the model experiments of Vanderkooi and Martonosi,[12] it is known that conditions leading to alteration in the microsomal phospholipids significantly diminish the hydrophobic nature of microsomal membrane, which reflects ANS^- fluorescence decrease. Our experiments with ANS^- indicate a decrease in the hydrophobity of microsomal membrane (Fig. 1), damage of its phospholipid component as a result of prolonged CCl_4 intoxication.

The results indicate the possible participation of CCl_4-induced lipoperoxidation in the development of structural impairments of microsomal membrane and, also show a decrease in the amount of P-450 and drug-metabolizing activity connected with this hemoprotein. Apart from all the studied characteristics in cirrhotic rats undergo an obvious "improved" rise, in comparison to 3-week-CCl_4 microsomes under conditions of prolonged effect of toxic agent supply. The formation in the liver of cirrhotic rats of a certain "resistance factor" to the destructive action of CCl_4, perhaps the activation of lipoperoxidation in the endoplasmic reticulum membranes.

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