acetoxyl substituent was at either C-2′ or C-3′. The model compound 1-phenyl-2-pentanol acetate (V) showed a methine shift at δ 5.07. However, 1-phenyl-3-pentanol acetate (VI) showed a methine shift at δ 4.87 (quintet, J = 6Hz), thus establishing IIIa as the structure of the second metabolite.

The configuration of the hydroxyl groups on C-1′ and C-3′ is not known[11].

Zusammenfassung. (−)-Δ^8-Tetrahydrocannabinol ist einer der Aktivstoffe in Marihuana (*Cannabis sativa* L.). Inkubation dieser Verbindung mit der überstehenden Zentrifugationsfraktion (9000 *g*) aus männlicher Hundeleber ergab zwei Hauptmetaboliten, welche durch Massenspektrometrie und Kernresonanzspektroskopie identifiziert wurden. Den beiden Verbindungen werden die Strukturen des 1′-Hydroxy-Δ^8-tetrahydrocannabinols und des 3′-Hydroxy-Δ^8-tetrahydrocannabinols zugeordnet.

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Changes in Microsomal Lipids of Rat Liver after Chronic Carbon Tetrachloride Intoxication

Previous studies from our laboratory[1,2] described changes in the fatty acid composition of rat liver microsomal lipids following acute carbon tetrachloride intoxication. The observed decrease in arachidonic acid was interpreted on the basis of CCl_4-induced lipid peroxidation. This interpretation is supported by several lines of evidence which demonstrate the occurrence of such a process in the liver cell structural lipids of carbon tetrachloride poisoned rats[3–7]. It is suggested that this reaction plays an important role in the pathogenesis of the hepatotoxic effect of the poison.

Recently it has been shown[8] that the hepatic triglyceride accumulation resulting from chronic carbon tetrachloride administration to rats is due to an inhibition of the secretion of low density lipoproteins, i.e. to the same mechanism leading to fatty liver following acute CCl_4 poisoning[9,10].

Therefore it seemed to be of interest to investigate whether the alterations of liver microsomal lipids induced by acute CCl_4 intoxication could still be detected (and if so, to what extent) after chronic carbon tetrachloride treatment. Positive evidences for changes, possibly related to a lipoperoxidation process, would support a common origin for both acute and chronic CCl_4-induced fatty liver.

Female Wistar rats maintained on a pellet diet (Piccioni, Brescia, Italy) were used. CCl_4 was mixed with an equal volume of olive oil and the mixture was administered s.c., twice weekly, in a dose of 0.25 ml/100 g body weight. Control rats received an equal volume of olive oil. Since a study of a long term effect of CCl_4 was planned, the treatment was prolonged for 8 months. About 50% of the CCl_4-treated rats died throughout the experimental period.

Eight randomly chosen survivors and as many controls were used for the present study. They did not receive injections 3 days in advance and were starved 15 h before sacrifice. The mean body weight at the start of the CCl_4 treatment was 162 ± 4 g for the control and 166 ± 6 g for the experimental group. The final body weight was 268 ± 6 and 265 ± 16 g for each group, respectively.

The fatty acid composition of microsomal phospholipids was determined by gas-liquid chromatography as previously reported[1,11]. Heptadecanoic acid (GLC grade, Sigma Chem. Co.) was used as an internal standard.

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Fatty acid composition of microsomal phospholipids of rat liver after chronic carbon tetrachloride intoxication

	Group	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:4}$	$C_{22:6}$
Composition (%)	Control	0.2 ±0.02	17.4 ±0.7	0.8 ±0.1	28.8 ±0.8	7.0 ±0.4	9.4 ±0.2	30.2 ±0.3	6.4 ±0.3
	CCl_4	0.3 ±0.05	23.4 ±1.2[a]	1.4 ±0.2[b]	23.8 ±0.5[a]	11.0 ±0.3[a]	10.4 ±0.3	24.9 ±0.9[a]	4.7 ±0.9[b]
mg/microsomes/ g liver	Control	0.01 ±0.002	1.16 ±0.08	0.05 ±0.01	1.91 ±0.08	0.46 ±0.05	0.63 ±0.04	2.02 ±0.10	0.41 ±0.02
	CCl_4	0.01 ±0.002	1.13 ±0.09	0.07 ±0.01	1.15 ±0.09[a]	0.54 ±0.05	0.51 ±0.04[c]	1.22 ±0.12[a]	0.23 ±0.02[a]

Results are expressed as mean of 8 rats each group ± S.E. [a] $P < 0.001$. [b] $P < 0.01$. [c] $P < 0.05$.

Microsomal lipids were analyzed for the absorption over the UV-range according to the method of RECKNAGEL et al.[6,7], with minor modifications. Liver triglyceride concentration was determined by the method of VAN HANDEL and ZILVERSMITH[12].

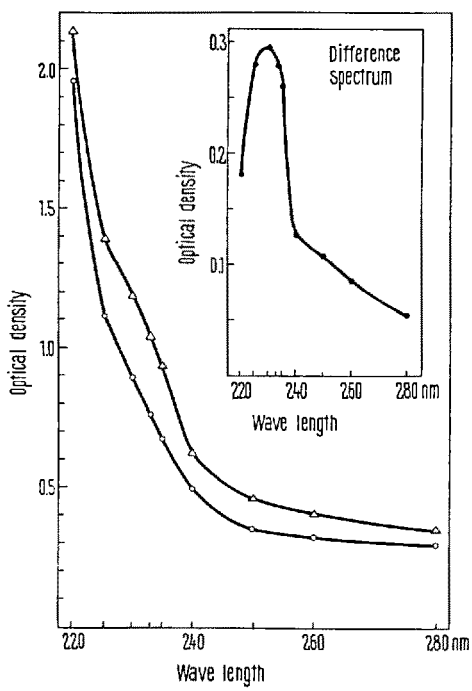
Livers from CCl_4 treated rats were markedly enlarged (13.4 ± 1.4 versus 8.6 ± 0.5 g in control animals). Histological examination showed a very severe cirrhosis as well as a massive fatty infiltration in livers of all rats given carbon tetrachloride. A normal liver structure was observed in control animals.

Hepatic triglyceride concentration was markedly enhanced in the CCl_4-treated group (68.13 ± 3.18 versus 13.80 ± 2.56 mg/g liver in control rats).

The fatty acid changes occurring in liver microsomal phospholipids of CCl_4-treated rats, as compared to controls, are reported in the Table. A clear per cent decrease in stearic, arachidonic and docosahexaenoic acids was observed; on the other hand, palmitic, palmitoleic and oleic acids were increased. The decrease in the percentage of arachidonic acid is larger than that found in the phospholipids of rat liver microsomes at the early stages (2 and 4 h) after acute CCl_4 intoxication[2]; however it is comparable to the decrement in $C_{20:4}$ occurring at the late times (12 h) after the poisoning[1,2].

Phospholipids of microsomes equivalent to 1 g liver were found to contain smaller amounts of $C_{18:0}$, $C_{18:2}$, $C_{20:4}$ and $C_{22:6}$ in the rats chronically treated with CCl_4, as compared to the controls; other variations were not statistically significant. The larger decrement was found in docosahexaenoic acid (43.9%) and the lower one in linoleic acid (17.4%).

As demonstrated for the acute CCl_4 hepatotoxicity[5–7], liver microsomal lipids from animals given chronic carbon tetrachloride exhibited the typical diene conjugation absorption of peroxidized lipids[18] (Figure). The difference spectrum is little increased with respect to that found in our[14] as well as in other laboratories[7] at the earliest stages after acute intoxication with carbon tetrachloride.



Diene conjugation absorption in rat liver microsomal lipids, after chronic carbon tetrachloride intoxication. ○—○, control rats; △—△, CCl_4 treated rats.

The changes observed in the fatty acid composition of liver microsomal phospholipids after a prolonged CCl_4 treatment may be explained by several hypotheses. Since docosahexaenoic and arachidonic acids are the most unsaturated components of the fatty acid spectrum of microsomal phospholipids, the possibility exists that the decrease in these moieties may be related to a long-term effect of the CCl_4-induced lipid peroxidation. The decrease in stearic acid is difficult to explain. However, a decrease of this saturated component was observed in the phospholipids of erythrocytes peroxidized in vitro[15]. Furthermore, in some experiments in which liver microsomes were peroxidized in vitro for different lengths of time, at pH 7.4, with $10^{-4}M$ ascorbic acid, the largest decrements of the content (by weight) of each fatty acid were found in $C_{22:6}$, $C_{20:4}$ and $C_{18:0}$ (76.7, 54.5 and 42.8%, respectively, after 20 h of incubation at 37 °C)[16]. So the major changes in the fatty acid pattern of liver microsomal phospholipids of rats chronically treated with CCl_4 are somewhat comparable to those occurring in microsomes peroxidized in vitro for many hours.

Alternatively, different factors may cause the changes reported above in the fatty acid composition of liver microsomal phospholipids; namely the fatty acid pattern could be affected 1. by an activation of liver phospholipases normally bound to lysosomes[17], mitochondria[18] and microsomes[19], or 2. by a CCl_4-induced alteration in the synthesis of some fatty acids.

However, the absorption of conjugated dienes was detected in liver microsomal lipids of rats given chronic carbon tetrachloride. Therefore peroxidative decomposition of lipids of the endoplasmic reticulum appears to persist also after a very prolonged treatment with the poison. It is conceivable that the polienoic fatty acids of the phospholipid molecule are partially destroyed by lipid peroxidation.

Riassunto. Un prolungato trattamento con tetracloruro di carbonio, tale da indurre una grave cirrosi epatica, determina una diminuzione degli acidi grassi più insaturi (acido arachidonico ed acido docosaexenoico) dei fosfolipidi dei microsomi di fegato di ratto. L'ipotesi che tale diminuzione sia dovuta ad una parziale distruzione di questi acidi grassi attraverso un processo di perossidazione lipidica indotto dal CCl_4, è avvalorata dalla presenza, nei lipidi microsomiali, di una banda di assorbimento caratteristica dei «dieni coniugati».

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