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Effect of propyl gallate on carbon tetrachloride induced fatty liver

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IT HAS been suggested that carbon tetrachloride-induced fatty liver is mediated by the stimulated lipid peroxidation.[1–6] Hepatic fat infiltration has been found to be both partially prevented by tocopherol[7] and inhibited by compounds other than antioxidants.[8] These findings do not seem to fit with the hypothesis that lipid peroxidation plays the most important role in the pathogenesis of the CCl_4-induced fatty liver. Propyl gallate is a water soluble antioxidant which has been shown to

improve the survival of mice after whole body X-irradiation,[9–11] to protect DNA molecules against denaturation caused by free radicals[10, 12] and to prevent liver lipase from the *in vitro* inactivation by carbon tetrachloride.[13] The experiments reported here deal with the effect of propyl gallate on the triglyceride accumulation in the liver after CCl_4 poisoning.

Male Wistar rats, 145–190 g, were used throughout all the experiments. The animals were starved for 18 hr before the intoxication but had free access to water. The hepatic triglyceride content was determined 4 hr after carbon tetrachloride dosing in rats previously treated with several doses of propyl gallate either by stomach tube or via the intraperitoneal route. Triglycerides were estimated with the method of Van Handel and Zilversmit[14] on the fraction separated by thin layer chromatography from the lipids extracted with chloroform : methanol (2:1).[15] The findings reported in Fig. 1

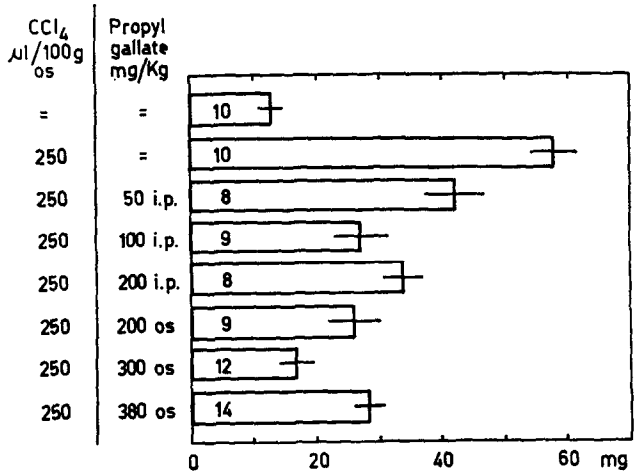

FIG. 1. Hepatic triglyceride content estimated 4 hr after carbon tetrachloride poisoning.

Abscissa: mg of liver triglycerides per 100g of body weight. Mean ± S.E.M. The number of the animals is reported inside the blocks.

Ordinate: Treatment schedule. Rats were given 50 up to 300 mg propyl gallate per kg of body weight, in saline, by either stomach tube (os) or intraperitoneum injection (i.p.). 45–60 min later, 250 µl CCl_4/100 g body wt., as 1:1 (v:v) mixture with mineral oil, were administered by mouth. The animals of the last group were dosed with propyl gallate 24, 18, 12 and 1 hr before and then again 1 hr after poisoning (whole dose: 380 mg per kg of body weight). Controls received either saline or mineral oil alone. The sacrifice followed the CCl_4- feeding 4 hr later.

show that the triglyceride accumulation in the liver is prevented by oral administration of 300 mg propyl gallate per kg of body weight. Both the intraperitoneal injection and the repeated feedings of the free radical scavenger, which account for a whole dose of 380 mg per kg, lower but do not inhibit the hepatic fat infiltration.

Furthermore, the time course of fatty changes occurring in rat liver after dosing with propyl gallate has been studied during later stages of the poisoning. Eight and twelve hours after CCl_4-feeding, the free radical scavenger does not prevent fatty liver but lowers the hepatic triglyceride accumulation. Additional doses of antioxidant, administered after CCl_4 poisoning, lead to better effects (Table 1).

The *in vitro* lipid peroxidation was estimated with the 2-thiobarbituric acid method[16] on duplicate samples of liver homogenate, after either *in vivo* feeding normal rats or *in vitro* treatment with propyl gallate. A decrease in the *in vitro* production of malonyldialdehyde by liver homogenate has been found to occur under the experimental conditions. Lipid peroxidation is inhibited either by 300 mg propyl gallate per kg of body weight, given by mouth 60 min prior the sacrifice, or when the antioxidant is added *in vitro* at the final concentration of 10^{-3} M (Fig. 2).

TABLE 1. EFFECT OF PROPYL GALLATE ON HEPATIC TRIGLYCERIDE CONTENT AFTER CARBON TETRACHLORIDE POISONING, UP TO 12 hr

Treatment		Hepatic triglyceride content (mg/100 g b.w. Mean ± S.E.M.)			
CCl_4* (μl/100 g body wt.)	Propyl gallate* (mg/100 g body wt.)	Controls	Intoxicated animals		
			4 hr	8 hr	12 hr
none	none	13·12 ± 1·90 A (10)	—	—	—
250	none	—	57·84 ± 3·33 B (10)	84·25 ± 2·10 D (11)	139·23 ± 12·75 F (9)
250	30†	—	16·87 ± 3·07 C (12)	56·85 ± 2·20 E (9)	102·34 ± 9·17 G (9)
250	70‡	—	—	—	75·85 ± 6·79 H (9)

*fed by stomach tube.
†30 mg/100 g body wt. 1 hr before poisoning.
‡30 mg/100 g body wt. 1 hr before and then 20 mg/100 g body wt. 3 and 7 hr after poisoning.
The number of the animals is reported in parenthesis.
Statistical significance of the differences, P value by *t*-test:
C–A: n.s. B–C: < 0·001 D–E: < 0·025
F–G: < 0·05 F–H: < 0·001 G–H: < 0·05

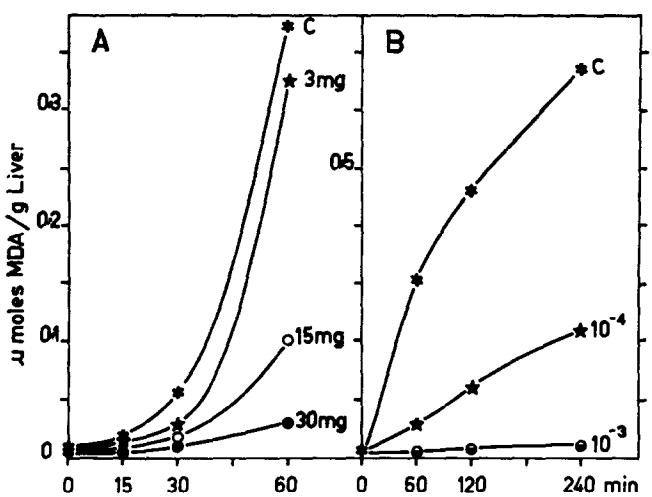

FIG. 2. (A) *In vitro* peroxidation of the homogenate of liver from rats fed 3, 15 and 30 mg propyl gallate per 100 g of body weight, respectively, 60 min prior the sacrifice. Controls received saline alone.
(B) Lipid peroxidation of normal rat liver homogenate incubated at 37° in the presence of propyl gallate (final concentration: 10^{-3} and 10^{-4} M; C: no propyl gallate added).
Ordinate: malonyldialdehyde produced by 6 per cent liver homogenate prepared with 0·1 M KCl in 0·05 M phosphate buffer, pH 5·6.
Abscissa: incubation time.

The results show that propyl gallate is able to prevent rat liver from triglyceride accumulation during the earliest stages of the poisoning. Furthermore, this free radical scavenger succeeds in inhibiting the *in vitro* lipid peroxidation of the liver soon after feeding, and its antioxidant activity is confirmed by the *in vitro* experiments. It is a noteworthy fact that the water soluble antioxidant does not maintain the liver triglyceride content at the normal level but succeeds in lowering the accumulation. The peroxidative mechanism in the pathogenesis of CCl_4-induced fatty liver may be

supported by both these findings and the failure of propyl gallate to affect the hepatic uptake of the administered $^{14}CCl_4$.[17] A possible competitive effect of propyl gallate on the metabolism of carbon tetrachloride by the endoplasmic reticulum, during this short term experiment, could not be excluded, since the hexobarbital sleeping time has been found to be longer in the rats treated for 5 days with either propyl gallate or *N-N'*-diphenyl-*p*-phenylenediamine.[17] The partial failure to prevent intoxication during the later phases could be explained by comparing the actual levels of both the antioxidants and carbon tetrachloride (and/or the CCl_4-metabolites) in the liver. In fact, smaller doses of the poison (e.g. 25μl/100 g of body weight) allow propyl gallate protection better than those reported here, although the antioxidant properties found in the liver appear to be progressively exhausted at 5 up to 11 hr after dosing.[17]

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Stimulation of renal *p*-aminohippurate transport by folic acid*

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THE KIDNEY responds with a marked increase in DNA synthesis to variety of stimuli, including unilateral nephrectomy,[1] temporary ischemia,[2] metabolic acidosis,[3] mercuric chloride necrosis,[4] and folic acid administration.[5] It has been shown that a single injection of folic acid causes an increase in kidney weight and RNA synthesis within 6 hr after injection, while DNA synthesis and dry weight increase within 24 hr and reach a maximum level by 96 hr.[5–7] The increase in DNA content and the associated increase in kidney mass after administration of folic acid appear to be due to hyperplasia of the renal tubules.[5] Since folic acid appears to be a specific growth stimulant for the kidney,[5] it

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