Since NAD^+ is a powerful electron transfer agent and as an obligate coenzyme is intimately involved in many oxidative metabolic processes its possible further role in questions of radiation response is worthy of further detailed investigation.

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Carbon tetrachloride induced peroxidation of liver lipids in vitamin E pretreated rats

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IT HAS been demonstrated that carbon tetrachloride induces a peroxidative reaction of the structura lipids of the hepatic cell.[1–6] It has also been suggested that lipoperoxidation is an important factor in the pathogenesis of CCl_4 hepatotoxicity.[7–9] Recently McLean[10] observed that α-tocopherol (vit. E), which is an antioxidant, afforded only minor protection against the CCl_4-induced liver damage. It was thus deduced that lipid peroxidation does not play a major role in the hepatotoxic effect of carbon tetrachloride.

It seemed, therefore, of interest to us to investigate whether lipid peroxidation can still be detected in liver cells after pretreatment of rats with vit. E and subsequent poisoning with carbon tetrachloride. Should vit. E prevent lipid peroxidation, then the biochemical changes produced by CCl_4 cannot be related to a peroxidative hypothesis, but if not then the alterations produced by carbon tetrachloride, may well be mediated through lipoperoxidation.

Male Sprague–Dawley rats were used. The animals to be pretreated with vit. E were given *dl*-α-tocopherol acetate (Roche) in a small volume of olive oil, by mouth. Two levels of vit. E were examined: 25 mg/100 g body wt. (an experimental situation identical to that of McLean's experiments) and 75 mg/100 g body wt. Twenty-four hr after vit. E pretreatment, one-half of the rats received CCl_4 (0·25 ml/100 g body wt.) by gastric intubation, the others mineral oil. These rats not pretreated with vit. E received an equal volume of olive oil and then either CCl_4 or mineral oil as above. All rats were starved for 18 hr before CCl_4 administration. Spectrophotometric analysis of microsomal lipids over the u.v. range was performed according to the method of Recknagel and Ghoshal[3,4] with minor modifications. Liver triglycerides were determined by the method of Van Handel and Zilversmit.[11]

One hr after CCl_4 administration, liver microsomal lipids of the rats pretreated with 25 mg/100 g body wt. of vit. E showed the diene conjugation absorption characteristic of peroxidized lipids (Fig. 1 b). The spectral changes are identical to those observed in rats not pretreated with vit. E (Fig. 1 a). However, when the dose of vit. E was increased to 75 mg/100 g body wt., the absorption of "conjugated dienes", although still evident, was reduced, as shown by the reduced magnitude of the difference spectrum (Fig. 1 c). The same results as shown in Fig. 1, were found for the u.v. absorption of microsomal lipids, 5 min after CCl_4 dosing. Even at 4 and 6 hr after poisoning, microsomal lipid peroxidation was not prevented by prior administration of 25 mg/100 g body wt. of vit. E.

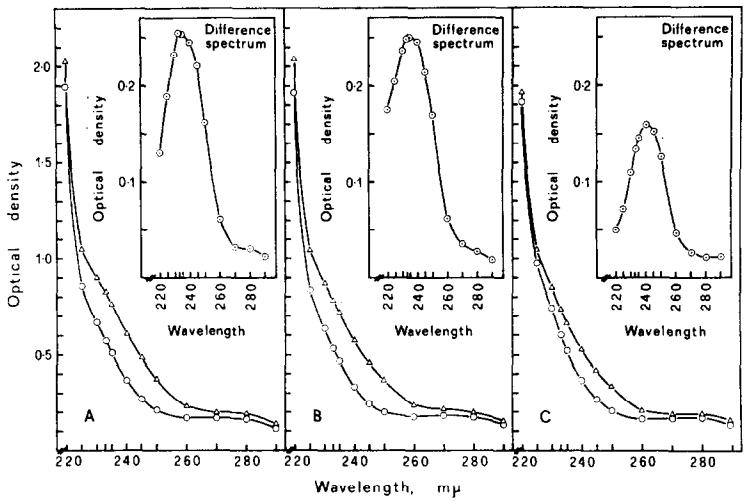

FIG. 1. Diene conjugation absorption in rat liver microsomal lipids, 1 hr after carbon tetrachloride administration. ○——○ Control rats; △——△ CCl_4 poisoned rats. (A) Rats not pretreated with vit. E. (B) Rats pretreated with vit. E (25 mg/100 g body wt.). (C) Rats pretreated with vit. E (75 mg/100 g body wt.).

In the non-pretreated rats CCl_4 administration resulted in a 209 per cent increase in liver triglyceride level 4 hr after poisoning (Table 1). Pretreatment with vit. E at the dose of 25 mg/100 g body wt. had no effect on the CCl_4-induced liver steatosis. However, liver steatosis was significantly reduced when the amount of vit. E administered as preventive treatment was increased to 75 mg/100 g body wt.

TABLE 1. HEPATIC TRIGLYCERIDE CONTENT IN RATS PRETREATED WITH VITAMIN E AND POISONED WITH CCl_4*

Group	Pretreatment	Liver triglyceride	
		mg/100 g body wt.	% Increase
Controls	None	12·07 ± 1·16	
CCl_4	None	37·36 ± 4·02	209·5
Controls	Vit. E 25 mg/100 g body wt.	14·51 ± 1·85	
CCl_4	Vit. E 25 mg/100 g body wt.	39·55 ± 4·81	172·6
Controls	Vit. E 75 mg/100 g body wt.	13·73 ± 1·84	
CCl_4	Vit. E 75 mg/100 g body wt.	27·52 ± 2·45†	100·5

* The results are the means of six animals each group ± S.E.
† Significantly different from the CCl_4-treated animals of the other group ($P < 0·05$).

In agreement with McLean's findings, moderate amounts of vit. E (25 mg/100g body wt.) do not prevent CCl_4-induced fatty liver. However, neither is lipid peroxidation prevented. So the peroxidative hypothesis for CCl_4 hepatotoxicity is not ruled out by McLean's observations. On the contrary, a correlation between fatty liver and lipid peroxidation seems to exist in our experimental conditions, since the higher dosage of vit. E employed (75 mg/100 g body wt.) significantly reduces both the extent of the peroxidative reaction and the severity of liver steatosis.

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Studies on the metabolism of sulfametoxipirimidine in endotoxin tolerant mice

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IN A PREVIOUS paper[1] we demonstrated that the sulfametoxipirimidine is potentially toxic in endotoxin treated animals. We suggested that this phenomenon was due to the blockade of its various metabolical transformations and decreased urine elimination. Aware of the role of the spleen and liver in detoxification, we studied the metabolism of sulfametoxipirimidine in acute endotoxin toxemia and toxemia induced in endotoxin tolerant animals.

180 H strains of mice, males, weighing 18–22 g were divided into groups of 30 animals. The sulfametoxipirimidine was given orally in single doses of 100 mg/kg weight 1 hr after LD_{50} endotoxin. To

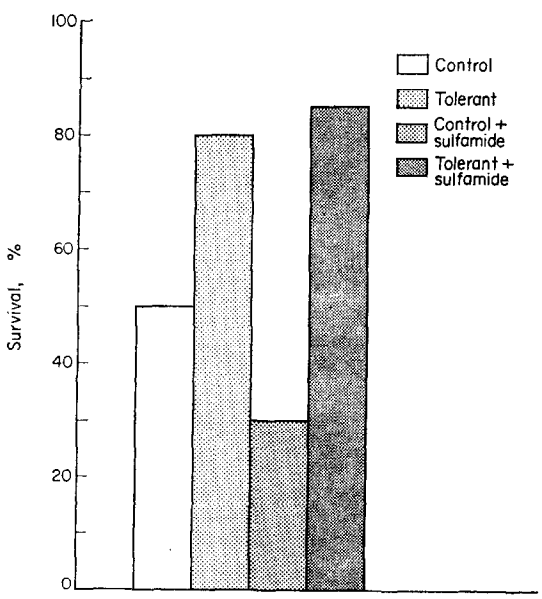

FIG. 1. Endotoxin induced toxicity in endotoxin tolerant and non-tolerant mice in association with sulfametoxipirimidine (30 mice for each series).