THE EFFECT OF DIET AND VITAMIN E ON LIVER INJURY DUE TO CARBON TETRACHLORIDE

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In the last 2 yr there has been a revival of interest in the idea that carbon tetrachloride causes liver damage by causing lipid peroxidation inside cells (Slater, 1966 for review). This hypothesis is based on 2 lines of evidence. First, it is claimed that vitamin E and antioxidants protect against CCl_4 injury (Hove, 1948; Gallagher, 1961; Di Luzio, 1966). Second, spectral changes that suggest the presence of peroxidised lipids have been found in the microsomes from poisoned rats, and carbon tetrachloride has been found to promote lipid peroxidation of microsomes *in vitro* (Recknagel and Ghoshal, 1966*a*, *b*).

However Krone (1952) found no protection by vit. E against CCl_4 liver injury. All the experiments showing protection by vit. E and antioxidants are open to objections which will be enumerated in the discussion. It seemed worth knowing whether vit. E really gave protection against CCl_4 injury, so experiments were designed to answer this question. The effect of vit. E on liver injury due to CCl_4 was observed in circumstances that were known to affect the intensity of the injury.

In previous papers (McLean and McLean, 1965, 1966) we showed that rats were resistant to CCl_4 poisoning when the activity of the microsomal enzymes that metabolise CCl_4 was lowered by feeding a protein free diet. When the synthesis of these hydroxylating enzymes was stimulated by injection of DDT the rats became sensitive to CCl_4. It was also shown that fasting for 18 hr. increased sensitivity to CCl_4 without affecting microsomal hydroxylating activity.

In the present experiments some rats were given DDT and others fasted. The effect of vit. E on the CCl_4 liver damage produced in resistant and sensitive rats was then observed.

MATERIALS AND METHODS

Male Wistar rats of a Porton strain were used. They were made vit. E deficient by feeding a 30 per cent casein diet (McLean and McLean, 1966), without added vit. E, for 4 weeks from weaning. They then weighed 120–160 g. and were divided into 2 groups. One group was given 60 mg. α-tocopherol acetate (Roche) mixed with 60 mg. olive oil, by mouth. The other group was given 120 mg. olive oil.

The efficacy of this regimen of vit. E depletion and of vit. E repletion after dosing was checked by confirming that liver slices from deficient animals were unable to reaccumulate potassium after cooling (McLean, 1963). The liver preparation was able to transport potassium again 24 hr. after dosing with vit. E. Red cell haemolysis at 43° gave a similar confirmation of the vit. E status of these 2 groups (Christensen, Dam, Gortner and Sonder gaard, 1956).

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The groups of animals were then subdivided. One half of the animals had food removed on the evening after dosing with tocopherol (30 per cent casein fasted), the other group were left with food (30 per cent casein fed).

The next morning, (18 hr. after removal of food, and 24 hr. after dosing with α-tocopherol) all the animals were given CCl_4. The dose was given by mouth 2·5 ml. CCl_4/kg. body weight mixed in an equal volume of liquid paraffin. Food was returned to the cages of the fasted group 4 hr. later.

The animals were killed 24 hr. after dosing with CCl_4 and plasma bilirubin and isocitric dehydrogenase were measured and calculated as previously described (McLean and McLean, 1966). Liver water and fat content were also measured.

In other experiments male rats weighing 120–150 g. were fed on stock cube diet 41B (Bruce and Parkes, 1956) and given oral CCl_4. Some were given a single subcutaneous injection of DDT, 100 mg./kg. 1 week before dosing with CCl_4. Others had no DDT but were starved for 18 hr. before CCl_4.

Rats on stock diets are mildy deficient in vit. E. One half of each of the stock groups was given α-tocopherol acetate, 35 mg., by mouth, 24 hr. before giving CCl_4. The controls were given 35 mg. olive oil.

The rats were killed 24 hr. after dosing with CCl_4 and plasma bilirubin, isocitric dehydrogenase, liver water and fat content were measured.

Plasma isocitric dehydrogenase activities are expressed in logarithmic terms, since these enzyme activities are log normally distributed (Heath, 1967).

RESULTS

Table I shows that when vit. E deficient rats are given carbon tetrachloride there is the usual rise of plasma bilirubin and enzyme activity, and this is prevented to only a slight, though statistically significant extent, by a dose of vit. E. This protective effect is absent when the degree of injury is made more severe by fasting the rats before giving them CCl_4. The rise of liver water content is not affected by vit. E deficiency or dosage. There is some reduction in liver fat in the groups given vit. E. In rats fed stock diets there is no significant protection against CCl_4 injury by dosing with vit. E.

DISCUSSION

The present experiments have shown that in vit. E deficient rats, giving vit. E leads to a small but statistically significant decrease in some indices of liver damage measured 24 hr. after giving CCl_4. The extent of protection is far smaller than the striking reduction in liver damage that is obtained in rats fed a protein free diet (McLean and McLean, 1965, 1966). When histological observations are made it is not possible to pick out the animals given vit. E.

Some previous experiments have shown much greater apparent protection by vit. E and antioxidants.

Hove (1948) and Seward, Vaughan and Hove (1966) compared CCl_4 injury in vit. E deficient rats, and rats given vit. E over a long period. These groups differed not only in vit. E status but also in growth and probable food intake.

Gallagher (1962) found protection on injecting vit. E but none when oral dosage was used. Oral dosage is a good way of converting the liver from a deficient state to a high vit. E state in 4 hr. (McLean, 1963), so in Gallagher's experiment the injections must have functioned in some other way. It is probable that the protection he found was of the type found by Ravdin, Vars and Goldschmidt (1939) and Calder (1942) when injection of xanthine crystals and carbon particles protected against liver injury (see McLean, McLean and Judah, 1965).

TABLE I.—*The Effect of Vit. E on Liver Injury due to CCl_4 in Rats Fed a Vit. E Deficient Diet*

Treatment	Vit. E dosage	n	Log plasma isocitric dehydrogenase activity m. μmoles/ml./min.	Plasma bilirubin mg./100 ml.	Liver water g./g. fat free dry wt.	Liver fat mg./g. fat free dry wt.
Fed	−	6	1·95±0·32	0·36±0·09	3·52±0·13	347±129
Fed	+	6	1·47±0·33*	0·21±0·07†	3·48±0·29	302± 96
Fasted	−	6	2·59±0·27	1·70±0·25	3·63±0·15	394± 45
Fasted	+	6	2·58±0·26	1·34±1·29	3·57±0·14	281± 52†
[No CCl_4 controls]	±	6	0·35±0·02	0·17±0·06	2·53±0·09	93± 50

Rats were fed a vit. E deficient 30 per cent casein diet for 4 weeks. They were given CCl_4 2·5 ml./kg. by mouth and killed 24 hr. later. Results are expressed as means ± one standard deviation.

† Indicates a result significantly different from its control value in the group without vitamin E (P < 1 per cent) (* indicates 1 per cent < P < 5 per cent). The analytical and dietary techniques are described in the section on Methods.

TABLE II.—*The Effect of Vit. E on Plasma Isocitric Dehydrogenase and Bilirubin and Liver Fat and Water in Rats Treated with CCl_4 2·5 ml./kg.*

Diet and treatment	Vit. E dosage	n	Log Plasma isocitric dehydrogenase activity m. μmoles/ml. min.	Plasma bilirubin mg./100 ml.	Liver water g./g. fat free dry wt.	Liver fat mg./g. fat free dry wt.
Cube diet Fasted	−	5	2·72±0·11	1·39±0·33	3·42±0·26	377±136
	+	5	2·59±0·15	1·09±0·28	3·58±0·10	261± 29
Cube diet + DDT	−	5	2·59±0·37	1·22±0·50	3·92±0·44	516± 70
	+	5	2·38±0·58	1·31±0·50	3·77±0·22	514±109
Cube diet (No CCl_4 controls)	−	8	0·15±0·14	0·19±0·02	2·61±0·10	71± 23

Rats were given CCl_4 2·5 ml./kg. orally and killed 24 hr. later.
Results are expressed as means ± standard deviation. The analytical and dietary methods are described in the section on Methods.

Di Luzio (1966) found protection using antioxidant mixture containing butylated hydroxy toluene. CCl_4 requires activation in the microsomal hydroxylation system to exert its toxic effects (McLean and McLean, 1965, 1966). Competitors for the hydroxylation system such as aniline and phernergan reduce CCl_4 metabolism (Seawright and McLean, 1968) and it seems likely that butylated hydroxy toluene, which is such a substrate (Gilbert and Goldberg, 1966) will be a competitive inhibitor of CCl_4 metabolism.

Recknagel and Ghoshal (1966, *a*, *b*) have produced evidence that there is lipid peroxidation in CCl_4 poisoning. The question is whether this is a major factor in liver injury, or one of the incidental events that occur during liver cell breakdown. The absence of a major protective effect when one gives vit. E, or of a major worsening of CCl_4 injury in vit. E deficient animals suggests that lipid peroxidation of the autocatalytic type (Farmer, Koch and Sutton, 1943) is not an important step in CCl_4 injury.

The steps by which CCl_4 is activated and causes liver damage remain obscure. The amount of CCl_4 metabolised is about 0·5 per cent of a dose of 2·5 ml./kg. This means that a dose of 20 mg./kg. body weight of CCl_4 metabolites is produced inside the liver cells. If either a CCl_3 free radical (Wirtschafter and Cronyn, 1964) or phosgene ($COCl_2$) are produced in this order of amount, the resulting damage needs no multiplying effect of lipid peroxidation.

SUMMARY

Carbon tetrachloride was fed to rats and the amount of liver damage assessed.

A single oral dose of 35 mg. vit. E (α-tocopherol acetate) did not reduce the liver damage found 24 hr. after CCl_4 in rats fed stock diets.

In rats fed a vit. E deficient diet there was some slight protection against CCl_4 poisoning by an oral dose of vit. E.

It seems unlikely that lipid peroxidation plays a major part in the toxic effects of CCl_4.

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