3. The ε-amino group availabilities of the 640 Å, 220 Å and structureless fibrils were also identical (69·5 %) on dinitrophenylation at higher ionic strengths.

4. When dinitrophenylated in water, the proportions of reactive ε-amino groups in the 640 Å and the two long-spacing aggregates were considerably less (approx. 44 %) than those of the same aggregates in 1 % sodium chloride.

5. As the ionic strength of the aqueous dinitrophenylation medium was decreased, the molar ratio of lysyl to hydroxylysyl ε-amino groups available to fluorodinitrobenzene in the 640 Å and the two long-spacing aggregates also decreased.

6. Most samples of the fibrous long-spacing aggregate contained about 10 % of the glycoprotein used in their preparation.

7. The significance of these findings is discussed in relation to the calcification of collagen fibrils *in vitro*.

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Changes in Liver Nucleotide Concentrations in Experimental Liver Injury

1. CARBON TETRACHLORIDE POISONING

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A single dose of carbon tetrachloride administered to rats causes centrilobular necrosis and fatty degeneration of the liver (for early references see Van Oettingen, 1955). After a suitable enteral dose, large increases in liver fat can be detected at 6 hr. and hepatic necrosis is easily recognizable at 12 hr., reaching a maximum 1 day after administration.

Much biochemical work has been done in attempts to elucidate the pathogenesis of the

injury produced by carbon tetrachloride (for references see Dianzani, 1955; Recknagel & Lombardi, 1961; Brody & Maickel, 1963; Rees & Shotlander, 1963). Early views as to the site of initial injury, leading to fat accumulation and necrosis, favoured the mitochondria (Christie & Judah, 1954; Dianzani, 1955), but although some early mitochondrial changes have been reported (Rossi & McLean, 1963; Judah, Ahmed & McLean, 1963) these would appear to be neither as early nor as widespread as is the damage to the endoplasmic reticulum (Bassi, 1960; Recknagel & Lombardi, 1961; Richter, 1962; Smuckler, Iseri & Benditt, 1962; Rees & Shotlander, 1963). However, although there is early damage to, and disturbance in, the endoplasmic reticulum this does not exclude the possibility that damage to other intracellular sites is important in the consolidation and ramifications of the initial disturbance. Further, the major symptoms of poisoning that appear may not follow a common pathway of development, or spread from a single locus of injury either in character or in time. For example, Rees, Sinha & Spector (1961) have shown that two major features of carbon tetrachloride poisoning, the widespread centrilobular necrosis and the extensive fat accumulation in the liver, can be dissociated from one another by administering the antihistamine drug Phenergan [N^{10}-(2-dimethylamino-*n*-propyl)phenothiazine hydrochloride] concomitantly with the carbon tetrachloride. Phenergan prevents the appearance of necrosis while having little effect on the accumulation of fat. Various other experimental treatments also effect a partial or complete dissociation of the necrotic process from that resulting in a fatty liver, i.e. adrenalectomy (Recknagel, Stadler & Litteria, 1958) and cordotomy (Brody & Maickel, 1963). Thus it seems possible that the sequences of reactions in liver tissue sparked off by the administration of carbon tetrachloride may follow several main and divergent paths.

Many of the reactions affected in liver by the administration of carbon tetrachloride are intimately associated with the nicotinamide–adenine dinucleotides (NAD, $NADH_2$, NADP and $NADPH_2$): for example, lipid metabolism (Schotz & Recknagel, 1960; Robinson & Seakins, 1962; Maling, Frank & Horning, 1962; Rees & Shotlander, 1963), nucleic acid metabolism (Richter, 1962; Leevy, George, Deysine & Gnassi, 1962; Smuckler & Benditt, 1963), detoxication reactions (Neubert & Maibauer, 1959) and mitochondrial respiratory activity (Recknagel & Lombardi, 1961; Reynolds, Thiers & Vallee, 1962; Artizzu & Dianzani, 1962). Thus an early feature of carbon tetrachloride poisoning might be expected to be a disturbance in the concentrations and proportions of the nicotinamide–adenine dinucleotides. Further, bearing in mind the rapid development of some of the above changes, the nucleotide alterations might well occur during the initial stage of the injury process. The present paper is concerned with work aimed at answering three major questions relevant to such a possibility: (i) are there any changes in the concentrations of NAD and $NADH_2$, or of NADP and $NADPH_2$, in the rat liver during the early phases of carbon tetrachloride poisoning? (ii) if so, are these changes among the earliest biochemically detectable lesions produced by the poison? (iii) if changes occur are they reversed by the concomitant administration of Phenergan at a dose known to prevent the development of necrosis?

METHODS

Rats used were adult albino females, body wt. 130–150 g.; they were killed by cervical dislocation and the livers quickly removed, and samples were analysed for NAD, $NADH_2$, NADP and $NADPH_2$ by the method of Slater, Sawyer & Sträuli (1964). Approx. 0·8 g. of liver was used for each extract. Calibration points were obtained by using NAD and NADP (purest grade; Sigma Chemical Co., St Louis, Mo., U.S.A.). In a few experiments, NADP was extracted with 0·02 N-H_2SO_4–0·1 M-Na_2SO_4 after first freezing the tissue in liquid nitrogen (Burch, Lowry & Van Dippe, 1963); NADP estimated in such extracts is called 'total NADP'.

Student's *t* test was used to evaluate the significance of differences between liquid paraffin control groups and CCl_4-treated groups in Tables 1–3; *** indicates $P < 0.05$ for a particular pair of means, and * signifies $0.10 > P > 0.05$; no marking indicates that $P > 0.10$ for the pair of means concerned.

The CCl_4 was administered as a mixture with liquid paraffin (1 vol. of CCl_4 + 3 vol. of liquid paraffin) by stomach intubation with the rat under light ether anaesthesia; 0·5 ml. of this mixture was given/100 g. body wt. Control rats were given an equivalent volume of liquid paraffin by the same procedure. In experiments involving the use of Phenergan this was injected intraperitoneally (2·5 mg./100 g. body wt.) immediately before dosing with CCl_4.

Blood was obtained by severing the carotid artery with the rat under ether anaesthesia; nicotinamide–adenine dinucleotide estimations were carried out on extracts obtained from 1 ml. samples of serum. The acid-soluble nucleotide pattern of serum was obtained by the procedure of Hurlbert, Schmitz, Brumm & Potter (1954) by using Dowex 1 resin (formate form).

Bile samples were obtained from rats under ether anaesthesia; an abdominal incision was made just below the xiphisternum and a polythene tube was inserted into the left hepatic duct; the bile was collected in tubes cooled in ice. Bile samples (0·1 ml.) obtained from normal rats or from rats poisoned 1 hr. before with CCl_4 were mixed with 2·5 ml. of 20% (v/v) perchloric acid. After standing in ice for 20 min. the mixtures were centrifuged and the supernatant solutions each diluted with 3·0 ml. of ethanol. The solutions so obtained were recentrifuged to clear the slight

haze that developed. The extinction at 260 mμ was measured with an Optica spectrophotometer. The bile flow rate after dosing with CCl_4 was not substantially different from the normal value (0·29 ml./hr.).

RESULTS

The values found for the NAD, $NADH_2$, NADP and $NADPH_2$ contents in rat liver at various times after dosing with liquid paraffin or a carbon tetrachloride–liquid paraffin mixture are shown in Tables 1 and 2. The results are expressed both in terms of the nucleotide 'concentration' in liver tissue (i.e. μg./g. wet wt. of liver) and as the total nucleotide content in the whole liver (i.e. μg. of nucleotide/whole liver/100 g. body wt.) To obtain the latter value the determined total liver wt. was multiplied by [100 g./body wt. (g.) of rat] to minimize the effects of small variations in body wt. between different animals. Both the changes in 'concentration' and in the total organ content of any component must be considered since, for instance, extensive alteration in the blood content of the organ would make results expressed in terms of 'concentration' alone difficult to interpret.

Oxidized and reduced nicotinamide–adenine dinucleotide. Compared with untreated controls, rats dosed with liquid paraffin showed no significant alterations in either NAD or $NADH_2$ concentration (μg./g. wet wt.) during the period studied (Table 1). When the results were expressed as μg./whole liver/100 g. body wt., however, it was seen that there was a decrease in the NAD content in the liquid-paraffin-treated animals 1–3 hr. after dosing ($P < 0·05$); there were no significant alterations in $NADH_2$ content when expressed in this manner, nor did the NAD:$NADH_2$ ratio show any significant differences compared with the control value throughout the period studied.

When the values obtained from the carbon tetrachloride-treated rats are compared with the liquid-paraffin-treated groups serving as controls (Table 1), the only significant change found was a transient decrease in NAD concentration 1 hr. after dosing ($P < 0·05$); this resulted in a similarly significant decrease in NAD + $NADH_2$ concentration at this time. These effects are not detectable when the results are expressed in terms of μg./whole liver/100 g. body wt.

Oxidized and reduced nicotinamide–adenine dinucleotide phosphate. The administration of liquid paraffin resulted in greater disturbances in NADP and $NADPH_2$ than those described for NAD and $NADH_2$. It was seen (Table 2) that, although the NADP concentration was unaffected by dosing with liquid paraffin, the NADP content in the whole liver fell rapidly and remained significantly depressed for 2 hr. ($P < 0·05$). The content of

Table 1. *Variation in the contents of oxidized and reduced nicotinamide–adenine dinucleotide in rat liver after the administration of carbon tetrachloride*

Values are expressed both as μg./g. wet wt. of liver and as μg./whole liver/100 g. body wt. Details of the CCl_4 dosage and administration etc. are given in the Methods section. Values are given as means ± S.E.M.

Time after dosing (hr.)	Treatment†	No. of rats	NAD		$NADH_2$		NAD + $NADH_2$		NAD:$NADH_2$ ratio
			μg./g.	μg./liver	μg./g.	μg./liver	μg./g.	μg./liver	
0	None	7	330 ± 12	1830 ± 58	79 ± 8	411 ± 43	416 ± 23	2254 ± 165	4·1 ± 0·47
0·5	CCl_4	3	356 ± 18	1640 ± 56	79 ± 9	364 ± 40	435 ± 18	2055 ± 63	4·6 ± 0·68
	LP	3	373 ± 33	—	107 ± 5*	—	481 ± 28	—	3·5 ± 0·44
1·0	CCl_4	5	289 ± 13	1610 ± 130	71 ± 5	391 ± 26	360 ± 18	2002 ± 145	4·1 ± 0·23
	LP	4	350 ± 22***	1611 ± 83	89 ± 12	383 ± 59	439 ± 26***	1994 ± 25	4·1 ± 0·61
2·0	CCl_4	6	343 ± 19	1507 ± 122‡	109 ± 9	455 ± 31‡	452 ± 26	1938 ± 143‡	3·2 ± 0·32
	LP	4	322 ± 15	1460 ± 182	93 ± 7	363 ± 35*	398 ± 17	1671 ± 209	3·7 ± 0·38
3·0	CCl_4	6	315 ± 16	1325 ± 90	74 ± 7	315 ± 39	389 ± 22	1640 ± 120	4·4 ± 0·48
	LP	5	320 ± 19	1442 ± 24	79 ± 4	354 ± 9	399 ± 22	1796 ± 30	4·1 ± 0·10
5·0	CCl_4	6	346 ± 19	1760 ± 192‡	82 ± 3	405 ± 27‡	428 ± 19	2160 ± 208‡	4·2 ± 0·28
	LP	4	312 ± 11	1740 ± 64	77 ± 2	431 ± 23	389 ± 11	2171 ± 77	4·0 ± 0·18
18·0	CCl_4	7	355 ± 17	1903 ± 170‡	67 ± 6	346 ± 18‡	421 ± 20	2249 ± 188‡	5·5 ± 0·38
	LP	4	362 ± 13	1747 ± 122	80 ± 3	387 ± 20	442 ± 10·3	2135 ± 131	4·5 ± 0·32
42·0	CCl_4	2	272	—	53	—	325	—	5·0

* $0·10 > P > 0·05$; *** $P < 0·05$.
† CCl_4, administration of CCl_4–liquid paraffin (3:1, v/v) mixture; LP, administration of liquid paraffin alone.
‡ Four animals only in this group.

$NADPH_2$ in the whole liver was, in contrast, unaltered, but the concentration of $NADPH_2$ was temporarily raised 30–60 min. after dosing ($P < 0.05$). These two opposite effects, i.e. decreased NADP and increased $NADPH_2$ concentrations, led to a significant change in the $NADPH_2$:NADP ratio in the liquid-paraffin-treated group 1 hr. after dosing compared with that of untreated animals.

Table 2 shows that the administration of carbon tetrachloride produces marked alterations in the $NADPH_2$ and NADP concentrations compared with those of the liquid-paraffin-treated controls. There is a tendency for the NADP concentration to be higher in the carbon tetrachloride-treated groups but this is only significant ($P < 0.05$) for the 1 hr. group. The $NADPH_2$ concentration is decreased in the carbon tetrachloride-treated groups compared with the liquid-paraffin-treated controls throughout the period studied both on a μg./g. wet wt. and μg./whole liver/100 g. body wt. basis. The latter effect is significant ($P < 0.05$) for the 30 min., 1 hr. and 2 hr. groups. These changes in NADP and $NADPH_2$ concentrations reinforce one another to produce very marked changes in the $NADPH_2$:NADP ratio in the carbon tetrachloride-treated groups compared with the control animals: there is a very significant difference ($P \ll 0.001$) in this ratio 1 hr. after dosing with carbon tetrachloride compared with the liquid-paraffin-treated group. The ratio is also significantly decreased in the 2 hr. and 3 hr. groups ($P < 0.05$). A few experiments were performed on rats 1 hr. after dosing with liquid paraffin or the carbon tetrachloride–liquid paraffin mixture to see if any changes in 'bound NADP' (see Burch *et al.* 1963) occurred similar to those described above for $NADPH_2$. Two groups of four rats each were used. The mean values obtained for 'bound NADP' (i.e. 'total NADP' measured after weak acid extraction minus 'free NADP' measured after extraction with 1N-perchloric acid) were 60 ± 11 μg./g. wet wt. of liver and 73 ± 3 μg./g. wet wt. for the control and carbon tetrachloride-treated groups respectively. Thus 'bound NADP' shows a similar tendency to increase after dosing with carbon tetrachloride to that found for 'free NADP' assayed as a routine in the present investigation. The increase in 'bound NADP' is not commensurate with the decrease found in $NADPH_2$ (Table 2).

Table 3 shows the effect of administering Phenergan concomitantly with either the carbon tetrachloride–liquid paraffin mixture or with liquid paraffin alone on the $NADPH_2$:NADP ratio in liver samples obtained 1 hr. after dosing. Phenergan not only prevents the $NADPH_2$ loss found after the administration of carbon tetrachloride but also increases the $NADPH_2$:NADP ratio to an unusually high value.

Table 2. *Variation in the contents of reduced and oxidized nicotinamide–adenine dinucleotide phosphate in rat liver after the administration of carbon tetrachloride*

Values are expressed both as μg./g. wet wt. of liver and as μg./whole liver/100 g. body wt. Details of the CCl_4 dose and method of assay are given in the Methods section. Values are given as means ± S.E.M.

Time after dosing (hr.)	Treatment†	No. of rats	NADP		$NADPH_2$		NADP + $NADPH_2$		$NADPH_2$:NADP ratio
			μg./g.	μg./liver	μg./g.	μg./liver	μg./g.	μg./liver	
0	None	10	27 ± 1·7	142 ± 8§	190 ± 6	1017 ± 71§	219 ± 7·2	1160 ± 48§	7·2 ± 0·56
0·25	CCl_4	6	26 ± 3·8	123 ± 20	178 ± 8	922 ± 71	206 ± 11	984 ± 81	7·7 ± 0·81
	LP	6	23 ± 1·5	100 ± 8	191 ± 12	912 ± 58	214 ± 12	956 ± 52	8·7 ± 0·91
0·50	CCl_4	7	25 ± 2·7	106 ± 4	195 ± 6	822 ± 39	219 ± 7	927 ± 38	7·8 ± 0·49
	LP	6	24 ± 4·3	94 ± 11	243 ± 18***	1054 ± 79***	264 ± 26*	1100 ± 106	8·9 ± 1·07
1·0	CCl_4	12	31 ± 2·1	133 ± 9	159 ± 9	720 ± 39	188 ± 10	855 ± 41	6·2 ± 0·70
	LP	10	23 ± 1·7***	107 ± 6***	230 ± 15***	1072 ± 23***	247 ± 18***	1173 ± 87***	10·6 ± 0·73***
2·0	CCl_4	4	28 ± 2·5	135 ± 12	149 ± 11	736 ± 35	177 ± 14	871 ± 46	5·5 ± 0·23
	LP	8	24 ± 1·9	112 ± 7	195 ± 10***	952 ± 58***	221 ± 11***	1018 ± 72	8·3 ± 0·50***
3·0	CCl_4	6	40 ± 2·8	161 ± 12	178 ± 15	790 ± 74	217 ± 16	958 ± 85	4·7 ± 0·33
	LP	5	31 ± 3·1*	132 ± 12	199 ± 17	902 ± 38	231 ± 19	1047 ± 48	6·4 ± 0·54***
5·0	CCl_4	4	32 ± 2·6	163 ± 7	162 ± 18	796 ± 94	194 ± 19	959 ± 87	5·1 ± 0·58
	LP	4	27 ± 3·1	150 ± 15	202 ± 19	1045 ± 111	214 ± 22	1191 ± 121	7·0 ± 0·51*
18·0	CCl_4	4	30 ± 2	154‡	129 ± 8	661‡	160 ± 10	814‡	4·3 ± 0·23
	LP	2	27	127	233	1092	260	1218	8·6
24·0	CCl_4	2	26	124	109	514	135	638	4·7
42·0	CCl_4	2	33	—	86	—	119	—	2·6

* $0.10 > P > 0.05$; *** $P < 0.05$.
‡ Two animals only in this group.
† CCl_4, administration of CCl_4–liquid paraffin (3:1, v/v) mixture; LP, administration of liquid paraffin alone.
§ Five animals only in this group.

Table 3. *Effects of various treatments on the contents of oxidized and reduced nicotinamide–adenine dinucleotide phosphate in female rat liver*

Values are given as μg./whole liver/100 g. body wt. The methods of administration and assay procedures are given in the Methods section. All animals were killed 1 hr. after the appropriate treatment. The carbon tetrachloride–liquid paraffin mixture (groups E–G) and the liquid paraffin (groups C and D) itself were given by stomach tube with the animal under ether anaesthesia. Injections of Phenergan (groups B, D and G) or an equal volume of water (group F) were given intraperitoneally under ether anaesthesia. Group H gives results obtained from rats given undiluted CCl_4 by stomach tube under ether anaesthesia; the dose was the same as that given as a routine in admixture with liquid paraffin (0·125 ml./100 g. body wt.).

Group	Treatment	No. of rats	NADP	$NADPH_2$	NADP + $NADPH_2$	$NADPH_2$:NADP ratio
A	None	5	142±8	1017±71	1160±48	7·2±0·56
B	Phenergan	3	111±9	917±67	1028±66	8·4±0·90
C	Liquid paraffin	10	107±6	1072±23	1173±87	10·6±0·70
D	Phenergan + liquid paraffin	4	126±9	1017±53	1144±49	8·2±1·1
E	CCl_4–liquid paraffin (3:1, v/v) mixture	12	133±9	720±39	855±41	6·2±0·70
F	CCl_4–liquid paraffin (3:1, v/v) mixture + water	3	164±11	748±32	912±21	4·6±0·50
G	CCl_4–liquid paraffin (3:1, v/v) mixture + Phenergan	6	87±6	1039±63	1127±56	12·0±0·90
H	CCl_4 alone	4	105±16	657±20	762±20	6·7±1·24

The concentrations of the nucleotides in serum were measured in attempts to decide if the decreased liver $NADPH_2$ content after dosing with carbon tetrachloride was due to leakage of this nucleotide. The only nucleotide detected in normal serum was $NADH_2$ (mean value, 6 μg./ml. of serum); the serum from rats poisoned 1 hr. previously with carbon tetrachloride contained approx. 3 μg. of both NAD and $NADH_2$/ml. of serum. These values are only approximate owing to the very low concentrations of nucleotide present, and are the means of only two animals in each case. Thus there is no evidence that leakage of $NADPH_2$ into the serum is a major factor in the decreased liver content of $NADPH_2$. Examination of the acid-soluble nucleotide pattern of serum obtained from rats 3 hr. after dosing with carbon tetrachloride showed that only AMP and NAD were present in detectable amounts (6 μg./ml. and 2·5 μg./ml. of serum respectively).

Measurement of the extinction at 260 mμ of acid-soluble extracts of bile (see the Methods section) did not indicate that this excretory route was the cause of the decreased $NADPH_2$ content in liver after dosing with carbon tetrachloride. The mean extinction at 260 mμ for bile samples from three normal rats was 0·84; bile samples from two rats poisoned 1 hr. before with carbon tetrachloride gave a mean extinction value of 0·68.

DISCUSSION

Earlier systematic studies of the effects of carbon tetrachloride injection on mice have included determinations of the NAD and $NADH_2$ contents in liver in the course of the injury (Frunder, Fischer & Bornig, 1956; Frunder, Bornig, Richter & Stade, 1959; Huller & Frunder, 1959). A decrease in the NAD:$NADH_2$ ratio was found to occur some 2 hr. after the intraperitoneal injection of a carbon tetrachloride–plant oil mixture although there was no change in the NAD + $NADH_2$ concentration (in μg./g. of liver) at this time. At later stages (4–12 hr.) of carbon tetrachloride poisoning the NAD + $NADH_2$ concentration (in μg./g. of liver) was considerably decreased in the carbon tetrachloride-treated groups of mice (Frunder *et al.* 1959). No similar extensive investigations on liver nucleotide alterations in early carbon tetrachloride poisoning have been performed on rats (see Dianzani, 1955; Gallagher & Rees, 1960). Thus, although most biochemical and histological investigations on the pathogenesis of carbon tetrachloride poisoning have been with rats, comprehensive data for the time-sequence of the changes in the liver nicotinamide–adenine dinucleotides are lacking. No data at all for any species appears to have been published on the changes occurring in liver NADP and $NADPH_2$ during carbon tetrachloride poisoning.

It is important, if one wishes to draw satisfactory comparisons between the time of onset of changes in the liver nucleotides and previously studied alterations in other biochemical or histological parameters, that all studies used in such comparisons should have been performed on the same species and with the same dose and route of administration of the toxic substance. The present investigation has attempted to supply the necessary data on the liver nicotinamide–adenine dinucleotides for such comparative purposes; the dose of carbon tetrachloride given and the route of

administration are the same as those used in many previous studies with the rat.

Oxidized and reduced nicotinamide–adenine dinucleotide. During the first few hours of carbon tetrachloride poisoning (i.e. 0–5 hr.) there are only slight disturbances in the liver NAD and $NADH_2$ contents. During this period many important biochemical perturbations are known to occur (e.g. see Smuckler *et al.* 1962; Rees & Shotlander, 1963; Judah *et al.* 1963). Even late in the course of the injury resulting from the administration of 0·125 ml. of carbon tetrachloride/100 g. body wt. there seems to be little disturbance in the concentrations of NAD and $NADH_2$ in the liver (Table 1); this dose results in extensive centrilobular necrosis readily detectable 18 hr. after dosing. Thus it would appear that changes in liver NAD and $NADH_2$ concentrations are not a necessary factor in the development of this form and degree of necrosis. The marked decreases previously reported for liver NAD and $NADH_2$ concentrations in carbon tetrachloride poisoning in rats (Dianzani, 1955) are possibly reflexions of the higher doses given and the different route of administration (subcutaneous).

Oxidized and reduced nicotinamide–adenine dinucleotide phosphate. In contrast with the lack of changes found in the contents of NAD and $NADH_2$, there are rapid and extensive alterations in the concentrations of NADP and $NADPH_2$ in the liver after the administration of carbon tetrachloride. There is a rapid decrease in the $NADPH_2$ content in liver samples from the carbon tetrachloride-treated rats. This decrease is most readily apparent when the $NADPH_2$ values in μg./whole liver/100 g. body wt. are considered. Further, although liver NADP values tend to be higher in the carbon tetrachloride-treated groups than in the control animals (Table 2, columns 3 and 4) this increase is of a much smaller magnitude than the decreases occurring in the $NADPH_2$ concentration so that there is a net decrease in the NADP + $NADPH_2$ content after the administration of carbon tetrachloride. This net decrease is statistically significant as early as 1 hr. after dosing with carbon tetrachloride.

These differences in the NADP and $NADPH_2$ concentrations in rat liver after the ingestion of carbon tetrachloride are somewhat magnified by considering the $NADPH_2$:NADP ratio (Table 2, column 10). Statistically significant changes occur in this ratio very early after the administration of carbon tetrachloride, By considering the rate at which carbon tetrachloride reaches the liver after dosing (Recknagel *et al.* 1958; Artizzu & Dianzani, 1962; Dawkins, 1963) it would seem that the changes found must be developing almost concurrently with the accumulation in the liver of appreciable concentrations of the toxic substance.

A decrease in the liver $NADPH_2$ content could result from leakage of the coenzyme into bile or serum, but no changes in either bile or serum could be detected after dosing with carbon tetrachloride. Two other possible factors that could result in a decreased liver $NADPH_2$ content are an increased catabolism or a decreased synthesis of the coenzyme during the early phase of the injury. Although Dianzani (1955) reported that the corresponding synthesis and breakdown of NAD were unaffected by carbon tetrachloride poisoning no work of this nature has been done with $NADPH_2$ (or NADP).

It is, of course, possible that the changes in NADP and $NADPH_2$ concentrations are unrelated to the disturbances produced by carbon tetrachloride that culminate in widespread necrosis. They could be only one aspect of a more general cell disturbance and secondary to the necrogenic reactions. However, the results found after the administration of the antihistamine drug Phenergan concomitantly with carbon tetrachloride support the hypothesis that the changes in liver NADP and $NADPH_2$ concentrations are indeed an integral part of the necrogenic chain of reactions.

Large doses of Phenergan prevent, or at least substantially delay, the appearance of necrosis resulting from the administration of carbon tetrachloride (Rees & Spector, 1961; Rees *et al.* 1961; see also Gallagher, Gupta, Judah & Rees, 1956) and inhibits the leakage of liver enzymes into the serum, a characteristic feature of severe liver injury (Rees & Sinha, 1960; Rees *et al.* 1961). Phenergan also prevents the early changes in $NADPH_2$ concentration and in the $NADPH_2$:NADP ratio produced by carbon tetrachloride poisoning (Table 3). However, despite these actions of Phenergan in delaying necrosis and preventing a decreased $NADPH_2$ content in the liver, many other actions of carbon tetrachloride are unaffected by the antihistamine drug. For instance, Phenergan does not prevent the accumulation of fat in the liver or the decreased incorporation of amino acids into protein (Rees *et al.* 1961; K. R. Rees, personal communication). Thus it seems possible that the changes found in NADP and $NADPH_2$ concentrations after the administration of carbon tetrachloride, and which are prevented by Phenergan, are part of the series of disturbances that lead to necrosis rather than the response that results in fatty liver.

Whatever the relationship of the changes in NADP and $NADPH_2$ concentrations to liver necrosis, it seems likely that the important period to study is the first 30 min. after enteral dosing with carbon tetrachloride. Previous studies on the protective action of Phenergan in carbon tetrachloride poisoning have concentrated on effects (i.e. leakage of enzymes into serum) that are appreciable only

some hours after dosing. Thus the time-scales not only of the necrogenic reactions themselves but also of the protective action of Phenergan need re-evaluation.

SUMMARY

1. The concentrations of NAD, $NADH_2$, NADP and $NADPH_2$ in rat liver were determined at various times after enteral dosing with a carbon tetrachloride–liquid paraffin mixture (dose of carbon tetrachloride, 0·125 ml./100 g. body wt.).

2. Compared with control groups receiving liquid paraffin alone, the administration of carbon tetrachloride had little significant effect on the concentrations and relative proportions of NAD and $NADH_2$ during the period studied (0–18 hr.).

3. In contrast, the administration of carbon tetrachloride resulted in rapid changes in the NADP and $NADPH_2$ concentrations. There were falls in $NADPH_2$ and rises in NADP contents respectively. These changes resulted in considerable variations in the $NADPH_2$:NADP ratio.

4. Administration of the antihistamine drug Phenergan prevented these changes in NADP and $NADPH_2$ concentrations resulting from the administration of carbon tetrachloride.

5. The probable implications of the changes in $NADPH_2$ and NADP concentrations to the mechanisms of necrosis are discussed. It is concluded that during the first 30 min. after the ingestion of carbon tetrachloride the changes that are found in the NADP and $NADPH_2$ concentrations are related in a direct manner to the necrogenic chain of reactions.

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