A re-examination of the effect of vitamin B_{12} concentrate on the hepatic injury produced by carbon tetrachloride

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It has been stated that the damaging effect of carbon tetrachloride (CCl_4) on the liver of rats—as measured by the degree of bromsulphalein retention, the increase in the level of liver fat, and the histologically demonstrable liver-cell damage—can to some extent be inhibited by the administration of vitamin B_{12} given in relatively large doses for a few days before the injection of a single dose of CCl_4 (Popper, Koch-Weser & Szanto, 1949; Koch-Weser, Szanto, Farber & Popper, 1950; Popper & Schaffner, 1957). Vitamin B_{12} is even stated to increase the amount of CCl_4, given in a single dose, that can be tolerated by a rat (Koch-Weser *et al.* 1950). Some of these findings have been confirmed by other workers. Hove & Hardin (1950), for instance, have concluded that 'vitamin B_{12} afforded some protection against carbon tetrachloride'. Similarly, Mushett (1950) has reported a protective effect of vitamin B_{12} against the '. . . fatty metamorphosis, the hydropic change and the depletion of cytoplasmic ribonucleic acid . . .' caused by a single dose of CCl_4, but he also noted that vitamin B_{12} increased the size of the liver and the degree of fatty change when CCl_4 was given over a more prolonged period.

More recently Wolff (1957), in his review of the part played by the liver in the metabolism of vitamin B_{12}, has, on the basis of a very small series of experiments, accepted the inhibitory action of vitamin B_{12} on the hepatotoxic effects of CCl_4 as first claimed by Popper and his associates. This claim has also been accepted by several other workers, for instance by Vorhaus & Vorhaus (1954), and has even led to the therapeutic use of vitamin B_{12} in disorders of the liver (for instance by Campbell & Pruitt, 1955). There is, however, little experimental evidence that vitamin B_{12} exerts a protective effect against other hepatotoxic agents such as, for instance, selenium (Rigdon, Couch, Brashear & Qureshi, 1955). On the other hand, Wachter (1955), who used different criteria, has concluded that vitamin B_{12} has no effect on the liver damage caused by CCl_4. Attempts to demonstrate in vitro an effect of vitamin B_{12} on some of the enzyme systems of the liver of normal rats, and of rats treated with CCl_4, have also not been successful (Rahman & Ahmad, 1955). It may be of interest in this connexion to point out that no satisfactory explanation of the inhibitory effect of vitamin B_{12} on the hepatotoxic action of CCl_4 has been advanced, even by those workers who have reported it. According to Popper *et al.* (1949) a primary lipotropic effect of vitamin B_{12} cannot be invoked, because it is only seen in animals fed on a diet low in protein. These workers at first supposed that vitamin B_{12} influenced the

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metabolism of nucleic acids, but abandoned this view later in favour of a possible vasodilatory effect of the vitamin (Koch-Weser *et al.* 1950).

A proper understanding of the inhibitory effect of vitamin B_{12} on the damaging effects of CCl_4 might conceivably throw some light on the mode of action of one of the most widely used hepatotoxic agents; it was, therefore, thought of interest to re-examine the question. In view of the statement of Meites (1952) and Meites, Feng & Wilwerth (1957) that the requirements for vitamin B_{12} are increased by the administration of cortisone to experimental animals, the possibility was also considered that any differences caused by the administration, or lack, of vitamin B_{12} might be accentuated by simultaneous treatment with cortisone.

EXPERIMENTAL

Animals and their treatment. Male rats of a strain bred in the Department of Anatomy, Birmingham, numbering 235, were used. Their body-weights ranged from 105 to 250 g, but this variation was largely offset by the litter-mate arrangement used

Table 1. *Stock rat-cake diet and protein-free diet (Kosterlitz, 1947) given to the rats*

Rat-cake diet (Expt 1, values as percentages)			
Ground oats	17·7	Meat-and-bone meal	8·8
Wheat offal	17·9	White fish meal	4·5
Ground wheat	17·7	Dried yeast	1·2
Full-cream dried milk	14·0	Cod-liver oil	0·4
		Salt	0·4
Protein-free diet (Expt 2)			
Agar (%)	2	Pyroxidine (μg/10 g)	30
Lard (%)	6	Riboflavin (μg/10 g)	50
Maize starch (%)	64	Calcium D-panthothenate (μg/10 g)	100
Sucrose (%)	25	Nicotinic acid (μg/10 g)	100
Salts (Glaxo Laboratories Ltd) (%)	3	Inositol (mg/10 g)	1
Thiamine (μg/10 g)	30	Choline chloride (mg/10 g)	17·3

throughout the experiment. It consisted in comparing in each group brothers only, so that the effect of each factor examined could be checked in brothers treated with another factor or not treated at all. With, for instance, four litter-mates it was thus possible to compare the effect of CCl_4 with or without vitamin B_{12} in rats also given injections of cortisone. Two major experiments were done; they differed only in the diet (Table 1) given to the animals. In Expt 1 the rats were fed on the stock rat-cake diet, but in Expt 2 they were given for 7 days a diet similar to the protein-free diet of Kosterlitz (1947) before being treated with CCl_4 by either subcutaneous or intraperitoneal injection. The subcutaneous dose of CCl_4 was always 0·15 ml/100 g body-weight, and one, four or twelve injections at intervals of 3 days were given to the animals on the normal diet. Some animals of the last two groups were also given a total dose of 97·5 mg cortisone acetate (Cortone, Merck and Co. Ltd) for the last 10 days of the experiment. Rats fed on the protein-free diet received only a single subcutaneous injection of CCl_4. Other groups of rats, in both major experiments, were given a single intraperitoneal injection of either 0·03, 0·2 or 0·4 ml CCl_4 in mineral oil

per 100 g body-weight, as reported by Koch-Weser *et al.* (1950). Half the animals in each group of both major experiments were, in addition, treated with vitamin B_{12} concentrate (Cytamen, Glaxo Laboratories Ltd) in a dose of 10–30 μg/100 g body-weight. It was given either in four or, with the multiple injections of CCl_4, ten consecutive daily injections, in such a manner that the last injection of vitamin B_{12} always coincided with the last or the only injection of CCl_4. Most animals were allowed free access to food throughout the experiment, but some were fasted for 19–24 h before being killed by bleeding under light ether anaesthesia. Rats given the larger, lethal, intraperitoneal injections of CCl_4 (0·4 ml) were allowed to die spontaneously, and the time of death was recorded as nearly as possible for a study of the survival times (Table 4). The livers of animals that had been killed were quickly removed, weighed, frozen in liquid nitrogen, and stored at $-30°$, or immediately dried. Residual moisture after preliminary drying was removed by heating and drying to constant weight under reduced pressure over phosphorus pentoxide, and the water content was taken as the difference between the wet and the dry liver weight. Lipids were extracted from the dry residue by a hot mixture of 2 parts methanol and 1 part chloroform, and the percentage of fat was calculated from the difference between the dry weight and the weight of the fat-free residue.

Histological examination. Small specimens of the liver were fixed by the method of freezing and drying of Altmann–Gersh (Gersh, 1948) or, in a few instances, by immersion in Bouin's alcoholic fluid. Sections were stained with haematoxylin–eosin for a general survey, with the Hotchkiss–McManus Periodic Acid Schiff (PAS) reagent for polysaccharides, and, sometimes, with toluidine blue or methyl green–pyronin for nucleic acids. Some sections were also stained with iron haematoxylin for mitochondria.

Statistical treatment. The statistical methods used in the analysis of these experiments require some comment. Since litter-mate arrangements were used in the designs of most of the experiments, it was necessary to equalize the numbers of animals in each treatment group. Unequal numbers arose either through extra littermates being included or through animals dying before the end of the experiments. Excess animals in any litter were discarded, the choice of these animals being made in a random manner. Litters without representatives in more than half the treatment groups were also discarded. When any of the remaining litters were incomplete the statistical analysis was adjusted appropriately. The number of animals used in the tables presented here relates therefore only to the numbers of animals used for statistical analysis, although the total number of animals whose livers were examined was somewhat larger.

The values for times of survival were analysed by a method suggested to us by Dr M. R. Sampford to whom we are indebted.

RESULTS

Expt 1. Effect of carbon tetrachloride with and without vitamin B_{12} concentrate on the liver of rats given the normal diet

After the injection of single or repeated doses of CCl_4, certain characteristic changes take place in the liver which can range, briefly, from fatty changes and loss of nucleic

acids and of glycogen to necrosis and 'hydropic degeneration' or 'ballooning' of the liver cell. Since these changes have been described in detail by many workers (for literature see, for instance, Drill, 1952), a further enumeration here is not necessary. It will be sufficient to draw attention briefly to the difficulty encountered in comparing the damage caused by CCl_4 in individual animals, since it is well known that there is considerable variation in response. The litter-mate arrangement chosen for these experiments to some extent reduced this difficulty. The histological features found most useful for comparative purposes of the type studied here were the extent of 'hydropic degeneration' of the liver cell and the degree of glycogen depletion in the rats with free access to food. Goldschmidt, Vars & Ravdin (1939) have suggested that the loss of glycogen from the liver of animals treated with chloroform can best be explained by the inability of the damaged liver cells to retain glycogen. It is probable that this explanation also holds for the liver-cell damage by CCl_4. Since the method of fixation by freezing and drying used by us is the method of choice for the histo-

Table 2. *Mean values with their standard errors for the fat content of the liver of normal rats fed or fasted for 24 h, showing lack of effect of fasting*

Treatment	No. of animals	Mean fat content (as percentage of wet weight)
Fed	16	$4{\cdot}87 \pm 0{\cdot}145$
Fasted	13	$4{\cdot}99 \pm 0{\cdot}379$

chemical demonstration of glycogen, making it possible to detect relatively small changes in the amount and distribution of that substance by means of the PAS method (Aterman, 1952), a fairly reliable estimate of the degree of liver damage caused by CCl_4 could be obtained. When glycogen had been depleted by a preceding fast, attention was also paid to the degree of eosinophilia of the hepatocellular cytoplasm, and to the behaviour of the 'basophile inclusion bodies' of the liver cells.

When these histological criteria were applied to liver sections from rats treated with carbon tetrachloride little, if any, difference could be seen in either the acutely or the subacutely poisoned animals by comparison with their litter-mates treated with vitamin B_{12} as well. The failure of vitamin B_{12} to inhibit the histological manifestations of damage by CCl_4 was seen in fasted as well as in fed rats, and is borne out by a study of the level of hepatic lipids, and of the survival time of the rats. It can be seen from Tables 2 and 3 that, in fed or fasted rats, treatment with vitamin B_{12} did not significantly alter the degree of fatty change that follows a single or repeated injection of CCl_4. Also, the administration of cortisone made little difference to the failure of vitamin B_{12} to affect the course of poisoning with CCl_4. This failure of vitamin B_{12} was also seen when the absolute or relative liver weights were compared, or when the survival time of those rats given intraperitoneal injections of a single toxic dose of CCl_4 was studied (Table 4). Although a preliminary experiment had suggested (Table 4, *A*) that vitamin B_{12} given before treatment with CCl_4 might have a slight delaying effect in these animals, a second, larger experiment (Table 4, *B*) did not bear out this possibility.

Table 3. *Expt* 1. *Effect of one, four or twelve injections of carbon tetrachloride* (0·15 *ml*/100 *g body-weight*) *alone or with vitamin* B_{12} (15 μg/100 *g body-weight*) *or cortisone* (97·5 *mg*) *or both on the fat content of the liver of rats, expressed as a percentage of the wet weight*

Treatment	No. of animals	Doses of CCl_4	Vitamin B_{12}	Cortisone	Mean fat content	Error mean square	d.f.
Fed	7	0	N.G.	N.G.	4·77*		
	13	1	N.G.	N.G.	5·72	1·03	12
	13	1	G.	N.G.	5·20		
Fasted	7	0	N.G.	N.G.	5·14		
	8	1	N.G.	N.G.	5·69	0·82	13
	9	1	G.	N.G.	5·13		
	7	4	N.G.	N.G.	7·92		
	7	4	G.	N.G.	8·81		
	5	4	N.G.	G.	6·67	5·58	16
	7	4	G	G.	8·54		
	9	12	N.G.	N.G.	7·36		
	9	12	G.	N.G.	7·62		
	8	12	N.G.	G.	5·80	3·75	22
	8	12	G.	G.	5·37		

G., given; N.G., not given.

* Standard error of the mean = 0·25.

Table 4. *Expt* 1. *Effect of vitamin* B_{12} *injection* (30 μg/100 *g body-weight*) *on the survival time of litter-mate rats fed on a normal diet and given a single intraperitoneal injection of carbon tetrachloride* (0·4 *ml*/100 *g body-weight*)

		No. found dead at								No. that survived
	Treatment	0–4 h	4–8 h	8–12 hr	12–16 h	16–20 h	20–24 h	24–36 h	36–48 h	48 h
A	CCl_4	6	2	0	0	0	0	2	0	0
	CCl_4 and vitamin B_{12}	0	4	2	3	0	0	1	0	0
B	CCl_4	1	6	8	1	0	0	1	0	3
	CCl_4 and vitamin B_{12}	3	3	7	3	0	0	1	1	2

Expt 2. *Effect of* CCl_4 *with and without vitamin* B_{12} *concentrate on the liver of rats given the protein-deficient diet*

Thus, on the one hand, we have the failure of vitamin B_{12} to influence the damage caused by CCl_4 in the normal rats of our experiments; on the other is the statement by Popper *et al.* (1949) and Koch-Weser *et al.* (1950) that the inhibitory effect of vitamin B_{12} reported by them could not be attributed to a sparing action of vitamin B_{12} on lipotropic agents such as choline, since this effect was only seen in animals fed on a diet deficient in protein. These findings suggested a study of the effect of vitamin B_{12} on the course of poisoning by CCl_4 in rats fed on such a diet. But first the possibility had to be considered that the discrepancy between the findings presented here and those of Popper and his associates could perhaps be attributed to some defect in the

preparation of vitamin B_{12} used. An assay of the preparation, undertaken after the results of Expt 1 had been analysed, had shown it to be fully active. (We are indebted to Glaxo Laboratories Ltd for this information.) It was, therefore, necessary to establish whether the inhibitory effect of vitamin B_{12}, claimed by Popper's group, could be demonstrated in rats depleted of protein by ingestion of a protein-free diet for 7 days before treatment with CCl_4. Here, however, the difficulty is encountered that the depletion of protein produces, by itself, certain characteristic changes in the liver cell which have to be considered before the effect of CCl_4 can be fully assessed. Such changes were first noted by Afanassiew (1883), but have more recently been studied in greater detail by Elman, Smith & Sachar (1943), Wang, Hegsted, Lapi, Zamcheck & Black (1949) and, particularly, by Kosterlitz (1947). Histologically, the liver cells assume a peculiar rarefied appearance which has led several authors to speak of 'plant-like' or even 'hydropically changed' cells. They are of normal, or smaller than normal, size with an apparently normal nucleus and prominent nucleolus. Clumps of cytoplasm are clustered around the nucleus and pressed against the periphery of the cells. As a result of this distribution of the stainable cytoplasm, the liver cells depleted of protein, in contrast to normal cells, appear to have in freeze-dried sections a distinct cellular membrane. The cell body contains, in sections stained with haematoxylin–eosin, irregular 'empty' spaces which can easily be mistaken for vacuoles but which, on staining with Best's carmine or with the PAS reagents, can be shown to contain ample amounts of glycogen. It is the formation of these spaces that gives the liver cell of the animal depleted in protein its characteristic rarefied appearance. Although there exists a certain superficial resemblance between this change and the 'ballooning' of the liver cells caused by CCl_4—the term 'hydropic change' has indeed been applied by different workers to both these phenomena—these changes are easily distinguishable. Not only does almost every cell in the liver lobule show this 'plant-like' appearance, in contrast to the limited number of cells showing the true 'hydropic change' caused by CCl_4, but also there is the presence of glycogen in the 'plant-like' cells, in contrast to its invariable absence in the true 'hydropic' change. The morphological changes, briefly outlined here, are always accompanied by certain biochemical changes in the composition of the liver cells. These have been summarized by Kosterlitz (1947) as a loss of 'labile cytoplasm'. Of interest for the purpose of the present paper is the fact that the fat content of the liver of animals fed on a diet deficient in protein can increase in comparison with that of normal controls (Kosterlitz, 1947; Best, Hartroft, Lucas & Ridout, 1955; Johnson, Firth & Mistry, 1955). A slight increase in the mean fat content was indeed seen in the untreated rats given the protein-free diet (Table 5) but it was not statistically significant. After the injection of CCl_4 a significant ($P < 0{\cdot}001$) further increase in the level of lipids in the liver took place. Treatment with vitamin B_{12} before the administration of CCl_4 reduced this rise in the amount of fat present in the liver which, however, still remained at a higher level than in the untreated litter-mates fed on the protein-free diet. Although statistically this reduction was only significant at the 5% level, it would seem that in the rat fed on the protein-free diet and treated with CCl_4 vitamin B_{12} may have some lipotropic effect, in contrast to its ineffectiveness in the rat fed on a normal diet.

Histological examination, however, showed that the slight lipotropic action of vitamin B_{12} in these animals was not accompanied by any corresponding inhibitory effect against the other manifestations of poisoning with CCl_4. Neither the degree of glycogen depletion nor the other cytological criteria used were significantly influenced by the prior administration of vitamin B_{12}. This finding is the more significant as the histological changes caused by CCl_4 in these animals were considerably less pronounced than in the rats fed on the normal diet and treated with approximately the same dose of CCl_4. Hydropic degeneration or 'ballooning', for instance, was only rarely seen and, if present, tended to occur more in isolated cells than in clusters and rows, as in the rat fed on the normal diet. The failure of vitamin B_{12} to alter materially the course of poisoning with CCl_4 was also clearly demonstrated by its lack of effect on the survival time of rats given a single toxic (0·4 ml) dose of CCl_4 intraperitoneally (Table 6).

Table 5. *Expt 2. Effect of a single subcutaneous dose of carbon tetrachloride* (0·15 *ml/*100 *g body-weight*) *with and without vitamin* B_{12} (15 μg/100 *g body-weight*) *on the fat content of the liver of rats fed on a protein-free diet, expressed as a percentage of the wet weight*

Treatment	No. of animals	Mean fat content	Significance of difference between adjacent rows
Normal diet	8	4·99	
			0·4 > *P* > 0·3
Protein-free diet	13	5·39	
			0·001 > *P*
Protein-free diet; CCl_4	13	6·83	
			0·05 > *P* > 0·02
Protein-free diet; CCl_4 and vitamin B_{12}	12	6·06	

Error mean square 0·85; d.f. = 30.

Table 6. *Expt 2. Effect of vitamin* B_{12} *injection* (30 μg/100 *g body-weight*) *on the survival time of rats fed on a protein-free diet and given a single intraperitoneal injection of carbon tetrachloride* (0·4 *ml/*100 *g body-weight*)

	No. found dead at								No. that survived
Treatment	0–4 h	4–8 h	8–12 h	12–16 h	16–20 h	20–24 h	24–36 h	36–48 h	48 h
CCl_4	0	2	2	1	1	0	0	0	1
CCl_4 and vitamin B_{12}	1	1	2	1	1	0	1	0	0

The findings so far presented suggested the possibility that the partial inhibitory effect of vitamin B_{12} seen here was not a real inhibitory effect as far as the hepatotoxic action of CCl_4 was concerned, but that it was more likely to be an expression of the primary lipotropic effect of vitamin B_{12} postulated in the literature and dismissed by Popper *et al.* (1949) as an explanation of their findings. This possibility was tested by studying the effect of vitamin B_{12} alone, without the simultaneous treatment with CCl_4, in rats fed on the protein-free diet. It can be seen from Table 7 that treatment with vitamin B_{12} alone produced a slight, but fairly consistent, decrease in the fat content of the liver (0·05 > *P* > 0·02). This finding, in conjunction with the other observations reported here and in the literature, lends some support to the view that

under the conditions of this experiment any inhibitory effect of vitamin B_{12} need not necessarily be attributed to its effect on the hepatotoxic action of CCl_4.

Table 7. *Expt 2. Effect of vitamin B_{12} injection (15 μg/100 g body-weight) on the fat content of the liver of litter-mate rats fed on a protein-free diet, expressed as a percentage of the wet weight*

Protein-free diet	Protein-free diet; vitamin B_{12}	Difference
4·90	4·13	0·77
5·24	4·26	0·98
5·32	4·91	0·41
4·23	4·37	0·14
5·60	4·42	1·18
4·82	4·47	0·35
Mean 5·01	4·42	0·59 ± 0·196 ($0·05 > P > 0·02$)

DISCUSSION

The findings presented here are at variance with those reported by Popper *et al.* (1949). It is, of course, always possible that variations in the experimental conditions, such as differences in the strain and sex of the rats used, could account for this discrepancy. Attention should, on the other hand, be drawn to the fact that a satisfactory explanation of the postulated inhibitory effect of vitamin B_{12} has so far not been advanced. It is, indeed, difficult to understand why animals fed on an adequate diet should respond favourably to the administration of an excess of this vitamin. A cursory attempt to find other instances of a favourable effect of vitamin B_{12} in animals fed on a more or less adequate diet has so far not been successful. Rigdon *et al.* (1955), for instance, found no effect of vitamin B_{12} on the hepatic necrosis caused by the ingestion of selenium. Similarly, Arrigo & Montini (1950) were not able to detect any effect of vitamin B_{12} on the fatty changes in the liver after partial hepatectomy, and no effect was seen in the present experiments on the loss of body-weight and on the level of hepatic lipids of rats treated with CCl_4 and given cortisone at the same time. The lipotropic effect of vitamin B_{12} is on occasion even missing with diets that cannot be considered to be nutritionally adequate. This question has been discussed by Shils, De Giovanni & Stewart (1955). Lipotropic effects of vitamin B_{12} have, on the other hand, been reported with experimental diets clearly deficient in vitamin B_{12}. This is, presumably, the explanation of the changes reported by Hedin & Schultze (1955), as well as of those found in our experiments. To some extent the discrepancy between the findings reported here and the results of the other workers quoted above is reminiscent of the controversy concerning the favourable effects of supplements of vitamin B_{12} in clinical nutrition. Whereas early reports had suggested that treatment of children with vitamin B_{12} improved their nutritional status (Wetzel, Fargo, Smith & Helikson, 1949), some later reports failed to confirm this finding (Benjamin & Pirrie, 1952). It has been suggested that this occasional lack of a favourable effect could be explained by the absence of a significant deficiency of this vitamin (Pink & Wokes, 1952; Wokes & Picard, 1955) and that, indeed, in the absence of such a deficiency a favourable effect of supplements of vitamin B_{12} could not be reasonably

expected (Howe, 1958). This conclusion could equally well be applied to our results and may, perhaps, explain the discrepancy between them and those of Popper and his associates. These workers, however, have been careful to stress that a vitamin effect was unlikely since the doses of vitamin B_{12} used in their experiments were 'pharmacological'. But whatever the explanation of the reported lipotropic effect of vitamin B_{12} may be, our findings also suggest that this action is not synonymous with an 'inhibition' of the hepatotoxic functions of CCl_4. Despite a slight lowering action on the level of liver lipids in the protein-depleted rats, vitamin B_{12} has no significant effect on either the histological picture of the liver or the survival time of rats treated with CCl_4. In the absence of further evidence, the inhibitory effect of vitamin B_{12} on the hepatotoxic action of CCl_4, as opposed to its possible lipotropic effect, can therefore not be considered to have been proved beyond doubt. Further experiments are needed.

The difference in the histological picture after treatment with CCl_4, in the rats fed on the normal diet and in those fed on the diet free of protein, was pronounced. The dependence of the effect of hepatotoxic agents on dietary factors is well known. For some time it had been considered that a high content of glycogen in the liver exerted some protective effect, but Goldschmidt *et al.* (1939) have maintained that, at least as far as the effects of chloroform are concerned, protein in the diet 'decidedly decreases' the degree of hepatic injury. Similarly, Miller, Ross & Whipple (1940) have reported a protective effect of cystine given to dogs depleted of protein. From these and related observations one would have expected to find in our experiments a greater degree of liver damage in the rats fed on the protein-free diet than in those fed on the normal diet, but it was not found. Similar results have been reported by Campbell & Kosterlitz (1948), and by Drill (1952), who has published a detailed review of the part played by dietary factors in the injury produced by hepatotoxic agents. According to this worker the best protection against such liver damage is offered by diets rich in carbohydrates. At a constant dietary level of fat, damage was greater in animals fed on a diet with a 'normal' content of protein than in animals fed on a diet low in protein but high in carbohydrate. The nature of this protective effect of diets low in protein is not well understood and deserves further investigation, particularly with regard to changes in liver function which have so far received only comparatively little attention.

SUMMARY

1. Male rats, fed on a normal or a protein-deficient diet, were treated with single or with multiple injections of carbon tetrachloride. Their litter-mates were also given injections of vitamin B_{12}, and the effect of this treatment on the hepatotoxic actions of CCl_4 was studied, since a protective action of this vitamin has been claimed by some workers.

2. In contrast to these reports in the literature, vitamin B_{12} was found to have no effect on the histological picture, the fat content, or the survival time of acutely poisoned rats fed on a normal diet.

3. In rats fed on a protein-free diet for some days before injection of a single dose of CCl_4, vitamin B_{12} produced a slight decrease in the level of lipids in the liver, but

had no effect on the histological picture, or on the survival time after treatment with toxic doses of CCl_4.

4. Since vitamin B_{12} also exerted a slight 'lipotropic' effect in rats given only the protein-free diet, but not treated with CCl_4, a distinction has to be made between the 'lipotropic' action of vitamin B_{12} and the alleged inhibitory action on the hepatotoxic effect of CCl_4.

5. The difference in response seen in the normal and in the protein-depleted rat is compared to the differences reported in the response of children given supplements of vitamin B_{12}, which have been attributed by some workers to the alleviation of a deficiency of that vitamin.

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