Effect of Carbon Tetrachloride Injury on Plasma and Liver Vitamin B_{12} Levels. (22990)

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Serum vit. B_{12} level of patients with certain liver diseases is increased(1,1a). In the very early stage of viral hepatitis there is an abrupt decrease of vit. B_{12} binding capacity in serum. Since liver is a main site of B_{12} storage it seemed of interest to investigate the effect of liver damage by carbon tetrachloride upon B_{12} serum levels, and on B_{12} absorption.

Methods. (1) *Administration of carbon tetrachloride.* To produce liver damage in rats, one ml of a solution of carbon tetrachloride dissolved in olive oil was injected subcutaneously to adult male animals of McCollum strain weighing approximately 400 g. The carbon tetrachloride in the one ml olive oil solution was such that each rat received 0.035 to 0.10 ml of carbon tetrachloride per 100 g body weight. (2) *Microbiological determinations of total and alkali-unstable vitamin B_{12} activity in serum or plasma and liver.* Blood was obtained by cardiac puncture under light ether anesthesia. Twenty-five thousandths, 0.05 and 0.075 ml of serum or plasma separated in the usual manner was added directly to 5 ml of Skeggs' media(2). Growth of *Lactobacillus leichmannii* No. 4797 was estimated titrimetrically, after 64 hours incubation and was taken as a measure of total vit. B_{12} activity. To determine vit. B_{12} activity in liver, the organ was weighed and homogenized with one part liver and 19 parts water. An aliquot of the homogenate was mixed with acetate buffer, heated in boiling water bath, diluted to appropriate concentrations, and then added to test media for assay. Since vit. B_{12}, unlike other substances with growth promoting activities, is unstable toward alkali(3), liver or serum extracts prepared by heating specimens in acetate buffer at pH $= 4.5$ and subsequent removal of proteins by centrifugation were heated at 100° for 30 minutes at 0.20 N NaOH concentration. Alkaline solutions were subsequently neutralized and assayed for vit. B_{12} activity as described above. (3) *Determination of radioactivity of vit. B_{12} in urine and feces.* Adult rats were given, either orally or subcutaneously, radioactive vit. B_{12} labeled with Co^{60}, with specific activity of 1100 μc/mg. The animals were kept in individual metabolism cages. Urine and feces were collected for at least two 24 hour periods. Urine and washings were evaporated to 50 ml in a graduated 100 ml brown bottle. Feces were collected for 4 consecutive days, moistened with small amounts of distilled water and finally homogenized with concentrated sulfuric acid. The homogenate was made up to 50 ml volume. Radioactivity in the bottles containing urine or feces specimens was measured with scintillation counter. (4) *Composition of diets.* All animals were fed a casein diet, or soybean meal diet, both consisting of 4% corn oil, 4% salt mixture IV(4) and vitamin supplement containing all known essential vitamins(5) except vit. B_{12}. The casein diet contained 20% crude casein and 72% sucrose, while the soybean diet contained 68% soybean meal and 24% sucrose. The remaining 8% is made up of 4% corn oil and 4% salt mixture IV.

Results. Effect of carbon tetrachloride administration on plasma vit. B_{12} activity. In study one, 15 adult rats were divided equally into 3 groups. Two of them received 0.035 and 0.07 ml carbon tetrachloride/100 g body weight, the third group received olive oil alone and, therefore, served as control. All animals were bled at 24 hours and again 7 days after administration of carbon tetrachloride. Plasma specimens were analyzed for vit. B_{12} activity. After the second bleeding, the animals were sacrificed; their livers removed and assayed for vit. B_{12} content. The results in Table I indicate that the plasma vit. B_{12} level was increased markedly

TABLE I. Effect of CCl_4 Administration on Plasma and Liver Vit. B_{12} Levels in Rat.

Dose of CCl_4 (ml/100 g body wt)	Plasma B_{12} level (mγ/ml)		Liver B_{12} level (mγ per g wet wt) 7 days
	24 hr after CCl_4	7 days	
.00	.656 ± .024*	.775 ± .025	202 ± 57
.035	.871 ± .082	.809 ± .031	
.07	1.174 ± .105	.857 ± .025	190 ± 26

Each group contained 5 rats.
* Avg and stand. error of mean.

upon administration of carbon tetrachloride and the increase was greater the higher the dose of carbon tetrachloride given. The increase was observable within 24 hrs, but the levels returned to the control values by the seventh day, at which time the B_{12} levels in livers of both control and treated groups were essentially the same. To ascertain whether the increase in vit. B_{12} activity in plasma was due to the vitamin itself or other substances with vit. B_{12}-like activities, a second study was conducted in which the alkali stable fraction was also measured. Three groups of rats (Table II) were bled 24 hours after administration of carbon tetrachloride. The results again indicate elevation of vit. B_{12} serum level after carbon tetrachloride administration. The amount of alkali stable fraction in serum remained the same after this treatment. The elevated total activity was destroyed with NaOH.

Effect of previous dietary history and plasma vit. B_{12} elevation. Increase in the plasma vit. B_{12} level is probably due to its release from storage organs. It is, therefore, of interest to compare the effect of carbon tetrachloride administration on plasma B_{12} levels of rats fed B_{12}-containing and vit. B_{12}-deficient diets. To this end, 2 groups of weanling rats were placed on casein and on soybean diets, respectively. The protein contents of both diets were approximately 20%. After 3 months of feeding on these diets, the B_{12} contents in livers of the 2 groups of rats were assayed and found to be 230 ± 46 and 122 ± 31 mμg/g wet liver for casein and soybean fed rats, respectively. The remaining animals were subdivided into 2 groups. One subgroup received carbon tetrachloride and the other received olive oil alone. Twenty-four hours after injection, the animals were bled and the plasma specimens were assayed for vit. B_{12} activity. Our results demonstrate a marked increase in plasma level of vit. B_{12} in the group fed the casein diet from 0.75 mμg/ml for untreated group to 0.99 ± 0.073 mμg/ml of the treated group. No significant change was observed in the group receiving the soybean diet; the B_{12} serum levels were 0.50 mμg/ml for both treated and untreated groups. The low vit. B_{12} content in the vegetable protein was reflected in low plasma vit. B_{12} level.

TABLE II. Effect of CCl_4 Administration on Serum Total Vit. B_{12} and Alkali Stable B_{12} Levels (24 Hr after CCl_4 Admin.).

Dose of CCl_4 (ml/100 g wt)	No. of rats	Total serum B_{12} level (mγ/ml)	Alkali-stable B_{12} activity (mγ/ml)
.00	5	.401 ± .008	.210 ± .016
.05	5	.659 ± .059	.235 ± .008
.10	6	.737 ± .051	.236 ± .012

To test the hypothesis that increase in plasma vit. B_{12} is due to release of vit. B_{12} from the storage organs or tissues, 10 rats (experiment A) were given subcutaneously 20 mμg of radioactive vit. B_{12} for 3 consecutive days. Urine collection was started 48 hours after the last injection of the radiovitamin. A small amount of radioactivity was found in the urine. These animals were then divided into 2 groups of 5 each. One group received carbon tetrachloride in olive oil and the other group received olive oil alone. Urine and feces collections were continued for the measurement of radioactivity. The results shown in Table III indicate that urinary but not fecal excretion of radioactivity increased markedly in animals treated with carbon tetrachloride.

In another experiment (B), the test animals were divided into 2 groups, one group received carbon tetrachloride and the other received olive oil alone. Twenty-four hours later, both groups of animals were given 20 mμg of radioactive vit. B_{12} subcutaneously. Urine and feces were collected for 4 days for radioactivity measurement. The results again demonstrate that the injection of carbon tetrachloride resulted in greater excretion

TABLE III. Excretion of Radioactive Vit. B_{12} (Each Group Contained 5 rats).

Exp.	Dose of CCl_4 (ml/100 g wt)	Route of B_{12}* admin.	Time of B_{12}* admin.	Urinary excretion of radioactivity (mγ) 0-24 hr	24-48	48-96	Total, 4 day	Fecal excretion (mγ) 4 day
A	.00	Subcut.	Prior to CCl_4 inj.	.47 ± .05	.41 ± .037	.41 ± .049	1.30 ± .089	6.60 ± .15
	.05			.90 ± .038	.57 ± .049	.71 ± .053	2.29 ± .107	6.25 ± .25
B	.00	"	After CCl_4 inj.	1.04 ± .054	.24	.36	1.64 ± .07	2.51 ± .15
	.05			1.46 ± .078	.27	.23	1.98 ± .17	1.94 ± .15
C	.00	Oral	After CCl_4 inj.	.16 ± .04	.24	.46	.86 ± .06	19.5 ± 1.8
	.15			.38 ± .03	.26	.44	1.08 ± .13	24.8 ± 1.2

of radioactive vit. B_{12} in urine and slightly less excretion in feces.

The effect of carbon tetrachloride on the absorption of orally administered B_{12} was also studied. Ten adult rats (experiment C) were divided into 2 groups of 5 each. One group was treated with carbon tetrachloride and the other, with olive oil. Twenty-four hours afterwards, 50 mμg of radioactive vit. B_{12} in one ml was given by a stomach tube to every animal. The radioactivity in urine and fecal specimens was measured. The results demonstrate a larger amount of radioactivity in urine specimens of treated rats. A slight increase in fecal excretion was likewise observed.

Discussion. Vit. B_{12} serum level may be useful in determining the reserve of this vitamin in subjects with different diseases. It can be categorically stated that in clinically recognizable vit. B_{12} deficiency, as in patients with either Addisonian pernicious anemia or anemia due to fish tapeworm infestation or to poor absorption following total gastrectomy or to the dietary lack of vit. B_{12} (vegans), the vit. B_{12} serum level is always extremely low. On the other hand, there are also instances where vit. B_{12} deficiency is believed to exist clinically and yet the plasma level of vit. B_{12} is elevated above normal. For example, diabetics with retinopathy(6) possess higher vit. B_{12} levels than clinically healthy non-diabetics. Elevation of vit. B_{12} level in sera of subjects with leukemia has also been reported(7,8). Our studies suggest that the interpretation of vit. B_{12} levels in serum must take into account the existence of an agent (chemical or physiological) which may temporarily release vit. B_{12} from storage organs. On the other hand, a low level of vit. B_{12} may be considered as a measure of poor reserve. A high level, particularly those with abnormally elevated values, need not indicate sufficiency of vit. B_{12}.

The results of our experiments lead us to believe that administration of carbon tetrachloride causes liver injury with consequent release of vit. B_{12}, although our data do not rule out the possibility that other organs for B_{12} storage, *e.g.*, kidneys might have been

damaged in a similar manner. B_{12} enters the blood, thus resulting in elevation of vit. B_{12} and in a concomitant increase in urinary excretion. This injury provoked with the amounts of carbon tetrachloride used is apparently only temporary. The elevation of vit. B_{12} activity in serum is most likely due to an increase in vit. B_{12} *per se*, rather than alkali stable factors such as desoxyribosides.

Summary. (1) Administration of carbon tetrachloride increased the serum level of vit. B_{12} of rats fed a casein diet. No similar increase was observed in rats previously offered a low vit. B_{12} diet. (2) Administration of carbon tetrachloride likewise increased the urinary excretion of parenterally administered vit. B_{12} or of absorbed vit. B_{12} following oral administration. (3) Physiological significances of these findings are discussed.

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Interrelationship of Murexine, Dihydromurexine and Human Cholinesterases.* (22991)

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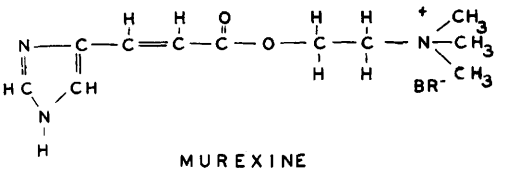
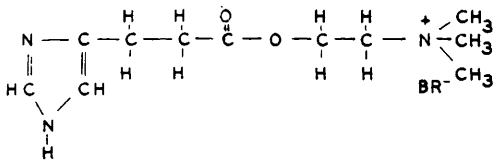
It has been shown by Vincent and Julien (1) and later by Erspamer(2) that extracts from the hypobranchial glands of *Murex trunculus* contain several pharmacologically active agents. One of these, murexine (Mur) was identified as (β-(4-Imidazolyl)acrylyl) choline or the choline ester of urocanic acid (3).† The compound was first synthesized by Pasini *et al.*(4). Mur has a nicotinic effect on the autonomic ganglia and blocks myoneural transmission in laboratory animals(5) and man(6). Dihydromurexine (DhMur), (β-(4-Imidazolyl)propionyl) choline, the reduced derivative of Mur. (Fig. 1) is about 4 times as potent as Mur, at the autonomic ganglia and at the neuromuscular



MUREXINE



DIHYDROMUREXINE

FIG. 1. Structural formulae of murexine and dihydromurexine.

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† Murexine, dihydromurexine and urocanic acid (impurity less than 1%) were synthesized by Hoffmann-LaRoche, Nutley, N. J. and supplied by Dr. Leo A. Pirk.