17300. Protective Effect of Vitamin B_{12} Upon Hepatic Injury Produced by Carbon Tetrachloride.*

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The relation between vitamin B_{12} and ribonucleic acid compounds is, at present, being investigated.[1-4] Vitamin B_{12} has been reported to prevent the disappearance of liver basophilia which occurs when soybean oil meal is the sole source of protein in the diet of rats.[5] This basophilic hue of the cytoplasm of liver cells is due to the presence of ribonucleic acid compounds.[6] In addition, it has been shown that ribonucleic acid decreases or disappears in the initial stages of carbon tetrachloride intoxication,[7,8] as well as in other types of hepatic injury.[9,10] These findings suggested an investigation of the effect of vitamin B_{12} upon acute hepatic injury due to CCl_4 intoxication in rats as an easily reproducible example of hepatic injury.

Methods. A total of 69 female rats, 2 to 3 months old, on a well balanced diet with a relatively high protein content received intraperitoneally 1 cc of mineral oil containing 0.033 cc of CCl_4 per 100 g of body weight. Of these, 38 received 15 μg of vitamin B_{12}‡ per 100 g of body weight, divided into 4 doses, injected subcutaneously 72, 48, and 24 hours prior to, and simultaneously with, the injection of the CCl_4. As controls 7 normal rats of the same strain and age were used for chemical and histological examination of the liver and 25 for the bromsulphalein (BSP) retention test.

To evaluate the degree of liver damage 48 hours after the administration of the CCl_4 (the time of the most marked deposition of fat[11,12]) the following tests were done:

1. Immediately before sacrificing the animals, the serum BSP concentration was determined[13] in 1.5 cc of blood obtained by heart puncture 30 minutes after intraperitoneal administration of 5 mg of dye per 100 g body weight. The results were expressed in mg of BSP per 100 cc of serum.

2. The total liver lipids were determined after extraction with absolute alcohol and reextraction of the alcoholic extract with ether according to Bloor.[14] The results were recorded both in grams per 100 g of liver and milligrams per 100 g of body weight.

3. The amount of fat was graded from 0 to 3+ in Sudan III stained slides of formalin-fixed material. As the fat deposition starts around the central vein, the amount is reflec-

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‡ Vitamin B_{12} concentrate (Rubramin) in ampuls containing the equivalent of 15 μg of vitamin B_{12} was generously supplied by E. R. Squibb & Sons, New York.

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TABLE I.
Bromsulphalein Retention, Total Liver Lipids, Amount of Fat and Ribonucleic Acid Depletion with Standard Deviation in Normal Rats and Rats Intoxicated with CCl_4 with and without Previous Administration of Vitamin B_{12}.

No. of rats	Administration of		BSP retention in mg %		Total liver lipids				Histologic fat grade		Ribonucleic acid depletion grade	
					g % liver		mg % body wt					
	Vit. B_{12}, μg/100 g	CCl_4, cc/100 g	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
25			0.29	0.26								
7					4.8	1.4	166	24.2	0.15	0.28	0.65	0.75
31		0.033	2.12	0.97	8.8	1.4	366	61.6	2.00	0.70	3.60	0.87
38	15	0.033	0.90	0.60	6.2	1.4	236	41.5	0.83	0.60	2.50	0.83

ted in the distribution. Therefore the criteria used can simply be described as follows: 0 - absence of fat; 1+ – fat deposition in the central zone; 2+ – fat deposition in the central and intermediary zone; 3+ – diffuse fat deposition.

4. The ribonucleic acid depletion of the cytoplasm was graded from 0 to 5+ in methyl green-pyronine stained slides of Carnoy-fixed material. The specificity of the stain for ribonucleic acids was confirmed by digestion with ribonuclease.[15] Again the distribution was graded as a measure of the intensity of the depletion as follows: 0 — no depletion; 1 + – partial depletion of the central zone; 2 + – marked depletion of the central zone; 3 + – depletion of the central and intermediary zone; 4 + – depletion of the central and intermediary zone and partial depletion of the peripheral zone; 5 + – diffuse depletion.

In addition, hematoxylin-eosin stained sections were studied for confirmation.

Results. The rats intoxicated with CCl_4 showed the characteristic histologic alterations.[11] In comparison to the controls, there was increased deposition of lipids (demonstrated histologically and chemically), increased BSP retention and ribonucleic acid depletion. Intoxicated rats which previously had received vitamin B_{12} showed significantly less histologic alterations than the non-treated, CCl_4 intoxicated rats. In the treated group, the increase in BSP retention and in fat deposition was only one-third and the ribonucleic acid depletion about one half that observed in the non-treated group. In general, the 4 criteria studied quantitatively varied in a parallel fashion in individual animals.

Discussion. The previous administration of vitamin B_{12} protects rats from CCl_4 intoxication to a considerable degree. This is evidenced by 4 criteria selected for quantitative studies, 2 of them histologic and 2 of them biochemical. On the average, the vitamin B_{12} treated animals showed approximately one-third of the changes observed in non-protected rats. Although the criteria selected concerned different hepatic functions, there was marked parallelism in the degree of alterations in the individual animals, the intoxicated as well as the protected. The dose of vitamin B_{12} used was excessively large when compared to the effective dose in anti-anemic therapy. However, the smallest effective dose in hepatic injury is yet to be determined. The dose in absolute weight is much smaller than that of any other substance used in lipotropic or protective therapy of the liver.[16]

The mechanism of action of vitamin B_{12} in ameliorating the effect of CCl_4 intoxication upon the liver is not yet fully elucidated. Since the animals were on a balanced diet, a primary lipotropic effect, comparable to that of choline and methionine, is doubtful; the latter occurs, if at all, only in animals on low protein diet.[17] If, as originally assumed, the protective effect through the ribonucleic acid metabolism could be further confirmed, vitamin B_{12} therapy would be promising in other types of hepatic injury. It is possible

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that the influence upon nucleic acids is the basic function of this vitamin. This may be the mechanism of its action as the maturation factor of erythrocytes.

Summary. Administration of 15 μg per 100 g body weight of vitamin B_{12} to rats preceding acute CCl_4 intoxication inhibits the development of histologic changes, especially fatty metamorphosis and depletion of ribonucleic acid. In addition, there is less deposition of lipids determined biochemically and less BSP retention than in the intoxicated controls. These results are tentatively related to an effect of vitamin B_{12} on cytoplasmatic ribonucleic acid, which has been shown to disappear early in hepatic injury.

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17301. Persistence of Desoxycorticosterone-Induced Hypertension in the Nephrectomized Rat.

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The administration of desoxycorticosterone acetate (DCA)* provokes hypertension characterized by persistent elevation of systolic and diastolic blood pressures. This has been observed in rats[1-4] and in dogs.[5] Elevation of blood pressure to hypertensive levels in patients with Addison's disease,[6,7] and also aggravation of essential hypertension[8,9] have been observed following DCA treatment.

Animals treated with this hormone develop nephrosclerosis[1,10] and periarteritis nodosa,[11] vascular lesions which are frequently observed in hypertension induced experimentally by various surgical interventions on the kidney.[12] This raises a question as to the mechanism whereby DCA exerts its effect. The most commonly encountered views are either that DCA causes the development of hypertension which then results in widespread vascular damage, or that the vascular tissue is affected first with hypertension developing as a sequel to interference with intrarenal haemodynamics.[5,13,14]

Whether or not the hypertensive effect of DCA is mediated via the kidneys is a question of fundamental importance in the interpretation of experimental results involving this hormone and is also intimately concerned with the nature of hypertension accompanying adrenal cortical hyperfunction.

Hypertension resulting from the application of a constricting ligature to one renal artery subsides completely and promptly to normotensive levels following total nephrec-

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