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EFFECT OF CARBON TETRACHLORIDE ADMINISTRATION ON THE METABOLISM OF VITAMIN B_{12} IN THE RAT

II. EXCRETION OF RADIOACTIVE VITAMIN B_{12}

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It has been demonstrated in the previous report that the plasma B_{12} level of rat is elevated at the height of carbon tetrachloride intoxication, and returns to the normal level in a due course of time. It was also postulated that this increase of vitamin B_{12} is brought about as a result of liver cell injury, probably by the liberation of this vitamin from the damaged cells. In order to ascertain such a hypothesis, further experimentation was required to study the deranged pattern of B_{12} metabolism in carbon tetrachloride poisoning.

Radioactive vitamin B_{12} (B_{12}*) has been widely used as a tool to follow the metabolism of this vitamin in animals as well as in humans. Since the specific activity of radioactive B_{12} has recently been raised by the improvement of its production, it is now possible to detect and measure a minute quantity of B_{12}, even less than one millimicrogram. One of the advantages of using such highly rabioactive B_{12} is that the experimental conditions, particularly the dose of B_{12} to be administered as tracer, can be made very similar to the quantity that is normally metabolized.

In this study, therefore, Co^{60} labeled vitamin B_{12} with a high specific activity was employed to investigate into the dynamic phase of vitamin B_{12} metabolism under the influence of CCl_4 in the rat. The results of such a study are presented in this report.

EXPERIMENTAL

Adult male rats of *McCollum* strain were used throughout the experiments. They were raised on the stock diet.

Administration of CCl_4 was done in the same way as in the previous report.

Administration of radioactive vitamin B_{12}: Aqueous solution of Co^{60}-lebeled vitamin B_{12} with a specific activity of approximately 1000 μc per mg was used. B_{12}* was administered to rats by either subcutaneous injection or oral feeding through a stomach tube.

Determination of radioactive B_{12}: The rats were kept in individual metabo-

lism cages during the experiment for collection of urine and feces. Urine was collected for two 24 periods and one 48 hour period following the administration of B_{12}*. Urine and washings were pooled and condensed down to 50 ml in a 100cc bottle and counted in a well-typed γ-scintillation counter. Feces was collected for 4 days, homogenized with concentrated sulfuric acid, made up to the volume of 50 ml and counted for radioactivity. The details of this technique for the measurement of γ radiation of B_{12}* were described elsewhere[1]

The design of individual experiments was as follows:

Experiment 1: Ten rats, five in each group, were given 20 mγ B_{12}* by subcutaneous injection three times in four days. On the fifth day, CCl_4 at the level of 0.05 ml per 100 gm body weight was given to one group. The urinary excretion of radioactivity was measured from the time of CCl_4 administration. The B_{12}* injected prior to CCl_4 administration was considered to have been fixed in tissues by the time of CCl_4 treatment.

Experiment 2: In this experiment, CCl_4 was first given at the dose of 0.05 ml per 100 gm body weight, followed by administration of a single dose of 20 mγ B_{12} by subcutaneous route 24 hours later. The urine collection was started from the time of B_{12}* Administration.

Experiment 3: In order to administer vitamin B_{12} under natural conditions, B_{12}* was given in this experiment by oral route, 50 mγ in 1 ml solution through a stomach tube, 24 hours after CCl_4 administration. The urinary excertion as well as fecal excretion were measured for 4 days following oral administration.

RESULTS AND DISCUSSION

The results of each experiment are presented in the table with a corresponding number. The radioactivity recovered in the urine, feces and organs were calculated in terms of mγ of B_{12}* originally administrated.

The deta shown in table 1 demonstrate that urinary excretion of radioactive B_{12} was increased by CCl_4 administration over the control receiving no CCl_4. The increased urinary excretion of B_{12}* lasted at least 4 days, since a difference was obtained not only in the first 24 hour urine but also in 48–97 hour specimen. Separate studies[2] on oral administration of B_{12}* indicated that 20–30 mγ out of a single dose of 50 mγ are absorbed under normal conditions in rats. Hence, a single injection dose of 20 mγ which was used in this experiment should be considered a physiological amount of B_{12} an adult rat can handle. It will also be reasonable to expect that most of the B_{12} given prior to CCl_4 administration had been fixed in the tissue by the time of CCl_4 injection. The increase of urinary excretion in the experimental animals, therefore, seems to be due to B_{12} derived, probably liberated, from the damaged tissue.

It is of interest to note that none of the CCl_4 treated animals died and that

they did not lose weight at the end of the experiment. Remarkably high figures in fecal excretion rate of the injected B_{12}* in both groups seem to be a new finding which has never been reported elsewhere.

TABLE I

Excretion of radioactive B_{12} parenterally administered prior to CCl_4 injection.

Dose of CCl_4 (ml/100 gm Body Wt)	Body Weight (gm)		Urinary Excretion of B_{12}*(mγ)				Fecal Excretion (mγ)	Organ B_{12}* Content (mγ)
	Initial	4 Days after CCl_4 Adminis.	0–24 Hours	24–48	48–96	Total in 4 Days	in 4 Days	Liv. Kid.
0	331	350(+19)	0.47±0.05	0.41±0.04	0.41±0.05	1.30±0.09	6.60±0.15	4.95 4.61
0.05	349	355(+ 6)	0.90±0.04	0.67±0.04	0.71±0.05	2.29±0.11	6.25±0.25	5.21 4.97
t			6.7	6.8	4.2	5.1		

Five rats in each group.

When B_{12}* was given 24 hours after CCl_4 injection, a similar difference was demonstrated as shown in table II. However, the difference in urinary excretion was obtained only in the first 24 hour period in contrast to the previous experiment. One of the plausible explanations will be that the damaged liver temporarily loses its normal affinity for B_{12} to result in prolonged circulation of free B_{12} in the blood and subsequent overflow of this B_{12} into the urine. This finding

TABLE II

Excretion of radioactive vitamin B_{12} parenterally administered after CCl_4 injection.

Dose of CCl_4 (ml/100gm Body Wt.)	Urinary Excretion of B_{12}* (mγ)				Fecal Excretion (mγ)
	0–24 Hours	24–48	48–96	Total in 4 Days	in 4 Days
0	1.04±0.05*	0.24	0.36	1.64±0.07	2.51±0.15**
0.05	1.46±0.08*	0.27	0.23	1.98±0.17	1.94±0.15**

* t=4.4; ** t=2.7

might therefore indicate another possible mechanism in which the B_{12} binding capacity of the liver influences the uninary excretion rate. A greater excretion rate of B_{12}* in the feces than in the urine is the same finding as in the previous experiment.

In the last experiment, the absorption of orally administered B_{12} as well as the

urinary excretion of the absorbed B_{12} were measured (Table III) The fecal excretion of the unabsorbed B_{12}* was higher in CCl_4 treated group, indicating lower intestinal absorption rate in CCl_4 poisoning. The nrinary excretion was greater in CCl_4 treated rats, although CCl_4 treated group absorbed less B_{12}. The difference in urinary excretion between the two groups could have been even greater if the same amount of oral B_{12}* dose was absorbed. The difference in organ B_{12}* content will probably be correlated to the different rates of intestinal absorption.

TABLE III

Excretion of radioactive B_{12} administered orally following CCl_4 injection.

Dose of CCl_4 (ml/100 gm Body Wt)	Body Weight (gm)			Urinary Excretion of B_{12}* (mγ)				Fecal Excretion (mγ)	Onrgan B_{12}* Cotent (mγ)	
	Initial	24 Hous After CCl_4	4 Days After CCl_4 Inject.	0–24Hours	24–48	48–96	Total in 4Days	in 4 Days	Liver	Kidney
0	398	385(-13)	399(+ 1)	0.16±0.04*	0.24	0.46	0.86±0.06	19.5±1.8**	4.52	3.35
0·05	402	357(-45)	376(−26)	0.38±0.03*	0.26	0.44	1.08±0.13	24.8±1.2**	3.64	2.71

* t=4.3, ** t=2.5

Unlike the first experiment, two of seven rats receiving CCl_4 died in this experiment and the survived animals lost weight considerably.

From the evidence presented in this paper, it is plausible to hypothesize that CCl_4 causes damage to the liver cells which have high concentration of B_{12}, and subsequent liberation of tissue B_{12} into the blood stream. The increased level of B_{12} in plasma, probaly of free form, further results in an increase of urinary excretion. The radioactivity retained by the liver was, however, found to be much higher than the urinary excretion. Therefore, even though the main mechanism for tht increase of B_{12} level is due to the liberation of B_{12}, the quantitative relation of this mobilizable B_{12} to the total B_{12} seems to be very small. It is not likely that the liver cell breakdown products of high B_{12} concentration are transported into the liver capillaries as was discussed in the preceding report. Probably, the histological structure of the liver would prevent direct transfer into the blood of the cell components such as protein to which B_{12} is loosely bound.[3]

Besides this mechanism, the affinity of the liver for B_{12} also seems to participate to some extent in this metabolism pattern. Under normal circumstances, there is a constant shift of B_{12} in the body from the intestine to organs and from organs to organs as pointed out by *Chow et al.*[4] More of the B_{12} coming into the blood will be spilled over into the urine if the liver does not take up blood B_{12} rapidly.

A very similar feature has been known in the metabolism of amylase. The increase of plasma level and urinary excretion of amylase in acute pancreatic

injuries has been described in the text-book and is of diagnostic value. It is not at all unlikely that acute liver injury in humans also results in increased levels of B_{12} in the blood and urine by the mechanism postulated This hypothesis seems to be supported by *Lear et al* who reported unusually high serum B_{12} levels in some of the cirrhotic patients but failed to explain it.

The last experiment demonstrated a decrease in oral absorption of B_{12} by CCl_4 treated rats. Although there is no evidence that this decrease is the result of liver injury itself, this finding is in agreement with the author's observation that patients with liver diseases did not respond to B_{12} oral tolerance test[6] as frequently as the control normal subjects.

There have been several papers which suggested the effect of vitamin B_{12} to protect the liver from experimental injury. Whether the difference in mortality of animals between experiment 1 and the other experiments is due to the effect of multiple doses of B_{12}* remains as yet to be studied.

It is also interesting to note that after parenteral administration of B_{12}*, the fecal excretion of radioactivity was far greater than the urinary excretion as shown in tables I and II. The difference in fecal excretion rate between CCl_4 treated and untreated groups in experiment 2 might indicate the role of the liver in excretion of B_{12} through the bile-feces route which was predicated by *Okuda* and *Gräsbeck*[7] lately.

Conclusion

When radioactive B_{12} was given to rats shortly before and after carbon tetrachloride administration, urinary excretion of radioactive B_{12} was increased. Absorption of orally administered radioactive B_{12} as well as fecal excretion of radioactive B_{12} was decreased in carbon tetrachloride treated rats. Feces seems to be an important excretion route of vitamin B_{12}, since more of the parenterally administered radioactive B_{12} appeared in the feces than in the urine.

The mechanism involved in carbon tetrachloride induced derangemeat of B_{12} metabolism was discussed, and its entire pattern was compared to that of amylase in pancreatic injury.

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