Effects of Intrinsic Factor on the Adsorption of Vitamin B_<12> to Organs other than Intestine I). Intrinsic Factor-mediated Vitamin B_<12> Adsorption to Kidney and Placenta

Tohoku Journal of Agricultural Research
Vol. 24, No. 2, August 1973
Printed in Japan

Effects of Intrinsic Factor on the Adsorption of Vitamin B_{12} to Organs other than Intestine

I). Intrinsic Factor-mediated Vitamin B_{12} Adsorption to Kidney and Placenta

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(Received May 28, 1973)

It was found that vitamin B_{12}, which was administered orally or parenterally, deposits in the kidney and placenta in a higher concentration than in liver or blood. Accordingly, the effects of gastric intrinsic factor on the adsorption of vitamin B_{12} to such organs were investigated. Consequently, as in the case of the small intestine, the adsorption of vitamin B_{12} was remarkably enhanced by the addition of intrinsic factor source. It was also recognized that reincubation in the physiological saline solution added with ethylenediaminetetraacetic acid (EDTA) added caused a release of a large amount of vitamin B_{12} from organs with previous vitamin B_{12} adsorbtion, and that therefore the presence of bivalent cations was required for adsorption.

Pendl and Franz[1] investigated the maturation of erythrocytes obtained from pernicious anemia patients *in vitro*, and found that the addition of normal human serum to the incubation medium caused the conversion of megaloblast, which is a characteristic of the pernicious anemia, to normoblast. Alpha-gloublin was known to be particularly effective in normal human serum. Other investigators also recognized a similar phenomena. These observations seem to lead to the conclusion that alpha-gloublin contains a carrier which binds vitamin B_{12}, transfers it to peripheral tissues and plays roles, just as those of intrinsic factor (IF) in the small intestine, when the vitamin is taken up by the cells. Recently, the carrier in the serum has been called transcobalamin and, the studies on its properties and purification are under way (2, 3).

We found that a fairly large amount of the vitamin B_{12} administered orally to pregnant rats or mcie moves into the embryos (Table 1). It is well known that

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embryos obtain all of their needs through the placenta from their mother's body. Therefore, our findings mentioned above suggest that the placenta is able to take up the vitamin B_{12} which is bound to a carrier protein in the mother's blood and actively send it into the embryos.

It was also observed that the kidney takes much of the vitamin B_{12} which was injected intraperitoneally with or without IF or which was injected into the intestinal loop with IF source (Tables 2 and 3).

Then, it was investigated whether or not the adsorption of vitamin B_{12} to the placenta or kidney, which are able to uptake much vitamin B_{12} bound to a carrier protein *in vivo*, can be enhanced by the addition of gastric IF *in vitro*. It was also of interest whether or not the carrier protein in the blood had some functions in common with gastric IF.

Materials and Methods

Wistar strain albino rats and DD strain albino mice fed on commercial diets (NMF; product of Oriental Yeast Co. Ltd., and F-1; product of Funabashi Farmstead Co. Ltd.) were used throughout the study. The smear test was applied to know on what day the animals became pregnant. About five thousands $\mu\mu$g of ^{57}Co-vitamin B_{12} per 100 g body weight of the animals, namely about 15000 $\mu\mu$g for the rats and about 1500 $\mu\mu$g for the mice, were administered in three divided doses for three days beginning on the seventeenth day of gestation. The vitamin was dissolved in 0.6 ml of physiological saline solution and was fed by stomach tube. The administration of the vitamin in to the intestinal loop was performed about the same as the method described by Okuda (4). Intraperitoneal administration was carried out as follows. From 1500 $\mu\mu$g to 7500 $\mu\mu$g of ^{57}Co -vitamin B_{12} was dissolved in physiological saline solution or in physiological saline solution with IF added just enough to bind the vitamin to be dissolved. These solutions were injected intraperitoneally with syringes in a single dose or in several doses for a few days.

The Animals were sacrificed 24 hours after the last administration and the distribution of ^{57}Co-vitamin B_{12} was investigated. Organs and embryos were resected, weighed and then solubilized with 30% KOH solution. The solutions were filled up to the determined amount and in a part of them radioactivity of the ^{57}Co-vitamin B_{12} was measured in a well type scintillation counter. The placentae were placed in a counting tube *in toto* without solubilization and the radioactivity was measured.

The methods for the preparations of the IF source and tissue homogenates, and for the measurement of the amount of vitamin B_{12} adsorbed to the tissue homogenates were almost same as those of Castro and Glass (5, 6) with some modifications described elsewhere (7).

Results

1. *Distribution of Vitamin B_{12} Administered Orally or Intraperitoneally:*

As shown in Table 1, when vitamin B_{12} was administered orally to pregnant rats or mice, more than one thrid deposited in the embryos. The fairly large amounts of vitamin B_{12} found in the gastrointestine of mice accounts for the part which was not absrobed. The Vitamin B_{12} in the organs, exclusive of gastrointestine, amounts in all to 59.7% of that administered. This value is identical to that which has been found when vitamin B_{12} was administered to normal animals by mouth (4). But the amount of vitamin B_{12} in kidney of pregnant animals was less than that of normal animals.

TABLE 1. *Distribution of Orally Administered Vitamin B_{12} in Pregnant Rats and Mice*

Organs	Amount of vitamin B_{12} found in organs as per cent of oral dose (%)	
	Rats	Mice
Blood		1.7
Liver		2.2
Kidney		0.5
Spleen		0.1
Gastrointestine		17.8
Womb		2.4
Placenta	5.6	16.0
Amniotic fluid	0.1	0.4
Embryo	35.3	36.4

About five thousands $\mu\mu$g of ^{57}Co-vitamin B_{12} per 100 g body weight of the animals, namely about 15000 $\mu\mu$g for the rats and about 1500 $\mu\mu$g for the mice, were administered in three divided doses for three days beginning on the seventeenth day of gestation. The vitamin was dissolved in 0.6 ml of physiological saline solution and fed by stomach tube. Twentyfour hours after the last administration the animals were sacrificed and the distribution of ^{57}Co-vitamin B_{12} was investigated.

Table 2 shows the distribution of intraperitoneally administered vitamin B_{12} with or without IF. Most vitamin B_{12}, about one fifths, was found in the kidney and followed that found in the liver. The distribution of vitamin B_{12} was identical whether it was injected with IF or without IF. This fact suggests that the IF which was injected with vitamin B_{12} intraperitoneally had no effect on the metabolism of vitamin B_{12} and was probably treated as a contaminant. Moreover no difference was seen between the distribution of vitamin B_{12} after it was administered in a single dose and that after it was administered in several divided doses for a few days. Thus it was indicated that the period required for vitamin B_{12} to attain equilibrium among the tissues is not so long. The fact that a considerable amount of the vitamin B_{12} which was administered intraperitoneally

TABLE 2. *Distribution of Intraperitoneally Administered Vitamin B_{12} with or without Intrinsic Factor*

Materials	Amount of vitamin B_{12} as per cent of intraperitoneal dose (%)			
	Administered in a single dose		Administered in several doses	
	With IF	Without IF	With IF	Without IF
Urine	0.6	1.4	0.9	1.5
Fece	1.3	2.4	0.8	2.3
Liver	4.9	5.8	9.0	8.3
Kidney	19.7	19.2	25.5	26.0
Spleen	1.2	0.7	0.0	0.4
Womb	0.9	0.7	0.6	0.6
Gastrointestine	5.5	5.7	3.6	4.5
Cecum and Colon	4.9	6.4	3.5	3.5
Blood	0.5	0.8	0.7	0.6

From 1500 $\mu\mu$g to 7500 $\mu\mu$g of ^{57}Co-vitamin B_{12} was dissolved in physiological saline solution or in physiological saline solution with IF added just enough to bind the vitamin to be dissolved. These solutions were injected intraperitoneally with syringes in a single dose or in several doses for a few days. Animals were sacrificed 24 hours after the last administration, and the distribution of ^{57}Co-vitamin B_{12} was investigated.

TABLE 3. *Uptake by Organs of Vitamin B_{12} Absorbed from Intestinal Loop*

Organs	Amount of vitamin B_{12} as per cent of that absorbed (%)
Liver	7.2
Kidney	12.7
Spleen	0.6
Blood	4.0

The Administration of vitamin B_{12} in the intestinal loop was performed nearly according to the method described by Okuda (4). The animals were sacrificed 24 hours after the administration, and the distribution of vitamin B_{12} was investigated.

moved into the gastrointestine, cecum, colon and fece, supports the view that the digestive tract is one of the main routes of vitamin B_{12} excretion (8).

In Table 3, the uptake by organs of vitamin B_{12} absorbed from intestinal loop with the aid of IF was shown. Absorption from the intestinal loop can be thought of as a model of typical intestinal absorption, in which the effects of food and other factors are omitted. The total amount of vitamin B_{12} absorbed from the intestinal loop equaled 57.8% of the amount injected into the loop, which agrees with the value obtained for normal animal vitamin B_{12} administration by mouth (4). Here again, the uptake of vitamin B_{12} by the kidney was the greatest and that by the liver second.

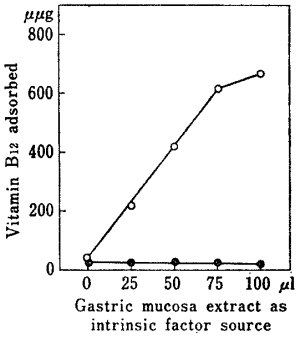

Fig. 1

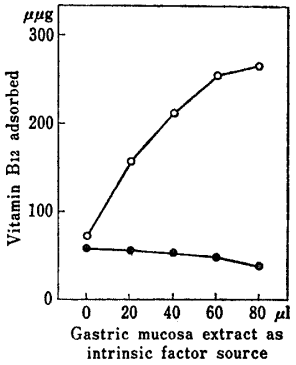

Fig. 2

FIG. 1. IF-mediated vitamin B_{12} adsorption to kidney homogenate and the effect of EDTA washing.
Vitamin B_{12} adsorbed with the aid of IF ○——○, vitamin B_{12} remaining after tissue homogenate adsorbed with vitamin B_{12} was washed with physiological saline solution added with EDTA ●——●. About 200 mg of kidney in wet weight was used in each test.

FIG. 2. IF-mediated vitamin B_{12} adsorption to placenta homogenate and the effect of EDTA washing.
Vitamin B_{12} adsorbed with the aid of IF ○——○, vitamin B_{12} remaining after tissue homogenate adsorbed with vitamin B_{12} was washed with physiological saline solution with EDTA added ●——●. About 100 mg of placenta in wet weight was used in each test.

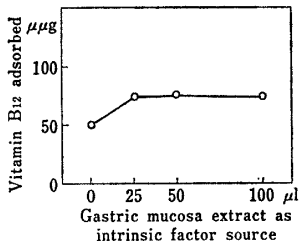

FIG. 3. IF-mediated vitamin B_{12} adsorption to kidney slice.
Kidney slices with a thickness of 0.1 mm were placed in test tubes to attain a wet weight of about 200 mg.

2. *Action of Gastric IF on the Adsorption of Vitamin B_{12} to the kidney and Placenta*:

As shown in Fig. 1, the amount of vitamin B_{12} which was adsorbed to the kidney homogenate *in vitro* increased in proportion to the increasing amount of gastric mucosa extract added as the source of IF. After the tissue homogenate adsorbed with vitamin B_{12} was washed with physiological saline solution with EDTA added, the vitamin B_{12} which had been adsrobed with the aid of IF was completely detached from the tissue homogenate. These results resemble those obtained when the adsorption of vitamin B_{12} to intestinal mucosa homogenate was investigated (7). Also in the case of placenta homogenate, similar phenomena were observed (Fig. 2).

Fig. 3 shows the effects of IF on the adsorption of vitamin B_{12} to kidney slice.

In this experiment, kidney slice with a thickness of 0.1 mm was used instead of kidney homogenate as a receptor for IF-vitamin B_{12} complex. Adsorption of vitamin B_{12} was enhanced by the addition of IF only in a limited range, and the amount of vitamin B_{12} was by far less than that when kidney homogenate was used.

Discussion

In the present studies, it was found that gastric IF, which promotes intestinal absorption of vitamin B_{12} *in vivo* and stimulates vitamin B_{12} adsorption to intestinal mucosa homogenate *in vitro*, also enhances vitamin B_{12} adsorption to kidney and placenta *in vitro*. FI-mediated vitamin B_{12} adsorption to such organs, as in the case of intestine, needed the presence of bivalent cations.

Although the nature of transcobalamin which binds vitamin B_{12} in blood and mediates vitamin B_{12} uptake by peripheral tissues has not yet been clearly elucidated, the present studies indicated that IF and transcobalamin possibly occupy two common structures. One is of course the site which binds vitamin B_{12} and the other is the site which fits in receptors of organs. The possibility was also shown that the intestine reccptor of IF and that of organs such as kidney and placenta of transcobalamin have a very similar structure and function.

About the origin of transcobalamin, there have been three views. The first attributes the transcobalamin in or wholely to the IF which was absorbed with vitamin B_{12} through the intestinal mucosa. At the present, it is obscure whether this is true or not, because there has been contradictory information concerning the absorption of IF through intestinal mucosa. Some investigators showed results suggesting that IF cannot be absorbed (9–12). Some reported that IF can be found with its physiological activity in the cells of intestinal mucosa (13, 14). Other investigator exhibited results which can be considered to be one evidence of the absorption of IF through intestinal mucosa (15). The second view is that transcobalamin originates in internally secreted IF. According to this view, a part of the IF which has been synthesized in the chief cells of the stomach is not secreted into the lumen of the stomach but subjected to intracellular digestion and then released intravesculary to form transcobalamin (16, 17). The third considers that transcobalamin is synthesized in the liver (18, 19). We have obtained experimental results which seem not to support the view that transcobalamin takes its rise in IF absorbed through intestinal mucosa.*

The Data shown in Table 2 indicated that the distribution of vitamin B_{12} was similar after it was administered in a single dose and after it was administered in several doses for a few days. This fact seems to suggest that the vitamin B_{12} was gathered in the kidney not only to be excreted, but with some physiological

* Yamada, S. and Kimura, S., Unpublished data.

significances.

The difference in vitamin B_{12} adsorption to the kidney homogenate and to the kidney slices may be due to the difference in the number of receptors which function *in vitro*. More receptors for IF-vitamin B_{12} complex which presumably localize in cell surface must be exposed to the reaction mixture in the homogenate system than in the slice system.

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