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

II). Adsorption of Vitamin B_{12} to Liver and Effects of Intrinsic Factor

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Summary

The circumstances of vitamin B_{12} adsorption to the liver and the effects of intrinsic factor (IF) on it were essentially different from those found in the case of the small intestine, kidney and placenta. Centrifugal fractionation of liver homogenate, which had been previously incubated with vitamin B_{12} in the presence of IF, suggested that the receptor for IF-vitamin B_{12} complex in the liver does not exist or that it exists only in the portion which remains in the supernatant after centrifuged at $6000 \times g$ for 10 minutes, as contrasted with the receptor of the small intestine which localizes in the fraction precipitated by the centrifugal force of $1300 \times g$.

In general, it has been considered that vitamin B_{12} absrobed from the small intestine first enters the liver via portal vein. This conception leads to the speculation that the liver is able to take up vitamin B_{12}, which presumably is bound to a certain kind of macromolecular originating in the IF absorbed through the intestinal mucosa or in *de novo* synthesized protein, from portal blood.

In the previous paper (1), we reported that vitamin B_{12}, which had been administered orally or parenterally, deposited in the kidney and placenta in higher concentrations than in the liver or blood. Moreover, the adsorption of vitamin B_{12} to the kidney and placenta was found to be remarkably enhanced by the addition of IF source *in vitro*.

Then interest has been taken in the effect of IF on the adsorption of vitamin B_{12} to the liver *in vitro*.

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Materials and Methods

Wistar strain albino rats fed on commercial diet (NMF; product of Oriental Yeast Co. Ltd.) were used as the experimental animals throughout the study. The methods for the preparation of stomach extract as an IF source and for the measurement of the amount of vitamin B_{12} adsorbed to the tissue were almost the same as those of Castro and Glass (2, 3) with the modifications described elsewhere (4). Gastric juice was collected by the methods of Shay *et al* (5, 6) and also used as an IF source in some experiments. The liver homogenate was prepared as follows: Immediately after the rats had been sacrificed by decapitation, the liver was resected and immersed in a cold physiological saline solution. It was homogenized in a Waring blendor at high speed for 60 sec, then centrifuged at 1300 $\times$g for 10 min. The resulting sediment was then washed with physiological saline solution by the disperse-centrifuge method and then resuspended in physiological saline solution. In some experiments, a liver slice with a thickness of 0.1 mm was used instead of liver homogenate.

In the experiments to investigate the distribution of vitamin B_{12} among the centrifugal fractions, liver homogenate and intestinal mucosa homogenate were prepared without any centrifugation. The tissue homogenate prepared in this way was added to the incubation medium containing ^{57}Co-vitamin B_{12} without or with IF source enough to bind the vitamin. The incubation was performed at 37.0°C for 1 hour with mechanical shaking. After incubation, the mixture was centrifuged at 1300 $\times$g for 10 min. and resulting precipitate was washed twice by repeating disperse-centrifuge in a physiological saline solution containing 10 mM of $CaCl_2$. The supernatant was then centrifuged at 3500 $\times$g for 10 min. and the precipitate was washed as above. The supernatant obtained was again centrifuged at 6000 $\times$g for 10 min. and the precipitate treated as above. Each fraction was assayed for ^{57}Co-vitamin B_{12} in a well type scintillation counter.

Results and Discussion

1. *Effects of IF on the Adsorption of Vitamin B_{12} to Liver*

Fig. 1 shows the effect of IF on the adsorption of vitamin B_{12} to liver homogenate. The amount of vitamin B_{12} adsorbed to liver homogenate did not increase in proportion to the increasing amount of IF source added. Moreover, the absolute quantity of vitamin B_{12} adsorbed to liver homogenate was far less than that found in the case of intestinal mucosa, kidney or placenta homogenate. The results obtained when liver slice was used in stead of liver homogenate were essentially the same (Fig. 2). Thus it was indicated that IF is unable to enhance vitamin B_{12} adsorption to liver.

We have reported (7) that in the small intestine of unweaned rats, vitamin B_{12} adsorption occurs independent of IF and so bivalent cations are not needed.

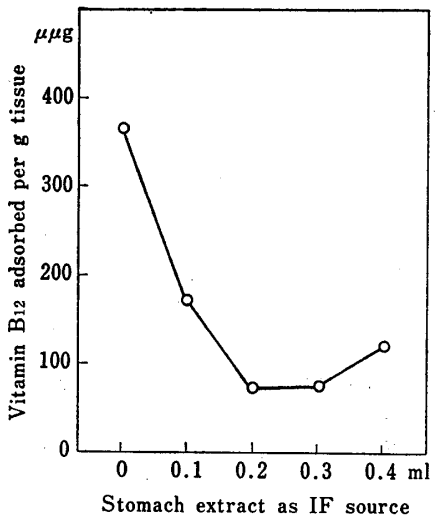

FIG. 1. The effect of IF on the adsorption of vitamin B_{12} to liver homogenate. Liver homogenate prepared from 50 mg wet tissue was incubated with vitamin B_{12} and varying amounts of IF source at 37.0°C for 1 hour with mechanical shaking. The results were calculated as $\mu\mu$g of vitamin B_{12} adsorbed per g tissue.

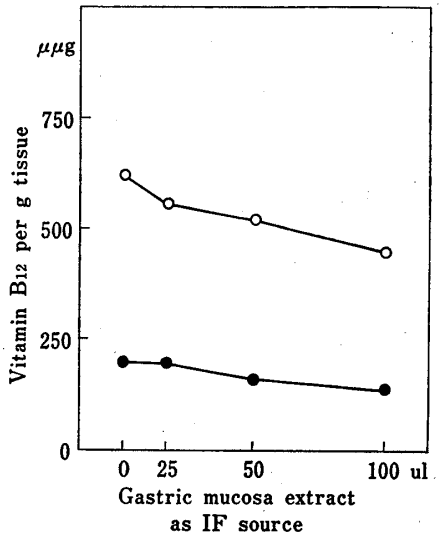

FIG. 2. The effect of IF on the adsorption of vitamin B_{12} to liver slice and the release of the vitamin after EDTA washing. Liver slice with a thickness of 0.1 mm was placed in each test tube to attain a wet weight of about 300 mg. The results were calculated as $\mu\mu$g of vitamin B_{12} per g tissue. Liver slice was incubted with vitamin B_{12} and varying amounts of IF source at 37.0°C for 1 hour with mechanical shaking, and the amount of vitamin B_{12} adsorbed was measured ○—○. Then reincubated in saline solution with EDTA added sufficiently to inactivate total Ca^{++} and Mg^{++} ions contained in incubation medium, and then the remaining vitamin B_{12} was measured ●—●.

Therefore, reincubating the intestine, which had once adsrobed vitamin B_{12}, in saline solution with ethylenediamine tetraacetic acid (EDTA) added did not cause a release of the vitamin. In the case of liver reported here, vitamin B_{12} adsorption was also independent of IF, but about two-thirds of the vitamin B_{12}

TABLE 1. *Distribution of Vitamin B_{12} among Centrifugal Fractions of Liver Compared with Intestine after Incubated with Vitamin B_{12} in the Presence or Absence of IF*

Fraction	Liver		Intestine	
	IF added (%)	IF not added (%)	IF added (%)	IF not added (%)
1300×g ppt.	0.3	0.7	5.6	3.3
3500×g ppt.	0.1	0.3	2.7	2.1
6000×g ppt.	0.1	0.3	1.2	1.0
6000×g sup.	99.6	98.7	90.5	93.6

Tissue homogenates prepared without any centrifugation were incubated with vitamin B_{12} in the presence or absence of IF source at 37.0°C for 1 hour with mechanical shaking. After incubation, the mixture was centrifuged by turns at 1300×g, 3500×g and 6000 ×g for 10 min. The amount of vitamin B_{12} in each fraction was measured, and expressed as per cent of total vitamin B_{12} added to incubation mixture.

once adsorbed was released by reincubation in saline solution with EDTA added. This phenomenon suggests the possibilities that the presence of bivalent cations are needed for IF-independent vitamin B_{12} adsorption in liver, and that there may be a system to adsrob vitamin B_{12}, which is different from those found in small intestine, kidney or placenta.

2. *Distribution of Vitamin B_{12} among Centrifugal Fractions of Liver Homogenate Compared with Intestinal Mucosa Homogenate*

In general, the amounts of vitamin B_{12} found in the precipitate fractions were very little (Table 1). But in comparing the amounts between each fractions it was elucidated that in the small intestine, the addition of IF source caused an increase of vitamin B_{12} in the fraction precipitated at 1300 ×g. In contrast, the addition of IF source rather decreased the amount of the vitamin in the liver fraction. The results suggest that a receptor for IF-vitamin B_{12} complex does not exist in the liver or exists in smaller portion from that in the small intestine, kidney or placenta. This may be related to the fact that the distribution of vitamin B_{12} among subcellular fractions in liver is different from that in kidney (8), although at present, we must leave this conclusion for a future study.

The IF source used in this experiment was prepared from the supernatant obtained after gastric mucosa extract was centrifuged at 6000 ×g for 30 min. (4), and so the supernatant fraction in this experiment contains not only free vitamin B_{12} but also IF-bound vitamin B_{12} which was not adsorbed to the precipitate fraction.

In the past, assay procedures for IF activity were devised by Herbert (9) and by Miller and Hunter (10) using liver slice. They indicated that hog IF stimulated vitamin B_{12} uptake by rat liver slice. Moreover, Miller and Hunter (11)

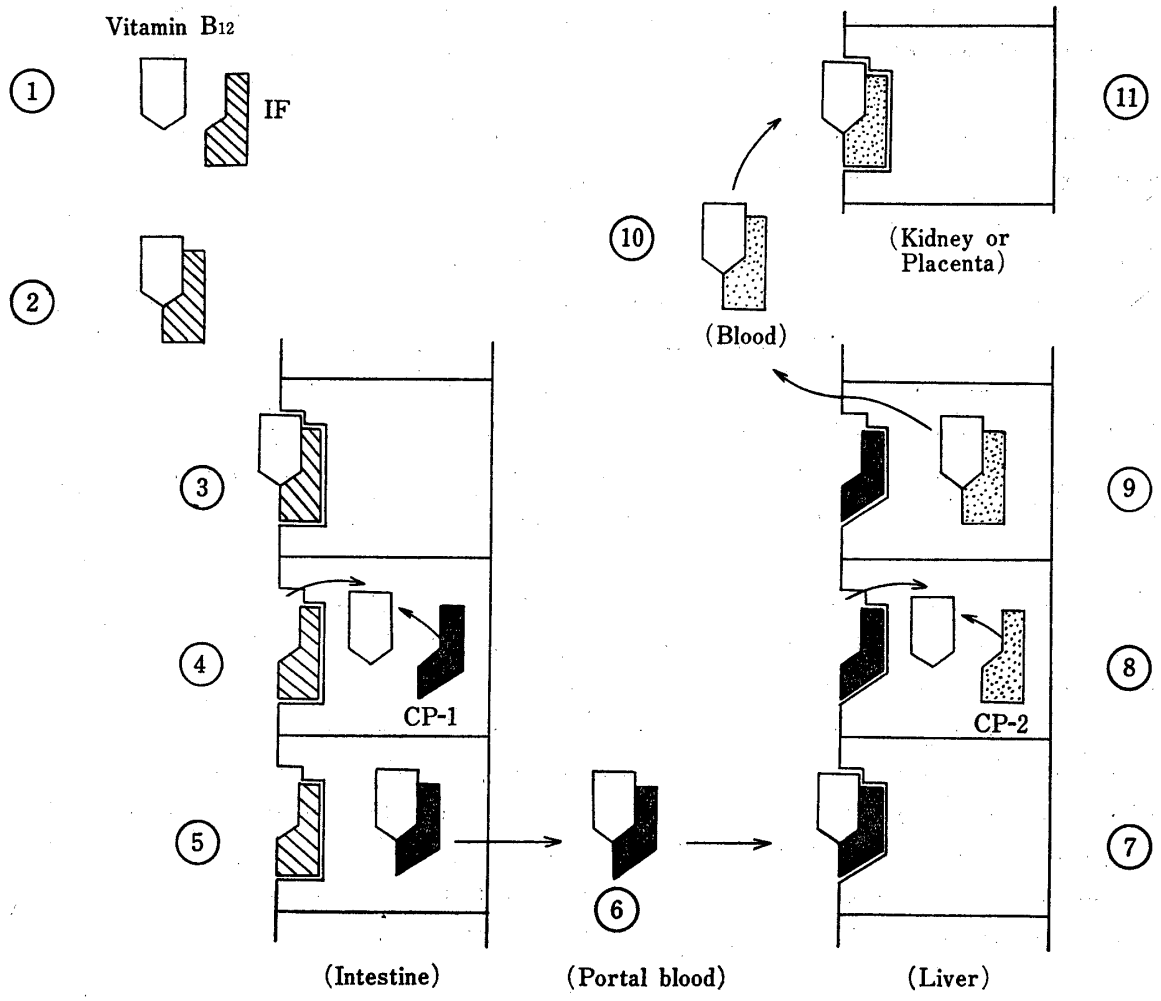

FIG. 3. A possible schematic model of vitamin B_{12} transport. Vitamin B_{12} from food and IF secreted from stomach join together and make complex ①–②. The complex attaches to receptor of intestinal epithelial cells ③. Vitamin B_{12} is absorbed through membrane and is combined with carrier protein (CP-1) synthesized in intestinal cells ④. The CP-1-vitamin B_{12} complex gets out of intestinal cells and enters portal blood ⑤–⑥. The complex attaches to receptor of liver ⑦. Vitamin B_{12} enters hepatic cells and is combined with another carrier protein (CP-2) ⑧. The CP-2-vitamin B_{12} complex gets out of the hepatic cells and enters blood vessel ⑨–⑩. The complex attaches to receptor of kidney or placenta which simulates that of intestine ⑪.

reported that hog IF enhanced vitamin B_{12} adsorption to rat liver homogenate. However, these results are somewhat questionable, because at present it is well known that hog IF is of no effect on rat intestine (12). In addition, these investigators observed only a slight enhancement of vitamin B_{12} uptake produced by a large amount of hog IF. A careful examination on data reported by Miller and Hunter (11) reveals obscurity of fractional operation after incubated with vitamin B_{12} and hog IF, and so it is unclear what fraction contains IF-vitamin B_{12} complex which is not adsorbed to rat liver homogenate.

Glass, in his review on IF (8), has pointed out that incompletion still remains with regard to investigations on the action of IF using liver slice or homogenate.

Putting this all together, one can say that liver slice or homogenate are considered to be unsuitable for an assay of IF activity.

The effect of gastric IF on the uptake of vitamin B_{12} by liver *in vivo* were reported by Okuda *et al* (13), by Okuda and Gräsbeck (14) and by Brody *et al* (15). They found that more vitamin B_{12} was taken up by liver when the vitamin was injected intraveneously with IF than when vitamin B_{12} alone was injected, and that the uptake was completed within a few minutes. These observations lead to the speculation that receptors for IF-vitamin B_{12} complex or, in some cases, for carrier portein-vitamin B_{12} complex exist in liver and that they are localized in the portion which is easily washed away from slice or homogenate during *in vitro* experiments.

It is also conceivable that vitamin B_{12} in portal blood is combined with a carrier portein, the function and structure of which are different from those of IF. The carrier protein-vitamin B_{12} complex is able to attach to the liver receptor, and those vitamin B_{12} gets into liver. When vitamin B_{12} goes out of the liver, it is combined with another carrier protein which was probably synthesized in the liver and plays roles when the vitamin is taken up by peripheral tissues such as kidney or placenta. Assuming that the function and structure of the second carrier protein simulate those of IF, the phenomenon that IF enhances adsorption of vitamin B_{12} to intestine, kidney and placenta but not to liver can be explained (Fig. 3).

References

1) Yamada, S., and Kimura, S., *Tohoku J. Agr. Res.*, **24**, 41 (1973)
2) Castro-Curel, Z., and Glass, G.B.J., *Proc. Soc. Exptl. Biol. Med.*, **112**, 715 (1963)
3) Castro-Curel, Z., and Glass, G.B.J., *Clin. Chim. Acta*, **9**, 317 (1964)
4) Yamada, S., and Kimura, S., *Tohoku J. Agr. Res.*, **23**, 167 (1972)
5) Shay, H., Komarov, S.A., Fels, S.S., Meranze, D., Gruenstein, M., and Siplet, H., *Gastroenterology*, **5**, 43 (1945)
6) Shay, H., Sun, D.C.H., and Gruenstein, M., *Gastroenterology*, **26**, 906 (1954)
7) Yamada, S., Yokoyama, Y., and Kimura, S., *Tohoku J. Exptl. Med.*, **104**, 215 (1971)
8) Glass, G.B.J., *Physiol. Rev.*, **43**, 529 (1963)
9) Herbert, V., *Proc. Soc.Exptl. Biol. Med.*, **97**, 668 (1958)
10) Miller, O.N., and Hunter, F.M., *Proc. Soc. Exptl. Biol. Med.*, **96**, 39 (1957)
11) Miller, O.N., and Hunter, F.M., *Proc. Soc. Exptl. Biol. Med.*, **97**, 863 (1958)
12) Wilson, T.H., and Straus, E.W., *Am. J. Physiol.*, **197**, 926 (1959)
13) Okuda, K., Wider, J.A., and Chow, B.F., *J. Lab. Clin. Med.*, **54**, 535 (1959)

14) Okuda, K., and Gräsbeck, R., *Proc. Soc. Exptl. Biol. Med.*, **111**, 808 (1962)
15) Brody, E.A., Estren, S., and Wasserman, L.R., *Proc. Soc. Exptl. Biol. Med.*, **120**, 436 (1965)