The Mode of Action of Intrinsic Factor in Vitamin B_{12} Absorption by Rat Intestine

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LITTLE is known about the mode of action of intrinsic factor in vitamin B_{12} absorption by rat intestine. In order to obtain some information about this problem, we have studied the intestinal and hepatic uptake of this vitamin at different intervals of time (from thirty minutes up to seventy-two hours after intubation) in adult white rats receiving 100 mμg. of Co^{60}-vitamin B_{12} by gastric intubation. Identical studies were made in totally gastrectomized rats, and the results of this comparative study are given here.

MATERIAL AND METHODS

The experiments were made with a rat gastric-mucosal extract, which was prepared by homogenizing the gastric mucosa in a Potter Elvejhem homogenizer in twelve parts of saline. The homogenate was centrifuged to give a clear solution. After repeated freezing and thawing of this solution, small amounts of insoluble matter could be separated. The final solution no longer contained insoluble material.

After severing the ligated part of the intestine and after thorough washing with saline, the whole intestine was dissolved in NaOH solution by heating in a water bath. The tube containing the intestine material was inserted into a well scintillation counter.

Total gastrectomy was performed according to the procedure of Machella and Griffith[1] and of Nieweg et al.,[2] as modified by Nabet et al.[3] The small intestine, not including the duodenum, was excised from the rat and submitted to three washings of thirty minutes each, with 50 ml. of Krebs glucose-phosphate medium at 37°C., in the Warburg apparatus. The purpose of the washings was to eliminate traces of vitamin B_{12} solution retained by capillarity between the intestinal villi. A preliminary control study afforded evidence that oxygen uptake was not altered by this procedure and that during these washings unbound vitamin B_{12} gradually disappeared.

Radioactivity measurements were performed on the Co^{60} salt, obtained by wet washing of the intestine, corrections being allowed for self-absorption and background countings. The use of a windowless flow counter ensured satisfactory recoveries with low counting rates.

RESULTS

The results obtained on normal and gastrectomized rats are given in Table I.

Two stages in the uptake of the vitamin B_{12} by rat intestine at various time intervals may be differentiated. In the first stage, there is a rapid increase of vitamin B_{12} uptake followed by a rapid* but incomplete decrease which slackens from the third to the seventy-second hour after oral administration.

The first stage (rapid increase) bears no relationship to the evolution of the hepatic Co^{60}-vitamin B_{12}. In the second stage, the slow decrease of intestinal Co^{60}-vitamin B_{12} is accompanied by an hepatic uptake of this vita-

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* In normal rats, Booth et al.[4] observed a similar maximal uptake of vitamin B_{12} in the small intestine (between fifteen minutes and two hours after the oral dose).

TABLE I

Intestinal and Hepatic Uptake of Co^{60}-vitamin B_{12} After Oral Intubation in Normal and Gastrectomized Rats

Time Elapsed (hr.)	Normal Rats	Gastrectomized Rats	Gastrectomized Rats Plus Intrinsic Factor
Intestinal Uptake of Vitamin B_{12} ($m\mu g.$)			
1/2	2.90 (1)	...	...
1	12.06 ± 3 (7)	5.66 (1)	...
3	1.46 ± 0.28 (5)	0.10 (1)	...
24	1.36 ± 0.43 (6)	0.105 ± 0.035 (2)	0.38 (1) 1.00 (1)
48	1.20 ± 0.25 (7)	...	...
72	0.80 ± 0.34 (5)	...	...
Hepatic Uptake of Vitamin B_{12} ($m\mu g.$)			
1/2	0.08 (1)	...	...
1	0.08 ± 0.004 (7)	0.12 (1)	...
3	0.77 ± 0.13 (5)	0.35 (1)	...
24	1.97 ± 0.47 (6)	0.13 ± 0.03 (2)	0.35 (1) 1.75 (1)
48	2.40 ± 0.25 (7)	...	...
72	2.72 ± 0.24 (5)	...	...

* Numbers between parentheses indicate the number of animals. The results are given with the standard error of the mean.

min in amounts of the same magnitude as those which disappear from the gut. These results visualize the moving of the vitamin B_{12} from the intestinal mucosa to the liver.

In gastrectomized rats, the first stage is also observed although to a lesser degree. In the second stage, extending from the third to the seventy-second hour, the previously absorbed Co^{60}-vitamin B_{12} disappears almost completely from the intestine.

From these results, it may be inferred that intrinsic factor does not interfere with the intestinal uptake of vitamin B_{12} but that it is essential for the retention of the absorbed vitamin in the gut.

In normal animals, a part of the absorbed vitamin B_{12} is retained in the intestine; from there it is removed to the liver. In gastrectomized rats, the intestine is no longer able to retain the vitamin and it flows back to the intestinal lumen from where it is excreted by the feces.

In the liver of gastrectomized rats, in contrast to what occurs in normal animals, no increase of Co^{60}-vitamin B_{12} may be noted. The amount of vitamin B_{12} in the liver remains low.

In gastrectomized animals, receiving intrinsic factor by oral administration, the intestinal as well as the hepatic uptake of vitamin B_{12} after twenty-four hours tends to become normal.

CONCLUSION

These results are in accord with the fact that the intestinal uptake of orally administered vitamin B_{12} is not dependent on the presence of intrinsic factor, but that its role is essentially that of retaining the already absorbed vitamin B_{12} in the intestinal mucosa and preventing it from overflowing into the intestinal lumen and being excreted by the feces.

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