The Uptake of Radioactive Vitamin B_{12} by Perfused Rat Intestine *In Situ*

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PREVIOUS work on gastrectomized rats has demonstrated that intestinal uptake of vitamin B_{12} takes place only when the intrinsic factor preparation of the rat is administered by intubation at the same time as vitamin B_{12}.[1] Under these circumstances, it is possible to observe vitamin B_{12} absorption by the intestinal mucosa in as little as twelve to fifteen minutes.

In a study of *in situ* absorption of vitamin B_{12} by the small intestine of normal rats (severed at the stomach and cecum by ligature), Holdsworth and Coates[2] in an eighteen to twenty-four–hour study observed the intrinsic factor-controlled absorption of this vitamin. This was demonstrated by the amount of vitamin B_{12} in the cecum and the liver and, more recently, in an intestinal loop.[3]

Upon examination of the results of these two experimental procedures, it appeared that, in the latter, a rapid intrinsic factor-dependent uptake of vitamin B_{12} might possibly occur. A short time experimental procedure on normal rats, analogous to that outlined by Holdsworth and Coates, but extending over no more than a two-hour period (the duration of a Nembutal® anesthesia), was likely to ensure satisfactory results.

This proved to be true. In the absence of intrinsic factor, the intestinal uptake amounts to an average of 1.7 per cent of the administered dose of vitamin B_{12}, and in the presence of intrinsic factor, the uptake increases to an average of 17 per cent.

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EXPERIMENTAL PROCEDURE

Perfusion

An intraperitoneal injection of Nembutal was given to rats weighing 200 to 300 gm. that had fasted twenty-four hours. Anesthesia occurred rapidly and lasted from one and a half to two hours. When necessary, a second injection with half the amount of Nembutal was given after one and a half hours to ensure complete anesthesia for at least two hours.

The lower portion of the abdomen was opened by a midline incision and the small intestine was ligated 2 to 3 cm. from the pylorus in the vicinity of the cecum. At the two ends, the intestinal wall was opened and a glass canula was inserted into each end. The abdomen was then closed with clips and the intestine was perfused with 100 ml. of 37°C. saline. The perfusion was controlled by a drip device and the saline preheated in a thermostat. A washing of about ten minutes was ample to deplete the intestine of traces of intrinsic factor.

After closing the rubber tubing with a clamp at each end, a mixture of 1 ml. of a known dilution of radioactive vitamin B_{12} and 1 ml. of rat gastric mucosa preparation was injected through the proximal rubber tubing into the intestinal lumen. This was followed by 1 ml. of saline. The solution remained in the intestinal lumen for forty-five minutes.

Following that time, a slow perfusion of 50 ml. of saline was started and continued over a period of thirty minutes to distribute the vitamin B_{12} intrinsic factor solution along the intestinal wall.

This procedure was followed by the removal of all traces of vitamin B_{12} remaining in the intestinal lumen or by capillarity in the interstices of the villi by three rapid washings per-

TABLE I
Intestinal Uptake of Co^{60}-Vitamin B_{12} Alone and in the Presence of Rat Stomach Mucosa

Per cent Vitamin B_{12} Alone (8 rats)	Per cent Vitamin B_{12} Intrinsic Factor (17 rats)	
2.48	28.58	7.42
3.44	5.83	18.80
2.14	13.10	34.70
0.88	4.86	12.50
0.63	31.70	21.30
1.23	19.10	22.20
1.13	26.30	22.36
1.93	14.45	26.90
		22.90
1.73 ± 0.293*	20.76 ± 8.94*	

* Average.

formed with 50 ml. of saline, each for a duration of five to seven minutes. All perfusion liquids were collected separately for control.

The rat was then sacrificed and the intestine was severed distal to the pylorus and removed from the animal. All superfluous mesentery and fat were removed. The intestine was opened along the line of mesenteric attachment, washed three times in 0.9 per cent sodium chloride and divided into small sections. The whole intestine was put into a glass tube, dissolved by heating the tube in a water bath in the presence of 1 ml. of concentrated sodium hydroxide, and then inserted into a scintillation well counter.

Correction was provided for background and the height of the solution in the tube. The results were calculated in per cent of the amount of administered vitamin B_{12} taken up by the intestine.

Solution of Co^{60} Labeled Vitamin B_{12}

Radioactive vitamin B_{12} was obtained by a fiftyfold dilution from a standard solution of Merck & Co., the concentration of which was 1 ml. = 21 mγ and the specific activity 1 μc. per ml. Each milliliter of this diluted solution had 13,000 counts per minute.

Intrinsic Factor

The supernatant of a centrifuged homogenate in saline of the mucosal part of rat intestine was used. The suspension was obtained by homogenizing twelve glandular parts of rat stomach in 60 ml. of saline, in a Potter-Elvejhem homogenizer. One milliliter of the supernatant corresponded to 0.2 rat stomach. Table I shows the results obtained.

COMMENTS

There is a considerable difference between the amount of vitamin B_{12} taken up by the rat intestine in the absence of intrinsic factor and in the presence of intrinsic factor. Whereas the scattering is small in the intrinsic factor-depleted intestine, it becomes large in the presence of intrinsic factor. The irregular gradient concentration of vitamin B_{12} and intrinsic factor along the intestinal wall, and the difficulty to warrant reproducibility, may, at least in part, be responsible for this irregularity. Even with such a wide scattering there is no example of overlapping, and it seems likely that with a statistical mean obtained on four animals, a clear-cut effect of intrinsic factor may appear.

We hope to quantitate the action of intrinsic factor in a more reproducible procedure.

CONCLUSION

The small intestine of normal rats, isolated by ligature and maintained *in situ* with all its connections, and depleted from intrinsic factor by perfusion, takes up added vitamin B_{12} in an amount which depends greatly on the added intrinsic factor.

A method is described whereby rat intrinsic factor activity of gastric mucosal extract may be demonstrated in a two-hour test on normal anesthetized rats.

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