I. E. M., Castro, D. P., Forattini, O. P., Rabello, E. X., Am. J. Trop. Med. & Hyg., 1965, v14, 156.

2. Roca-Garcia, M., J. Infect. Dis., 1944, v75, 160.

3. Casals, J., Whitman, L., Am. J. Trop. Med. & Hyg. 1960, v9, 73.

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Absorption of Vitamin B_{12} in a Rectal Suppository. (31527)

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Physiological absorption of vitamin B_{12} requires intrinsic factor (IF). In conditions where IF activity is lacking in the gastrointestinal tract, a therapeutically significant absorption of B_{12} occurs only when given in large quantities. Such absorption seems to be independent of IF, and constitutes the basis of the oral B_{12} therapy in massive dosages for pernicious anemia and agastric B_{12} deficiency. Rectal absorption of large doses of B_{12} in solution has been noted by Ungley(1) and Brigeau *et al*(2), but the results have been inconsistent. The mechanism for the non-IF-mediated absorption has not yet been elucidated, although a physical phenomenon like diffusion has been suggested, and some observations are rather against such a mechanism(3).

In this study, radioactive B_{12} in 2 extremely different doses was given to human subjects *per rectum* in suppositories and absorption was measured in comparison with oral administration of the same dosage. The demonstrated rectal absorption was further corroborated in animals.

Methods. Hospital patients with minimal disease not involving the intestine or the kidney were used. Shortly after a morning bowel movement, a suppository containing Co^{57}-cyanocobalamin ($Co^{57}B_{12}$)* was placed in the rectum to be retained for at least 4 hours. Two hours after the rectal administration, 1 mg of unlabeled B_{12} was injected subcutaneously for flushing and 24 hours' urine collection was made as in the Schilling test(4). In some subjects, feces were also collected for 3 days for measurement of the unabsorbed portion. For comparison, the regular Schilling test was carried out in a separate group using the same doses of $Co^{57}B_{12}$ in aqueous solutions *per os* and the same flushing procedure.

The suppository, weighing 2 g, consisted of the base of isococoa butter and a small amount of polyoxyethylene lauryl ether as the emulsifier. Aqueous $Co^{57}B_{12}$ with a specific activity of about 14 μc per μg was mixed with either lactose powder or crystalline B_{12}, dried *in vacuo*, pulverized, redried, and thoroughly pulverized $Co^{57}B_{12}$ powder was suspended in the suppository base melted at 50°C. Two extreme doses of B_{12}, 10 mμg and 2,000 μg, were used, but the radioactivity was so adjusted that each suppository contained 20,000-100,000 cpm as measured in a well type gamma scintillation counter.

The radioactivity in each suppository was measured in the same geometry before use, and the variation in count was found to be within 10% of the average. One suppository with about the average count was dissolved in 100 ml of warm 80% ethanol and quantitatively transferred to measuring tubes and bottles as the standards. Corrections were made from the differences in count of the used suppositories from the original standard suppository. Urine was condensed, feces were homogenized with water and radioactivities in aliquots were determined.

For the animal study, adult rabbits were used. A suppository, half in size and containing 1,000 μg $Co^{57}B_{12}$ was placed in the rectum, the anus was ligated for the prevention of ejection, and the animal was sacrificed 15 hours later. The contents of the colon after homogenization, as well as the

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TABLE I. Urinary Excretion of Radioactivity in 24 Hours Following Administration of 2 Doses of $Co^{57}B_{12}$, *per rectum* vs *per os*.

Dosage	Subjects	Sex	Age	Urinary excretion (%)	Fecal excretion (%)
2,000 μg *per rectum*	M.Y.	♂	27	1.70	95.5
	N.Y.	♂	51	1.66	91.6
	F.H.	♀	31	1.61	97.0
	S.S.	♂	41	.32	98.3
	T.H.	♂	29	1.17	96.8
	T.K.	♂	27	2.41	88.2
	K.N.	♂	33	1.41	
	Y.K.	♂	42	.98	
	S.O.	♂	28	.34	
	Y.M.	♀	38	1.86	
	M.S.	♂	36	.40	
	Y.S.	♂	31	.95	
	n = 12			Avg 1.15 ± .19	
2,000 μg *per os*	n = 7			Avg .39 ± .07	
10 mμg *per rectum*	M.T.	♂	28	.27	
	Y.U.	♂	37	.26	
	T.Y.	♀	25	.39	
	N.M.	♂	19	.15	
	S.K.	♂	44	.16	
	K.N.	♂	27	0	
	n = 6			Avg .21 ± .04	
10 mμg *per os*	n = 6			Avg 18.1 ±2.67	

liver and kidneys solubilized with 30% NaOH, were measured for radioactivity.

Results. Table I represents the urinary excretion after rectal administration of $Co^{57}B_{12}$ and flushing, in comparison with oral administration of the same dosage. There was a significant difference at the dose of 2,000 μg, and the rectal route was superior to the oral in terms of urinay excretion. In 6 subjects, fecal recovery of radioactivity was found to be about 90%. It was realized that under these conditions, the calculated net absorption was not accurate because the percentage recovery was close to 100 and a small percentage error in measurement would be exaggerated in the process of subtraction. In this respect, urinary excretion provides a better assessment of absorption.

Of interest was the finding that when the dose was reduced to 10 mμg, urinary excretion rate was also reduced in the case of suppositories, whereas in the oral administration, the percentage excretion was increased to more than 18 for the dose of 10 mμg from 0.39% for 2,000 μg.

The rabbit study likewise demonstrated that the rectally administered dose of 1,000 μg was absorbed to a significant extent as evidenced by the radioactivity found in the liver and kidneys, which are the major organs in terms of B_{12} deposit following administration (Table II). At least in one animal, some of the radioactivity was regurgitated further back into the cecum making the recovery much smaller than it should be (Rabbit #2). Again, the calculated net absorption from the fecal recovery seems to be inaccurate for the same reason as above. The net absorption could roughly be estimated by multiplying the liver uptake precentage by the factor of 10-15, the figure based on the result of a separate study in which the ratio of hepatic uptake

TABLE II. Rectal and Colonic Absorption of $Co^{57}B_{12}$ and Organ Uptake in Rabbits.

Rabbit No.	Fecal recovery from colon (%)	Calculated absorption (%)	Organ uptake (%) Liver	Kidney
1	85.4	14.6	.39	.05
2	22.3*	?	1.72	.28
3	89.2	10.8	.48	.61
4	—	—	1.92	.41
5	—	—	1.10	.58
Avg		12.7	1.12 ±.31	.39 ±.10

* Probably regurgitated into the cecum.

to a parenteral dose of $Co^{57}B_{12}$ was determined in the range of 10-100 μg.

Discussion. Vitamin B_{12} is absorbed from the small intestine by the aid of IF under physiological conditions, and the large intestine has been shown to be incapable of absorbing small doses of B_{12}(5). Our earlier study(6) showed that IF directly applied into the colon in man did not enhance absorption of the coadministered $Co^{57}B_{12}$. Therefore, the mucosa of the large bowel is so different from that of the small bowel that no IF-mediated type of absorption takes place there. The demonstration that a large dose of B_{12}, but not a small dose, was absorbed from the rectum seems to support the concept of passive transport for the non-IF-mediated type of absorption in which a concentration gradient between the mucosa and lumen is important. The reversed dose-absorption relationship in the case of oral administration cannot be explained on the same premise, because of the complicating factors such as the transit of the dose.

The passive transport theory for the non-IF-mediated absorptian has been questioned on several grounds. Berlin *et al*(7) used doses in the range of 100-100,000 μg *per os* and found a rather constant absorption of 1%. Abels *et al*(8) demonstrated a marked reduction in absorption upon slight lowering of the body temperature in rats, which would suggest a metabolic process. However, we have demonstrated earlier in rats that this type of absorption, unlike the IF-dependent absorption, is not at all influenced by EDTA (9). It is possible, therefore, that a passive mechanism is also operative following ingestion of a large amount of B_{12} by mouth but other factors are limiting the overall absorption.

The present data clearly indiate that the rectal route is an efficient one for large dosages of B_{12}. Although the urinary excretion rate is an indirect measure of absorption, it is more or less proportional to the net absorption and there is no difference whether the major absorption site is the rectum or the ileum in that respect. Massive B_{12} doses in rectal suppositories may be indicated under certain circumstances.

Summary. Absorption of Co^{57}-cyanocobalamin in a rectal suppository was measured in man and rabbits by the fecal and urinary excretion test. Two extremely different doses, 10 mμg and 2,000 μg were employed in man, and 1,000 μg in rabbits. It was found that vit B_{12} in the large dose was absorbed to a significant extent, urinary excretion being greater than after oral administration of the same dose. Absorption of the small dose of 10 mμg per rectum was negligible. The demonstrated relationship between the dose and absorption for the rectal administration has been the reverse of that for oral administration. This difference is discussed with reference to the intrinsic factor—independent absorption mechanism.

1. Ungley, C. C., Nutr. Abst. Rev., 1951-2, v21, 1.
2. Brigeau, J., Lambin, S., German, A., Compt. Rend. Soc. Biol., 1956, v150, 78.
3. Glass, G. B. J., Physiol. Rev., 1963, v43, 529.
4. Schilling, R. E., J. Lab. Clin. Med., 1953, v42, 860.
5. Ross, G. I. M., Mollin, D. L., Cox, E. V., Ungley, C. C., Blood, 1954, v9, 473.
6. Okuda, K., Sasayama, P., Am. J. Physiol., 1965, v208, 14.
7. Berlin, M., Berlin, H., Brante, G., Sjöberg, S. G., Vitamin B_{12} und Intrinsic Factor, 2nd Europ. Symposium, Stuttgart, Ferdinand Enke, 1961, 485.
8. Abels, J., Vegter, J. J. M., Woldring, M. G., Jans, J. H., Nieweg, H. O., Acta Med. Scand., 1959, v165, 105.
9. Okuda, K., Am. J. Physiol., 1960, v199, 84.

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