ABSORPTION OF VITAMIN B₁₂ FROM THE JEJUNUM IN MAN STUDIED WITH A THREE LUMEN TUBE

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Intestinal absorption of vitamin B₁₂ (B₁₂) has been the subject of rigorous studies. However, due to technical difficulty, absorption capacity of individual intestinal segments has not been fully elucidated. Booth and Mollin (1) determined distribution of radioactivity in the bowels following oral administration of B₁₂-Co⁶⁰ in patients during laparotomy. They found a large portion of radioactivity in the ileum three hours after the administration and presumed that the ileum was the site of absorption. Studies of similar designs in animals indicated the middle to distal segments of the small bowel to be the major absorptive area (2—4). The intestinal physiology is such that the bowel content stagnates in the distal ileum and the interpretation of such data is rather limited.

Our studies have shown that B₁₂ ingested with a meal is absorbed to a much greater extent than B₁₂ given in an empty stomach (5), its transit in the upper intestine is rapid in the latter situation, and the speed of B₁₂ passage is inversely related to absorption (6). The possibility remains, therefore, that the proximal bowel of man is capable of absorbing B₁₂ under physiological conditions. In the present study, the absorption capacity of the upper jejunum was measured in man with a three lumen tube.

EXPERIMENTAL

Young cooperative patients in convalescence who had no gastrointestinal disease or malabsorption as studied with I¹³¹-triolein, were used. After overnight fasting, a three lumen tube with an inflatable balloon at the tip was inserted to a distance of 150 cm from the mouth, the balloon inflated to prevent descent of fluid, and a test solution warmed to body temperature was instilled through an opening at 50 cm from the tip. The test solution consisted of 100—200 mμg of cyanocobalamin-Co⁶⁰ ²(specific activity, 1 μc/μg) or 10 mμg of cyanocobalamin-Co⁵⁷ ²(sp. activity, 13 μc/μg) and 100 mg of bromsulfalein (BSP), made up to 100 ml with a physiological saline. Two ml of fluid were drawn from a hole at the foot of the balloon at 30

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minutes' interval for a period of three hours, and the concentration ratio of radioactivity to BSP was determined and compared with that of the original solution which was expressed as unity. The decrease of this ratio should be proportional to absorption of B_{12} , assuming that no BSP was absorbed. This method has been used for the absorption study of other substances (7). Radiocobalt was measured with a well-type gamma scintillation counter as described (8), and BSP, spectrophotometrically after alkalization.

In some subjects, the intestinal fluid was drained at the end of three hours, the intestine was washed with 100 ml of warm saline three times, and then with a mixture of 100 ml saline and 60 ml of 4% neutralized disodium ethylenediaminetetraacetate (EDTA). In each washing the BSP- B_{12} ratio was measured and the recovery was calculated. Schilling test was also carried out after the washing for the measurement of 24 hour urinary excretion of radioactivity.

For comparison with B_{12} , absorption of 50 μ c of I^{131} -human serum albumin (RISA) dissolved in 1% saline gelatine solution containing 100 mg BSP was studied under the identical conditions. A preliminary study to compare BSP with polyethyleneglycol as unabsorbable reference has indicated that, in spite of its minimal absorbability (9), the former is superior because of the simplicity and accuracy of measurement; turbidimetric determination of the latter in intestinal fluid (10) was quite inaccurate.

Two sources of intrinsic factor (IF) were employed; (a) a purified hog stomach preparation (WES #942)³ which is active in pernicious anemia patients at the dose of a few milligrams and has a B_{12} binding power of 270 μ g/mg, and (b) fresh human gastric juice which was immediately neutralized; its B_{12} binding capacity was measured by a dialysis method (11).

RESULTS

Fig. 1 represents the curve for the B_{12} : BSP ratio determined in 15 subjects with the dose of 200 μ g B_{12} -Co⁶⁰, in comparison with that for the RISA: BSP in 20 subjects. The absorption of B_{12} was very small and slow as contrasted by the sharp decline of the RISA: BSP ratio. When the B_{12} dose was reduced to 10 μ g and the ratio determined in three subjects, practically no difference was noted. Neither the addition of 10 ml of human gastric juice (180–300 μ g binding capacity) to 100 μ g B_{12} -Co⁶⁰ nor 1 to 5 mg purified IF (270–1350 μ g binding capacity) to 200 μ g B_{12} -Co⁶⁰ increased absorption (Fig. 2).

The changes of B_{12} : BSP ratio in the washings of the intestine at the end of the test in 13 subjects are given in Table 1 together with percentage recoveries. It is noted that while the ratio did not change with washing in the majority, the last washing with EDTA yielded more B_{12} than the previous washing and increased the ratio markedly in 4 and moderately in 2 subjects, the largest increase being more than four fold.

The urinary excretion of radioactivity by the Schilling method varied; it was negligible when EDTA had yielded an increased B_{12} : BSP ratio, and there was a

³ Kindly supplied by Dr. L. Ellenbogen, Lederle Laboratories, Pearl River, N. Y

significant excretion, and therefore absorption, when EDTA had not. The high figure for subject #12 was due to breakage of the balloon and the figure for #5 was probably due to incomplete inflation of the balloon. The fluid drained from subjects #7, #11 and #16 and containing approximately 50% of the test dose was readministered to three separate subjects through a tubing directly into the proximal jejunum and absorption of B₁₂ was assessed by the Schilling test. The 24 hour urinary excretions were 6.5, 19.7 and 12.6%, respectively.

FIG. 1 Decrease of B₁₂: BSP Ratio in Jejunal Fluid (upper curve) in Comparison with that of RISA:BSP (lower curve)
Vertical bars represent standard error of the mean.

FIG. 2 Effect of the Addition of Human Gastric Juice (G.J.) and Purified Hog Intrinsic Factor (I.F.) on B₁₂ Absorption from the Proximal Jejunum, Estimated from B₁₂:BSP Ratio
Each curve represents average of three tests. Doses: 10 ml G.J. + 100 mμg B₁₂-Co⁶⁰ and 1-5mg I.F. + 200 mμg B₁₂-Co⁶⁰.

TABLE 1
B₁₂: BSP Ratios in the Washings and Recoveries

Test dose	Subject No.	B ₁₂ : BSP ratio ^a					Recovery		Schilling test
		Drained fluid	Washing#1	#2	#3	#4(+EDTA)	BSP	B ₁₂	
							<i>per cent</i>	<i>per cent</i>	
B ₁₂ -Co ⁶⁰ 200 mμg	1	0.82	0.75	0.7	0.8	>4.0	99	86	0.2
	3	0.79	0.73	0.7	0.6	0.6	101	69	3.2
	5	0.80	0.75	0.7	0.8	0.7	86	68	6.2
	7	0.78	0.88	0.8	0.8	1.1	97	80	
	8	0.93	0.91	0.8	0.7	3.0	82	76	0.6
	9	0.91	0.82	0.7	0.9	0.8	87	69	
	10	0.75	0.70	0.6	0.7	0.7	95	73	
B ₁₂ -Co ⁶⁰ 200 mμg + IF 5 mg	11	0.98	0.87	0.7	0.8	2.2	95	85	0.5
	12	0.80	0.74	0.7	0.7	>4.0	— ^b	—	6.9
B ₁₂ -Co ⁶⁰ 200 mμg + IF 1 mg	15	0.83	0.72	0.7	0.6	0.6	94	76	1.4
B ₁₂ -Co ⁶⁰ 100 mμg + gastric juice 10 ml	20	0.80	0.66	0.6	0.5	1.7	96	92	0.2
B ₁₂ -Co ⁵⁷ 10 mμg	16	0.85	0.89	0.8	0.8	1.5	96	86	0.4
	18	1.06	1.08	1.0	1.0	1.1	— ^b	—	

^a Ratio of the original solution: 1.0

^b Balloon was broken during test.

DISCUSSION

The data demonstrated that absorption of B₁₂ from the upper jejunum is very poor even with the allowance of three hours for absorption, a time sufficient for B₁₂ to go through a step of absorptive process. Furthermore, the real absorption of B₁₂ could have been even smaller than what the curve of B₁₂: BSP ratio suggests, because a small fraction of BSP might have been absorbed. The reported duodenal absorption of B₁₂ (12) was based on a dog study in which a high dose of B₁₂ was used. The doses employed in the present study were within the physiologic range.

The results of the Schilling test performed after the complete removal of the test solution from the jejunum at the end of three hours indicated a minimal to small absorption of B₁₂. Addition of human gastric juice or purified hog IF did not increase absorption, suggesting that the poor absorption was not due to lack of IF in the upper jejunum. A separate study has suggested the presence of IF activity in the small bowel in man (13).

The use of EDTA increased the B₁₂: BSP ratio in the washing of the jejunum in about one half of the tests. The inconsistent figures might be due to irregular extent of washing and regurgitation of EDTA back toward the duodenum. It is of interest, however, to postulate that in those subjects a small portion of B₁₂ had been adsorbed onto the duodeno-jejunal mucosa and EDTA released it. EDTA has been shown to inhibit mucosal attachment of IF-bound B₁₂ (14), and participation of divalent cations in this process has been proposed (15). The consistent lower

recoveries of B₁₂ in comparison with BSP is in line the mucosal adsorption and/or absorption.

The presence in the gastrointestinal tract of inhibitory B₁₂ binders has been suggested (16). However, no evidence was obtained that B₁₂ which was not adsorbed to the mucosa and drained with the jejunal fluid was in an unabsorbable form, because normal absorption of such B₁₂ occurred when instilled into the jejunum, avoiding digestion in the stomach.

SUMMARY

Absorption of vitamin B₁₂ from the proximal jejunum was studied and compared with that of albumin in man employing a three lumen tube with an inflatable balloon. A test solution containing physiological doses of radioactive cyanocobalamin (Co⁵⁷, Co⁶⁰) or I¹³¹-human albumin and bromsulfalein (BSP) as an unabsorbable reference, was instilled in the upper jejunum and the decrease of B₁₂: BSP or I¹³¹: BSP was determined in the fluid withdrawn at intervals. It was found that absorption of B₁₂ was minimal during the test period of three hours, whereas I¹³¹-human albumin was absorbed quickly and to a much greater extent. After drainage and washing of the jejunum at the end of the test, infusion of EDTA released B₁₂ further and increased the B₁₂: BSP ratio in about one half of the test subjects, and the results of Schilling test carried out then were consistent with the finding.

REFERENCES

1. Booth, C. C., and Mollin, D. L., *Lancet* **1**, 18 (1959).
2. Boass, A., and Wilson, T. H., *Proc. Soc. Exptl. Biol. Med.*, **203**, 14 (1963).
3. Okuda, K., *Amer. J. Physiol.*, **199**, 84 (1960).
4. Baker, S. J., Mackinnon, N. L., and Vasudevia, P., *Indian J. Med. Res.*, **46**, 812 (1958).
5. Siegel, S., Chow, B. F., Okuda, K., Chen, S., and Hanus, E. J., *Amer. J. Clin. Nutr.*, **9**, 705 (1961).
6. Okuda, K., Duran, V. E., and Chow, B. F., *Proc. Soc. Exptl. Biol. Med.*, **103**, 588 (1960).
7. Hendrix, T. R., *Amer. J. Digest. Dis.*, **2**, 643 (1957).
8. Okuda, K., *J. Nutr.*, **77**, 131 (1962).
9. Lorber, S. H., Oppenheimer, M. J., Shay, H., Lynch, P., and Siple, H., *Amer. J. Physiol.*, **173**, 259 (1953).
10. Hyden, S., *Ann. Royal Agricul. Coll. Sweden*, **22**, 139 (1955).
11. Okuda, K., *Clin. Chim. Acta*, **7**, 780 (1962).
12. Rosenthal, H. L., and Hampton, Jr. J. K., *J. Nutr.*, **56**, 67 (1955).
13. Okuda, K., and Sasayama, K., *Amer. J. Physiol.*, **208**, 14 (1965).
14. Herbert, V., *J. Clin. Invest.*, **38**, 102 (1959).
15. Herbert, V., and Castle, W. B., *J. Clin. Invest.*, **40**, 1978 (1961).
16. Latner, A. L., and Merrills, R. J., Vitamin B₁₂ und Intrinsic Factor, Enke, Stuttgart, 201 (1957).