Intestinal Absorption of Vitamin B_{12} in Liver Cirrhosis

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The liver is one of the few organs where vitamin B_{12} is present in high concentrations, and for this reason, it is generally considered as a store house of this vitamin. Acute damage to the liver parenchym is manifested by a sharp rise of vitamin B_{12} in the blood as well as in the urine as first demonstrated by the author.[1] This phenomenon is probably due to a sudden release of cytoplasm bound vitamin B_{12} into the blood stream as a result of increased permeability of cell membrane. A number of attempts have since been made to utilize this finding for differentiating various hepatic diseases,[2,3,4,5] and determination of serum vitamin B_{12} is now among recognized diagnostic measures in clinical practice. However, tissue metabolism of vitamin B_{12} has not yet been fully understood even in normal physiology, much less in liver diseases. And the turnover of a vitamin in tissues will never be quantitative unless its intestinal absorption is known. The present study, therefore, aimed at elucidating changes, if any, in intestinal absorption in patients with impaired liver functions.

Since the use of radioactive vitamin B_{12} is not ideal because of a possible radiation hazard, nonradioactive vitamin B_{12} was employed in this study to measure the intestinal absorption. The technique used was that developed by Lang and Okuda[6] in which a large dose of cyanocobalamin is given by mouth and absorption is assessed by an increase in serum vitamin B_{12} level which ensues. Inasmuch as serum vitamin B_{12} levels are markedly elevated in acute phase of viral hepatitis which constitutes the majority of acute liver dieases, and interpretation of this test in such cases will be limited, chronic liver cirrhosis was chosen for this investigation.

MATERIALS AND METHODS

Ten patients with liver cirrhosis in the Ward of Chronic Division, Baltimore City Hospital, were chosen at random. Two cases were alcoholic and therefore the nature of the disease was thought to be alcoholic. The rest of the patients were either of Laennec or postnecrotic type. The diagnosis was established in all patients on the basis of needle biopsy study. No biliary cirrhosis was included. The control subjects were chosen from adult volunteers of both sexes who had no history of chronic or acute diseases. They were given in the morning, while fasting, 1,000

μgm of unlabeled cyanocobalamin in 20 ml of aqueous solution from a small glass, which was subsequently washed twice with water to make the total fluid intake 50 ml. Blood specimens were withdrawn before, one and a half hours and three hours after the administration. Sera were separated at room temperature and subjected to the microbiological assay for vitamin B_{12}. If the assay was not carried out immediately, the sera were stored in a deep freezer.

The serum was processed according to the technique of Okuda et al.[7] which is a modification of Rosenthal's[8], and was assayed with Skeggs' medium[9] and Lactobacillus leichmannii 4797 as the test organism. No cyanide was added for the liberation of vitamin B_{12} from serum proteins. The average of serum vitamin B_{12} levels measured with this technique in 50 healthy young adults was 210 $\mu\mu$g/ml.

RESULTS

The results of the oral tolerance test performed on 10 healthy control subjects and 10 cirrhotics are presented in Table I. In nine out of the ten controls, the serum vitamin B_{12} level increased by more than 150 $\mu\mu$g/ml above the basal level either 1.5 hours or 3 hours after the administration of the test dose. It has been our experience that in the majority of normal individuals the increase is more than 150 $\mu\mu$g/ml, and hence the critical increment for normal absorption has tentatively been set at this value. In a distinct contrast, none of the cirrhotics showed increases exceeding 150 $\mu\mu$g/ml. As a matter of fact, the largest increase was 90 $\mu\mu$g/ml (Case #4) and, in most of the patients, the increase was 85 $\mu\mu$g/ml or less. Case #1 who had a high basal level in blood failed to show any response at all following the test dose.

The number of the tests performed on normal individuals has been increased, and the cummulative results in 35 are tabulated in comparison with the results for the cirrhotic in Table II. It is obvious from the table that, following administration of 1,000 μgm of cyanocobalamin by mouth, serum vitamin B_{12} levels in cirrhotics did not increase as much as in healthy individuals.

DISCUSSION

The oral tolerance test was given to patients with hepatic cirrhosis and the results demonstrated that serum vitamin B_{12} concentrations were not elevated in cirrhotics as much as healthy individuals. This indicates that intestinal absorption of the test dose was poor in the former. Another possibility may be that intestinal absorption of vitamin B_{12} was normal but the absorbed molecules were taken up by tissues to such an extent as to mask the increase in serum vitamin B_{12}. Glass et al.[10] studied hepatic uptake of radioactive vitamin B_{12} in liver disease patients by his surface scintillation counting technique and found it to be reduced in most of them. Since

the dose used in the present investigation was large and the absolute amount of vitamin B_{12} absorbed was expected to be proportionally great, it is very unlikely that tissue uptake is enhanced in liver cirrhosis to minimize the increase in serum vitamin B_{12}. Therefore, the results are to be taken as evidence for impaired intestinal absorption.

There are two known mechanisms for intestinal absorption of vitamin B_{12}; the physiological absorption is for smaller amounts of the vitamin and requires Castle's intrinsic factor, whereas absorption of this vitamin in a concentrated state is effected by diffusion without involving intrinsic factor.[11,12] Since the oral tolerance test uses high doses of vitamin B_{12}, the absorption measured by this technique gives indication for the latter type of absorption. The demonstrated malabsorption of vitamin B_{12} in hepatic cirrhosis, therefore, may suggest that the mucosa of the small bowel is altered functionally in this disease, and substances of small molecular size are not readily diffusible across the mucosal barrier. Although whether this assumption holds for other vitamins and minerals awaits further elucidation, there is evidence that intestinal mucosa is morphologically altered in advanced dirrhosis with portal hypertension.[13] According to Krasnow et al.[14], megaloblastic anemia occurs in about two per cent of cirrhotic patients. Even though Schilling test was within normal limits in a few cases they studied, it by no means precludes impaired absorption of nutrients in general. The results of the Schilling test have to be evaluated with reservation, unless the fecal excretion test was performed simultaneously. Many factors could influence the results, and reduced tissue uptake will make absorbed radioactive vitamin B_{12} readily exchangeable with the flushing dose to result in a greater urinary excretion. The demonstrated impairment of intestinal permeability for nutrients has to be given a serious consideration in the management of liver cirrhosis.

SUMMARY

Intestinal absorption of vitamin B_{12} was studied in ten biopsy-proven cases of liver cirrhosis employing the oral tolerance test, and the results were compared with those obtained in 35 healthy individuals. All the cirrhotic patients were found to absorb the test dose of vitamin B_{12} very poorly, suggesting that intestinal absorption of nutrients in general is also impaired. The clinical implications have been discussed.

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Table I. Oral tolerance test performed on healty and cirrhotic individuals.

Group	Subject (No.)	Sex	Age	Serum B_{12} level* ($\mu\mu$g/ml)	Maximum increase ($\mu\mu$g/ml)	Remark
Control	1	M	25	235, 385, 480	245	
	2	M	31	185, 580, 530	395	
	3	M	29	210, 840, 645	630	
	4	F	21	340, 485, 570	230	
	5	F	24	196, 340, 318	144	
	6	M	35	348, 1280, 978	932	
	7	F	37	205, 438, 378	233	
	8	M	19	180, 428, 410	248	
	9	F	28	238, 390, 385	162	
	10	M	40	158, 820, 645	662	
Cirrhotic	1	M	41	530, 525, 485	0	CCF 4+, TT 7, prothrombin 45%
	2	F	39	146, 152, 175	29	CCF 4+, bilirubin 1.5/2.7mg, ascites
	3	F	22	245, 350, 310	65	CCF 4+, TT 9.5, A/G 0.62
	4	M	37	350, 420, 440	90	CCF 3+, bilirubin 0.8/1.4
	5	M	39	430, 505, 515	85	CCF 4+, TT 6, A/G 0.8 (alcoholic)
	6	M	60	64, 87, 105	41	CCF 4+, bilirubin 0.6/1.0mg, ascites
	7	F	42	165, 215, 205	50	CCF 4+, prothrombin 51%, A/G 0.71
	8	F	39	235, 235, 320	85	CCF 4+, TT 7, ascites
	9	M	35	621, 582, 635	14	CCF 3+, TT 9, ascites (alcoholic)
	10	M	38	105, 138, 150	45	CCF 4+, A/G 0.58, bilirubin 2.1/5.0mg
				Average	50	

* The figures are before, 1.5 hours and 3 hours after administration of the test dose of 1 mg, respectively.

Table II. Analysis of the oral tolerance test.

Group	Number of subjects tested	Increase in serum vitamin B_{12} ($\mu\mu$g/ml)			Number of subjects showing increase above 150 $\mu\mu$g/ml
		Minimum	Maximum	Average	
Control	35	84	1400	315	29 (83%)
Cirrhotic	10	0	90	50	0 (0%)

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