

BACTERIAL CONTAMINATION OF THE JEJUNUM AND VITAMIN B₁₂ ABSORPTION

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Macrocytic anemia due to stasis of the contents of the small intestine is a well known clinical entity. It has been reported in association with improperly drained intestinal segments at all levels of the small bowel, including partially obstructed afferent loops after gastrectomy,¹ multiple jejunal diverticula,¹⁻³ and with poorly draining ileal segments.^{4, 5}

There is now strong evidence that bacterial overgrowth in such intestinal segments plays a major role in the etiology of the hematologic syndrome.^{2, 4-7} At present, however, the exact mechanism by which this bacterial proliferation leads to a malabsorption or malutilization of vitamin B₁₂ is still not clear. In an effort to throw further light on this matter, segments of jejunum were interposed into the colon. We hoped by this technique to provide some information on two separate but related aspects of the relationship of bacterial proliferation to malabsorption. First, because the segment of small intestine subjected to continuous fecal contamination would be out of the upper alimentary stream, any defect in absorption of vitamin B₁₂ could then be considered to occur as a result of a circulating substance such as a toxin. Second, it could be observed whether prolonged bacterial contamination of the mucosa of the upper intestinal tract could cause recogniza-

ble histologic changes suggestive of a malabsorptive defect.

Materials and Method

Healthy, dewormed mongrel dogs were used. During the study, the animals were fed 1 can of Pard daily to which 100 gm. of lard was added. This high fat diet was given as part of a study which will be reported separately. All animals handled the diet well and none developed steatorrhea by actual stool fat measurement. On the eighth day of this diet, each animal was fed a capsule containing 0.67 μ g. of vitamin B₁₂ tagged with radioactive cobalt 57. The radioactivity of these capsules had been previously measured in a large crystal scintillation counter. After ingestion of the capsule, a total stool collection was carried out over the next 72 hours while the animal was maintained on the same diet. The radioactivity of the total stool sample was then measured. Collections over a longer period of time failed to yield a significantly increased amount of tracer material. The amount of vitamin B₁₂ excreted was then calculated as a percentage of that given.

Ten unoperated animals were used as controls. Three animals had a 30 cm. segment of upper jejunum interposed into the colon, and 3 animals had a 60 cm. segment interposed. The isolated segment on its mesentery was interposed isoperistaltically in an end-to-end fashion with a standard 2-layer technique (fig. 1). After recovering from surgery, the animals were sent to the animal farm for 3 to 4 months. Upon return to the laboratory, similar metabolic studies were again carried out. The animals were reexplored and biopsy specimens were taken from the transplanted segment.

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Results

In the 10 control animals, the average B₁₂ excretion in the stool collection was 28 per cent (S.D., 7.6) of the ingested dose. This is similar to the figures of 33 per cent⁸ and 10 to 47 per cent⁹ excretion reported in normal humans.

In group 1, those animals with 30 cm. in-

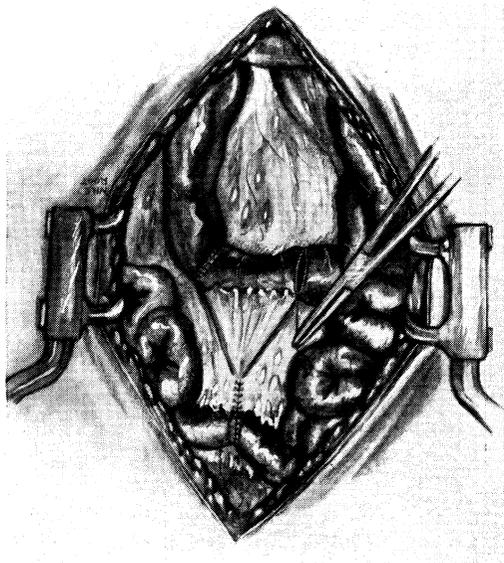


FIG. 1. Segment of jejunum on its mesentery interposed into colon.

TABLE I

Fecal excretion of a test dose of cobalt labeled vitamin B₁₂ in intact dogs and in dogs with ileal segments interposed in the colon.

	No. of dogs	Length of interposed loop cm.	Vitamin B ₁₂ in stool	
			Dose administered preoperatively %	Dose administered postoperatively %
Control	10		28	
Group 1		30		
F-11	3		27	28
F-15			27	39
F-19			23	28
Group 2		60		
F-33	3		20	39
F-34			13	28
F-37			27	26

terposition, both pre- and postoperative B₁₂ excretions were all within the normal range. The animals with 60 cm. interpositions had similar results (table 1).

At reexploration, the interposed loops were filled with fecal contents as expected. Histologic study of the contaminated jejunum failed to reveal any consistent or marked change in the histologic pattern of the jejunal mucosa.

Discussion

The anemia associated with stasis in the upper intestinal tract was investigated by Seyderhelm¹⁰ in 1924. He produced partial obstruction by suturing a band of the abdominal wall fascia around the terminal ileum, and postulated that the anemia thus produced was due to autointoxication from the chronically distended bowel. Several years later, Tonnis⁴ suggested that overgrowth of bacteria in the chronically obstructed loop might be important as a cause of the anemia.

More recently, Toon and Wangenstein⁷ and Watson and Witts,¹¹ by using rats with surgically created blind loops as the experimental animal, were able to offer strong evidence that bacterial overgrowth in the loops was intimately associated with the ensuing macrocytic anemia. There has been no lack of clinical support for this concept. The reports of Goldstein,² Scudamore,³ and Adams¹ are particularly important, because, in their cases, there is no possibility of the alimentary stream bypassing a significant portion of the terminal ileum which is now known to be the primary site of vitamin B₁₂ absorption.¹²

The exact mechanism by which bacterial overgrowth may interfere with the absorption or utilization of vitamin B₁₂ is not clear. One theory holds that there is bacterial competition for the available vitamin B₁₂.

A second popular theory and one that is under investigation here is that a bacterial toxin is elaborated in and absorbed from the blind intestinal loop.¹³ Presumably, such a toxin might act to block B₁₂ metabolism within the hematopoietic tissues.^{14, 15} Clinical data, however, support the concept that

the block is at the absorptive rather than the utilization level. Because of this, it has been suggested¹⁶ that a circulating toxin may somehow interfere with the ability of the mucosal surface of the ileum to properly absorb vitamin B₁₂. Because it is known that the bacterial flora of the stagnant loop is similar to that of the colon,^{2, 11, 17} it was hoped that interposing a segment of jejunum into the colon would demonstrate whether bacterial overgrowth in such a length of small intestine alone could interfere with the absorption of vitamin B₁₂. If this occurred, it would have to be by means of a circulating toxin because the remainder of the small intestine was not exposed to any spillover of bacteria from the contaminated loop.

It is evident from the results of this study that if a toxin is elaborated, it is not produced by organisms normally present in the colon under the conditions of this experiment.

Although there are recognizable histologic changes present in many of the malabsorptive states,^{18, 19} the role that bacteria play, if any, in these morphologic changes is still not certain. Some authors have felt that antibiotics, by virtue of altering the intestinal flora, were helpful in tropical sprue.²⁰ Others have not been able to reproduce the same results in such a striking fashion.^{21, 22} With the administration of neomycin, Jacobson *et al.*²³ produced a malabsorptive syndrome associated with changes in jejunal morphology suggestive of early sprue. The relationship of their findings to changes in intestinal flora is uncertain, however. More recently, Schiffer²⁴ reported a case of "blind loop syndrome" associated with multiple diverticulitis of the jejunum in which peroral biopsy showed findings resembling those of early tropical sprue and celiac disease.

Thus, there is apparently at this time no clear cut agreement on whether changes in bacterial flora alone can lead to abnormalities in mucosal morphology. Multiple sections of jejunum from each animal in this study failed to reveal any abnormality even though they had been under continuous fecal contamination for upwards of 4 months.

Summary

An attempt was made to see if continuous massive bacterial contamination of the upper jejunum would adversely affect the absorption of vitamin B₁₂ or cause recognizable mucosal histologic changes, or both. This was done by interposing jejunal segments into the colon for periods of up to 4 months. Vitamin B₁₂ uptake by the fecal excretion method before and after this operation was measured. No effect on vitamin B₁₂ absorption was evident. There was, in addition, no apparent effect on jejunal morphology of the interposed segment grossly or microscopically.

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