ABSORPTION OF RADIOACTIVE VITAMIN B_{12} IN PATIENTS WITH DISEASE OF THE SMALL INTESTINE: RELATION TO MACROCYTIC ANEMIA

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In 1895, Faber[1] described the case of a young woman with pernicious anemia in whom a stricture of the ileum with subacute obstruction was found at autopsy. During the ensuing sixty years further cases have been reported and several reviews[2,3,4] on the subject have been written. Up to now 76 cases of macrocytic anemia associated either with an intestinal stricture or a blind loop from an anastomosis, have been reported.

The anemia is indistinguishable from addisonian pernicious anemia, with similar blood and bone marrow findings. Subacute combined degeneration of the spinal cord, glossitis and icterus all may occur. Liver therapy was found to be just as effective as in pernicious anemia in most cases, and surgical correction of the intestinal abnormality, if successful, may result in permanent cure of the anemia. The term "Intestinal Macrocytic Anemia" has been used for the syndrome and this appears to be an appropriate one.

Etiology

In considering the basic mechanism of this disease, a mere coincidence of the intestinal abnormality with pernicious ane-

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mia must be considered. That this is not the case is evident by the fact that in a majority of the cases free hydrochloric acid has been found in the gastric secretion, and intrinsic factor[5,6] has also been demonstrated when the gastric juice has been tested for it.

The next possibility is that there is a general defect in intestinal aborption associated with the underlying intestinal lesion, and that the disease is therefore similar to sprue. Against this possibility is the fact that studies of intestinal absorption have shown it to be good, especial in respect to glucose. Although steatorrhea has been found in about one fifth of the cases, it was pointed out by Cameron, Watson and Witts in their review of 61 cases in 1949[4] that most of the cases with steatorrhea were associated with gastro-colic or other high intestinal anastomoses in which steatorrhea would be expected, regardless of anemia. Since steatorrhea is not a very common feature of the syndrome, it seems probable that a defect in the ability of the intestinal mucosa to absorb hematopoietic agents along with fat, as one assumes to be the case in sprue, is not the primary basis of the anemia.

Every writer on the subject, beginning with Faber in 1895, has suspected that bacteria were somehow mplicated in the etiology of this syndrome. Luxuriant bacterial growth has always been noted in the stagnant areas of bowel above the stricture or in a blind loop. In 1924 Seyderhelm[7] produced intestinal strictures in ten dogs. In two out of the ten severe hyperchromic anemia developed. In these two dogs there were numerous bacteria above the stricture but in the dogs who did not become anemic the bowel remained sterile. Renshaw and his co-workers,[8] studying gastro-colic fistula, noted that two out of seven dogs in which it was experimentally produced developed macrocytic anemia. They emphasized that in this syndrome stomach contents do not readily pass into the colon but that colonic material does easily pass into the stomach. This led to the hypothesis that the basis for the symptoms was contamination of the stomach and small intestine with bacteria from the colon, rather than direct pas-

sage of food from stomach to colon, which would by-pass the digesting and absorbing surfaces of the small intestine.

Studies over the past few years on the effect of antibiotics in nutrition, and in hematopoiesis, provide an important chapter in the attempts to elucidate some of the relationships between bacteria and nutrition. In respect to anemia Lichtman et al[9] reported partial hematologic responses in four patients with pernicious anemia and one with nutritional macrocytic anemia treated with aureomycin. Foy and Kondi[10] were able to produce dramatic improvement in 16 cases of nutritional macrocytic anemia occuring in Africans in Keyna by penicillin alone. Siurala and Kaipainen[11] obtained marked hematologic responses in two patients with intestinal macrocytic anemia associated with blind loops, by aureomycin alone.

An experimental approach to the problem was made by Cameron, Watson and Witts in 1949[12] and by Toon and Wangensteen in 1950.[13] Both of these groups of investigators constructed retroperistaltic intestinal cul-de-sac placed high in the small intestine of rats. A considerable percentage of the rats which survived six months or more developed macrocytic anemia. This anemia responded to aureomycin treatment, or was prevented if aureomycin was added to the diet starting with the operation.

Judging from these clinical and experimental reports there is little doubt that an abnormal bacterial flora may have an adverse effect on hematopoiesis and that antibiotics may favorably influence it, apparently by altering in some way the utilization or absorption of vitamin B_{12}, folic acid, or both.

In intestinal macrocytic anemia no data was available relative to the use of vitamin B_{12} or folic acid until 1953. Since that time several reports have appeared indicating that vitamin B_{12} usually produced a hematologic response.[14,15] Information with respect to folic acid is not as clearcut, although it appears that some patients have responded to this agent.

Vitamin B_{12} Absorption in Small Intestinal Disorders

In order to test more directly the concept that the absorption or availability of vitamin B_{12} may be altered by an abnormal intestinal bacterial flora we have carried out vitamin B_{12} absorption tests, with and without aureomycin, in six patients with a variety of intestinal diseases. A brief summary of the history of each patient follows:

Case No. 1 J.S., a 24 year old Mexican hospital laboratory worker had several operations for intestinal obstruction, and to correct internal fistulae which developed after appendicits at the age of 13. After this he was en-

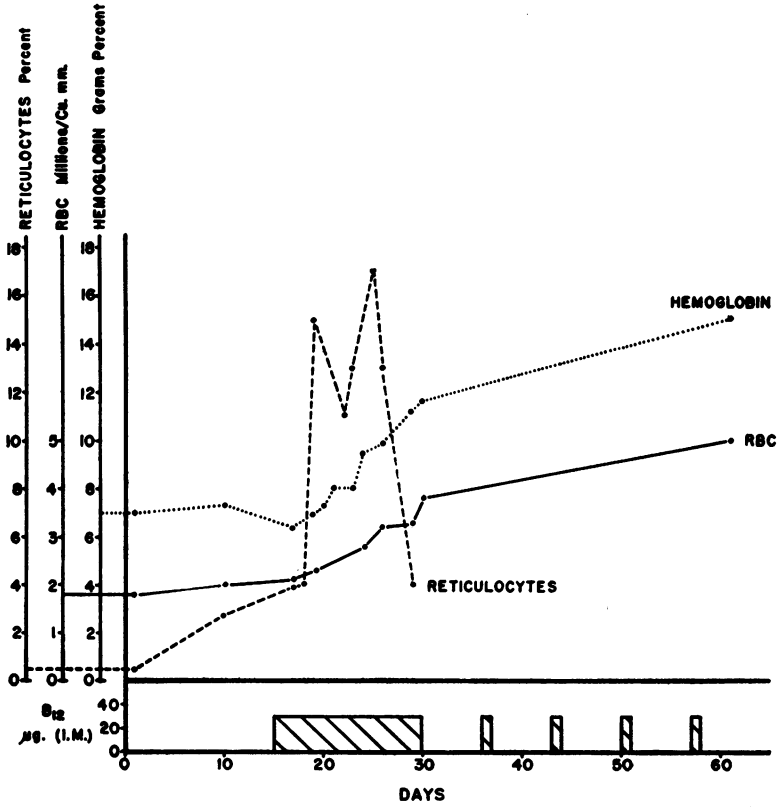

Figure 1. Graph showing the hematopoietic response to vitamin B_{12} in Case No. 1.

tirely well until 1952, when in the army in Korea, at which time he developed weakness, pallor and a sore tongue. Studies revealed a severe anemia and free HCl in the gastric juice. Bone marrow smear revealed some degree of megaloblastosis. He was treated for six months with parenteral B_{12} with complete relief of symptoms. The injections were stopped and he remained well for 16 months at which time weakness and sore tongue recurred. He was then admitted to Wadsworth V. A. Hospital in January 1954. The examination revealed pallor, mild icterus, and a normal neurological examination. There was free HCl in the gastric juice. Icterus index was 23 on admission, falling to 9 at discharge. An oral glucose tolerance test was normal. The hemoglobin was 6.4 gm. per 100 cc., red cell count 1.8 million and hematocrit 19.5 per cent. (MCV = 108, MCH = 35, MCHC = 30). A small bowel barium study revealed dilated loops of ileum with stagnation of barium in the pelvic portion of the ileum for 24 hours. The patient was treated with vitamin B_{12} with a marked hematologic response. (See Figure 1) At the present time he is entirely well and receives 30 μg. of B_{12} intramuscullarly once a month. The diagnosis of intestinal macrocytic anemia associated with intestinal anastomoses and a blind loop seems undoubted.

Case No. 2. W.M., a 60 year old man with extensive diverticulosis involving stomach and small bowel, as well as urinary bladder and urethra, first had gastro-intestinal symptoms in 1942, consisting of attacks of diarrhea alternating with constipation. He had several attacks of small bowel obstruction thought to be caused by jejunal diverticulitis, and treated medically. In 1949 a jejunal diverticulum perforated and a volvulus of the terminal ileum and cecum occurred. A resection of two feet of ileum with the cecum was performed, with end-to-side anastomosis of ileum to hepatic flexure. This resulted in a blind pouch of ascending colon.

In January 1952 the patient entered the hospital and was found to have a mild macrocytic anemia. The bone marrow smear revealed megaloblastosis. The hemoglobin was 8.0 gm. per 100 cc., red cell count 2.49 million and hematocrit 27 per cent. (MCV = 108, MCH = 32, and MCH = 33) Gastric analysis revealed free HCl. Stool examination did not show an increased amount of fat. Treatment with vitamin B_{12} parenterally resulted in a hematologic response, with reticulocyte rise to 6 per cent. (See Figure 2). Later he was given folic acid without further effect on reticulocytes or blood count.

Upon discharge he ceased taking vitamin B_{12} but continued to take folic acid orally. In May 1954 he was readmitted with recurrent diarrhea, pallor, weakness and weight loss of 25 pounds. The red-cell count was 3.0 million, hemoglobin 12.0 gm. per 100 cc., and hematocrit 33 per cent. (MCV = 110, MCH = 40, MCHC = 36). An oral glucose tolerance test was normal. With parenteral vitamin B_{12}, 30 μg. daily, the reticulocytes rose to 4.8 per cent with a slow rise in hemoglobin, red count and hematocrit. Administration of aureomycin resulted in a marked improvement in the patient's diarrhea. It is believed that this patient represents a case of intestinal macrocytic

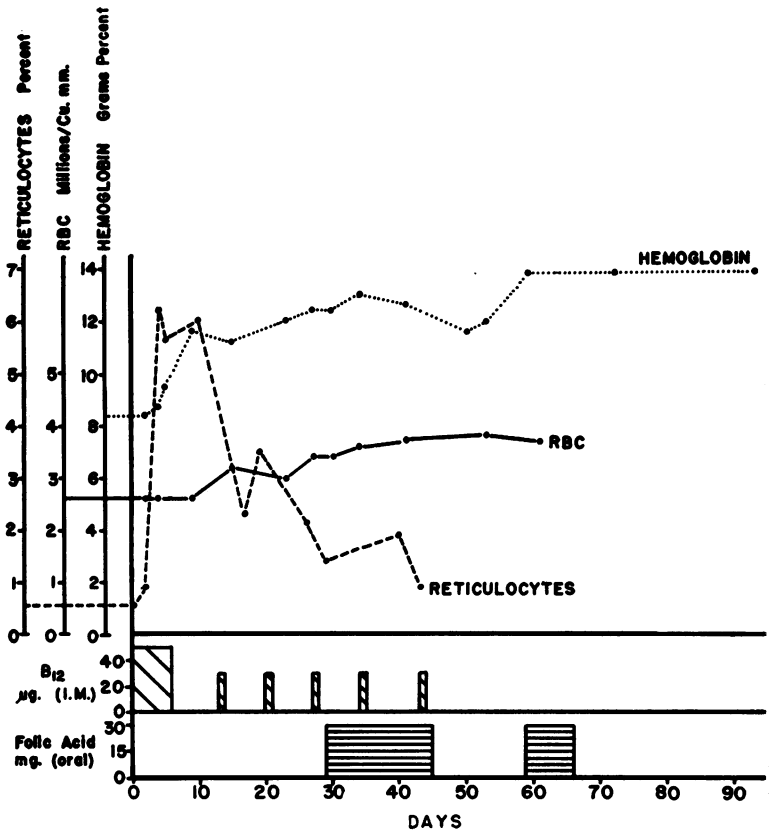

Figure 2. Graph showing the hematopoietic response to vitamin B_{12} in Case No. 2.

anemia associated with small bowel diverticula which acted similarly to "blind loops".

Case No. 3. C.B. was a 58 year old man who has had recurring attacks of pancreatitis since 1945. At the present time he has steatorrhea associated with calcification in the pancreas. A fat balance study carried out in 1952 indicated that when the patient was on a diet containing 95 grams of fat he excreted about 50 per cent of this fat daily in the stools. When he was given pancreatic extract (Viokase), in large doses, the fat excretion decreased to 20 per cent of the fat intake. During the studies with radioactive vitamin B_{12} the patient was taking Viokase and felt quite well. He did not have diarrhea. He has no anemia and is able to lead a moderately active existence.

Case No. 4. This is a 32 year old married woman who developed nontropical sprue in 1945. She has had three relapses, the last one occurring in January 1953. At this time severe normocytic anemia occurred which did not respond to folic acid, B_{12} or iron, but gradually cleared up with her general improvement. The relapse lasted for six months with gradual improvement from cortisone therapy.At the time of the radioactive vitamin B_{12} tests, the patient weighed 158 pounds, which was her normal weight, and she had no anemia or other nutritional abnormalities. She had one to two formed bowel movements daily which were grossly fatty. The small bowel series revealed a typical deficiency pattern and she had a flat oral glucose tolerance curve. She had been entirely well for a year prior to the vitamin B_{12} absorption studies and was following a gluten free diet.

Case No. 5. B. D. was a 43 year old male who entered the hospital in January 1954 with complaints of weakness and diarrhea. It was noted that his skin was markedly pigmented. Intermittent diarrhea and pigmentation had begun to appear in 1945. Studies in the hospital revealed that the patient had a macrocytic anemia with a red blood count of 2.37 million, hemoglobin of 10.3 gm. per 100 cc. and hematocrit of 29 per cent. The stools were grossly fatty. A glucose tolerance curve was flat and the small bowel x-ray examinations revealed a disordered motor pattern. A diagnosis of nontropical sprue was made. The patient made a good response to vitamin B_{12} therapy, with reticulocyte response up to 8.3 per cent on the seventh day. The hemoglobin, hematocrit, and red blood cell count gradually rose to normal.

Case No. 6. P. T. This is a 24 year old negro male who developed regional enteritis in 1951. In July 1952 a resection of two feet of the terminal ileum was performed because of obstructive symptoms. Following this he improved but after six months he had a relapse with crampy abdominal pains, diarrhea and weight loss. He entered the hospital in November 1953, and on this occasion it was found he had a normocytic anemia. The hemoglobin was 10 grams per 100 cc., and the hematocrit 31 per cent. The anemia failed to respond to vitamin B_{12}, folic acid or iron, and at the time of the vitamin B_{12} absorption studies hemoglobin was 11 grams per 100 cc. He had slight diarrhea.

Method of Determining Vitamin B_{12} Absorption

Since vitamin B_{12} contains cobalt as an essential part of the molecule it is possible to make radioactive vitamin B_{12} by using cobalt^{60} in its synthesis. Although the half life is 5.3 years the minute amounts which can be used in the tracer

technique are considered safe.* This can be detected in minute amounts in the feces, owing to intense gamma emission of isotopic cobalt, by means of the sensitive scintillation counter.

In 1952, Heinle and his co-workers[16] demonstrated that patients with pernicious anemia failed to absorb cobalt^{60} labeled vitamin B_{12}. These workers were able to recover in the stools most of the administered radioactivity contained in a dose of 0.5 μg of cobalt^{60}-labeled vitamin B_{12}, over a four or five day period following its administration. When a source of intrinsic factor was given with the test dose the fecal excretion of radioactive vitamin B_{12} decreased to around 30 per cent. This is interpreted as meaning that 70 per cent was absorbed. Swendseid, Halsted and Libby[17] showed that patients who had had a total gastrectomy behaved similarly to patients with pernicious anemia. They were able to recover nearly 100 per cent of the administered radioactivity in such patients. If a source of intrinsic factor was given with the test dose only 10 to 30 per cent of the radioactivity was recovered in the stools. These findings indicate that intrinsic factor is necessary for the absorption of vitamin B_{12}, and also that the stomach is the principal if not the only site of secretion of intrinsic factor. Halsted, Gasster and Drenick[18] reported that normal individuals excreted on the average 32 per cent of a test dose of 0.5 μg., the smallest amount excreted being 19 per cent and the greatest amount 57 per cent. Callender, Turnbull and Wakisaka[19] found that ten normal individuals excreted almost identical amounts, the average being 31 per cent, with lower and upper limits of 14 and 45 per cent respectively. Further studies in this laboratory indicate that when fecal excretion tests with 0.5 μg. of radioactive viatmin B_{12} are made in the same individual under similar conditions, the greatest variation in fecal excretion between the two tests which occurred in any patient was 18 pr cent. (See Table I) Within this limitation, the test may be considered quantitative.

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*U. S. Atomic Energy Commission Authorization No. 17055.

TABLE I

PER CENT FECAL EXCRETION OF 0.5 μG $CO^{60}B_{12}$ IN PATIENTS WITH REPEATED TESTS

Case No.	*Diagnosis*	*Test No. 1*	*Test No. 2*	*%Difference*
1	Normal	53	48	5
2	Normal	27	25	2
3	Normal	32	29	2
4	Normal	27	30	5
5	Normal	32	30	2
6	Normal	26	40	14
7	Enteritis	53	80	17
8	Sprue	58	44	14
9	Sprue	74	86	12
10	Pancreatitis	76	72	4
11	Enteritis	77	82	5
12	Blind Loop	59	77	18
13	Jejunal Diverticula	84	98	14
14	Enteritis	64	71	7

Average difference in 6 normals—5.0 %.

Average difference in 8 patients with small bowel abnormalities—12.6%.

In other words, when the same dose is repeated in a patient, a change in fecal excretion of at least 18 per cent must occur, before one can attribute a change in absorption to the effect of an agent used in the second test for the purpose of influencing absorption, such as intrinsic factor or an antibiotic.

Details of the method of carrying out the test in our laboratory have been described elsewhere.[18]

In the present study 0.5 μg. of the cobalt[60]-labeled vitamin B_{12} with a specific activity of 220 microcuries per milligram were administered to the six patients described above who had various disturbances of small bowel function. The test was repeated at least once, after which it was again performed with a source of intrinsic factor administered with the test dose. Then, either aureomyicn or achromycin were given in doses of 2 grams daily for three days, at which time the test dose of radioactive vitamin B_{12} was again administered. After

collecting stools until radioactivity no longer appeared, (usually four days) the test does was again repeated while continuing the administration of antibiotic. This was given in amounts of one gram daily throughout the period of stool collections. The total duration during which antibiotic therapy was administered ranged from ten to fourteen days for the two tests.

Results

The fecal excretion of radioactive vitamin B_{12} expressed in per cent of the test dose of 0.5 μg. is recorded for each patient in Table II, and is represented graphically in Figure 3.

TABLE II

THE EFFECT OF INTRINSIC FACTOR AND OF ANTIBIOTICS ON THE FECAL EXCRETION OF 0.5 μG. $Co^{60}B_{12}$ IN PATIENTS WITH DISORDERS OF THE SMALL BOWEL

				Responded to	% Fecal Excretion $Co^{60}B_{12}$			
					Date		With I. F.	During Antibiotic
Case	*Diagnosis*	*Anemia*	B_{12}	*Folic Acid*	*1954*	*Alone*		
J.S.	Intestinal Anastomosis	Macrocytic	+	-	2- 9	59		
					2-16	77		
					2-25		92	
					3-19			25*
					3-26			15
					9-14	72		
W.M.	Jejunal Diverticulosis	Macrocytic	+	0	6- 4	98		
					6- 8		95	
					6-14			44*
					6-19			65
					7-26	84		
					8- 4			63**
					8-10			60
					9-13	91		
					9-27	87 (Codeine)		

TABLE II (Continued)

C.B.	Pancreatic Insufficiency (Steatorrhea)	None	-	-	3–10	7		
					7–12		7	
					7–21			45**
					7–27			55
E.M.	Non-Tropical Sprue	None	-	-	3–15	58		
					4–23			44*
					4–30			27
					8– 2	44		
B.D.	Non-Tropical Sprue	Macrocytic	+	-	3–10	66		
					3–30		86	
					4–12		74	
					4–26			61*
					5– 4			52
P.T.	Regional Enteritis	Normocytic	0	0	4– 5	53		
					4–26	67		
					5– 3	80		
					5–21		87	
					7–30			45**

* Aureomycin
**Achromycin

In recording differences in fecal excretion from test to test we have compared the test in each of the three categories—"given alone", "with intrinsic factor," and "with antibiotic"—in which the least difference occurred, instead of averaging the tests in each category when more than one was performed. Thus differences noted become more significant.

In five of the six patients the effect of intrinsic factor was tested. 50 mg. of a hog mucosa extract* was mixed with the test dose of Co^{60} B_{12}. In none of the patients was there any

*Kindly supplied by Dr. R. W. Heinle of Upjohn Co., Kalamazoo, Michigan.

decrease in fecal excretion. In fact excretion was even greater in three of the five cases. In the other two there was no essential change.

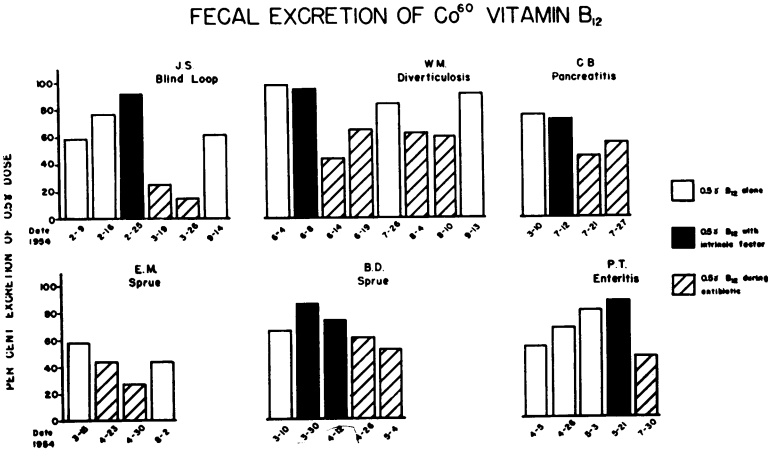

Figure 3. Effect of intrinsic factor, and of an antibiotic on fecal excretion of Co^{60} labeled vitamin B_{12} in six patients with small bowel disorders.

When an antibiotic was given there was a significant decrease in fecal excretion in two of the cases, and a possibly significant decrease in a third. In the other three cases the changes were not greater than might occur spontaneously. (See Table I) In the case of J. S., excretion of 59 and 77 per cent occurred when the test dose was administered alone. During aureomycin administration the excretion was only 25 and 15 per cent in two tests performed six days apart. Six months later, the patient being entirely well with normal blood count, the fecal excretion was again measured and found to be 72 per cent. Thus the decreased fecal excretion during the antibiotic administration was very marked, there being a difference of at least 34 per cent.

In the case of W. M. two experiments were carried out. In the first, 98 per cent of the test dose was excreted; then, with aureomycin 44 and 65 per cent was excreted in two consecutive tests. This represents a decrease of at least 33 per

cent. In the second experiment to test the effect of antibiotic administration achromycin was used. Here the change was less marked, being a 21 per cent decrease. Because the patient had diarrhea, which was markedly ameliorated on both occasions by the antibiotics used, it was thought that the improved absorption might be due to slowing of the intestinal rate. Therefore another test was performed while the patient was under the influence of codeine, which also stopped his diarrhea. Here the excretion was 87 per cent, indicating that diarrhea by itself was not the reason for the high excretion.

In the case of C. B. there was 67 per cent excretion when the test dose was given alone, and 55 per cent when it was given with aureomycin, a decrease of 21 per cent which is of borderline significance.

In the cases of E. M., B. D., and P. T. the changes in excretion with aureomycin were not greater than could occur spontaneously, i.e. less than 18 per cent. For these three cases the smallest differences in excretion were 0, 5, and 8 per cent respectively.

Discussion

All six patients excreted radioactive vitamin B_{12} in increased amounts, the average for the group being double that of normals. The first two patients (J.S. and W.M.) had intestinal stasis with B_{12} deficiency. It can be concluded that an abnormal small intestinal bacterial flora existed in these patients, since there is ample clinical and experimental evidence that such is the case in the syndrome of intestinal macrocytic anemia, which these patients had. The sixth patient (P.T.) had regional enteritis with a blind loop. Although there was no clinical evidence of B_{12} deficiency it is probable that an abnormal bacterial flora existed. The third, fourth and fifth patients (C.B., E.M., and B.D.) had steatorrhea associated with pancreatic insufficiency and sprue respectively. Here the existance of an abnormal small intestinal flora can only be conjectured.

The impaired absorption of radioactive B_{12} in these patients with evidence of an abnormal growth of bacteria in the small intestine suggest that bacteria may adversely affect intestinal absorption, perhaps through utilization of B_{12} by the bacteria themselves. Certain strains of E. coli have a marked avidity for B_{12} even though it may not be a growth requirement for these strains.[12]

That there was a decrease in the fecal excretion of radioactive B_{12} in the two patients with intestinal stasis when aureomycin or achromycin was administered, indicates that utilization or absorption of vitamin B_{12} was favorably influenced by affecting the bacterial flora. Krevans, Conley and Sachs[12] have reported similar findings in one patient with multiple diverticula of the jejunum and a megaloblastic anemia.

Present clinical evidence indicates that in macrocytic anemia associated with intestinal stricture or anastomosis, a B_{12} deficiency often occurs, since several cases have been reported recently responding to vitamin B_{12}. The B_{12} absorption tests in the two cases of this syndrome reported here also indicate B_{12} deficiency. Furthermore the pronounced increase in vitamin B_{12} absorption occurring when aureomycin was administered supports the previous clinical and experimental evidence that the fundamental mechanism in the syndrome of macrocytic anemia associated with intestinal stasis is bacterial interference with the absorption of vitamin B_{12}. The fact that a marked hematologic response occurred in the two patients of Siurala and Kaipainen[12] treated only with aureomycin, provides additional support for this concept. Whether or how folic acid enters the picture is not yet clear, although the complex metabolic interrelationships between it and vitamin B_{12} make it likely that there is also some disturbance in the utilization or absorption of this substance as well.

There are certain similarities between the macrocytic anemia associated with intestinal stricture or anastomosis, and the macrocytic anemia of *Diphyllobothrium latum* infestation. In both syndromes the clinical and hematologic picture is identical with addisonian pernicious anemia. In both, free HCl

and intrinsic factor can be demonstrated in the gastric juice. The fish tapeworm anemia may be cured by getting rid of the worm, just as intestinal macrocytic anemia may be relieved by surgical correction of the lesion causing stasis and bacterial growth or, apparently, by treatment with certain antibiotics.

A plausible explanation for the increased absorption of vitamin B_{12}, and for the hematologic response in macrocytic anemia which may occur with aureomycin administration, is that bacterial competition for hematopoietic agents is decreased. However, alternative possibilities exist. The antibiotic might exert its action by changing the flora in such a way that increased synthesis of hematopoietic material occurs. This could occur if organisms were destroyed which produced a hemolytic toxin or in some other way antagonized hematopoiesis. There might be effects on amino acid metabolism or methyl group synthesis or other metabolic changes which could lead to increased utilization or absorption of essential hematopoietic factors. Certain antibiotics might have a specific hematopoietic action, although there is no evidence for this. Unfortunately quantitative studies of the bacterial flora in our patients were not carried out. Further work relating the identity, as well as the numbers of bacteria present, to vitamin B_{12} absorption, is in progress.

Apparently only a few patients with intestinal stasis from stricture or anastomosis develop a macrocytic anemia, and numerous factors must enter the picture, such as the type and number of bacteria in the stagnant area and their needs for B_{12}, storage of vitamin B_{12} in the body, and perhaps the patient's diet. Since we have shown that patients with sprue and regional enteritis also have impaired B_{12} absorption it is quite possible that when macrocytic anemia develops in these diseases it is the result of a bacterial flora in the small intestine interfering with normal utilization of hematopoietic factors in the diet. However, in these patients, (who differed from the first two patients in that there was no obstruction), the fact that aureomycin did not cause an increased absorption suggests that the defect may be an inability of the intestinal mucosa to absorb nutrient materials normally. Further study is required before this matter can be clarified.

Summary

1. The pathogenesis of the macrocytic anemia which occurs in certain individuals with intestinal stricture or anastomosis, is discussed. Evidence is cited which suggests that bacteria present in the absorbing areas of the gut interfere with normal utilization of hematopoietic agents.

2. The use of cobalt^{60} labeled vitamin B_{12} as a means of studying absorption of vitamin B_{12} from the gut is discussed.

3. The absorption of radioactive vitamin B_{12} was determined in six patients with various intestinal disorders, and was found to be significantly impaired in all six. Administration of intrinsic factor did not result in improved absorption. Administration of aureomycin or achromycin resulted in markedly increased absorption in two of the six patients. Both of these patients had macrocytic anemia associated with intestinal stasis with an obstructive element. In the others no obstruction occurred.

4. These studies provide additional evidence to the concept that abnormal bacterial growth in the small intestine may result in impaired utilization of vitamin B_{12} through bacterial competition for this substance, with development of macrocytic anemia in some instances.

REFERENCES

1. FABER, KNUD I.; Pernizios Anaemi Som Folg af Tarmlidelse. Hospitaltidende 4, Roekke 3 Bind 601, 1895.

2. MEULENGRACHT, E.; Pernicious Anemia in Intestinal Stricture. Acta Medica Scandinav. 72:231, 1929.

3. BARKER, W. H. and HUMMEL, L. E.; Macrocytic Anemia in Association with Intestinal Strictures and Anastomoses. Bull. of the Johns Hopkins Hosp. 64: 215, 1939.

4. CAMERON, D. G., WATSON, G. M., and WITTS, J. L.; The Clinical Association of Macrocytic Anemia with Intestinal Stricture and Anastomosis. Blood 4: 793, 1949.

5. VERLOOP, M. C. and FLORJN, E.; Megaloblastic Anemia in Intestinal Stricture and Anastomsis. Nederl, Tijdschr. Geneesk. 95: 454, 1951.

6. SCHLESINGER, A.; Nachweis des Antiperniciosa Prinzips in Magensaft Einer Patientin mit Perniziosen Anämischen Blutbild beim Dunndarm-Stenose, Klin. Wchnschr. 12: 298, 1933.

7. SEYDERHELM, R., LEHMANN, W. and WICHELS, P.; Experimentelle Intestinale Perniziose Anamie Beim Hund. Klinische Wochenschrift 2: 1439, 1924.

8. RENSHAW, R. J. F., TEMPLETON, F. E. and KISKADDEN, R. M. Gastro colic Fistula—A Clinical and Experimental Study. Gastroenterology 7: 511, 1946.

9. LICHTMAN, H., GINSBERG, V. and WATSON, J.; Therapeutic Effect of Aureomycin in Pernicious Anemia. Proc. Soc. Exp. Biol. & Med. 74: 884, 1950.

10. FOY, H. and KONDI, A. The Haematinic Action of Penicillin in Megaloblastic Anaemia and its Relationship to B_{12} Metabolism and the Intestinal Flora. Trans. Royal Soc. Trop. Med. and Hygiene 48: 17, 1954.

11. SIURALA, M., KAIPAINEN, W. J.; Intestinal Megaloblastic Anemia, Treated with Aureomycin and Terramycin. Acta Med. Scandinav. 147: 197, 1953.

12. CAMERON, D. G., WATSON, G. M., and WITTS, L. J. The Experimental Production of Macrocytic Anemia by Operations on the Intestinal Tract. Blood 4:803, 1949.

13. TOON, R. W. and WANGENSTEEN, O. H.; Anemia Associated with Blind Intestinal Segments and its Prevention with Aureomycin. Proc. Soc. Exp. Biol. & Med. 75: 762, 1950.

14. UNGLEY, C. C., COX, E. V., SCOFIELD, B and THOMPSON, R. B.; Megaloblastic Anaemia Following Operations on the Intestine. Gastroenterologia, 79: 338, 1953.

15. PEARSON, ROBERT D.; Macrocytic Anemia Associated with Intestinal Strictures and Anastomoses: Report of Two Cases. Ann. Int. Med. 40: 600, 1954.

16. HEINLE, R. W., WELCH, A. D., SCHARF, V., MEACHAM, G. C., and PRUSOFF, W. H. Studies of Excretion (and Absorption) of Co^{60} Labeled Vitamin B_{12} in Pernicious Anemia. Trans. Assoc. of Am. Phys. 65: 214, 1952.

17. SWENDSEID, M. E., HALSTED, J. A., and LIBBY, R. L.: Excretion of Co^{60} Labeled Vitamin B_{12} after Total Gastrectomy. Proc. Soc. Exp. Biol. Med. 83:226, 1953.

18. HALSTED, J. A., GASSTER, M. and DRENICK, E. J.: Absorption of Radioactive Vitamin B_{12} after Total Gastrectomy: Relation to Macrocytic Anemia and to the Site of Origin of Castle's Intrinsic Factor. New Eng. Jour. Med. 251: 161, 1954.

19. CALLENDER, S. T., TURNBULL, A. and WAKISAKA, G.: Estimation of Intrinsic Factor of Castle by Use of Radioactive Vitamin B_{12}. Brit. Med. Jour. 1:10, 1954.

20. BURKHOLDER, P. R.: Microbiological Studies on Materials which Potentiate Oral Vitamin B_{12} Therapy in Addisonian Anemia. Arch. Biochem. 39:322, 1952.

21. KREVANS, J. R., CONLEY, C. L. and SACHS, M.: Influence of Certain Diseases on the Absorption of Vitamin B_{12} from the Gastrointestinal Tract (Abst) Jour. Clin. Invest. 32: 949, 1954.

DISCUSSION

DR. CHESTER M. JONES (Boston): I think one of the points that Dr. Halsted has made is of interest in regard to the symptoms which we call sprue. It seems to me that information is beginning to accumulate that sprue is not a distinguishable disease in the ordinary use of the term; that it represents something much more fundamental—and I am talking now about the group of cases that might be called non-tropical sprue.

There may be a common denominator with the tropical group, but there is a good deal of evidence that there is a failure of absorption, as a great deal of things are taken in by mouth, including probably, among other things, iron, and very probably vitamin B_{12}. There is no doubt that there is a modification of the intestinal flora in patients with longstanding sprue. It is interesting that you were able to show very little difference before and after the use of aureomycin when you may have altered the bacterial flora in the two sprue cases.

We are beginning to believe that in sprue cases there is a fundamental defect which probably is based ver definitely upon intracellular make-up and the mucosal membrane, possible specific enzymes or groups of enzymes, and I think that if that is true, modifying the bacterial flora alone would probably not be effective, and I think this is evidence in that general direction. This is of some interest because we get little results with corticosteroids and most of these patients have improved in a great many factors and this sort of study may lead us a little bit closer to the time when we can be more specific in what goes on in what we call sprue.

DR. STEWART WOLF (Oklahoma City): I wonder if I could ask Dr. Halsted one question. I wonder if he has had experience with the other method of measuring vitamin B_{12} absorption; instead of excretion by the feces, trying to pick it up over the liver.

DR. HALSTED (Closing): I want to thank Dr. Jones for his discussion. I should like to add that our method of study of these patients was, of course, rather crude, in that we did not do careful, quantitative, bacteriological studies in these patients. It is possible that we can learn much more about the role of bacteria when we can study a few patients with much more

quantitative and detailed bacteriological study for which we did not have facilities at the time.

In regard to Dr. Wolf's question, we have had only a little experience with the method of determining B_{12} absorption by hepatic uptake of radioactivity, but we are beginning to look into it. There is also another method, reported by Schilling, from Madison, Wisconsin, in which he gives an injection of 1000 micrograms of non-radioactive B_{12} two hours after giving a dose of 2 micrograms of radioactive B_{12} by mouth. By this it is possible to "flush out" some of the radioactivity, which has been absorbed, through the kidneys where it can be measured in the urine. This is rather a neat way of determining whether or not vitamin B_{12} has been absorbed. We have a feeling that neither of these methods are as quantitative as actually measuring what comes out in the stools and subtracting from what goes in by mouth; but, as I say, we have not had enough experience to be sure about this yet.