THE PRESENT STATUS OF VITAMIN B_{12} IN PERNICIOUS ANEMIA*

EDWARD H. REISNER, JR.

WINTROBE states in his book, *Clinical Hematology*—"A confusing array of 'B vitamins' . . . have been shown to be related to blood formation." In the past pyridoxine, riboflavin, nicotinic acid and folic acid have been shown to play a role in hematopoiesis, and now vitamin B_{12} has been added to that group. It is the purpose of this paper to review briefly the steps leading up to the discovery of vitamin B_{12}, to discuss some of the facts we now know about it, and to show you the records of cases treated with it by Randolph West and myself.

Ever since the discovery of the effect of liver in pernicious anemia, successively more potent extracts of liver have been produced in the effort to isolate the active antianemic principle. These attempts were retarded by the necessity to test the potency of liver fractions by tedious clinical trial methods. With the discovery of folic acid a new technique of bioassay was developed, depending on the fact that certain bacteria required folic acid to achieve optimum growth. In 1947 Shorb[1] reported that the growth requirements of Lactobacillus lactis Dorner for liver extracts paralleled their antianemic potency. This provided an in vitro method of testing the potency of such extracts and expedited the research culminating in the isolation from liver last year by Rickes and his associates[2] of some reddish crystals, designated vitamin B_{12}. Simultaneously, the same substance was isolated by English investigators under Smith,[3] using the traditional clinical test methods.

Vitamin B_{12} is a red crystalline substance with a molecular weight of 1630.[4] It contains cobalt, nitrogen and phosphorus but no sulphur.[5] It is extracted from liver in exceedingly small amounts and can also be obtained from a variety of sources in nature, including the manure of cows, chicks and other species[6] and has recently been reported to be found in the liquor produced in the production of streptomycin. Its potency in the treatment of pernicious anemia was reported by West[7]

* From the Fourth Medical Division (New York University), Bellevue Hospital, N. Y. Given February 15, 1949 before the Section on Medicine of The New York Academy of Medicine.

and confirmed shortly by Spies[8] who also found it effective in sprue and nutritional macrocytic anemia. Its outstanding feature was its potency, doses as small as 4 micrograms being adequate to produce a maximal reticulocyte response.

The first patient treated was a 66 year old white female with pernicious anemia, a patient on the wards of the Kings County Hospital, made available to Dr. West through the courtesy of William Dock. Following a single injection of 150 micrograms a maximal reticulocyte response of 27 per cent was observed and the blood count rose to normal and is still over four million, ten months later, with no further therapy.

Having established the efficacy of the material, a series of trials was instituted to establish the limits of potency, using patients on the First and Fourth Medical Divisions of Bellevue Hospital. It was found that a single injection of as little as 3 to 6 micrograms produced a maximal reticulocyte response, and a total dose of 56 micrograms in one patient effected complete hematologic remission, which was maintained for four months before relapse, with no additional therapy. A dose of one microgram a day was found to give a maximal reticulocyte response and restore the blood count to normal. A daily injection of 1/10 of a microgram was ineffective, but remission occurred when this was increased to ¾ of a microgram daily. One-half a microgram daily gave a submaximal reticulocyte response. From these experiments the unit potency of B_{12} was fixed at approximately one unit per microgram, a unit being defined as the amount of antianemic substance required daily to effect and maintain hematologic remission in a patient with pernicious anemia.

The next problem to investigate was the effect of B_{12} on the neurologic lesions of combined sclerosis. One of the Bellevue patients had exhibited early cord lesions at the onset of treatment with one gamma a day, and at the end of fifty-three days of treatment had a negative neurological examination. Four patients with more severe neurological disease were treated at the Columbia-Presbyterian Medical Center by Dr. West. The dose employed here was 25 micrograms a week. There was marked improvement in ability to walk, and gain in subjective and objective motor strength and coördination. The neurologic signs such as Babinski and Romberg signs tended to diminish or disappear. Vibratory sense on the other hand improved slowly and only slightly. These

patients have now been followed for eight months, and on 25 micrograms a week have maintained their improvement and shown no sign of relapse. In general the neurological results of treatment with B_{12} seem to be as satisfactory as those that might have been obtained with vigorous liver therapy.

Meanwhile other workers have been investigating various aspects of B_{12} treatment and reporting their findings which will be briefly summarized. Hall[9] reported beginning disappearance of megaloblasts from the bone marrow as early as 18 hours after the injection of B_{12}. Bethell[10] demonstrated that a substance probably the same as B_{12} was excreted in the stools of patients with pernicious anemia in relapse, and that this substance when extracted from their stools and given parenterally, caused remission. Berk et al[11] reported that when B_{12} was given by mouth it was ineffective unless gastric juice was given with it, an observation confirmed by Hall.[12] From these facts it has been suggested that the role of the intrinsic factor of Castle may be to promote the absorption of B_{12} from the gut, and B_{12} has been suggested to be the extrinsic factor.

The question will at once be asked about the relationship of B_{12} to folic acid. Bethell[10] reported that the response to B_{12} was inhibited by folic acid antagonists. Sturgis[13] has reported that B_{12} is ineffective in the so-called pernicious anemia of pregnancy, which is cured by folic acid, and Luhby reports that B_{12} is likewise ineffective in the treatment of acute megaloblastic anemia of infancy, which also responds to folic acid. Folic acid and B_{12} probably operate at different levels in the process of hematopoiesis.

It has been shown that thymine in a ratio of several thousand to one can replace folic acid in the growth requirements of Streptococcus lactis R[14] and Lactobacillus casei.[15] Spies[16] gave thymine in similarly large doses to patients with pernicious anemia and sprue, and obtained restoration of the blood count. Like folic acid, however, thymine had no effect on the neurologic lesions in these conditions.[17] Wright[18] has shown that the growth requirements of L. lactis can be met by substituting thymidine for B_{12} in a ratio of 10,000 to 1. Thymidine is the desoxyriboside of thymine and from the foregoing evidence it is tempting to postulate that the role of folic acid is to act as a coenzyme in the formation of thymine, which is then converted to its desoxyriboside by the enzymatic action of B_{12}. We have attempted to treat patients

with pernicious anemia with thymidine in dosage up to 150 micrograms, without notable effect. While such an hypothesis is attractive it is probably oversimplified and fails to take into account a wealth of accumulated evidence concerning the role in hematopoiesis of other factors, including xanthopterin,[19] and other pterins. Most recently a highly potent hematopoietic substance called B_{14} [20] has been announced, but this claim is awaiting confirmation at present.

Finally, the presence of cobalt in B_{12} is certain to excite interest because of the fact that this element has been implicated for many years in the production of experimental polycythemia.[21] With the aid of its isotopes it is now possible to learn more about the fate of cobalt in the body,[22] and the presence of this inorganic ion in B_{12} may ultimately be of great help in studying the formation and role of this vitamin. Two of our patients were treated by West with cobaltous chloride with no effect, before they responded to B_{12}.

To summarize, it would appear that vitamin B_{12} is identical with the long sought anti-pernicious anemia fraction of liver. It has an anti-anemic potency of one unit per microgram of crystalline substance, and brings about complete hematologic remission, and improvement of the neurological lesions comparable to that obtainable with liver. In the light of recent observations it would appear that our twenty-year-old concept of extrinsic and intrinsic factors in pernicious anemia may have to be modified, and to end in the vein in which I started, the "array of B-vitamins" has been extended, and the confusion, which I have tried tonight to dispel to some extent, is still very dense.

REFERENCES

1. Shorb, M. S. Unidentified growth factors for Lactobacillus lactis in refined liver extracts, *J. Biol. Chem.*, 1947, *169:* 455.
2. Rickes, E. L., Brink, N. G., Koniuszy, F. R., Wood, T. R. and Folkers, K. Crystalline vitamin B_{12}, *Science,* 1948, *107:*396.
3. Smith, E. L. Purification of anti-pernicious anæmia factors from liver, *Nature,* 1948, *161:*638.
4. Smith, E. L. Presence of cobalt in the anti-pernicious anæmia factor, *Nature,* 1948, *162:*144.
5. Rickes, E. L., Brink, N. G., Koniuszy, F. R., Wood, T. R. and Folkers, K. Vitamin B_{12}, a cobalt complex, *Science,* 1948, *108:*134.
6. Shorb, M. S. Activity of vitamin B_{12} for the growth of Lactobacillus lactis, *Science,* 1948, *107:*397.
7. West, R. Activity of vitamin B_{12} in Addisonian pernicious anemia, *Science,* 1948, *107:*398.
8. Spies, T. D., Stone, R. E. and Aramburu, T. Observations on the anti-anemic properties of vitamin B_{12}, *South. M. J.,* 1948, *41:*522.

9. Hall, B. E. and Campbell, D. C. Vitamin B_{12} therapy in pernicious anemia; effect on hematopoietic system: preliminary report, *Proc. Staff Meet., Mayo Clin.*, 1948, *23*:584.
10. Bethell, F. H., Meyers, M. C. and Neligh, R. B. Vitamin B_{12} in pernicious anemia and puerperal macrocytic anemia, *J. Lab. & Clin. Med.*, 1948, *33*: 1477.
11. Berk, L., Castle, W. B., Welch, A. G., Heinle, R. W., Anker, R. and Epstein, M. Observations on the etiologic relationship of achylia gastrica and pernicious anemia; activity of vitamin B_{12} as food (extrinsic factors), *New England J. Med.*, 1948, *239*:911.
12. Hall, B. E., Morgan, E. H. and Campbell, D. C. Oral administration of vitamin B_{12} in pernicious anemia, *Proc. Staff Meet., Mayo Clin.*, 1949, *24*:99.
13. Sturgis, C. Advances in our knowledge concerning the etiology and treatment of hematological disorders, *Bull. New York Acad. Med.*, 1949, *25*:84.
14. Snell, E. E. and Mitchell, H. K. Purine and pyrimidine bases as growth substances for lactic acid bacteria, *Proc Nat. Acad. Sc.*, 1941, *27*:1.
15. Stokstad, E. L. R. Isolation of a nucleotide essential for the growth of Lactobacillus casei, *J. Biol. Chem.*, 1941, *139*:475.
16. Frommeyer, W. B., Jr., Spies, T. D., Vilter, C. F. and English, A. Further observations of anti-anemic properties of 5-methyl uracil (thymine), *J. Lab. & Clin. Med.*, 1946, *31*:643.
17. Spies, T. D. and Stone, R. E. Liver extract, folic acid, and thymine in pernicious anemia and subacute combined degeneration, *Lancet*, 1947, *1*:174.
18. Wright, L. D., Skeggs, H. R. and Huff, J. W. The ability of thymidine to replace vitamin B_{12} as a growth factor for certain lactobacilli, *J. Biol. Chem.*, 1948, *175*:475.
19. Simmons, R. W. and Norris, E. R. Xanthopterin, the fish anemia factor, *J. Biol. Chem.*, 1941, *140*:679.
20. Norris, E. R. and Majnarich, J. J. Vitamin B_{14} and cell proliferation, *Science*, 1949, *109*:32.
21. Shils, M. E. and McCollum, E. V. The trace elements in nutrition, *J.A.M.A.*, 1942, *120*:609.
22. Comar, C. L., Davis, G. K. and Taylor, R. F. Cobalt metabolism studies; radioactive cobalt procedures with rats and cattle, *Arch. Biochem.*, 1946, *9*:149.