1950

Vitamin B12 therapy in pernicious anemia

Henry Joseph Billerbeck
University of Nebraska Medical Center

VITAMIN B_{12} THERAPY IN PERNICIOUS ANEMIA

Henry J. Billerbeck

Senior Thesis

Presented to-

College of Medicine

University of Nebraska

Omaha, Nebraska

1950

VITAMIN B_{12} THERAPY IN PERNICIOUS ANEMIA

Minot and Murphy (1) in 1926 made the epochal discovery of the efficacy of a diet of liver in the treatment of pernicious anemia. This observation came after the basic work in experimental anemia of Whipple, Robschiet-Robbins and their coworkers. It completely revolutionized our knowledge of the cause and treatment of pernicious anemia and the relation of the diet, gastric secretion and the liver to normal hematopoiesis. In 1945 a substance was synthesized by Angier et al that was a potent nutritional factor. This substance was called folic acid. Spies (2) patients with pernicious anemia who were given folic acid gave a hematopoietic response. It was noted then that liver checked and often improved the neurological symptoms. Under folic acid therapy these symptoms progressed.

Research in Merck & Company Laboratories by Rickes et al (3) in 1948 resulted in isolating a crystalline compound from liver with microgram quantities and which produced positive hematological responses in initial tests in patients with pernicious anemia. Shorb and Briggs (4) collaboratively tested certain clinically highly active factors for growth activity for Lactobacillus lactis Dorner, LLD factor, and found them to be microbiologically active. This organism required two unidentified growth factors, one of them appearing to be related to the activity of commercial liver preparations used in pernicious anemia. Further, Rickes et al (3), purification of clinically active liver fractions has led to the isolation in minute amounts of a crystalline compound which was highly active

for the growth of Lactobacillus lactis Dorner. This compound was called Vitamin B_{12}.

Rickes et al (5) in their experiments with Vitamin B_{12} found that it appeared to be a cobalt coordination complex which, having six groups about the cobalt atom, could involve one or more organic moieties. The cobalt complex character is thought to give at least in part the red color to Vitamin B_{12}. The presence of cobalt in Vitamin B_{12} reflects significantly upon many biological studies which have shown that cobalt is an essential trace element in nutrition, and perhaps suggestions concerning cobalt as a trace substance in iron therapy of anemias. The average adult daily dietary intake of cobalt has been estimated at 100 micrograms. Spectrographic examination of Vitamin B_{12} also showed the presence of phosphorous. Tests for sulfur were negative but traces of nitrogen were found. The cobalt-complex nature of Vitamin B_{12} is one of its outstanding properties. Smith (6) obtained a red crystalline material by purifying liver extract. This material had a molecular weight of 1,500 and contained 4% cobalt.

The first source of Vitamin B_{12} was liver. Rickes et al (3) and Smith (6) isolated the red crystalline compound which produced positive hematological responses. Rickes et al (7) isolated a red crystalline compound from a grisein-producing strain of Streptomyces griseus. The refractive indices, spectrographic properties, analyses, solubility factor, microbiological assay and the "animal protein factor" activity were identically the same as those isolated from liver. West has tested these

crystals and found that the clinical response in pernicious anemia parallels that shown by Vitamin B_{12}. Erf and Wimer (8) found that in using the crystals from liver or from Streptomyces griseus, there was a similar hematologic response with the same dosage. Kaufman and Cooperberg (9) reported that Stokstad found that a non-motile rod shaped organism from hen feces could produce the "animal protein factor". Concentrations of this substance were standardized against refined liver extract and found effective in two patients with pernicious anemia. The substance is less potent than the liver extract.

There have not been any reports of Vitamin B_{12} giving any toxic effects. In those cases where neurologic symptoms manifest themselves, there is noted that these symptoms are exaggerated for the first few days of treatment. After a period of treatment with Vitamin B_{12}, these symptoms are relieved. Kaufman and Cooperberg reported that in their cases of pernicious anemia treated with Vitamin B_{12}, there was no evidence of any allergy to the purified substance though sensitivity to a test dose of liver extract persisted.

Berk et al (10) offer the theory that the food (extrinsic) factor may be identical with or closely related chemically to the anti-pernicious anemia principle of liver, which is itself presumably identical with Vitamin B_{12}. It is possible that the function of the intrinsic factor of normal human gastric juice is to facilitate the absorption by the intestine of Vitamin B_{12} or of chemically related compounds in the food, rather than to react with the extrinsic factor as hitherto assumed.

West (11) was one of the first to treat pernicious anemia patients with Vitamin B_{12}. He treated three patients with pernicious anemia in relapse with single intramuscular injections of Vitamin B_{12}. Three micrograms, six micrograms and 150 micrograms were given to the three patients respectively. He obtained good clinical and hematologic improvement with a reticulocyte peak up to 27% on the fifth day. At this time (1948) it was too early to determine the future use of Vitamin B_{12}, but West claimed that so far it had surpassed folic acid and various liver extracts.

More isolated cases of pernicious anemia treated with Vitamin B_{12} were reported late in 1948. Hall and Campbell (12) gave a preliminary report on their use of Vitamin B_{12}. They observed that when administered intramuscularly, there was a hematopoietic response. The reticulocyte response and the rate of rise in the blood level were comparable to those observed when liver therapy was employed. Extremely small doses of Vitamin B_{12} were effective. With parenteral administration, approximately one microgram was equivalent to one U.S.P. Unit of extracts of liver or stomach mucosa. Serial aspirations of the sternal marrow showed that erythrocyte regeneration from megaloblastic to normoblastic types of cells occurred in forty-eight to seventy-two hours when relatively large amounts of Vitamin B_{12} were administered.

Hall and Campbell also treated eleven patients with neurological manifestations. With the administration of Vitamin B_{12}, the patients showed improvement in strength, mental allertness, appetite, gain in weight and disappearance of glossitis.

Three patients with peripheral neuritis showed much improvement. Five of six patients with peripheral neuritis and combined degeneration of the cord showed improvement. In three, the rate of improvement was unusually rapid. These patients had not been followed long enough before the article was written to determine whether or not there would be complete recovery from their neurological involvments.

In another paper, Hall and Campbell (12) reported six cases of pernicious anemia with severe relapse and treated with Vitamin B_{12}. The reticulocyte count was normal in four to six weeks. The bone marrow showed a complete conversion from megaloblastic to normoblastic regeneration in from forty-eight to ninety hours after administration of Vitamin B_{12}. Dosages of 25 micrograms of Vitamin B_{12} of weekly intervals intramuscularly were given. Development of hypochromasia in the erythrocytes of two out of six patients during the period of rise in erythrocyte levels was noted. Glossitis in four patients disappeared. Four patients had combined degeneration of spinal cord. Two patients showed improvement with seventy-five micrograms of Vitamin B_{12} over a period of sixty-three days. One patient showed no improvement with 100 micrograms of Vitamin B_{12} over a period of sixty-two days. The other patient was not observed long enough.

Berk et al (14) had a patient with neurological symptoms of numbness and stiffness of the hands together with difficulty in their use of finer motions, pressure about the abdomen, unsteady gait, legs felt numb from knees down, positive Romberg, gross ataxia, defective position sense and absence of vibratory

sensation of the lower extremity. The patient was treated with five micrograms of Vitamin B_{12} daily for eight days. By the tenth day the neurological symptoms were leaving. The author's supply of the drug was depleted and without the administration of Vitamin B_{12}, they noted a definite set back of the patient. A few days later more Vitamin B_{12} was given and the patient showed much improvement by the twenty-fourth day of treatment.

Jones et al (15) treated eleven cases of pernicious anemia with Vitamin B_{12}. In eight cases of relapse there was a good hematologic response. Two patients with mild neurologic involvement were relieved by therapy with Vitamin B_{12} alone. Minimum dosage was three fourths microgram. One microgram promoted good erythropoiesis in one patient. Maximum rate of erythropoiesis was gotten with a dosage of approximately three micrograms. The authors concluded that the reticulocyte count was an unreliable quantitative criterion of activity or adequacy of therapy. Also that Vitamin B_{12} effects a reduction in the fecal urobilinogin output of patients with pernicious anemia. They suggested there may be hemapoietic factors in addition to Vitamin B_{12} which may be required by some patients to obtain maximum erythrocyte levels.

Hematologic and neurologic disorders in pernicious anemia were studied by Patterson, Stauffer and Freeman (16). They established the case as pernicious anemia by glossitis, gastric achlorhydria, hyperchromic macrocytic anemia with neutrophilic leukopena, posterior column affected, mental disorder and confusion. The patient was treated with Vitamin B_{12}, 25

micrograms every third day. On the first day, the patient was stuporous and could not walk. By the fourth day, he became alert and was sitting up. On the fifteenth day, he was rational but unable to walk. On the twenty eight day, patient had a normal mental status and could stand alone. By the seventh month, the patient showed only a slight spastic ataxic gait with all other symptoms having disappeared.

Reisner (17), 1949, gave the results of treatment of pernicious anemia with Vitamin B_{12}. Maximum reticulocyte responses were obtained from a single injection of as little as six micrograms. Vitamin B_{12} sufficed to bring about full remission of the blood count in patients maintained on it.

Spies et al (18) treated patients under a more strict regime and a selected group of patients who were definitely diagnosed as having pernicious anemia. The patients had to have the following laboratory findings:

1. Macrocytic hyperchromic anemia.
2. Red cell count of 2.5 million or less.
3. Color index of 1.0 or more.
4. Megaloblastic arrest of the sternal bone marrow.
5. Absence of free hydrochloric acid in the gastric contents after histamine stimulation.

All cases showed an increase of two million red blood cells or more with the administration of Vitamin B_{12} parenterally. There was a hemoglobin increase of three or more grams and the peak of rise of reticulocytes occurred on the seventh and eighth days. The patients were treated from twenty to fifty days with

a dosage ranging from six micrograms to twenty-five micrograms. Fourteen patients with neurologic symptoms showed improvement progressively during the first ten days of treatment with Vitamin B_{12}. Within two weeks after administration, a dramatic improvement in the observations of peripheral nerve and posterior column involvements were observed in each case.

Spies concluded from the above cases that -

1. Vitamin B_{12} is the only pure chemical substance known to be effective in relieving subacute combined degeneration in persons with pernicious anemia.
2. Reticulocyte count increase occurs and is followed by increase in erythrocytes, platelets and hemoglobin.
3. Patient feels better at time of reticulocyte response.
4. Appetite is much better.
5. Severe glossitis present heals spectacularly.
6. Dosage varies greatly.

Bethell, Meyers and Neligh (19) treated four pernicious anemia patients with one microgram of Vitamin B_{12} intramuscularly daily. There was a definite reticulocyte response in all patients treated. One patient received a milligram of folic acid antagonist, aminopterin daily for two days prior to Vitamin B_{12} and for the first fourteen days of treatment with Vitamin B_{12}. The reticulocyte response was delayed and suboptimal. When aminopterin was discontinued, a second reticulocyte response occurred with rapid elevation of red cell count.

Assays of feces of four patients with untreated pernicious anemia revealed high contents of growth-stimulating factor, Lactobacillus lactis Dorner. The content of Vitamin B_{12} equivalent ranged from .3 to 1.8 microgram per gram of dried feces. Thus the daily output of B_{12} by a patient with pernicious anemia appears to be many times greater than necessary to produce remission when introduced parenterally. This suggests that in pernicious anemia there may be a defective absorption of Vitamin B_{12} derived either from dietary sources or by intestinal bacterial synthesis.

Stone and Spies (20) treated a patient with pernicious anemia having mucous membrane lesions that were fiery red and very painful. Previously folic acid and thymine were administered. Vitamin B_{12} relieved the condition.

The two methods of administering Vitamin B_{12} are intramuscularly and orally. Parenteral injections were indicated previously owing to the scarcity of therapeutic material. Spies et al (21) stated it is a certainty that patients actually absorb Vitamin B_{12} and that a deranged tract could not serve as a block to the absorption. It also eliminates carelessness and forgetfulness. It takes 30 to 50 times as much material by mouth as by injection. Oral administration of an incubated mixture of Vitamin B_{12} and normal human gastric juice cuts down this amount to about five to ten times the parenteral dose. When two patients with pernicious anemia were given thirty micrograms of Vitamin B_{12} orally, they developed combined system disease. When thirty micrograms of the same material was incubated with human gastric juice and given to the same patient, the blood

responded and there was also a disappearance of the acute signs of combined system disease.

Hall (22), Campbell and Berk et al (11) demonstrated that Vitamin B_{12} was inactive orally unless given with normal gastric juice. They suggested that the extrinsic factor may be identical with or closely related chemically to the anti-pernicious anemic principle of liver which is itself presumably identical with Vitamin B_{12}. Gastric juice facilitates absorption of Vitamin B_{12} rather than to react with the extrinsic factor.

Hall et al (22) observed that the oral dosage did not give as great a hematologic response as when given intramuscularly. Doses much greater must be employed before conclusions can be reached that Vitamin B_{12} alone will not give a hematologic response but yet an intrinsic factor in gastric juice of human beings potentiates the hematopoietic activity of orally administered Vitamin B_{12} which appears to be established. Whether the intrinsic factor combines with the extrinsic factor (Vitamin B_{12}) to form a third substance or whether the intrinsic factor simply permits absorption of the extrinsic factor to take place in patients having pernicious anemia was undetermined by Hall and associates. They noted that it took from twenty five to one hundred cubic centimeters of gastric juice to give a hematopoietic response with oral Vitamin B_{12}.

From the treatment of pernicious anemia with Vitamin B_{12}, the following has been concluded:

1. Vitamin B_{12} administered intramuscularly effectively induces hematopoietic responses. The reticulocyte response and the rate of rise in the blood level

are comparable to those observed when liver therapy is employed.

2. Extremely small doses of Vitamin B_{12} are effective. With parenteral administration, approximately one microgram is equivalent to 1 U.S.P. unit of extract of liver or stomach mucosa.

3. Serial aspirations of the sternal marrow have shown that erythrocyte regeneration from megaloblastic to normoblastic types of cells may occur in forty-eight to seventy-two hours when relatively large amounts of Vitamin B_{12} were administered.

4. Patients with neurologic symptoms showed remarkable improvement.

5. Dosage of Vitamin B_{12} varies in different individuals. As low a dosage as one microgram gave a reticulocyte response while in some patients over a hundred micrograms were needed.

6. Severe glossitis present heals spectacularly.

7. Assays of feces of pernicious anemia patients untreated reveal high content of the growth-stimulating factor, Lactobacillus lactis Dorner.

8. Vitamin B_{12} administered orally must be given in conjunction with human gastric juice. It is thought that the human gastric juice aids only in absorption of the vitamin.

9. Vitamin B_{12} produces a hematologic response and relieves the neurologic manifestations. Folic acid failed to relieve the neurologic symptoms.

10. Dosages of thirty to fifty times as much Vitamin B_{12} are needed for oral administration as compared to injections to obtain the desired results.

Vitamin B_{12} in the treatment of pernicious anemia must be followed as it is yet too early to evaluate the prognosis of the patient now being treated. So far it has surpassed folic acid, thymine and the liver extracts in creating a hematopoietic response and relieving the neurologic manifestations. There have not been any side effects or complications attributed to the use of Vitamin B_{12}. Continued treatment with Vitamin B_{12} and time will indicate its further use in the treatment of pernicious anemia.

BIBLIOGRAPHY

1. Minot, G. R., & Murphy, W. P.: Treatment of Pernicious Anemia by Special Diet, J. Am. M. A., Vol. 87: pp 470-476, 1926.

2. Spies, Tom D.: Folic Acid, Year Book Publishers Inc., Chicago, 1947.

3. Rickes, E.; Brink, N.; Koniuszy, F. R.; Wood, T. R.; and Folkers, K.: Crystalline Vitamin B_{12}, Science, Vol. 107: pp 396, (April) 1948.

4. Shorb, M. S.: Activity of Vitamin B_{12} for the Growth of Lactobacillus Lactis, Science, Vol. 107: p. 397, 1948.

5. Rickes, E.; Brink, N. G.;Koniuszy, F. R.; Wood, T. R.; and Folkers, K.: Vitamin B_{12}, A Cobalt Complex, Science Vol. 108: p. 134, (Aug. 6) 1948.

6. Smith, E. L.: Purification of Anti-Pernicious Anemia Factors from Liver, Nature, Vol. 161: p. 138, 1948.

7. Rickes, E. L.; Brink, N. G.; Koniuszy, F. R.; Wood, T. R.; and Folkers, K.: Comparative Data on Vitamin B_{12} from Liver and from a New Source, Streptomyces Griseus, Science Vol. 108: pp. 634 - 635, (Dec. 3) 1948.

8. Erf, L. A., and Wimer, B. M.: Comparison of Vitamin B_{12} from Liver and from Streptomyces Griseus in the Treatment of Pernicious Anemia, Blood, Vol. 4: No. 7: pp. 845 - 857, 1948.

9. Kaufmann, J.; Cooperberg, A.: Vitamin B_{12} in Pernicious Anemia, Cand. M. Ass. J. Vol. 60: No. 6: pp. 552 - 555, (June) 1949.

10. Berk, L.; Castle, W. B.; Welch, A. D.; Heinle, R. W.; Anker, R.; and Epstein, M.: Activity of Vitamin B_{12} as Food (Extrinsic) Factor, New England J., Vol. 239: pp. 911 - 912, 1948.

11. West, R.: Activity of Vitamin B_{12} in Addisonian Pernicious Anemia, Science, Vol. 107: p. 398, 1948.

12. Hall, B., and Campbell, D. C.: Vitamin B_{12} Therapy in Pernicious Anemia, Proc. Mayo Clinic, Vol. 23: No. 25:, pp. 584 - 596 (Dec. 8) 1948.

13. Hall, B., and Campbell, D. C.: Effect of Vitamin B_{12} on the Hematopoietic and Nervous Systems in Addisonian Pernicious Anemia, J. Laborat. Clin. M., Vol. 33: No. 12: p. 1646 (Dec.) 1948.

14. Berk, L.; Brown, D.; Finland, M.; and Castle, W.: Effectiveness of Vitamin B_{12} in Combined System Disease, New. Eng. Journal of Med., Vol. 239: No. 9: 1948.

15. Jones, E.; Darby, W.; and Trotter, J.: Pernicious Anemia Treated with Vitamin B_{12}, Blood, Vol. 4: No. 7: pp. 827 - 843, (July) 1949.

16. Patterson, J.; Stauffer, R.; and Freeman, A.: The Effect of Vitamin B_{12} on Hematologic and Neurologic Disorders in Pernicious Anemia, Bulletin of the New. Eng. M. Center, Vol. 11: No. 3: pp. 120 - 125, (June) 1949.

17. Reisner, E.: Treatment of Pernicious Anemia with Crystalline Vitamin B_{12}, Am. J. Med., Vol. 6: No. 4: p. 505, (April) 1949.

18. Spies, T. C.; Suarez, R. M.; Garcia Lopez, G.; Milanes, F.; Stone, R. E.; Lopez Toca, R.; Aramburu, T.; and Kartus, S.: Tentative Appraisal of Vitamin B_{12} as a Therapeutic Agent, J. Am. M. Ass. Vol. 139: No. 8: pp. 521 - 525, (Feb. 19) 1949.

19. Bethell, F. H.; Meyers, M. C.; and Neligh, R. B.: Vitamin B_{12} in Pernicious Anemia and Puerperal Macrocytic Anemia, J. Lab. and Clin. Med., Vol. 33: p. 1477, 1948.

20. Stone, R.; and Spies, T.: The Effect of Liver Extract and Vitamin B_{12} on the Mucous Membrane Lesions of Macrocytic Anemia, J. Laborat. Clin. M., Vol. 33: No. 8: pp. 1019 - 23 (Aug.) 1948.

21. Spies, T.; Garcia Lopez, G.; Milanes, F.; Lopez Toca, R.; and Culver, B.: A Note on the Oral Versus Parenteral Administration of Vitamin B_{12}, South M. Journal, Vol. 42: No. 6: pp. 528 - 531, (June) 1949.

22. Hall, B.; Morgan, E.: and Campbell, D.: Oral Administration of Vitamin B_{12} in Pernicious Anemia, Mayo Clinic. Proc., Vol. 24: No. 4: pp. 99 - 107, (Feb. 16) 1949.