Pernicious Anemia

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MILESTONES

Whipple GH, Hooper CW, Robscheit FS: **Blood regeneration following anemia.** *American Journal of Physiology* 1920, 53:236-262

Minot GR and Murphy WP: **Treatment of pernicious anemia by special diet.** *Journal of the American Medical Association* 1926, 87:470-476

Castle WB: **Observations of the etiologic relationship of achylia gastrica to pernicious anemia.** *American Journal of the Medical Sciences* 1929, 178:748-763

George H Whipple

In 1855, Thomas Addison at Guy's Hospital described a lethal, idiopathic anemia that in 1872 was given the name pernicious anemia by Biemer. For decades following, a commonly held view was that pernicious anemia reflected the positive-acting, deleterious influence of an infectious agent or a microbial product. A popular concept was that the injurious agent caused excessive destruction of red blood cells.

Clinical and autopsy studies subsequently established that pernicious anemia was more than a disorder of red blood cells. Besides reduced numbers of blood erythrocytes and low concentrations of hemoglobin, patients were found to have excessive iron deposits in the liver, gastric glandular atrophy and achlorhydria, megaloblastic bone marrow hyperplasia, and profound demyelination and atrophy of sensory axons in the spinal cord. Under the microscope some cells of the body that normally turn over rapidly were found to be increased in size. Large erythrocytic precursors in the bone marrow were recognized early, but with time it became clear that the blood of patients with pernicious anemia contained giant granulocytes and platelets, and that large epithelial cells were present in their gastric and vaginal washings.

Beginning in the early 1900's anecdotal reports suggested to a few investigators that pernicious anemia might be a nutritional deficiency disease. In the 1920's two milestone discoveries were published, one by the pathologist George Whipple and colleagues at the University of Rochester[1], the other by two clinical investigators, George Minot and William Murphy, at Harvard Medical School[2]. Whipple showed that anemia could be the result of a nutritional deficiency. Minot and Murphy developed a special diet that could reverse the pathology of pernicious anemia and cure patients. This was a tremendous breakthrough because 1-2% of adults over age 50, primarily people of northern European ancestry, suffered from this fatal disease. In 1934, Minot, Murphy and Whipple shared the Nobel Prize in Physiology and Medicine for their discoveries. Their Nobel lectures – available on the Nobel website *(www.nobelprize.org)* – provide interesting historical information and data about their experiments. These milestone discoveries launched decades of investigations, notably by W.B. Castle[3], that uncovered the complex pathophysiological mechanisms underlying pernicious anemia. Castle's discoveries led to new treatments of the disease that were more effective, better tolerated by patients, and more affordable.

The importance of Whipple's work was that it firmly established on a quantitative basis that the properties of food influenced blood formation, a concept not previously accepted. Whipple was originally interested in the metabolism of biliary and blood pigments and had developed a model of chronic anemia in dogs by repeated phlebotomy. When he began to investigate factors that influenced blood regeneration in chronically anemic dogs, Whipple focused on diet. Of the various diets tested, he found that liver and liver extracts were the most effective, although feeding other meats – kidney, muscle, or brain – also stimulated hematopoiesis. The choice of liver was fortunate. As others later pointed out, had Whipple fed iron salts to the dogs, he likely would have observed the same result since the dogs he studied undoubtedly suffered from iron deficiency anemia. Whipple's findings on the effectiveness of liver feeding influenced Minot and Murphy to continue similar clinical studies they had been conducting in patients with pernicious anemia. A key element in the success of those studies was the reliance on blood reticulocyte counts to assess bone marrow responsiveness.

Once it was established that daily feedings of large amounts of liver or concentrated liver extracts induced varying degrees of remission in pernicious anemia, the central question became, "What is the active factor?" Castle[3] discovered that daily administration by gastric tube of liquefied stomach contents from a healthy person removed an hour after ingestion

of 300 grams of lean beef stimulated hematopoiesis in patients with pernicious anemia. Administration of gastric juice recovered from histamine-stimulated normal donors was ineffective. Administration of beef digested with pepsin was ineffective. Apparently, there was requirement for interaction between a factor in normal gastric juice and a factor in digested beef. The activity present in beef was designated as extrinsic factor; the activity present in normal gastric juice was designated as intrinsic factor. In retrospect, some of the dietary regimens in the clinical studies that led to the cure of pernicious anemia would probably raise eyebrows in today's Human Subject Committees.

Subsequent chemical analyses showed that extrinsic factor belonged to the cobalamin family of organometallic compounds. When it was shown that the active cobalamin was vitamin B12, therapy with vitamin B12 became standard treatment for pernicious anemia. Intramuscular treatment with vitamin B12 cured patients. To be effective by oral administration, vitamin B12 required the presence of normal gastric juice or massive doses of the vitamin. Later studies showed that intrinsic factor was a vitamin B12-binding protein produced by gastric gland parietal cells. Biochemical studies showed that vitamin B12 played a role in DNA synthesis, hinting at a mechanism that could account for the underproduction of red blood cell precursors in the bone marrow of patients with pernicious anemia.

Although curative treatment for pernicious anemia had been obtained, basic research in the area actually increased and publications continue through today. Uptake of vitamin B12 was shown to take place in the ileum via a specific mucosal receptor for the vitamin B12-intrinsic factor complex. New laboratory tests were developed to screen for pernicious anemia to distinguish it from other megaloblastic anemias. A great deal of effort was directed at understanding the basis for gastric gland atrophy and the loss of the intrinsic factor-producing parietal cells. The discovery of antibodies specific for parietal cells, intrinsic factor and other elements in the vitamin B12-uptake cascade have fostered the concept of pernicious anemia as an autoimmune disorder. Coming full circuit from the notion of a microbial etiology that was in vogue at the start of the 20th century and then discarded, it is now firmly established that *Helicobacter pylori* is a gastric pathogen that produces factors that are toxic for parietal cells.

Pernicious anemia is another example of a disease where an effective treatment came before an understanding of the underlying pathogenic mechanisms. It is another example where a disease, an experiment of nature, provided a powerful tool for biomedical discovery. Once the pathogenic mechanisms of pernicious anemia were understood, they provided critical insights into normal physiological processes, as well as the basis of other diseases. Minot, Murphy and Whipple worked without the highly specific, sensitive and sophisticated tools that are routine in today's biomedical research, yet they succeeded in making seminal discoveries, curing an incredibly complex disease, and launching the field of nutritional deficiency anemias.

References

1. Whipple GH, Hooper CW, Robscheit FS: *Am J Physiol* 1920, 53:236
2. Minot GR and Murphy WP: *JAMA* 1926, 87:470
3. Castle WB: *Am J Med Sci* 1929, 178:748

NEW DEVELOPMENTS IN THE DIAGNOSIS AND TREATMENT OF PERNICIOUS ANEMIA * †

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Criteria for the diagnosis of pernicious anemia must be reëvaluated in light of the changing pattern of this disease. The profound physical abnormalities and extreme hematologic aberrations associated with classic severe pernicious anemia are not often encountered in modern practice. There are two reasons for this alteration in the character of the disorder. In the first place, patients tend to consult their physicians early, at a time when symptoms may seem inconsequential. The only complaint may be slight weakness or fatigue, or perhaps soreness of the tongue. There may be no detectable pallor at this time, and even if the hemoglobin concentration is determined, anemia may be so mild in degree as to arouse little concern. The diagnosis at this stage is often overlooked. Second, patients with the early symptoms of pernicious anemia are very likely to receive therapeutic preparations which in varying degree are effective in overcoming the manifestations of the disease before the diagnosis has been established. The very nature of the initial symptoms makes it likely that a multivitamin or hematinic preparation will be prescribed, or even procured by the patient without medical advice. Most proprietary vitamin preparations contain folic acid, often in addition to vitamin B_{12}, intrinsic factor and a host of other substances. Administration of these preparations is followed by gratifying clinical improvement. Symptoms subside and anemia disappears. However, the need for adequate therapy for the duration of life is not recognized. Sooner or later relapse occurs, and often neurologic manifestations appear.[1]

In the past five years, 14 of the new cases of pernicious anemia seen at the Johns Hopkins Hospital have presented with crippling neurologic disease in the absence of an appreciable degree of anemia. In some of these the blood and bone marrow were entirely normal. All of these patients were seriously disabled; several were unable to stand or to control the bladder (figure 1). All have residual neurologic manifestations after prolonged and intensive parenteral therapy with vitamin B_{12}. In most cases it was definitely established that the patient had been taking a vitamin preparation containing folic acid.

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The possibility of pernicious anemia should be considered in any adult patient with an unaccountably subnormal hemoglobin value. Careful examination of the blood will show macrocytosis if anemia is present. However, it is important to remember that red cell abnormalities are slight, and the marrow pattern may not be diagnostic when anemia is mild. If there is a favorable response to treatment with parenterally administered vitamin B_{12}, the diagnosis is reasonably well established. A history of anemia which responded to treatment with a vitamin or hematinic preparation suggests the possibility of pernicious anemia. Appropriate tests should be performed to exclude this disease in any patient who complains of soreness of the tongue. Neurologic manifestations of subacute combined degeneration should always be considered as probably due to pernicious anemia, even though there are no hematologic abnormalities.

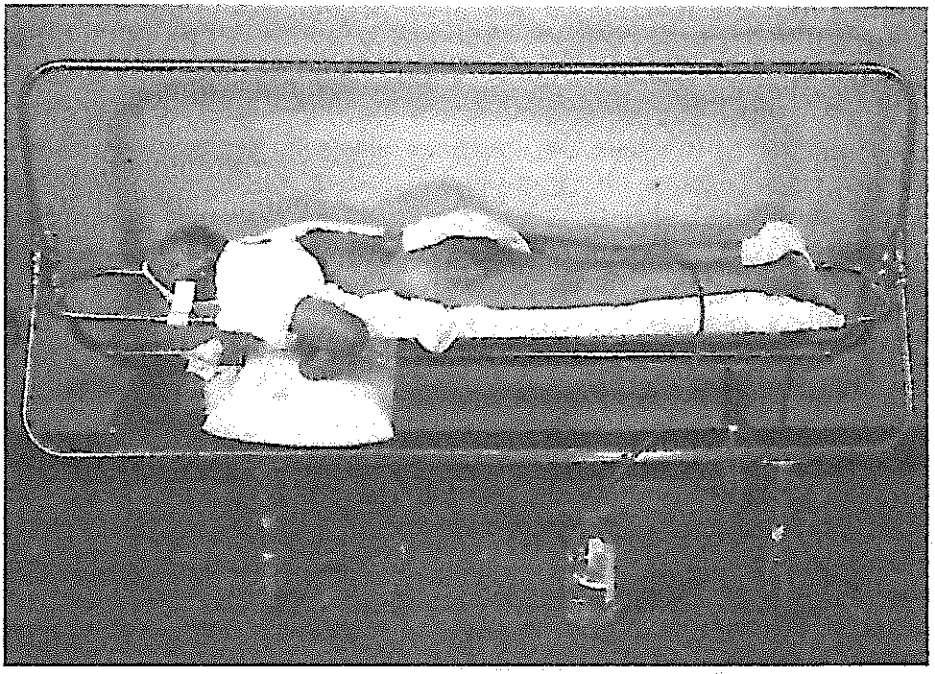

FIG. 1. This 66 year old woman for months had been taking a proprietary multivitamin preparation which contained folic acid. She had never previously had symptoms of pernicious anemia, but while receiving multivitamins rapidly developed extremely severe subacute combined degeneration. She was found to have achlorhydria, and the alimentary absorption of radioactive vitamin B_{12} was markedly impaired. After months of intensive parenteral therapy with vitamin B_{12} she has regained some control of bladder function, but it seems apparent that she will remain a helpless invalid because of permanent damage to the nervous system. A few millionths of a gram of vitamin B_{12}, administered parenterally, could have prevented this incapacitating disorder.

DIAGNOSIS OF PERNICIOUS ANEMIA

An extremely difficult problem is presented by the case in which the diagnosis of pernicious anemia was never definitely proved but in which

therapy has been adequate to induce or maintain a remission. The blood and marrow are normal, and the demonstration of gastric achlorhydria makes the diagnosis acceptable but by no means establishes it. If therapy is withheld, relapse may not occur for several years, so that this is an unsatisfactory diagnostic test. A very helpful solution to this problem has become available with the use of tracer tests employing vitamin B_{12} labeled with radioactive cobalt.[2-8] A small amount of the tagged vitamin is given orally and the fraction which is absorbed from the intestinal tract is measured. In pernicious anemia, absorption is impaired because of the deficiency of intrinsic factor.

Since 1951 we have used radioactive vitamin B_{12} in studies of more than 100 individuals. When an appropriate oral dose is administered, the presence of an absorption defect is readily demonstrated. Impaired absorption is regularly found in pernicious anemia and may also be encountered following total gastrectomy, in sprue and in association with lesions of the small intestine. Impaired absorption was not demonstrated in normal subjects of older age groups or in patients with a variety of diseases unrelated to pernicious anemia. Achlorhydria per se was not associated with reduced vitamin B_{12} uptake. In most of our studies the amount of radioactive material absorbed was determined by measuring the residual radioactivity of the stools. This is a time-consuming and laborious process, requiring total stool collections for not less than six days. The method has the advantage of permitting very accurate measurement. Schilling[9] devised an ingenious technic in which the radioactive material absorbed from the intestine is flushed out into the urine by means of a parenteral injection of a large amount of inert vitamin B_{12}. Radioactive measurements are then made on the urine rather than on the feces. This procedure is less precise, since all of the radioactive vitamin absorbed may not be excreted in the urine. It has the great advantage of simplicity, however, and the test is completed in only 24 hours. The Schilling test has been widely used, with extremely satisfactory results. We have found it to be a reliable clinical test of inestimable value in the diagnosis of pernicious anemia in patients who are in remission as a result of previous therapy. The procedure can easily be carried out in any laboratory in which there are facilities for radioactive measurements.

Treatment of Pernicious Anemia

When refined liver extract became available, completely satisfactory treatment for pernicious anemia was at hand. Patients adequately treated with parenteral liver preparations remained in complete remission throughout their lives. No form of treatment can accomplish more, and a number of therapeutic regimens frequently employed in recent years have accomplished much less. In particular, the use of folic acid in the past decade has in many instances permitted neurologic manifestations to develop while the blood remained normal.

The therapeutic effect of liver extract is attributable to the vitamin B_{12} which it contains. The results of treatment of pernicious anemia with vitamin B_{12} are no better than those obtained with liver extract. Some authors [10, 11] have reported that vitamin B_{12} does not provide complete replacement therapy in pernicious anemia, but the experience of other investigators does not support this contention.[12] More than 50 patients with pernicious anemia in the Hematology Clinic of the Johns Hopkins Hospital have had no therapy other than vitamin B_{12} in the past six years and all remain in complete remission. We have not encountered the hypoprothrombinemia * and macrocytosis which have been described by others during treatment with vitamin B_{12}.

Current interest in the treatment of pernicious anemia centers about the use of orally administered preparations. When vitamin B_{12} is combined with intrinsic factor, absorption of the vitamin is facilitated. Steady progress is being made in the purification of intrinsic factor, and preparations of considerable potency are now available. Several commercial preparations contain a mixture of vitamin B_{12} and a source of intrinsic factor for oral treatment of pernicious anemia. Preliminary observations indicate that

TABLE 1

Patients with Pernicious Anemia Treated with Orally Administered Vitamin B_{12}

Remission induced by oral therapy	17
Remission induced and subsequently maintained by oral therapy	14
Remission maintained by oral therapy	14
Total	45

these are satisfactory, although careful observation of a large group of patients over a long period of time will be required to establish that these preparations are as reliable as is the parenteral injection of vitamin B_{12}. It can be said with certainty that the amount of vitamin B_{12} which can be absorbed from the gastrointestinal tract under the most favorable conditions is far less than that which is customarily injected parenterally. Therefore, when intensive therapy is indicated, as in the patient with neurologic manifestations, parenteral therapy should always be used.

When very large amounts of crystalline vitamin B_{12} are given orally in the absence of intrinsic factor, satisfactory therapeutic responses may be obtained. The doses required for these effects are measured in milligrams rather than in micrograms.[13, 14, 15] In 1950 we initiated an experimental study to determine whether orally administered vitamin B_{12} alone is adequate treatment for pernicious anemia.[12] Forty-five patients have now been treated in this way † (table 1). Thirty-one were in hematologic

* Plasma prothrombin measured by the two-stage technic in 15 of our patients maintained for years on vitamin B_{12} alone was well within the normal range. The lowest values obtained were higher than values obtained in some normal subjects.

† The vitamin B_{12} used in these studies was generously provided by Merck and Co., Inc., Rahway, New Jersey.

relapse when first treated. Most of these patients were hospitalized and placed on a diet deficient in vitamin B_{12}. After completion of the initial studies, a single oral dose of vitamin B_{12}, ranging from 3,000 to 10,000 μg, was given in the morning with the patient fasting. After this single dose, additional therapy was withheld until it was clear that no further improvement was taking place. A number of patients were observed for more than a month before additional treatment was given. The clinical and hematologic responses to these large oral doses were in most instances entirely comparable to those seen after parenteral injection of 30 or more micrograms of the vitamin (figure 2). Soreness of the tongue and gastro-

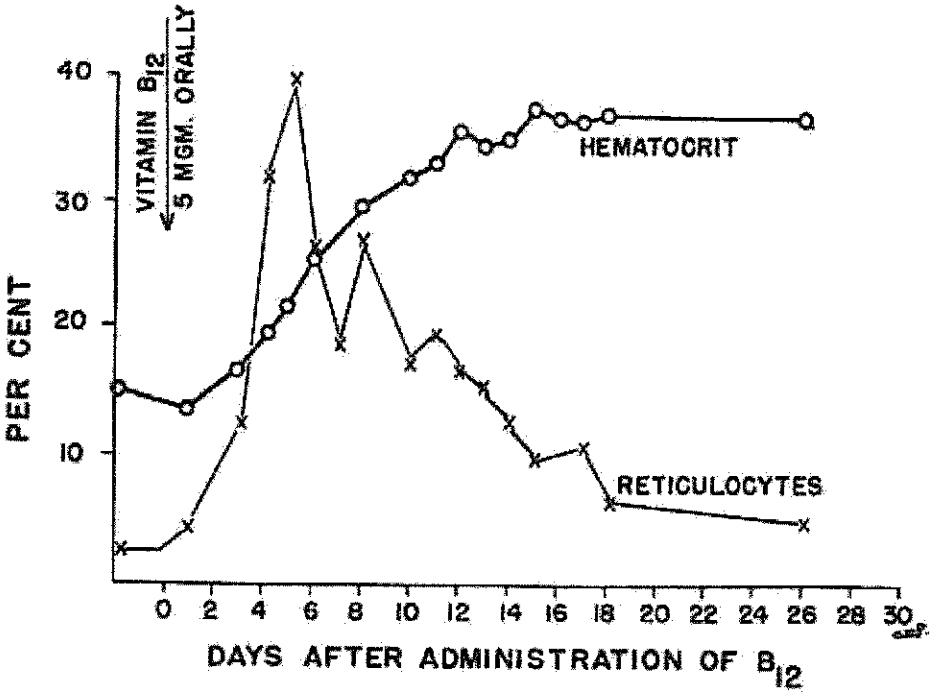

FIG. 2. Hematologic response of a patient with pernicious anemia to a single oral dose of 5,000 μg of vitamin B_{12}.

intestinal symptoms subsided. In several cases neurologic manifestations improved. Several patients developed a complete hematologic remission after a single oral dose. Three patients responded suboptimally to oral therapy. Parenteral injection of the vitamin in each of these three cases failed to accelerate the hematologic improvement. In two the retarded response was associated with infection. The third patient was subsequently allowed to go into relapse and on a second trial responded well to orally administered vitamin B_{12}. In addition to the cases of classic pernicious anemia, one patient with megaloblastic anemia following total gastrectomy and one with a pernicious anemia-like syndrome associated with multiple

TABLE 2

Patients Initially Treated and Maintained in Remission with Orally Administered Vitamin B_{12}*

Patient	Months	Hematocrit	
		Initial	Present
C. J. 55 CF	56	14.0	51.0
E. T. 44 WF	39	13.7	42.0
B. C. 61 CF	36	16.9	44.4
W. M. 84 WM	31	22.0	45.8
T. L. 54 WM	27	26.0	45.1
E. W. 68 CF	27	24.0	43.0

* These patients were brought out of relapse by oral therapy. The initial dose ranged between 3,000 and 10,000 μg of vitamin B_{12}. The maintenance dose in each case was 1,000 μg given as a single tablet once a week.

diverticula of the jejunum also responded well to single oral doses of the vitamin.

Information currently at hand makes it seem probable that extremely high concentrations of vitamin B_{12} in the intestine are required if adequate amounts are to be absorbed regularly in the absence of intrinsic factor. A single dose of less than 1,000 μg appears to be suboptimal, and some patients have failed to respond to daily doses of as much as 250 μg.[16, 17, 18] In attempting oral maintenance therapy, therefore, we decided to use a single large dose once a week, rather than smaller doses at daily intervals.

Of the 31 patients brought into remission by orally administered vitamin B_{12}, 14 have continued on oral therapy for periods ranging for from four months to almost five years. All 14 remain in complete remission, and eight have now been under continuous maintenance therapy for more than two years (table 2). The maintenance dose has been 1,000 μg of vitamin B_{12}, given as a single tablet once a week.

TABLE 3

Patients Maintained in Remission for More Than Three Years by Orally Administered Vitamin B_{12}*

(1,000 micrograms once per week)

Patient	Months	Hematocrit	
		Initial	Present
M. D. 63 WF	39	43.8	45.0
B. S. 73 CF	39	45.0	43.4
R. F. 49 WF	39	44.8	43.7
T. S. 50 WF	39	41.9	40.7
V. F. 48 CF	39	41.5	40.3
A. M. 56 WF	38	44.8	42.2
M. S. 67 WF	38	46.2	47.2
M. G. 70 CF	36	41.9	42.7

* These patients, previously maintained on parenteral therapy, were in remission at the onset of oral therapy. All have remained in complete remission.

HCT ↓

Fourteen additional patients had previously been maintained on parenteral therapy and were in remission at the time oral therapy was instituted. These patients have been receiving 1,000 μg of vitamin B_{12} orally once a week for 15 months or longer, and all remain in remission. Hematocrit values before and after oral maintenance therapy in those of this group treated for more than three years are shown in table 3.

The results of this and of comparable studies [19] indicate that patients with pernicious anemia can be satisfactorily treated by the oral administration of large amounts of vitamin B_{12} in the absence of intrinsic factor or other adjuvants. Therapeutic effects appear to be as good as those obtained with oral preparations containing concentrates of intrinsic factor. The

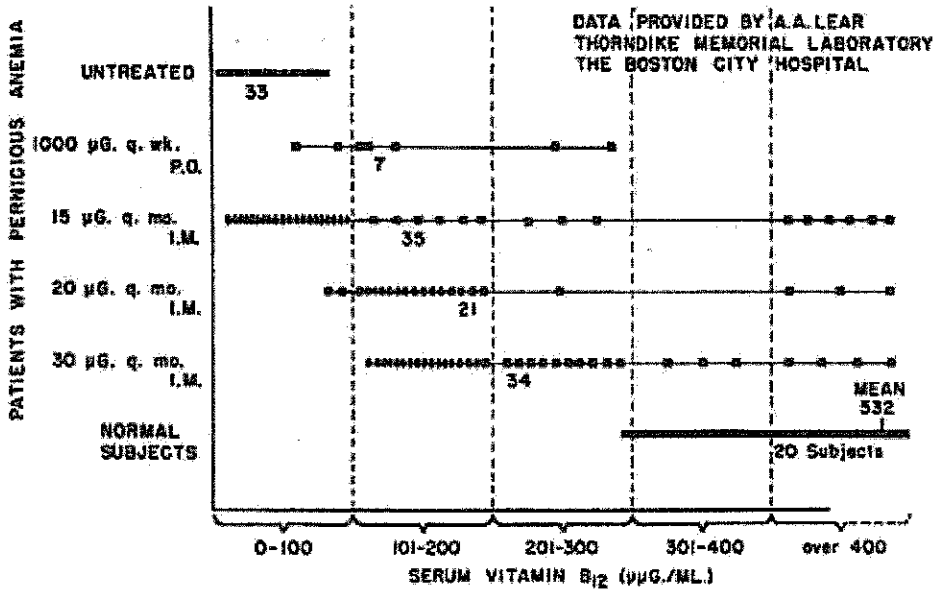

FIG. 3. The concentrations of vitamin B_{12} in the serum of seven patients maintained for prolonged periods on 1,000 μg of vitamin B_{12} orally once a week are compared with those of normal subjects and of patients with untreated and parenterally treated pernicious anemia. Blood for these determinations was drawn just prior to administration of a regularly scheduled dose. All of the treated patients were in clinical remission.

advantage of crystalline material is that it can be assayed by weight, whereas the potency of intrinsic factor preparations must necessarily be measured in terms of the response produced when administered to patients with pernicious anemia.

While these studies were in progress, Lear and his associates [20] were determining the serum vitamin B_{12} concentrations of a large group of patients at the Boston City Hospital. We were fortunate to have their collaboration in measuring the vitamin B_{12} levels in the serum of seven of our patients who had been maintained for prolonged periods of time on 1,000 μg of B_{12} orally once a week. The results can be compared directly with those obtained in other patients on various parenteral dosage schedules

(figure 3). It is clear that the amount of vitamin B_{12} absorbed from the intestine, even with these large doses, is not sufficient to restore the serum concentration to normal. Two patients had extremely low serum levels even though they appeared to be in complete clinical and hematologic remission. These data suggest that the oral dosage schedule employed, 1000 μg once a week, is suboptimal, and that larger amounts would be required to restore tissue saturation. Evidence has been provided by others [14, 19, 21] that the concentration of vitamin B_{12} in the serum of patients with pernicious anemia can be restored to normal levels if adequate oral doses are given.

It is important to emphasize that relatively little vitamin B_{12} can be absorbed from any orally administered preparation in contrast to the large amount which can be injected parenterally. Furthermore, the ability of various patients to absorb the vitamin differs. The complete effectiveness of parenteral therapy, which requires injections no more often than once a month, has been well established. At the present time it would seem wise for most patients with pernicious anemia to continue to receive parenteral therapy. However, if oral therapy is to be used, crystalline vitamin B_{12} alone in milligram doses appears to be as effective as smaller amounts of the vitamin combined with intrinsic factor.

Summario in Interlingua

Patientes monstrante le precoce manifestationes de anemia perniciose es frequentemente tractate con preparatos multivitaminic o hematinic que contine acido folic, mesmo ante le diagnose es definitemente establite. Per consequente, le curso clinic del morbo es alterate, e manifestationes neurologic pote resultar in le absentia de anemia. Le diagnose es frequentemente difficile a establir in patientes tractate in iste maniera. Le uso de tests a etiquettage con un forma radioactive de vitamina B_{12} es de specific valor diagnostic in tal casos.

Therapia parenteral con vitamina B_{12} remane le tractamento de selection, sed certe preparatos que es administrate per via oral pare therapeuticamente efficace.

In le presente studio 45 patientes con anemia perniciose esseva tractate con vitamina B_{12} in forma crystallin, administrate oralmente sin factor intrinsec. Quando 3 a 10 milles μg de vitamina B_{12} esseva administrate per via oral e in un sol dose a patientes in stato de recidiva, le responsas clinic e hematologic esseva simile al responsas resultante del therapia parenteral. Il esseva possibile mantener le patientes in stato de remission complete per le administration de un sol tabletta con 1000 μg B_{12} un vice per septimana. Per iste regime 11 patientes ha essite mantenite de maniera satisfactori durante periodos de plus que tres annos. Totevia, recente mesurationes del concentration seral de vitamina B_{12} in 7 de iste patientes indica que le nivellos seral que resulta de iste dosage es probabilemente suboptimal e que plus grande quantitates es requirite pro restaurar le saturation del texitos.

BIBLIOGRAPHY

1. Conley, C. L., and Krevans, J. R.: Development of neurologic manifestations of pernicious anemia during multivitamin therapy, New England J. Med. 245: 529, 1951.
2. Chaiet, L., Rosenblum, C., and Woodbury, D. T.: Biosynthesis of radioactive vitamin B_{12} containing cobalt^{60}, Science 111: 601, 1950.

3. Heinle, R. W., Welch, A. D., Scharf, V., Meacham, G. C., and Prusoff, W. H.: Studies of excretion (and absorption) of Co^{60}-labelled vitamin B_{12} in pernicious anemia, Tr. A. Am. Physicians 65: 214, 1952.
4. Meyer, L. M.: Oral administration of Co^{60} vitamin B_{12} in pernicious anemia, Proc. Soc. Exper. Biol. and Med. 82: 490, 1953.
5. Swenseid, M. E., Halsted, J. A., and Libby, R. L.: Excretion of cobalt^{60} labelled vitamin B_{12} after total gastrectomy, Proc. Soc. Exper. Biol. and Med. 83: 226, 1953.
6. Callender, S. T., Turnbull, A., and Wakisaka, G.: Estimation of intrinsic factor of Castle by use of radioactive vitamin B_{12}, Brit. M. J. 1: 10, 1954.
7. Krevans, J. R., Conley, C. L., and Sachs, M.: Influence of certain diseases on the absorption of vitamin B_{12} from the gastrointestinal tract, J. Clin. Investigation 33: 949, 1954.
8. Glass, G. B. J., Boyd, L. J., and Stephanson, L.: Intestinal absorption of vitamin B_{12} in humans as studied by isotope technic, Proc. Soc. Exper. Biol. and Med. 86: 522, 1954.
9. Schilling, R. F.: Intrinsic factor studies. II. The effect of gastric juice on the urinary excretion of radioactivity after the oral administration of radioactive vitamin B_{12}, J. Lab. and Clin. Med. 42: 860, 1953.
10. Owren, P. A.: The deficiency of a factor necessary for normal protein synthesis in the liver in pernicious anemia, Proceedings of the Third International Congress of the International Society of Hematology, 1951, Grune and Stratton, New York, p. 34.
11. Beard, M. F., and McIlvanie, S. K.: Vitamin B_{12} as a hemotherapeutic agent in pernicious anemia, Proceedings of the Third International Congress of the International Society of Hematology, 1951, Grune and Stratton, New York, p. 34.
12. Conley C. L., Green, T. W., Hartmann, R. C., and Krevans, J. R.: Prolonged treatment of pernicious anemia with vitamin B_{12}, Am. J. Med. 13: 284, 1952.
13. Ungley, C. C.: Absorption of vitamin B_{12} in pernicious anemia. I. Oral administration without a source of intrinsic factor, Brit. M. J. 2: 905, 1950.
14. Conley, C. L., Krevans, J. R., Chow, B. F., Barrows, C., and Lang, C. A.: Observations on the absorption, utilization and excretion of vitamin B_{12}, J. Lab. and Clin. Med. 38: 84, 1951.
15. Pitney, W. R., and Beard, M. F.: Serum and urine concentrations of vitamin B_{12} following oral administration of the vitamin, J. Clin. Nutrition 2: 89, 1954.
16. Spies, T. D., Stone, R. E., Lopez, G. G., Milanes, F., Toca, R. L., and Aramburu, T.: Vitamin B_{12} by mouth in pernicious and nutritional macrocytic anaemia and sprue, Lancet 2: 454, 1949.
17. Hall, B. E.: Studies on the nature of the intrinsic factor of Castle, Brit. M. J. 2: 585, 1950.
18. Meyer, L. M., Sawitsky, A., Cohen, B. S., Krim, M., and Fadem, R.: Oral treatment of pernicious anemia with vitamin B_{12}, Am. J. M. Sc. 220: 604, 1950.
19. Unglaub, W. G., and Goldsmith, G. A.: Oral vitamin B_{12} in the treatment of macrocytic anemias, South. M. J. 48: 261, 1955.
20. Lear, A. A., Harris, J. W., Castle, W. B., and Fleming, E. M.: The serum vitamin B_{12} concentration in pernicious anemia, J. Lab. and Clin. Med. 44: 715, 1954.
21. Ross, G. I. M., Mollin, D. L., Cox, E. V., and Ungley, C. C.: Hematologic responses and concentration of vitamin B_{12} in serum and urine following oral administration of vitamin B_{12} without intrinsic factor, Blood 9: 473, 1954.