THE ABSORPTION OF RADIOACTIVE VITAMIN B_{12} IN MEGALOBLASTIC ANAEMIA, WITH OBSERVATIONS ON THE DIAGNOSTIC VALUE OF THE SCHILLING TEST

J. METZ, M.D.* and D. HART, *Radioisotope Laboratory, South African Institute for Medical Research, Johannesburg*

The isolation of vitamin B_{12}[1,2] and the development of sensitive microbiologic techniques for the assay of the vitamin in body fluids have marked a significant advance in clinical medicine. Although the exact metabolic functions of vitamin B_{12} have not yet been fully elucidated, sufficient is known to indicate that it is concerned in fundamental processes in the cell, e.g. the synthesis of deoxyribose nucleic acid.[3] It is not surprising, therefore, that deficiency of the vitamin may result in severe pathological changes in Man, affecting many systems.

The major human dietary source of vitamin B_{12} is animal protein in foodstuffs, and the daily requirement, which is probably less than 1 μg.,[4] is derived from the diet. Diets markedly deficient in animal protein may nevertheless contain sufficient vitamin B_{12} to supply this small daily requirement, and severe deficiency of vitamin B_{12} is usually associated with malabsorption of the vitamin.

Assessment of the deficiency of vitamin-B_{12} absorption is therefore of considerable clinical importance and has been made possible by the introduction of radioactive-labelled vitamin B_{12}. By culturing vitamin B_{12}-producing bacteria in media enriched with one of the radioactive isotopes of cobalt, radioactive-labelled vitamin B_{12} can be produced. Various techniques for the quantitation of absorption following the oral administration of a test dose of radioactive vitamin B_{12} have been described, including: assay of unabsorbed radioactivity in the stools;[5] assay of urinary radioactivity after a large flushing dose of non-radioactive vitamin B_{12};[6] radiation counting over the liver;[7] assay of serum radioactivity;[8] and whole-body counting.[9]

The simplest method is the urinary excretion test (Schilling test). This procedure has been of value in the diagnosis of Bantu patients with various types of megaloblastic anaemia.[10-12] The test has also been used to investigate vitamin-B_{12} absorption in pernicious anaemia, neurological diseases and gastro-intestinal disorders. The results of these studies are presented here and their diagnostic value is discussed.

METHODS

Initially $^{60}CoB_{12}$ (half-life 5·3 years), kindly supplied by Dr. E. Lester Smith, was used, but since the introduction of $^{58}CoB_{12}$ with a shorter half life (72 days), all tests have been carried out with this radioisotope purchased from the Radiochemical Centre, Amersham, United Kingdom. Carrier vitamin B_{12} was added to make the specificity activity approximately 1 μc./μg. Initially the test dose used was 0·6 μg., but over the past few years the dose administered has been 1μg. of vitamin B_{12}, the differentiation between normal and sub-normal absorption being better with the larger dose.[13]

The Schilling test was carried out as follows: Vitamin-B_{12} therapy was withheld for at least 48 hours, and the patient had nothing to eat or drink from 10 p.m. the night before the test. In the morning the patient emptied the bladder and the urine was discarded. Water was added to the container with the radioactive B_{12}, and the patient drank directly from the container. The container was rinsed and the patient drank the rinsings. A total of approximately 100 ml. of water was administered. In some of the tests, a subcutaneous injection of 0·25 mg. of carbachol (carbamyl-choline chloride) was then administered. The reason for injecting carbachol was that absorption may be defective in some normal persons, but carbachol was found to correct this without increasing absorption in patients with pernicious anaemia.[14]

The patient had no food or drink for 2 hours, and after this period 1,000 μg. of vitamin B_{12} were administered by intramuscular injection, and all urine was collected for 24 hours. The smallest acceptable urine volume was 500 ml. When the effect of intrinsic factor on the absorption of radioactive vitamin B_{12} was studied, the test was repeated in the identical way, only a hog intrinsic factor concentrate was administered together with the radioactive vitamin B_{12}. The repeat test was carried out at least 2 days after the first test. The intrinsic factor preparations used were kindly supplied by Lederle Laboratories, and the potency of new batches was tested by measuring the enhancement of absorption of radioactive vitamin B_{12} in known cases of pernicious anaemia.

The radioactivity of the 24-hour urine sample was measured initially by counting one litre of urine in a flask placed over the well of a well-type scintillation counter containing a thallium-activated sodium iodide crystal. When the 24-hour specimen was less than 1 litre, water was added to make up to volume. More recently an aliquot (400 ml.) of urine has been counted in a volume counter comprising 2 sodium iodide crystals at a fixed distance apart. Standards corresponding to 5%, 10% and 20% of the administered dose were prepared, and counted with each assay of specimens. The percentage radioactivity in the urine was obtained from the graph, and corrected for the total volume of the urine specimen. Results are expressed as percentage of administered dose of radioactivity excreted in the urine, normal subjects excreting 5 - 30%.

MATERIAL

1. *Megaloblastic anaemia.* Ninety-four patients, who presented with anaemia associated with megaloblastic erythropoiesis in the bone marrow, were tested. A total of 176 tests were carried out on these patients.

2. *Non-anaemic patients in whom the diagnosis of pernicious anaemia was suspected.* Thirty-one non-anaemic patients, with a history of previous anaemia which the referring physicians regarded as possibly pernicious anaemia, were tested. Many patients in this group were receiving regular injections of vitamin B_{12} or liver extract. In this group 36 tests were carried out.

3. *Neurologic disease.* Thirteen patients with neurologic features suggestive of subacute combined degeneration of the spinal cord were tested. In this group 18 tests were carried out.

4. *Malabsorption syndrome.* Seven patients with lesions of the small intestine, in whom malabsorption of vitamin B_{12} was suspected, were tested. In this group 12 tests were carried out.

RESULTS

1. *Megaloblastic Anaemia*

(*a*) *Pernicious anaemia.* There were 50 patients in this group, 32 Bantu and 18 White. The results are shown in Fig. 1. The urinary excretion of radioactive B_{12} after the oral test dose ranged from 0·0% to 3·7% (mean 0·82 $\pm$ 0·139%); when the radioactive vitamin B_{12} was administered together with a source of intrinsic factor, the malabsorp-

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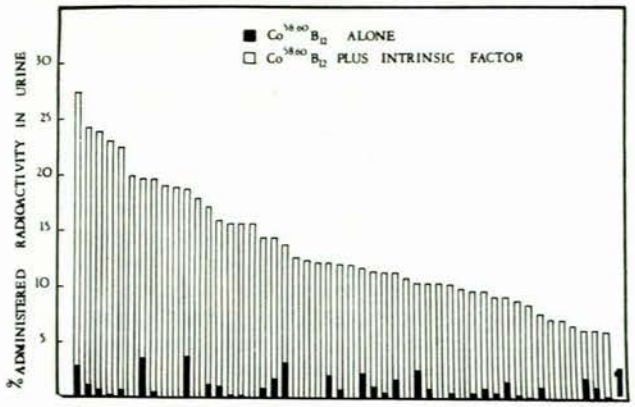

Fig. 1. The absorption of radioactive vitamin B_{12} in pernicious anaemia with and without oral intrinsic factor.

tion of vitamin B_{12} was corrected in all patients, the mean urinary excretion ranging from 6·0% to 27·4% (mean 13·19 ± 0·741%).

(*b*) *Megaloblastic anaemia associated with pregnancy.* This group comprised 40 Bantu females suffering from megaloblastic anaemia following pregnancy. The results are shown in Fig. 2. The urinary excretion of radioactive

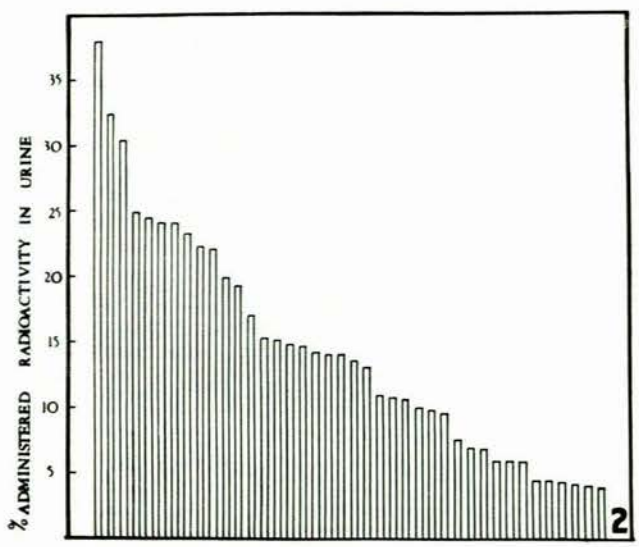

Fig. 2. The absorption of radioactive vitamin B_{12} in megaloblastic anaemia associated with pregnancy.

B_{12} ranged from 3·9% to 38·0% (mean 14·38 ± 1·350%). Of the group of 40 patients, 6 showed subnormal vitamin-B_{12} absorption, but the levels were not as low as in patients with pernicious anaemia.

(*c*) *Megaloblastic anaemia associated with liver disease.* This group comprised 3 White patients and 1 Bantu patient with megaloblastic anaemia associated with hepatic cirrhosis. The urinary excretion of $^{58}CoB_{12}$ after oral administration was 5·0%, 10·7%, 16·0% and 17·0%.

2. *Non-anaemic Patients*

In this group of 31 White patients, 6 showed malabsorption of vitamin B_{12}, which was corrected by the concomitant administration of intrinsic factor. In the other 25 patients, radioactive vitamin-B_{12} absorption was normal (range 6·3 - 31·2%; mean 15·61%).

3. *Neurologic Disease*

Of the 13 patients in this group 5 (3 Bantu and 2 White) showed malabsorption of labelled B_{12} (urinary excretion 0·0 - 0·13 - 0·6% of the administered dose), which was corrected by the concomitant administration of intrinsic factor (urinary excretion 8·1 - 10·3 - 12·5% of the administered dose). Of these 5 patients, 3 presented with megaloblastic anaemia and spinal-cord involvement; in 1 there was no anaemia, but the serum vitamin B_{12} was 10 $\mu\mu$g./ml.; and in 1 patient there was a history of anaemia treated by blood transfusion and liver injections.

In the other 8 patients, the absorption of radioactive vitamin B_{12} was normal (urinary excretion 7·1 - 16·3 - 29·4% of the administered dose).

4. *Malabsorption Syndrome*

This group consisted of 4 patients who had undergone resection of part of the small intestine, 2 patients with idiopathic steatorrhoea, and 1 patient with a history of tropical sprue. The 3 patients with resection of the small intestine, in whom part of the ileum was left intact, absorbed vitamin B_{12} in normal amounts (urinary excretion 14·0%, 15·0% and 9·4%); in one of these patients, the whole of the small bowel, with the exception of the duodenum, 3 feet of jejunum and 6 inches of terminal ileum had been resected. In the 4th patient, the whole of the ileum had been resected, and the urinary excretion was 0·0% of the administered dose. This malabsorption was unaffected by concomitant administration of intrinsic factor, or by sterilization of the bowel with antibiotics. Of the 2 patients with idiopathic steatorrhoea, one showed a urinary excretion of 0·3%, which was not corrected by concomitant administration of intrinsic factor, and in the other patient, who was receiving a gluten-free diet, the vitamin-B_{12} absorption was supranormal (urinary excretion 45%). The 1 patient with a history of tropical sprue many years previously, showed normal absorption (23·2%).

DISCUSSION

The urinary excretion test of Schilling is an adequate index of vitamin-B_{12} absorption, but does have minor drawbacks. To carry out the test it is necessary to administer a large dose of non-radioactive vitamin B_{12}, but this is rarely of practical importance, since the lesions causing vitamin-B_{12} malabsorption are not reversed by vitamin-B_{12} therapy. Loss of a specimen of urine during the collection period invalidates the result; however, even in the incontinent patient with neurologic disease, the test can be carried out using an indwelling catheter. The test is dependent on normal renal function, and in the patient with advanced renal failure, spuriously low results may be obtained.[15] A more valid index of vitamin-B_{12} absorption in patients with renal impairment is obtained if the urine collection period is extended to 48 hours.[16]

The results presented in this study illustrate many of the important applications of the Schilling test. While assay of the concentration of vitamin B_{12} in the serum is adequate to establish body deficiency of the vitamin, it

gives no indication of the pathogenesis of this deficiency. Severe deficiency is usually associated with malabsorption, which can be demonstrated by the Schilling test. While a single Schilling test will establish malabsorption of the vitamin, it will give no information about the site or nature of the lesion. If the Schilling test is abnormal then the test should be repeated with a dose of intrinsic factor. If the absorption is corrected by intrinsic factor it can be concluded that the malabsorption was due to lack of intrinsic factor. Intrinsic factor deficiency may be the result of: (1) a constitutionally determined failure in intrinsic factor secretion — the lesion of Addisonian pernicious anaemia; (2) surgical removal of the intrinsic factor secreting area of the stomach, i.e. gastrectomy; (3) destruction of the intrinsic factor secreting area by malignant disease or fibrosis.

In practice, the vast majority of patients with intrinsic factor deficiency have pernicious anaemia. In the present study, intrinsic factor deficiency was established in 61 patients, in all of whom the lesion was apparently constitutionally determined. It is of considerable importance to note that this intrinsic factor deficiency is permanent, and can be demonstrated in the pernicious anaemia patient equally well in relapse or remission. The Schilling test is invaluable in the study of non-anaemic patients in whom a history of previous anaemia has led to the suspicion of pernicious anaemia. In 80% of such patients investigated in this study, it was possible to exclude the diagnosis of pernicious anaemia, and thus obviate unnecessary maintenance therapy; similarly, patients in whom intrinsic factor deficiency is proved can be reassured that regular injections of vitamin B_{12} are necessary.

In the group of patients with pernicious anaemia, many were able to absorb some, although subnormal amounts, of the test dose of radioactive vitamin B_{12}, indicating that the intrinsic factor failure is rarely absolute. The apparent preponderance of Bantu patients is no reflection on the relative incidence of the disease among Whites and Bantu, in that the early part of the study was carried out at the Baragwanath and Coronation non-White Hospitals.

Study of the absorption of labelled vitamin B_{12} is of considerable value in the establishment or exclusion of the diagnosis of subacute combined degeneration of the spinal cord. While this complication of pernicious anaemia is almost invariably accompanied by the haematologic features of the disease, it may occasionally present without anaemia, or a history of anaemia, and in these patients study of the absorption of vitamin B_{12} is essential.

In terms of numbers of patients affected, the most important megaloblastic anaemia affecting adults in South Africa is that associated with pregnancy. In this condition the underlying deficiency is that of folate rather than vitamin B_{12},[17] but in some patients the serum vitamin-B_{12} levels may be subnormal.[18] Confusion with true pernicious anaemia should not occur, in that the disease usually affects Bantu females following pregnancy. It is well to remember, however, that Addisonian pernicious anaemia has been noted in young Bantu females.[19] In the present study Addisonian pernicious anaemia was readily distinguished from the folate deficiency group by means of the Schilling test.

Measurement of vitamin-B_{12} absorption is important in the study of patients with small-bowel lesions. Idiopathic steatorrhoea, which in South Africa is a rare cause of vitamin-B_{12} malabsorption, should not cause confusion with pernicious anaemia, since the malabsorption of vitamin B_{12} is not corrected by intrinsic factor. Patients in whom part of the small intestine has been resected, or in whom an anastomosis has been made which permits the bacterial flora of the large intestine to enter the small intestine, may develop vitamin-B_{12} malabsorption. In these patients, study of vitamin-B_{12} absorption can predict and therefore prevent, the development of deficiency. This is well illustrated in the one patient in this study whose ileum was resected, and who was unable to absorb vitamin B_{12}. Normal serum vitamin-B_{12} levels have been maintained for 5 years by injections of the vitamin.

The Schilling test is thus a valuable diagnostic aid in diverse clinical syndromes, and often yields information which cannot be obtained by other means.

SUMMARY

The results of 242 measurements of radioactive vitamin-B_{12} absorption (Schilling test) in 145 patients are presented.

In 50 patients with pernicious anaemia, vitamin-B_{12} absorption was subnormal, and was corrected by the concomitant administration of intrinsic factor.

In 40 patients with megaloblastic anaemia associated with pregnancy, 6 showed subnormal vitamin-B_{12} absorption, but the levels were not as low as in patients with pernicious anaemia.

In 4 patients with megaloblastic anaemia and hepatic cirrhosis, vitamin-B_{12} absorption was normal.

In 31 non-anaemic patients in whom a previous diagnosis of pernicious anaemia was suspected, the diagnosis was confirmed in only 6.

The diagnostic value of the Schilling test in patients with megaloblastic anaemia, neurologic disease and malabsorption syndromes, is discussed.

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