J. clin. Path. (1966), **19**, 606

Measurement of intestinal absorption of ^{57}Co vitamin B_{12} by serum counting

J. FORSHAW AND LILIAN HARWOOD

From Sefton General Hospital, Liverpool

SYNOPSIS The results of the measurement of vitamin B_{12} absorption by counting the radioactivity of 5 ml. serum obtained eight to 10 hours after the ingestion of an oral dose of 0·5 μg. vitamin B_{12} labelled with 0·5 μc. ^{57}Co are compared with those obtained with the urinary excretion (Schilling) test. Inadequate urine collection and impaired renal function were responsible for low results in the Schilling test in four of the 12 control subjects, and an incomplete urine collection in four patients with pernicious anaemia could have led to doubt about the validity of the low result.

The measurement of serum radioactivity for 1,000 seconds gave conclusive results, the range in the patients with malabsorption of vitamin B_{12} being between 0 and 24 counts per minute, and in the control subjects and other patients with megaloblastic anaemia between 28 and 64 counts per minute. The highest serum radioactivity level in a patient with pernicious anaemia was 19 counts per minute.

Serum counting is simpler than the Schilling test and may be done alone when the patient's renal function is known to be poor, when urine collection is expected to be unreliable, or when the flushing dose of vitamin B_{12} should be avoided. Otherwise there is an advantage in doing both tests together for confirmation.

It is necessary to measure the absorption of vitamin B_{12} from the small intestine in order to diagnose the cause of vitamin B_{12} deficiency with precision. The methods available for measuring the absorption of an oral dose of radioactive vitamin B_{12} include the estimation of radioactivity in the faeces (Heinle, Welch, Scharf, Meacham, and Prusoff, 1952), the urine (Schilling, 1953), the liver (Glass and Boyd, 1957), and the blood (Booth and Mollin, 1956). Before measuring the hepatic uptake of vitamin B_{12} the residual radioactivity in the bowel must be cleared by laxatives and an enema, and in the faecal excretion method the preparation of the specimens is unpleasant and it takes an average of seven days to complete the test. Because of these disadvantages the measurement of the excretion of radioactivity in the urine (Schilling test) has been the most widely adopted method. However, the Schilling test also has disadvantages: these are that the test depends upon renal function and a complete 24-hour urine collection, and that the large parenteral dose of non-radioactive vitamin B_{12} may occasionally obscure the diagnosis.

The measurement of radioactivity in a single sample of serum or plasma is clearly the simplest method. However, the amount of radioactivity that appears in the blood after oral doses of 0·5 to 1·0 μg. vitamine B_{12} is small and in the early studies in which vitamin B_{12} labelled with ^{56}Co, ^{58}Co, or ^{60}Co was used it was necessary either to obtain large quantities of serum or plasma, to count for long periods in very sensitive scintillation counters, or to use isotopes of high specific activity (Booth and Mollin, 1956; Doscherholmen and Hagen, 1957; Goldberg, Trivedi, and Oliver, 1957). In view of these disadvantages the counting of radioactivity in the blood was not regarded as a satisfactory method for measuring vitamin B_{12} absorption. However, the lower energy gamma radiation of ^{57}Co is counted with high efficiency by scintillation crystals (Eberle and Gleason, 1960), and when vitamin B_{12} labelled with ^{57}Co became available, blood counting was again investigated and satisfactory results were obtained using a dose of 0·5 μc. and a small volume of serum (Nelp, McAfee, and Wagner, 1963; McCurdy, 1965). As the results of serum or plasma counting using ^{57}Co vitamin B_{12} have not been reported by workers in this country we considered that it was important to determine whether this

Received for publication 9 May 1966.

method was feasible, using British radioactive counting equipment.

MATERIAL AND METHODS

Studies were made on 12 volunteer control subjects who were hospital patients with either respiratory infections or a myocardial infarction, and on 17 patients with megaloblastic anaemia.

Serum levels of vitamin B_{12} were assayed by the *Lactobacillus leichmanii* method (Matthews, 1962), and of folic acid activity by the *Lactobacillus casei* method (Spray, 1964). The normal range of the level of serum vitamin B_{12} is 150 to 850 pg. per ml., and of folic acid activity 2·1 to 12 ng. per ml.

The intestinal absorption of 0·5 μg. vitamin B_{12} labelled with 0·5 μc. ^{57}Co was measured. The oral dose of radioactive vitamin B_{12} was accompanied by either an intramuscular injection of 0·04 mg. histamine per kilogram of body weight or, if vitamin B_{12} absorption with histamine was low, by an oral dose of 60 mg. intrinsic factor preparation (Armour). The side-effects of histamine were prevented by giving an intramuscular injection of 100 mg. mepyramine beforehand. Two hours later a parenteral dose of 1 mg. non-radioactive vitamin B_{12} was given. The urine was collected for 24 hours and a 5 ml. sample of serum obtained between eight and 10 hours after the dose of radioactive vitamin B_{12}, as it has been shown that blood radioactivity reaches a maximum concentration at this time (Doscherholmen and Hagen, 1957).

An Ekco well-type scintillation counter (N664A) crystal (N597) and scaler (N610B) were used. Background counts were recorded for 1,000 seconds both before and after counting the samples. Three 5 ml. aliquots from each 24-hour collection of urine were counted for 300 seconds, and the 5 ml. samples of sera were counted for 1,000 seconds. The radioactivity in the urine was expressed as a percentage of the administered dose. There was little variation in the radioactivity of the doses of ^{57}Co vitamin B_{12}, and the serum radioactivity was therefore recorded directly as counts (serum counts minus background counts) per minute per 5 ml. When the serum radioactivity was in the marginal range (17 to 25 counts per minute) both the background and serum counting were repeated for a further 1,000 seconds.

RESULTS

The background radioactivity before and after counting each batch of urine and serum samples was fairly constant. During the period of this investigation, in which 19 batches of samples were counted, the background radioactivity ranged from 73 to 88 counts per minute (mean 80·8 c.p.m.). Before deducting the background counts, the level of radioactivity in the serum samples from the 12 control patients ranged from 105 to 147 counts per minute (mean 123·2 ± 8·2 c.p.m.), while levels in the 10 patients with pernicious anaemia ranged from 76 to 101 counts per minute (mean 87·6 ± 5·2 c.p.m.). Using a t test these differences were found to be significant at the 0·1% level.

The results are summarized in Tables I and II. In the 12 control subjects believed to have normal vitamin B_{12} absorption, the 24-hour urinary excretion of radioactivity was 6 to 29% of the administered dose, and the serum radioactivity was between 28 and 64 counts per minute. The urine contained less than 11% of the dose in four subjects, and this is below the normal range when a dose of 0·5 μg. vitamin B_{12} is used (Callender and Evans, 1955). One of these patients (no. 12), in whom only 6% of the administered radioactivity was excreted in the urine, had chronic pyelonephritis and the blood urea level was 250 mg. per 100 ml. In another patient (no. 7) only 200 ml. urine was collected, and in the other two patients (nos. 2 and 4) the small urine volumes suggest that these collections were also incomplete.

TABLE I

RESULTS OF VITAMIN B_{12} ABSORPTION TESTS IN 12 CONTROL SUBJECTS

Patient No.	Urine Volume (ml./24 hr.)	Vitamin B_{12} Absorption	
		Urine Radioactivity (% dose)	Serum Radioactivity (counts per minute)
1	1,000	16	30
2	915	10	56
3	1,800	22	45
4	640	10	28
5	1,950	25	48
6	990	29	55
7	200	8	29
8	1,120	14	31
9	710	14	64
10	1,950	23	43
11	1,500	18	37
12	2,260	6	34

In the 10 patients with pernicious anaemia the excretion of radioactivity in the urine ranged from less than 1% to 8%, and eight of the results were below 6%. For these patients serum radioactivity was from 0 to 19 counts per minute. When the tests were repeated with intrinsic factor, the radioactivity in the urine was from 10 to 24%, and in the serum from 24 to 47 counts per minute.

Two patients with idiopathic steatorrhoea (nos. 23 and 24) had normal vitamin B_{12} absorption, the radioactivity in the urine being 12 and 14% and in the serum 34 and 41 counts per minute. In the other two patients with idiopathic steatorrhoea (nos. 25 and 26) there was evidence of impaired absorption of vitamin B_{12}; the radioactivity in the urine was 8% in both patients, and the serum counts were 16 and 14 per minute. The test was repeated with intrinsic factor in one patient (no. 25) with no improvement,

TABLE II

DETAILS, INCLUDING RESULTS OF VITAMIN B_{12} ABSORPTION TESTS, OF 17 PATIENTS WITH MEGALOBLASTIC ANAEMIA

Diagnosis	Patient No.	Serum Vitamin B_{12} (pg./ml.)	Serum Folate (ng./ml.)	Vitamin B_{12} Absorption with Histamine			Vitamin B_{12} Absorption with Intrinsic Factor		
				Urine Volume (ml./24 hr.)	Urine Radioactivity (% of dose)	Serum Radioactivity (c.t.s./min.)	Urine Volume (ml./24 hr.)	Urine Radioactivity (% of dose)	Serum Radioactivity (c.t.s./min.)
Pernicious anaemia	13	40	2·4	770	8	9	1,020	24	31
Pernicious anaemia	14	30	6·9	1,680	3	0	2,300	16	24
Pernicious anaemia	15	20	4·5	820	3	8	860	13	24
Pernicious anaemia	16		5·4	1,450	3	6	1,100	15	29
Pernicious anaemia	17	60		550	6	19	800	15	40
Pernicious anaemia	18	15	3·3	475	5	17	700	10	47
Pernicious anaemia	19	10	10·0	820	< 1	0	1,250	16	33
Pernicious anaemia	20	10	10·0	800	4	12	810	18	25
Pernicious anaemia	21	10	5·5	1,525	5	0	1,500	20	24
Pernicious anaemia	22	25	14·5	1,110	2	0	630	16	37
Idiopathic steatorrhoea	23	170	0·2	1,375	12	34			
Idiopathic steatorrhoea	24	160		1,420	14	41			
Idiopathic steatorrhoea	25	20	1·6	2,020	8	16	2,180	8	9
Idiopathic steatorrhoea	26	210		2,300	8	14			
Partial gastrectomy	27	100	1·5	779	5	24	1,480	12	34
Nutritional anaemia	28	110	0·6	410	30	47			
Nutritional anaemia	29	190	1·0	2,070	16	36			

the urine radioactivity being 8% and the serum counts 9 per minute.

Two patients with nutritional megaloblastic anaemia had normal vitamin B_{12} absorption; the radioactivity in the urine was 30 and 18%, and in the serum 47 and 36 counts per minute. In one patient (no. 27) with megaloblastic anaemia following a partial gastrectomy, and in whom the serum vitamin B_{12} was 100 pg. per ml. and serum folic acid activity 1·5 ng. per ml., the excretion of radioactivity in the urine was only 5%, but the serum counts were 24 per minute. These figures rose to 12% and 34 counts per minute with intrinsic factor. The small collection of urine in this case suggests that the serum radioactivity was better than the urinary radioactivity as a measurement of vitamin B_{12} absorption, and the serum counts indicate only a partial deficiency of intrinsic factor.

DISCUSSION

An accurate result with the Schilling test depends on normal renal function and a complete collection of urine during a period of 24 hours. This is often impossible to achieve with unreliable elderly patients in an understaffed busy medical ward, and not infrequently the results of the Schilling tests were misleading. The radioactivity in the urine was low in four of the 12 control subjects as a result of an incomplete urine collection in three cases and impaired renal function in one case. Conversely the significance of a low result in a case of megaloblastic anaemia is uncertain when the collection of urine is probably incomplete, and this situation occurred in four patients with pernicious anaemia.

The serum counts were above 27 per minute in the 12 control subjects and below 20 per minute in the 10 patients with pernicious anaemia and in the two patients with idiopathic steatorrhoea who had low results in the Schilling test. These results show that the measurement of ^{57}Co vitamin B_{12} absorption by serum counting is quite feasible, and that the difference between the results in control subjects and patients with malabsorption of vitamin B_{12} is more definite than between those obtained in the Schilling test. Usually the serum counts in the patients with pernicious anaemia were below 10 per minute, but 17 and 19 counts per minute were obtained in two patients, in whom the diagnosis of pernicious anaemia was strongly supported by low serum vitamin B_{12} levels, improved vitamin B_{12} absorption with intrinsic factor and normal stool fat excretion. During the period of this investigation background counts varied between 73 and 88 per minute, and the ratio of serum to background radioactivity only ranged between 1·0 and 2·0. A misleading error could occur, therefore, with serum counts between about 17 and 25 per minute, and when results in this range are obtained both background and serum counting should be repeated.

Measurement of the serum radioactivity is simpler and gives more reliable results than the Schilling test, but in view of the possible errors when the result is marginal, it is usually advisable to do both tests. However, serum counting can be done without a Schilling test when the patient has impaired renal function, when a complete collection of urine is expected to be unsuccessful, or when the large flushing dose of vitamin B_{12} should be avoided.

REFERENCES

Booth, C. C., and Mollin, D. L. (1956). *Brit. J. Haemat.*, **2**, 223.
Callender, S. T., and Evans, J. R. (1955). *Clin. Sci.*, **14**, 295.
Doscherholmen, A., and Hagen, P. S. (1957). *J. clin. Invest.*, **36**, 1551.
Eberle, B. T., and Gleason, G. I. (1960). *Clin. Res.*, **8**, 208.
Glass, G. B. J., and Boyd, L. J. (1957). *Ann. intern. Med.*, **47**, 274.
Goldberg, S. R., Trivedi, B. K., and Oliver, L. (1957). *J. Lab. clin. Med.*, **49**, 583.
Heinle, R. W., Welch, A. D., Scharf, V., Meacham, G. C., and Prusoff, W. H. (1952). *Trans. Ass. Amer. Phycns*, **65**, 214.
Matthews, D. M. (1962). *Clin. Sci.*, **22**, 101.
McCurdy, P. R. (1965). *Ann. intern. Med.*, **62**, 97.
Nelp, W. B., McAfee, J. G., and Wagner, H. N., Jr. (1963). *J. Lab. clin. Med.*, **61**, 158.
Schilling, R. F. (1953). *Ibid.*, **42**, 860.
Spray, G. H. (1964). *J. clin. Path.*, **17**, 660.

The September 1966 Issue

THE SEPTEMBER 1966 ISSUE CONTAINS THE FOLLOWING PAPERS