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The reliability and reproducibility of the Schilling test in primary malabsorptive disease and after partial gastrectomy

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EDITORIAL SYNOPSIS A study of the reproducibility and reliability of the Schilling test in patients with primary malabsorptive disease and after partial gastrectomy is reported. The value of the test was assessed by repeated tests in each patient. Consistently normal or abnormal results were obtained in only one of the seven patients with primary malabsorptive disease and in only two of the eight patients who had undergone partial gastrectomy. From these results it is concluded that the result of a single test may be of little clinical value. Assessment of the results suggests that the mean value for a series of Schilling tests may give some indication of value clinically about the capacity to absorb radioactive vitamin B_{12} at the time of the tests at least in patients who have undergone partial gastrectomy. The significance of the findings is discussed, particularly in relation to the aetiology of post-gastrectomy megaloblastic anaemia.

Absorption tests using radioactive vitamin B_{12} may be of considerable value in establishing a precise diagnosis in conditions in which anaemia results from malabsorption. It is obviously important to appreciate the limitations of such tests. Of the various tests, the urinary excretion test introduced by Schilling (1953) is the most convenient for general use and has gained wide acceptance but its reliability and reproducibility in various conditions has not been fully established.

In this paper we report the results of an investigation into the reliability and reproducibility of the Schilling test in patients with primary malabsorptive disease and in patients who had evidence of malabsorption after partial gastrectomy.

PATIENTS, MATERIALS, AND METHODS

All patients with primary malabsorptive disease had come under observation with a megaloblastic anaemia and the Schilling tests were done as part of the diagnostic investigations. The post-gastrectomy patients had come under observation with anaemia or because of symptoms such as diarrhoea.

Schilling tests were performed at intervals of from three days to six months, the usual interval between the tests being one week. After an overnight fast the oral dose of 0·5 μg. 0·5 μc. ^{58}Co vitamin B_{12} was given in 200 to 400 ml. water; two hours later 1,000 μg. vitamin B_{12} was given intramuscularly and urine was collected for the subsequent 24 hours. The radioactivity in a 450 ml. aliquot was measured as described by Adams and Seaton (1961) and the total urinary radioactivity expressed as a percentage of the oral dose was calculated. The normal value was taken as $>$ 7·5% dose excreted. The ^{58}Co vitamin B_{12} solutions used were assayed microbiologically each week to detect any deterioration *in vitro*.

The amount of fat in three-day collections of faeces of the patients on a ward diet was estimated by the method of Harrison (1947), the normal value being less than 5 g. fat excreted per day. The amount of d-xylose in a five-hour collection of urine after an oral dose of 25 g. in 500 ml. water was estimated by the method of Roe and Rice (1948) the normal value being taken as more than 5 g. 'Blood sugar' concentrations before and after an oral dose of 50 g. glucose in 500 ml. water were estimated by the method of Somogyi (1952), the normal fasting value being taken as 65-100 mg./100 ml. and the upper limit of normal as 160 mg./100 ml. Serum vitamin B_{12} levels were estimated by a modification of the method of Hutner, Bach, and Ross (1956) using *Euglena gracilis* 3 strain as the test organism: the normal value was taken as $>$ 140 $\mu\mu$g./ml. The presence or absence of gastric acid was established by the augmented histamine test (Kay, 1953). Samples of gastric or intestinal mucosa were obtained by a biopsy tube (Shiner, 1956) or capsule (Crosby and Kugler, 1957), the position of the instrument being checked fluoroscopically. Other procedures were by standard laboratory methods.

RESULTS

The results of the Schilling tests are shown in Tables I and II and the results of other investigations in Tables III and IV. There was no evidence of a constant trend to an increase or decrease in the values for Schilling tests in any patient over the period of the investigation.

Deterioration of the ^{58}Co vitamin B_{12} solutions *in vitro* was not observed.

DISCUSSION

The striking feature of the results is that consistently normal or subnormal values for the Schilling tests were obtained in only one of the seven patients with primary malabsorptive disease (case M.2) and in only two of the eight patients who had undergone partial gastrectomy (cases PG.1 and 7). It is clear therefore that the test cannot be regarded as reliable or reproducible for practical clinical purposes. It may be the case that the result of each test does accurately reflect the capacity to absorb radioactive vitamin B_{12} at the time of the test and that the capacity to absorb radioactive vitamin B_{12} varies from day to day. Even if this were so it is still obvious from the results that a single test is valueless clinically. It may also be the case that a different technique might give different results: modifications of the test described by Schilling (1953) have been introduced by several workers, notably in respect of the mass of radioactive vitamin B_{12} orally and the mass and timing of the intramuscular dose, and it must be stressed that the results reported in this study were obtained using one of these modifications. Again this does not alter the fact that by the technique employed, which is in wide use, the result of a single test is of little value clinically.

TABLE II

RESULTS OF REPEATED SCHILLING TESTS IN CASE PG. 8[1]

Test No.	*Schilling Test Results (% dose excreted)*
1	0
2	5·6
3	9·7
4	7·8
5	7·4
6	14·7
7	4·6
8	9·7
9	9·9
10	7·4
11	3·4
12	3·6
13	6·7
14	12·6
15	8·8
16	17·0
17	7·4
18	5·9
19	9·1
20	11·7

	Mean	*S.D.*
All Tests	8·15	3·97
Excluding tests 2, 3, and 4	8·18	4·36

[1]The results are shown in chronological order extending over a period of two and a half years. Tests 2 and 3 were done with intrinsic factor and test 4 after four days' treatment with tetracycline, 0·25 g. q.i.d. orally.

It is logical to consider whether the mean value of a series of tests, as opposed to the result of a single test, is of any value in clinical practice. The most satisfactory approach to this problem is to consider the mean value of a series of Schilling tests in relation to unequivocal evidence of vitamin B_{12} deficiency and also, if possible, to unequivocal evidence of failure to develop vitamin B_{12} deficiency. Failure to develop vitamin B_{12} deficiency can only be determined by necessarily long-term follow-up

TABLE I

RESULTS OF SCHILLING TESTS IN CHRONOLOGICAL ORDER IN SEVEN PATIENTS WITH PRIMARY MALABSORPTIVE DISEASE (CASES M.1-7) AND IN SEVEN PATIENTS AFTER PARTIAL GASTRECTOMY (CASES PG.1-7)

Case	*Schilling Test Results (% Dose Excreted)*								*Mean*	*S.D.*
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*		
M.1	10·7	22·0	13·1	2·7	14·0	9·4	14·9	15·3	12·7	5·53
M.2	21·8	14·4	8·6	17·7	19·6	13·8			15·9	4·72
M.3	11·5	3·0	18·2	27·7	24·1	14·8			16·5	8·90
M.4	14·8	6·2	11·2	7·3	8·9	0			8·0	3·06
M.5	13·7	4·5	7·7						8·6	—
M.6	7·4	9·4	8·9	2·7	2·1	0·9			5·2	2·74
M.7	6·5	25·0	31·8	20·4	11·0				18·9	10·27
PG.1	21·2	22·9	20·7	12·9	14·1	8·0			16·6	5·85
PG.2	6·9	4·8	3·4	9·5	8·1	8·1			6·8	2·28
PG.3	3·9	7·6	5·5.	10·9	11·2	0			6·5	4·30
PG.4	1·6	10·5	18·8	26·6	13·9	5·7			12·8	9·04
PG.5	14·1	0	0	0	0	4·1			3·0	—
PG.6	1·8	16·5	7·3	5·5	0	0			5·1	5·74
PG.7	0	0	0	1·3	0	4·1			0·9	—

TABLE III

RESULTS OF VARIOUS INVESTIGATIONS IN PATIENTS WITH PRIMARY MALABSORPTIVE DISEASE

Case	Age (yr.)	Sex	Megaloblastic Anaemia			Faecal Fat (g./day)	Xylose Test (g./5 hours)	Glucose Tolerance (mg. %)	Intestinal Radiology	Gastric Acid	Intestinal Biopsy
			Hb (%)	Serum Vitamin B_{12} ($\mu\mu$g./ml.)	Therapeutic Response						
M.1	48	F	42	25	Complete to B_{12} only	2·6	2·7	—	Normal	Achlorhydria	Gastric normal, duodenal atrophic
M.2	44	F	44	170	Complete to B_{12} only	3·6	3·4	Fast 85 Peak 60 min. 123	Normal	Achlorhydria	Jejunal normal
M.3	33	F	68	—	Complete to B_{12} and folic acid together	10·0	3·3	Fast 81 Peak 60 min. 115	Flocculation pattern	—	Duodenal atrophic
M.4	71	F	43	119	Partial to B_{12} only	5·0	2·3	Fast 100 Peak 30 min. 150	Normal	Achlorhydria	—
M.5	72	F	31	190	Complete to B_{12} and folic acid together	1·5	1·7	—	—	Achlorhydria	Jejunal atrophic
M.6	59	M	78	—	None to B_{12}, complete to folic acid	6·2	1·3	—	Flocculation pattern	Present	Jejunal atrophic
M.7	70	F	37	—	Partial to B_{12} only	4·0	0·9	—	Normal	Present	Jejunal atrophic

TABLE IV

RESULTS OF VARIOUS INVESTIGATIONS IN POST-GASTRECTOMY PATIENTS

Case	Age (yr.) at Operation	Sex	Operations	Evidence of Vitamin B_{12} Deficiency	Faecal Fat (g./day)	Xylose Test (g./5 hours)	Glucose Tolerance (mg. %)	Intestinal Radiology	Jejunal Biopsy	Mean Schilling Value (% dose)
PG.1	60	M	Polya 6 years	None Hb 94%, serum B_{12} 370 $\mu\mu$g./ml. 6 yr. post-operatively	10·4	5·3	Fast 115, peak 30 min. 245, 120 min. 84	Clumping of barium in small gut	Normal	16·6
PG.2	57	M	Polya 15 years	+Megaloblastic anaemia Hb 62%, serum B_{12} 60 $\mu\mu$g./ml. 14 yr. post-operatively	10·6	6·2	Fast 83, peak 30/60 min. 118, 120 min. 45	Occasional dilated loops of small gut	—	6·8
PG.3	47	M	Polya 5 years	+Megaloblastic anaemia Hb 63%, serum B_{12} 140 $\mu\mu$g./ml. 5 yr. post-operatively	28·3	1·9	Fast 82, peak 15 min. 95, 120 min. 95	Normal	Atrophic	6·5
PG.4	53	M	Vagotomy 18 years, Polya 15 years	None Hb 98%, serum B_{12} 370 $\mu\mu$g./ml. 18 yr. post-operatively	5·6	6·1	Fast 116, peak 60 min. 165, 120 min. 35	Normal	Normal	12·8
PG.5	65	M	Polya 12 years	+Megaloblastic anaemia Hb 56%, serum B_{12} 25 $\mu\mu$g./ml. 10 yr. post-operatively	5·3	5·0	Fast 86, peak 30 min. 172, 120 min. 47	Dilated loops of small gut	Normal	3·0
PG.6	29	F	Polya 3 years, vagotomy 2 years	None Hb 94%, serum B_{12} 240 $\mu\mu$g./ml. 3 yr. post-operatively	51·0	5·2	Fast 69, peak 30 min. 69, 90 min. 27	Normal	Normal	5·1
PG.7	58	M	Polya 9 years	+Megaloblastic marrow Hb 91%, serum B_{12} 56 $\mu\mu$g./ml. 8 yr. post-operatively	22·6	7·2	Fast 84, peak 15 min. 156, 90 min. 33	Normal	—	0·9
PG.8	60	M	Polya 15 years	+Megaloblastic anaemia Hb 62%, serum B_{12} 48 $\mu\mu$g./ml. 12 yr. post-operatively	3·0	4·9	Fast 70, peak 30 min. 145, 120 min. 78	Normal	Oedematous villi	8·1

studies and even these may give fallacious results, particularly in the case of post-gastrectomy patients, because the capacity to absorb vitamin B_{12} may change as a result of atrophy of the mucosa of the gastric remnant occurring after operation, an occurrence which is not uncommon (Deller, Richards, and Witts, 1962). Some information, however, may be obtained from examination of values for repeated Schilling tests in patients who have presented with evidence of vitamin B_{12} deficiency: if subnormal mean values were obtained from a series of tests on patients who had developed vitamin B_{12} deficiency this would suggest that the mean value of a series of tests was of some diagnostic value; if normal mean values were obtained from a series of tests in such patients this would suggest that a series of tests was no more useful than a single test. Of the seven patients with malabsorptive disease, only one, case M.1, had unequivocal evidence of uncomplicated vitamin B_{12} deficiency and this patient had taken a diet grossly deficient in vitamin B_{12} for many years and the only evidence of malabsorptive disease was

an abnormal intestinal biopsy. Thus no conclusions can be drawn about the value of repeated Schilling tests in this group of patients. In the case of the eight patients who had undergone partial gastrectomy two of the mean values for the series of Schilling tests were normal (cases PG. 1 and 4), one was at the lower limit of normal (case PG. 8), and five were subnormal. The two unequivocally normal mean values were obtained from patients who had normal serum vitamin B_{12} levels five and 14 years after partial gastrectomy. The one mean value at the lower limit of normal was obtained from a patient who had unequivocal evidence of vitamin B_{12} deficiency when he came under observation. The five subnormal mean values were obtained from three patients who had developed unequivocal evidence of vitamin B_{12} deficiency at intervals of eight to 15 years after operation (cases PG. 2, 5, and 7), from one who had a serum vitamin B_{12} level at the lower limit of normal five years after operation (case PG. 3), and from one who had a normal serum vitamin B_{12} level three years after operation (case PG. 6), at which time it is unlikely that the body stores of vitamin B_{12} would have been depleted. These findings suggest that in patients who have undergone partial gastrectomy the mean value of a series of Schilling tests may be of value in assessing the capacity to absorb vitamin B_{12} at the time of the tests. The proviso is important because, as already stated, the capacity to absorb vitamin B_{12} may deteriorate due to atrophy of the gastric mucosa thus vitiating a prediction of continued capacity to absorb vitamin B_{12} made on the basis of the tests done soon after operation.

It might be considered that the issue has been unnecessarily complicated by adopting a rigid value, 7·5% dose of radioactivity excreted, to delineate normal and abnormal results and that the introduction of a 'doubtful range' would simplify the issue. There are sound reasons for our choice. First, in clinical practice it is helpful to adopt relatively fixed values; secondly it is obvious from the results that a 'doubtful range' would have to be so extensive to encompass the results in question that the vast majority of results would be meaningless. It is possible that the lower limit of the normal range of values for the Schilling test is higher in patients who have undergone partial gastrectomy than in subjects with intact gastro-intestinal tracts as suggested by the results in case PG. 8. There is some evidence, based on urinary excretion tests in studies in which radioactive vitamin B_{12} was introduced directly into the small bowel by intubation or time release capsules, that absorption may be greater when the stomach is bypassed (Citrin, DeRosa, and Halsted, 1957; Johnson and Berger, 1958) and this might occur after partial gastrectomy of the Polya type as had been performed in all our patients. Even if this were the case, and there are reasons for believing the reverse to be the case (see Deller *et al.*, 1962), it is obvious from the individual results that if a value much greater than 7·5% dose excreted was accepted as the lower limit of normal in post-gastrectomy patients, the result of a single test would still be valueless.

The wide range of results of Schilling tests in the post-gastrectomy patients raises further points relevant to the problem of establishing aetiological factors in megaloblastic anaemias occurring in patients who have undergone partial gastrectomy. It is generally held that post-gastrectomy megaloblastic anaemia due to vitamin B_{12} deficiency is commonly the result of deficiency of intrinsic factor (Herbert, 1959). This diagnosis can be established *in vivo* either in the patient under investigation by demonstrating that the failure to absorb orally administered radioactive vitamin B_{12} is corrected by the simultaneous administration of intrinsic factor, or by the cumbersome process of demonstrating that gastric juice from the patient under investigation does not contain intrinsic factor by a failure to restore absorption of orally administered radioactive vitamin B_{12} to normal in a patient with Addisonian pernicious anaemia. It is obvious from the results in cases PG. 2-6 and PG. 8 in particular that a normal result for a Schilling test can be obtained spontaneously from a post-gastrectomy patient under investigation and could be attributed to concurrent administration of intrinsic factor. It thus appears that a series of tests, with and without intrinsic factor, would be necessary to demonstrate intrinsic factor deficiency convincingly, and it is doubtful if such a tedious procedure is likely to find a place in routine clinical practice. The presence of a blind intestinal loop has been implicated as a factor in some cases of megaloblastic anaemia following gastrectomy. The diagnosis in such cases is most convincingly established by demonstrating that the failure to absorb orally administered radioactive vitamin B_{12} is corrected by the administration of certain antibiotics. Again, it is obvious from the results presented here that an apparent improvement in absorption as judged by a single Schilling test could be no more than a spontaneous variation. We stress these points because they are of some importance when using the test in the investigation of megaloblastic anaemias following partial gastrectomy.

Why such a wide range of results should occur after partial gastrectomy is not clear. In two patients who had undergone total gastrectomy it was found that there was a consistent failure of absorption of vitamin B_{12} as judged by repeated Schilling tests, and this was also found in seven patients who were diag-

nosed as having Addisonian pernicious anaemia and who presumably had gastric mucosal atrophy, although biopsy and endoscopic evidence was not sought (Adams and Seaton, 1961). This suggests that the secretion of intrinsic factor from the gastric remnant after partial gastrectomy may be episodic, This point might be clarified by repeating the Schilling tests using carbamylcholine chloride which is said to stimulate intrinsic factor secretion (Baker and Mollin, 1955). To settle this point a series of tests with and without carbamylcholine chloride stimulation would be necessary in each patient, and, as we have found the side-effects of carbamylcholine chloride to be common and not infrequently distressing, we have not investigated this point. There were no obvious exogenous factors which might have been responsible for the wide range of results in our cases. The role of exogenous factors has been emphasized by Deller, Germar, and Witts (1961) who observed that the effect of a vitamin B_{12}-free meal was to increase the absorption of radioactive vitamin B_{12}, as judged by single faecal excretion tests in post-gastrectomy patients but not in normal subjects or in patients with Addisonian pernicious anaemia. The effect of food was comparable to that of added intrinsic factor in some cases, and they therefore concluded that the administration of a fasting dose of radioactive vitamin B_{12} was not a reliable method of determining the ability of the post-gastrectomy patient to absorb vitamin B_{12}. Whether food affects the absorption of radioactive vitamin B_{12} as judged by the Schilling test, as opposed to the faecal excretion test, has not been established; until this has been done, it would be unwise to compare the two sets of results further. It is, however, plain from the results reported by Deller *et al.* (1961) and in this paper that the mechanisms which may occur after partial gastrectomy and lead to vitamin B_{12} deficiency are much more complex than has been believed hitherto, and still presents some unique problems worthy of investigation.

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