

Diagnostic value of the serum folate assay

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SYNOPSIS The diagnostic value of the serum folate assay has been assessed in 90 patients, each of whom had a macrocytic anaemia and a low serum vitamin B₁₂ level. Twenty-nine (32%) patients were found to have anaemia due primarily to folate deficiency. The cause of the low serum vitamin B₁₂ levels is uncertain in the 22 (25%) patients with normal or borderline vitamin B₁₂ absorption. The effect of folic acid therapy was studied in four of these patients, and in each case the serum vitamin B₁₂ rose slowly to a normal level.

The serum folate was low in only four (7.5%) of the 54 patients with pernicious anaemia, and the levels rose to normal on treatment with vitamin B₁₂ alone. A high serum folate occurred in eight (15%) pernicious anaemia patients. A normal serum folate indicated the diagnosis of pernicious anaemia or megaloblastic anaemia following partial gastrectomy. However, a normal serum folate and a very low vitamin B₁₂ level was found in two patients with idiopathic steatorrhoea.

It is concluded that the serum folate assay is a valuable routine test in patients who have a macrocytic anaemia and low serum vitamin B₁₂. A low folate level makes the diagnosis of pernicious anaemia unlikely and is a strong indication for full investigation of small intestinal function.

The significance of the serum and red cell folate concentration has been reviewed recently by Chanarin (1969). The serum folate was low in one third of 877 'normoblastic' hospital patients, whereas the red cell folate was low in only 17% of 161 'normoblastic' patients with a low serum folate level. On the other hand, the red cell folate was low in 60% of 161 patients with pernicious anaemia, but there was a low serum folate in only 10% of 361 pernicious anaemia patients. These observations show that the red cell folate gives a better assessment of the body folate status than does the serum folate, provided that vitamin B₁₂ deficiency has been excluded. However, some 47% of patients with megaloblastic anaemia due to folate deficiency have a low serum vitamin B₁₂ level (Mollin, Waters, and Harriss, 1962), and this may complicate the detection of vitamin B₁₂ deficiency. The serum folate, therefore, should be more valuable than the red cell folate in the investigation of patients with megaloblastic anaemia and low serum vitamin B₁₂ levels. The purpose of the present paper is to re-assess the diagnostic value of routine serum folate assays in such patients.

Materials and Methods

Ninety patients were studied, all of whom had a macrocytic blood film and a serum vitamin B₁₂ level below 150 pg per ml. The haemoglobin level was

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above 11.0 g per 100 ml in four patients, but the others were moderately or severely anaemic. The bone marrow was examined in 89 patients, and was overtly megaloblastic in 85. The final diagnoses were: pernicious anaemia 54 patients (41F, 13M); nutritional folate deficiency 18 patients (12F, 6M); idiopathic steatorrhoea 12 patients (7F, 5M); partial gastrectomy four patients (2F, 2M); anticonvulsant therapy one patient (M); unknown aetiology one patient (F).

The intestinal absorption of 0.5 µg vitamin B₁₂, labelled with 0.5 µC ⁵⁷Co, was measured in every case. The absorbed radioactivity was counted in the serum (Harwood and Forshaw, 1967) in 66 patients, and in a 24-hour collection of urine (Schilling, 1953) in the other 24. Secretion of intrinsic factor was stimulated by either histamine 0.4 mg or penta-gastrin 6.0 µg per kilogram of body weight. In 43 of the 70 patients with impaired absorption the test was repeated with an oral dose of 60 mg intrinsic factor preparation (Armour). A result below 0.5% of administered radioactivity per litre of serum or 10% in a 24-hour collection of urine indicates impaired absorption.

The levels of serum vitamin B₁₂ and folate were measured by microbiological assay using *Lactobacillus leichmanii* (Matthews, 1962) and *Lactobacillus casei* (Spray, 1964) respectively. In our laboratory the normal range for the serum vitamin B₁₂ level is 150 to 850 pg per ml and for the folate 2.1 to 10.0 ng per ml.

Serum intrinsic factor blocking antibody was assayed by the coated charcoal method (Gottlieb, Lau, Wasserman, and Herbert, 1965) and gastric parietal cells antibody by the immunofluorescent technique (Taylor, Roitt, Doniach, Couchman, and Shapland, 1962).

Results

RELATIONSHIP BETWEEN SERUM FOLATE LEVELS AND VITAMIN B₁₂ ABSORPTION

In Figure 1 the results of the serum folate assays and the vitamin B₁₂ absorption tests are compared. The 56 patients with a normal or raised serum folate had impaired vitamin B₁₂ absorption. These included 50 patients with pernicious anaemia, two

patients with idiopathic steatorrhoea, and three patients who had had a partial gastrectomy. In the other patient, who has been described previously (Forshaw, 1969), the impaired vitamin B₁₂ absorption was corrected by vitamin B₁₂ and the diagnosis was not certain.

There were 34 patients with a low serum folate, 20 of whom had normal vitamin B₁₂ absorption. This group of patients included 16 with nutritional folate deficiency, three with idiopathic steatorrhoea, and one on anticonvulsant therapy. Of the 14 patients with a low serum folate and abnormal vitamin B₁₂ absorption, seven had idiopathic steatorrhoea, four pernicious anaemia, one a partial gastrectomy, and there were two with nutritional folate deficiency in whom vitamin B₁₂ absorption

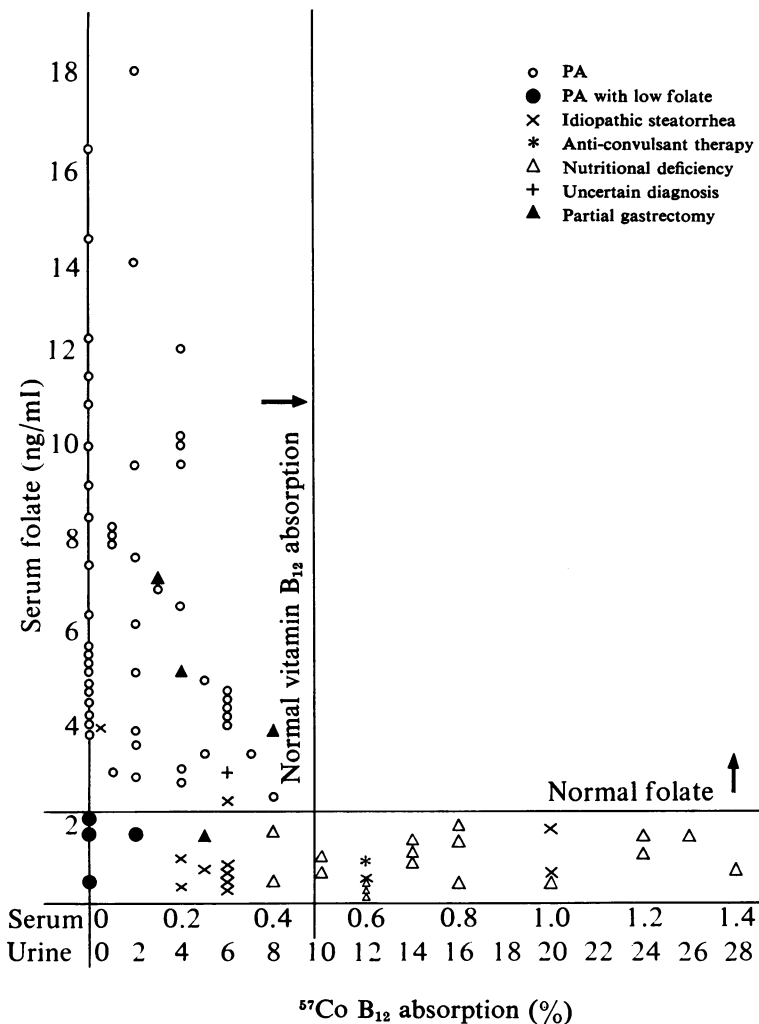


Fig. 1. Graph to show the relationship between serum folate and ⁵⁷Co vitamin B₁₂ absorption in 90 cases of megaloblastic anaemia.

was in the borderline range (0.4%/litre serum).

RELATIONSHIP BETWEEN SERUM FOLATE AND VITAMIN B₁₂ LEVELS

This is illustrated in Figure 2. None of the 56 patients with a normal or raised serum folate had a serum vitamin B₁₂ level above 90 pg per ml, and only one had a level above 70 pg per ml. The vitamin B₁₂ levels were more widely scattered in the patients with a low serum folate, but 15 of the 28 patients with either idiopathic steatorrhea or nutritional folate deficiency in this group had a vitamin B₁₂ level below 90 pg per ml. In four patients with nutritional folate deficiency and normal vitamin B₁₂ absorption, the serum vitamin B₁₂ levels were 45, 45, 30, and 20 pg per ml respectively

PERNICIOUS ANAEMIA

The mean serum folate level was 6.6 ng per ml in the 54 pernicious anaemia patients, which is significantly higher than the mean level of 4.7 ng per ml in control subjects. However, the levels ranged from 0.5 to 18.1 ng per ml, being above 10.0 ng in eight (15%) and below 2.1 ng in four (7.5%) patients. The details of the patients with a low serum folate are shown in Table I. They were all multiparous females, three of whom had gastric parietal cell antibodies in the serum, but none had intrinsic factor antibodies. In the patient with no antibodies, the diagnosis of pernicious anaemia depended on impaired vitamin B₁₂ absorption which was corrected by intrinsic factor. In each case vitamin B₁₂ therapy alone produced a complete haematological recovery,

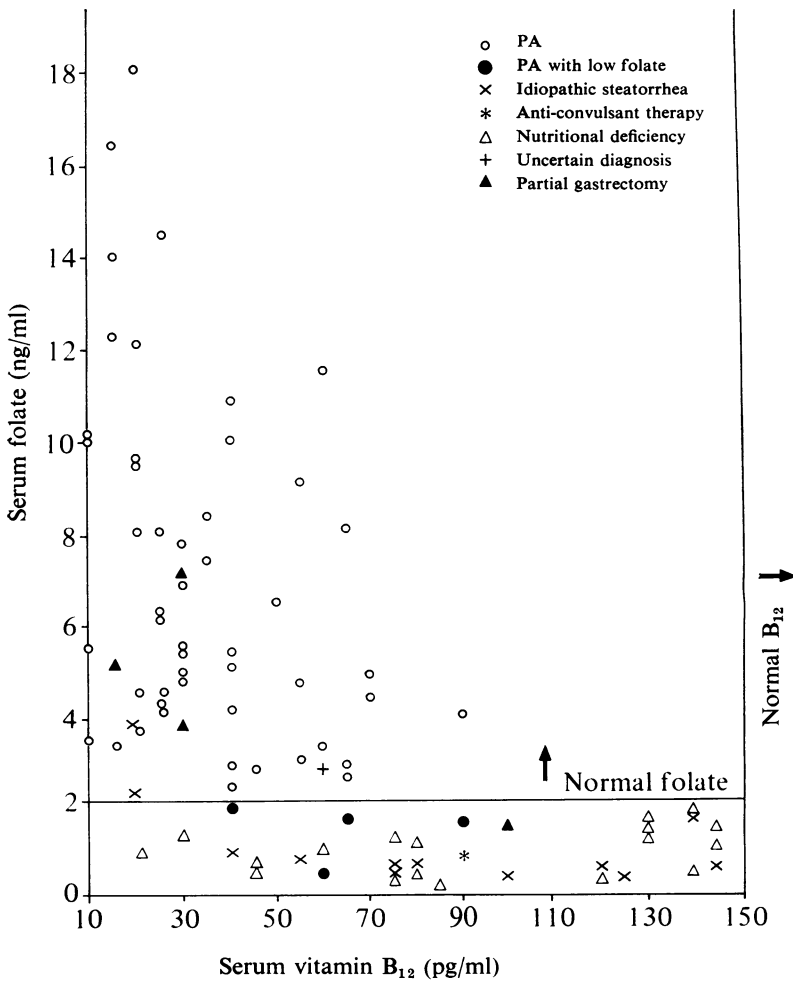


Fig. 2. Graph to show the relationship between serum folate and serum vitamin B₁₂ in 90 cases of megaloblastic anaemia.

and the serum folates rose to a normal level.

IDIOPATHIC STEATORRHOEA

We have used this term rather than coeliac disease, as we were unable to do a small intestinal mucosal biopsy in every case to confirm the diagnosis of

coeliac disease. However, radiographs of the small bowel were taken in each case and they showed no evidence of any other cause for the steatorrhoea.

Details of these 12 patients are given in Table II. The serum folate was low in 10 patients with a mean level of 0.7 ng per ml. The folate level was normal

Patient No.	Sex	Age	Hb (g/ml/100)	Serum Vitamin B ₁₂ (pg/ml)	Serum Folate (ng/ml)		Vitamin B ₁₂ Absorption (% dose per litre serum)		Serum Antibodies		No. of Pregnancies
					Before Vitamin B ₁₂ Therapy	After Vitamin B ₁₂ Therapy	Histamine or Pentagastrin	Intrinsic Factor	Gastric Parietal Cell	Intrinsic Factor	
1	F	74	11.0	60	0.5	4.1	0.0	0.8	-	-	4
2	F	37	8.5	90	1.6	3.0	0.1		+	-	2
3	F	71	6.5	65	1.7	2.5	0.0		+	-	2
4	F	61	5.7	40	2.0	3.2	0.0	0.5	+	-	13

Table I Details of patients with pernicious anaemia and low serum folate levels

Patient No.	Sex	Age (yr)	Hb (g/100 ml)	Serum Vitamin B ₁₂ (pg/ml)	Serum Folate (ng/ml)	Vitamin B ₁₂ Absorption (% dose per litre serum or 24-hr urine)		Fractional Test Meal	Stool Fat (g/24 hr)	Xylose Excretion (g/5 hr)	Jejunal Mucosa*
						Histamine or Pentagastrin	Intrinsic Factor				
5	F	57	5.5	120	0.6	0.5		Acid	9	1.6	
6	M	23	4.4	75	0.5	0.3		Acid	9	4.0	SVA
7	F	56	10.0	80	0.7	1.0			9		
8	M	67	3.8	20	2.3	0.3	0.4	Acid	13		
9	M	58	6.1 ¹	20	3.8	0.0	0.0	No acid	10	0.7	SVA
10	F	23	4.5	75	0.6	0.3	0.5	Acid	15	1.6	SVA
11	F	25	13.0	40	1.0	0.2	0.0		74	0.6	SVA
12	M	67	9.3	125	0.4	6.0(u) ²	10.0(u)	No acid	17		SVA
13	F	70	2.9	55	0.8	5.0(u)	5.0(u)	Acid	8		PVA
14	F	57	10.2	145	0.7	0.3			31	1.8	SVA
15	M	54	10.4	100	0.4	0.2	0.3		39	0.5	SVA
16	F	51	10.2 ¹	140	1.7	1.0			13	0.8	

Table II Details of patients with idiopathic steatorrhoea

¹Typical blood picture of hyposplenism ²u = urine tested
^{*}PVA = partial villous atrophy SVA = subtotal villous atrophy

Patient No.	Sex	Age (yr)	Hb (g/100 ml)	Serum Vitamin B ₁₂ (pg/ml)	Serum Folate (ng/ml)	Vitamin B ₁₂ Absorption (% dose per litre serum or 24-hr urine)		Fractional Test Meal
						Histamine or Pentagastrin	Intrinsic Factor	
17	F	56	7.0	30	1.4	0.8		
18	F	30	5.0	20	0.8	28.0(u)		Acid
19	M	78	4.4	80	0.5	0.4		No acid
20	F	76	8.0	130	1.3	0.7		
21	F	43	10.3	60	1.0	0.5		Acid
22	F	73	7.0	75	1.3	0.7		Acid
23	F	61	6.4	85	0.2	0.6		Acid
24	M	59	5.0	120	0.4	0.6		Acid
25	F	35	11.3	145	1.5	1.2		No acid
26	M	61	9.0	140	1.8	16.0(u) ¹		No acid
27	M	49	8.1	130	1.5	26.0(u)		No acid
28	F	81	5.7	75	0.4	16.0(u)		
29	F	64	11.9	80	1.2	14.0(u)		
30	F	74	8.7	145	1.1	1.2		
31	M	67	4.0	130	1.6	0.4		
32	M	70	4.8	45	0.5	1.0		No acid
33	F	64	7.3	45	0.7	0.5		Acid
34	F	80	4.0	140	0.6	0.6		No acid

Table III Details of patients with nutritional folic acid deficiency

¹u = urine tested

Patient No.	Sex	Age (yr)	Hb (g/100 ml)	Serum Vitamin B ₁₂ (pg/ml)	Serum Folate (ng/ml)	Vitamin B ₁₂ Absorption (% dose per litre serum or 24-hr urine)	
						Histamine or Pentagastrin	Intrinsic Factor
35	F	71	9.9	30	3.8	8.0(u) ¹	19.0(u)
36	F	62	9.9	30	7.1	3.0(u)	11.0(u)
37	M	60	10.4	15	5.1	0.2	
38	M	67	5.0	100	1.5	5.0(u)	12.0(u)

Table IV Details of patients with megaloblastic anaemia following partial gastrectomy

¹u = urine tested

in the other two patients (cases 8 and 9). In both these patients the serum vitamin B₁₂ was only 20 pg per ml, vitamin B₁₂ absorption was impaired, and vitamin B₁₂ therapy produced a complete haematological response without any subsequent fall in the serum folate level. The initial indications that these two patients might have idiopathic steatorrhoea rather than pernicious anaemia were that they both had moderate diarrhoea, one (case 8) had free acid in the gastric juice and the other (case 9) had the typical blood picture of hyposplenism.

MEGALOBlastic ANAEMIA DUE TO NUTRITIONAL FOLATE DEFICIENCY

This is in some respects an unsatisfactory diagnosis, as it depends on the exclusion of all other causes of megaloblastic anaemia and the demonstration of an inadequate dietary intake. The 18 patients in this group had no clinical or radiological evidence of steatorrhoea, but, as only two had a small intestinal mucosal biopsy, some cases of occult coeliac disease may possibly be included. All described a poor diet, and recovery was good on treatment with folic acid alone in eight patients and with folic acid and vitamin B₁₂ in seven patients. The other three patients showed a good initial response to folic acid therapy, but they ultimately deteriorated and were found to have carcinomas of the bronchus (case 24) caecum (case 27), and stomach (case 34).

The details of these patients are shown in Table III. The serum folate was, of course, low in all of them, the mean level being 1.0 ng per ml. Vitamin B₁₂ absorption was normal in 16 patients and slightly impaired in two. The serum vitamin B₁₂ was below 90 pg per ml in 10 patients, in four of whom it was below 50 pg. We observed the effect of folic acid therapy on the serum vitamin B₁₂ level in three patients (cases 18, 21, and 23) and in each case it rose slowly to within the normal range.

MEGALOBlastic ANAEMIA FOLLOWING PARTIAL GASTRECTOMY

The details of these four patients are shown in Table IV. There were three patients with a normal serum folate and a very low vitamin B₁₂ level. The

other patient (case 38) had a low serum folate and a vitamin B₁₂ level of 100 pg per ml.

MEGALOBlastic ANAEMIA ASSOCIATED WITH ANTICONVULSANT THERAPY

One patient, a man aged 42, was on treatment with phenobarbitone, phenytoin, and primidone, and he was also taking a very poor diet. The serum folate was 0.9 ng and the vitamin B₁₂ 85 pg per ml. There was free acid in the gastric juice and the vitamin B₁₂ absorption test showed 0.6% administered radioactivity per litre of serum. Radiographs of the gastrointestinal tract and jejunal mucosal biopsy were normal. After treatment with folic acid for two months the serum vitamin B₁₂ had risen to 180 pg per ml.

Discussion

We included in this investigation all patients attending Sefton General Hospital with a macrocytic anaemia and a low serum vitamin B₁₂, provided that they had had a vitamin B₁₂ absorption test and a suitable sample of serum for folate assay had been obtained. The inclusion of patients was as complete as possible, but some selection could not be avoided. Samples of serum for folate assay would tend to be collected more often from patients with serum vitamin B₁₂ levels above the usual pernicious anaemia range, but, on the other hand, several patients with a low serum folate were not included, as they had not had a vitamin B₁₂ absorption test. In spite of the fact that the patients were not entirely unselected, the value of a routine serum folate assay is emphasized by our finding a low folate in 34 (38%) patients, 29 (32%) of whom had anaemia due primarily to folate deficiency. These included 18 patients with nutritional folate deficiency, 10 with idiopathic steatorrhoea, and one on anticonvulsant therapy.

Low serum vitamin B₁₂ levels were observed in 47% of patients with megaloblastic anaemia due to folate deficiency, and 10% of these had levels below 100 pg per ml (Mollin *et al*, 1962). Our results have been similar, and our 18 patients with nutritional

folate deficiency formed 40% of the total group of such cases. The cause of the low serum vitamin B₁₂ levels is uncertain in both these patients and the three patients with idiopathic steatorrhoea, who had normal vitamin B₁₂ absorption. Previous observations on the effect of folic acid therapy on serum vitamin B₁₂ levels have been conflicting. The levels rose in one group of patients (Mollin *et al*, 1962) whereas they fell in another group on anticonvulsant therapy (Hunter, Barnes, and Matthews, 1969). Our patients with very low serum vitamin B₁₂ levels were usually treated with vitamin B₁₂ but we were able to study the effect of folic acid therapy on serum levels below 90 pg per ml in four patients, three with nutritional folate deficiency and one taking anticonvulsant drugs; in each case the serum vitamin B₁₂ level slowly rose to within the normal range.

Occasionally the anaemia in patients with a low serum folate is due primarily to vitamin B₁₂ deficiency, the incidence of such cases in our group being five (15%) out of 34. One of these patients had had a partial gastrectomy, and the other four pernicious anaemia. Low serum folates occurred in 7.5% of the 54 patients with pernicious anaemia, an incidence close to the 10% reported by Chanarin (1969). The five patients with low serum folates had serum vitamin B₁₂ levels (40, 60, 65, 90, and 100 pg/ml) above the mean level of 35 pg per ml in the pernicious anaemia and partial gastrectomy patients. Experimental evidence suggests that the low serum folates in gastrectomized subjects are the results of dietary deficiency (Markkanen, 1968). This may also explain the folate deficiency in pernicious anaemia as over one third of pernicious anaemia patients have anorexia and marked loss of weight (Seaton and Goldberg, 1960). Our four patients were anorexic, and as they were multiparous females, their body folate stores may have been depleted before the onset of pernicious anaemia. Their appetites improved immediately and the serum folates rose to normal levels within a few months of starting vitamin B₁₂ therapy.

A raised serum folate only occurred in pernicious anaemia (Fig. 2). A normal folate level indicated impaired vitamin B₁₂ absorption, but six of the 48 patients in this group did not have pernicious anaemia. They included three patients who had had a partial gastrectomy, one patient reported previously (Forshaw, 1969) in whom the impaired vitamin B₁₂ absorption was corrected by vitamin B₁₂ treatment, and two patients with idiopathic steatorrhoea. The last two are of particular interest as folate deficiency has been regarded as almost invariable in idiopathic steatorrhoea (Waters, 1963; Hoffbrand, Newcombe, and Mollin, 1966; Magnus, 1966). In a group of 28 patients with idiopathic steatorrhoea we found that

these were the only two without evidence of folate deficiency (Forshaw, 1970). Both patients had very low serum vitamin B₁₂ levels with impaired vitamin B₁₂ absorption, and they made a complete haematological recovery on vitamin B₁₂ therapy without a subsequent fall in the serum folate level. Impaired vitamin B₁₂ absorption is an indication that the mucosal damage has spread to the distal segments of the small bowel, and a direct correlation between the impairment of vitamin B₁₂ absorption and the severity of the steatorrhoea has been observed (Stewart, Pollock, Hoffbrand, Mollin, and Booth, 1967). However, our two patients only had mild diarrhoea and steatorrhoea, and we were unable to confirm a definite association between vitamin B₁₂ absorption and stool fat excretion in 23 patients with idiopathic steatorrhoea (Forshaw, 1970).

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