


Part II The contribution of folic acid deficiency to the anaemia

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EDITORIAL SYNOPSIS  Folic acid deficiency has been detected in 12% of randomly selected patients after partial gastrectomy. A megaloblastic reaction was found in seven of the patients of whom four had evidence of folic acid deficiency, one being regarded as due to primary folic acid deficiency. Attention is drawn to the dangers of therapy with folic acid and multi-vitamin preparations without full haematological investigation of the patient.

Megaloblastic anaemia following partial gastrectomy had usually been attributed to a deficiency of vitamin B12 (Badenoch, Evans, Richards, and Witts, 1955; Doig and Girdwood, 1960; Deller and Witts, 1962). The role of folic acid deficiency in the pathogenesis of anaemia following partial gastrectomy is uncertain, and only infrequently has folic acid deficiency been mentioned as a cause for the anaemia (Deller, 1962; Pitney, 1963). Until recent years the diagnosis of folic acid deficiency in cases of megaloblastic anaemia has rested upon the exclusion of vitamin B12 deficiency; by the finding of a normal serum level of vitamin B12; an inadequate response to vitamin B12; and a complete remission with folic acid therapy. More recently Chanarin, Mollin, and Anderson (1958) have used the rate of clearance from the plasma of intravenously administered folic acid as a measure of the folic acid stores, and they have reported an increased clearance rate in some patients with megaloblastic anaemia following gastric surgery. Rapid clearance of folic acid, however, was observed in haematological conditions other than folic acid deficiency.
The urinary excretion of formiminoglutamic acid following an oral dose of histidine has also been used as an index of folic acid deficiency (Lubhy, Cooperman, and Teller, 1959), and the urinary excretion of formiminoglutamic acid has been reported to be increased in some patients with megaloblastic anaemia or steatorrhoea following partial gastrectomy (Knowles and Prankerd, 1961; Kohn, Mollin, and Rosenbach, 1961; Chanarin, Bennett, and Berry, 1962). Elevated urinary levels have also been observed in patients with vitamin B₁₂ deficiency (Chanarin et al., 1962; Knowles and Prankerd, 1962).

Presumptive but inconclusive evidence would therefore appear to be present that folic acid deficiency may play a role in the pathogenesis of some cases of anaemia following partial gastrectomy. The microbiological assay of serum folic acid activity using *Lactobacillus casei* as the test organism promises to provide a more direct approach to the assessment of folic acid deficiency than the methods previously referred to (Herbert, 1961; Waters and Mollin, 1961). We therefore decided to estimate the serum folic acid activity in a randomly selected group of patients after partial gastrectomy in order to assess the contribution of folic acid deficiency to the pathogenesis of post-gastrectomy anaemia.

**MATERIALS AND METHODS**

One hundred patients, of whom 68 were men and 32 were women, were investigated. These were the same patients who had taken part in the study of the changes in the bones after partial gastrectomy, and the haematological investigations were performed concurrently with the other investigations. The Polya operation had been performed on 82 patients (59 men and 23 women) and on 18 (nine men and nine women) the Billroth I operation had been carried out. The mean interval between operation and the present investigation was 8-9 years. Twenty-one patients had been treated with iron at some stage between gastrectomy and examination in this survey. Two patients were currently being treated with vitamin B₁₂ and seven patients had received injections of vitamin B₁₂ more than one year before the investigation.

Each patient had the following investigations: blood count; serum level of folic acid activity; and serum vitamin B₁₂ concentration. Anaemia, which was defined as a haemoglobin level of less than 13-6 g. per 100 ml. in men and 11-6 g. per 100 ml. in women, was present in 33 patients (23 men and 10 women). Of these, 17 patients, including every patient in the anaemic group with a low level of serum vitamin B₁₂ or serum folic acid activity, had a bone marrow biopsy. Seven patients were found to have megaloblastic or partial megaloblastic changes in the marrow. These subsequently had a radioactive vitamin B₁₂ absorption test and an estimation of their faecal fat carried out.

**SERUM FOLIC ACID ACTIVITY** Serum folic acid activity was measured microbiologically with *Lactobacillus casei* as the test organism. The method described by Herbert (1961) was used for the assay of folic acid activity with only a few modifications. The syringes used for the collection of blood specimens were cleaned with alkali and rinsed in glass-distilled water. The containers into which the blood was placed had been cleaned with acid and rinsed in glass-distilled water. It was found necessary to use glass-distilled water as lower results were obtained for serum levels of folic acid activity if ordinary distilled water was used for rinsing.

The sera were diluted 1 in 11 with 0·1 M sodium phosphate buffer at pH 6-1, containing 150 mg. per millilitre of ascorbic acid. This resulted in a concentration of 15 mg. ascorbic acid per millilitre serum which gave optimal folic acid activity of the serum. The sera-buffer solutions were heated and not autoclaved to precipitate the protein.

The basal medium was stored in the deep freeze without the addition of a volatile preservative. This was found to be a more satisfactory method of storage, as in many cases some preservative remained after autoclaving which interfered with the growth of the bacteria.

**Recovery experiments** Recovery experiments were carried out with pteroylglutamic acid, although it appears likely that N⁴-methyl tetra-hydrofolic acid is the material in human serum which shows activity for *L. casei* (Herbert, Larrabee, and Buchanan, 1962). When 10 m₅g. per ml. of pteroylglutamic acid was added to pooled ‘low’ serum the mean recovery was 112·8±5·4% S.E., and when 20 m₅g. per ml. was added the mean recovery was 106·3±6·2% S.E.

*Variation within the same assay* The folic acid activity of serum from two patients was estimated repeatedly in the same assay. The coefficient of variation in the ‘high’ serum was 10·2% and in the ‘low’ serum 8·6%.

*Variation between different assays* Repeated assays on the same two sera were performed in six consecutive assays. With the ‘high’ serum the coefficient of variation was 13·6% and with the ‘low’ serum 20·5%.

**Serum folic acid activity in normal subjects** The level of serum folic acid activity in 60 normal subjects ranged from 2·3 to 28·6 m₅g. per ml. with a mean of 7·5 m₅g. per ml. ±0·6 m₅g. per ml. S.E. In this laboratory serum levels less than 2·0 m₅g. per ml. are found in patients with folic acid deficiency. The lower limit of normal for serum folic acid activity in this laboratory is lower than that reported by Herbert, Baker, Frank, Pascher, Sobotka, and Wasserman (1960) and Waters and Mollin (1961), but is similar to the findings of Grossowicz, Mandelbaum-Shavit, Davidoff, and Aronovitch (1962), and Arnold (1962).

**Serum vitamin B₁₂** Vitamin B₁₂ in the serum was assayed by the method of Hutner, Bach, and Ross (1956) using *Euglena gracilis* Z strain. Patients with pernicious anaemia have levels below 150 m₅g. per ml. Values of 150 to 200 m₅g. per ml. are indeterminate but may be indicative of ‘pre-pernicious anaemia’ (Wood, Cowling, Ungar, and Gray, 1960).

**Absorption of ¹⁵⁶CO-LABELLED VITAMIN B₁₂** The absorption of radioactive vitamin B₁₂ was measured using the...
urinary excretion test of Schilling (1953) as modified by Pitney and Stokes (1958). Carbachol (carbamylcholine chloride) was injected before the oral dose of labelled vitamin B₁₂ to ensure maximal secretion of intrinsic factor. One thousand µg. of non-radioactive vitamin B₁₂ was injected immediately after the oral dose of 0·6 µg. ⁵⁸Co-labelled vitamin B₁₂. One 24-hour urine collection was made. In normal subjects 9 to 29% (mean 16%) of the radioactivity is found in the urine in 24 hours. Patients with pernicious anaemia show a urinary excretion of radioactivity considerably below these levels.

**FAecal FAT** Faecal fat was estimated by the method of van de Kamer, Huink, and Weyers (1949) on three-day collections of faeces. A daily excretion of 7·0 g. or more was considered to indicate steatorrhoea.

**RESULTS**

**SERUM FOLIC ACID ACTIVITY** The levels of serum folic acid activity in the patients after partial gastrectomy ranged from 0·4 to 28·0 mµg. per ml. (mean 7·6 mµg. per ml.). Twelve patients had levels below 2·0 mµg. per ml., which has been set as the lower limit of normal in this laboratory. Reduced levels were more frequent in patients examined more than six years after operation (11 of 76 patients, or 14·5%) than in patients examined before this time (1 in 24, or 4·2%) (Fig. 1). Of the 33 patients with anaemia, five (15·2%) had low levels of serum folic acid activity as compared with seven of the 67 (10·4%) patients without anaemia. Statistical evaluation of reduced levels using the chi square test was carried out in respect of the following; sex; site of ulcer; type of operation; interval of time after operation; and the presence of anaemia. In none of these, however, was the 5% level of statistical significance attained.

**RELATION OF SERUM FOLIC ACID ACTIVITY AND SERUM VITAMIN B₁₂ CONCENTRATION** The serum vitamin B₁₂ levels were measured in 98 patients. In 10 patients the levels were less than 150 µµg. per ml. and in a further 14 the levels were 150 to 200 µµg. per ml. Of the patients with low or borderline serum vitamin B₁₂ levels, four showed a level of serum folic acid activity of less than 2 mµg. per ml., 16 had levels between 2 mµg. and 17·3 mµg. per ml., and four had levels greater than 17·3 mµg. per ml. (mean ± 2 S.D. for the control group = 17·3 mµg. per ml.).

**NATURE OF THE ANAEMIA** Thirty-three of the 100 patients were found to be anaemic; the haemoglobin levels were below 16·3 g. per 100 ml. in 23 of 68 men (33·8%) and below 11·6 g. per ml. in 10 of 32 women (31·2%). As judged by the blood count, serum vitamin B₁₂ concentration, serum folic acid activity, bone marrow biopsy, and response to treatment, the anaemia was considered to be due to iron deficiency in 26 patients, combined vitamin B₁₂ and folic acid deficiency in three, vitamin B₁₂ deficiency in three, and folic acid deficiency in one.

The bone marrow was found to be megaloblastic in three patients and partially megaloblastic in four (Table 1). Evidence of deficient absorption of radioactive vitamin B₁₂ was present in all of these seven patients, and partial or complete remissions were obtained after treatment with vitamin B₁₂. Four of the seven patients had reduced serum levels of folic acid activity and in two of these patients (D.C. and A.B.) treatment with folic acid was necessary to produce a complete haematological remission.

**RELATION OF ANAEMIA AND BONE DISEASE** Anaemia was a frequent finding in patients with bone disease.
Anaemia was detected in 22 of 50 patients with spinal rarefaction (44%), and in 11 of 50 patients with normal bones (22%). The increased incidence of anaemia in patients with bone disease was statistically significant (p < 0.05). Anaemia was present in nine of the 13 patients who were shown to have some of the features of osteomalacia as well as of osteoporosis. The appearance of the bone marrow in the nine patients was megaloblastic in one, partially megaloblastic in four, and normoblastic in four.

Discussion

Reduced serum levels of folic acid activity have been reported in the megaloblastic anaemias associated with idiopathic steatorrhoea, coeliac disease, pregnancy, cirrhosis, and nutritional deficiency (Herbert et al., 1960; Waters and Mollin, 1961; Dormandy, Waters, and Mollin, 1963). In these conditions the reduced levels have been interpreted as evidence of a deficiency of folic acid. In the present study reduced levels of serum folic acid activity were found in 12% of patients after partial gastrectomy, although in only one third of these was megaloblastosis in the bone marrow demonstrated. These findings are similar to those previously observed in studies of vitamin B₁₂ metabolism after partial gastrectomy, where low serum levels are not always associated with a frank vitamin B₁₂ deficiency anaemia (Deller and Witts, 1962; Jones, Williams, Cox, Meynell, Cooke, and Stammers, 1962).

As might be anticipated, there was evidence of multiple deficiencies in many of the patients, and so it may be difficult to ascribe a primary role in the aetiology of the anaemia to any particular deficiency. In primary vitamin B₁₂ deficiency the level of serum folic acid activity is either normal or increased (Waters and Mollin, 1961; Herbert and Zalusky, 1962). Reduced levels of both vitamin B₁₂ and folic acid activity were observed in a number of patients after partial gastrectomy in the present investigation and were probably indicative of a deficiency of both vitamins.

A frank or partially megaloblastic change in the bone marrow was found in four patients with anaemia and low serum levels of folic acid activity. One of these patients (A.B.) had been on treatment with vitamin B₁₂ for a number of months, so that the megaloblastic changes at the time of our examination could be ascribed with certainty to a deficiency of folic acid. In patient F.A. the serum vitamin B₁₂ concentration was 180 μg per ml., which is higher than that usually found in megaloblastic anaemia due to primary vitamin B₁₂ deficiency. In this patient also, the anaemia may have been the result of folic acid deficiency, although some degree of vitamin B₁₂ deficiency was present as well.

Although it is evident that folic acid deficiency plays some part in the pathogenesis of the anaemia after partial gastrectomy, it is noteworthy that the absorption of radioactive vitamin B₁₂ was impaired in every patient with megaloblastic changes in the bone marrow. Furthermore, the serum level of vitamin B₁₂ was reduced in six of the seven patients, the remaining patient already being on treatment with vitamin B₁₂. It follows from these investigations that the use of folic acid after partial gastrectomy in either the treatment of anaemia or as a multivitamin supplement is to be condemned unless vitamin B₁₂ deficiency has been excluded and treated. It is well known that although folic acid administration will often induce a temporary haematological remission in the patient who is deficient in vitamin B₁₂, this treatment is liable to be followed by the development or progression of subacute combined degeneration of the spinal cord and other neurological manifestations of vitamin B₁₂ deficiency.
Metabolic effects of partial gastrectomy with special reference to calcium and folic acid

(ROSS, BELDING, AND PAEGAL, 1948; CONLEY AND KREVANS, 1951). Furthermore, peripheral neuritis and spinal cord degeneration have been observed as late complications of partial gastrectomy (BASTRUP-MADSEN, 1954; ROBERTSON, 1959; DELLER, 1962).

The mechanism of folic acid deficiency following partial gastrectomy was not studied in detail. Nutritional deficiency appeared to be the explanation in three cases (D.C., E.C., F.A.) and impaired absorption may have been a factor in the patients with steatorrhea (E.C., F.A., A.B.). Folic acid deficiency on the basis of an inadequate diet was considered to be the cause of megaloblastic anaemia following partial gastrectomy in one third of cases studied by PITNEY (1963). However, the absorption of folic acid after partial gastrectomy has been reported to be normal (DOIG AND GIRDWOOD, 1960). This might not always be the case, as the method used to test absorption has involved doses of folic acid which are in excess of the amount generally present in the diet. Moreover, the absorption test does not measure the patient’s ability to absorb folic acid complexes in food.

SUMMARY

An investigation of the haematological status of 100 randomly selected patients after partial gastrectomy has shown low serum folic acid activity levels in 12%. Megaloblastic or partially megaloblastic anaemias have been found in seven of the patients of whom four had evidence of folic acid deficiency, one being regarded as due to primary folic acid deficiency.

Attention is drawn to the occurrence of combined vitamin B₁₂ and folic acid deficiency after operation, and to the dangers of therapy with folic acid and multi-vitamin preparations in the absence of a complete investigation of the haematological status of the patient.

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REFERENCES

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