VITAMIN B₁₂ AND FOLIC ACID IN MEGALOBlastic ANAEMIA AFTER TOTAL GASTRECTOMY

BY

NANCY S. CONWAY, M.B.

AND

HUGH CONWAY, M.B., B.Sc., M.R.C.P.

(From the Department of Surgery and the Gardiner Institute of Medicine, University of Glasgow, and the Western Infirmary, Glasgow)

Macrocytic anaemia following total gastrectomy has been described on a number of occasions. Many of the cases reported have, however, been inadequately studied; in particular, little attention has been paid to the possibility of dietary deficiency following operation, and special investigations such as reticulocyte counts and bone-marrow examinations have been few. There is no clear conception of the relationship of the anaemia to Addisonian pernicious anaemia. Wintrobe (1946) stated that "at least 10 acceptable cases with the typical blood picture of pernicious anaemia have been reported to date, and there is an equal number or more whose histories as recorded are less satisfactory." In the last four years at least six more cases have been published (Weir, 1946; Bethell, Meyers, Andrews, Swendseid, Bird, and Brown, 1947; Morgans, Rimington, and Whittaker, 1947; MacDonald, Ingelfinger, and Belding, 1947; Beebe and Meneely, 1949), but the haematological details are scanty.

Treatment with liver extracts has, in general, proved satisfactory. Folic acid has also been used with success (Morgans, Rimington, and Whittaker, 1947).

The present case of megaloblastic anaemia following total gastrectomy is reported because of its rarity and theoretical interest. The operation was performed for gastric contraction after acute corrosive poisoning. So far as is known this is the first recorded case of its kind to be treated with vitamin B₁₂.

Case Report

The patient, a labourer aged 47, drank, in mistake for cold tea, one-third of a cupful of solder "flux" on the evening of July 31, 1944. The "flux" consisted of zinc chloride 66%, acetic acid (90% glacial) 1%, and distilled water 33%. Immediately afterwards he felt sick but experienced no pain. He at once drank a strong solution of salt in water and began to vomit; the vomitus was at first watery but soon became blood-stained and very dark. An hour later gastric lavage was performed by his doctor, using a warm solution of sodium bicarbonate. Vomiting continued throughout the night, and he was admitted to the Western Infirmary, Glasgow, on August 1 in a state of shock. Acute phlegmonous gastritis developed, and by the end of August there was clinical and radiological evidence of gastric contraction. Jejunostomy was performed by Mr. A. W. Kay on September 10. His weight before operation was 105 lb. (47.62 kg.). In the next six weeks several attempts at oral feeding had to be abandoned because of vomiting. His weight fell gradually, and on October 8 radiological examination confirmed that gastric contraction was increasing.

Professor C. F. W. Illingworth performed a total gastrectomy on October 28. About 1 in. (2.5 cm.) of the duodenum and a small portion of the oesophagus were removed with the stomach, which was small, thick-walled, and extensively scarred; the lower end of the oesophagus was anastomosed to the jejunum. His weight before operation was 98 lb. (44.45 kg.). The post-operative course was uneventful, and in two weeks he could eat small quantities of solid food without discomfort or vomiting. He was discharged on November 21, when his weight was 100 lb. (45.36 kg.).

Subsequent Course

The patient reported at the follow-up clinic at regular intervals until the end of 1947. During 1948 and 1949 he failed to attend, but reappeared in June, 1950. Blood findings are shown in detail in Table I.

March, 1945.—Weight 118 lb. (53.52 kg.); appetite good, and able to eat an average meal; a feeling of emptiness between meals; no pain or vomiting; returned to work in January, 1945.

October, 1945.—Weight 121 lb. (54.88 kg.); symptom-free and taking normal meals; colour good; x-ray examination showed the barium passing direct into the jejunum, which dilated as it passed through.

TABLE I.—Haematological Details

Date	Hb (g.%)	P.C.V. (%)	R.B.C. (mill. per c.mm.)	M.C.V. (μ^3)	M.C.H.C. (%)	M.C.D. (μ)	W.B.C. per c.mm.	Icterus Index	Bone Marrow	Treatment* (μ g. Vitamin B ₁₂)	Remarks
16/10/44	12.0	—	4.60	—	—	—	—	—	Normal		Total gastrectomy on 28/10/44. Six pints of blood transfused
13/11/44	16.0	52	5.40	96	31	—	10,400	—			
6/10/46	12.3	40	3.51	113	31	—	8,400	—	Normoblastic but premature haemoglobinization of normoblasts		Liver therapy prescribed, but not more than 5 injections given during 1946-7
15/6/50	4.9	14	0.95	147	35	9.0	2,400	5	Megaloblastic	40	Blood film typical of Addisonian pernicious anaemia
22/6/50	6.2	22	1.39	158	28	9.0	3,100	5	No gross abnormality		
30/6/50	7.1	24	1.70	141	30	8.4	3,300	3		20	
5/7/50	8.4	30	2.30	130	28	8.4	—	—		20	
14/7/50	9.4	34	3.31	103	28	8.2	—	—		20	
21/7/50	10.4	34	3.62	94	30	8.2	—	—		20	
28/7/50	9.8	33	3.53	94	30	8.2	—	—		40	
4/8/50	10.4	34	3.71	92	29	8.2	5,300	3		40	
11/8/50	10.0	35	3.65	96	29	8.2	5,200	5		40	
18/8/50	10.8	36	3.80	95	30	8.0	—	3		40	
26/8/50	10.2	35	3.62	97	29	8.2	—	—		40	
2/9/50	10.3	35	3.75	93	29	8.1	—	3		40 μ g. + folic acid 20 mg. daily	
16/9/50	12.2	42	4.60	91	29	8.0	6,400	3		40	

* Treatment was given on dates shown after blood investigations were completed.

April, 1946.—Weight 126 lb. (57.15 kg.); taking ordinary diet without discomfort; no diarrhoea.

October, 1946.—Weight 129 lb. (58.51 kg.); no abnormal symptoms. Routine blood examination (Table I) revealed a slight macrocytic normochromic anaemia. Sternal marrow biopsy showed normoblastic erythropoiesis, but some of the normoblasts were prematurely haemoglobinized. An early stage of a "liver-factor" deficiency anaemia was diagnosed on the strength of the previous history, the macrocytosis and normal M.C.H.C., and the marrow appearances. Liver therapy was prescribed.

September, 1947.—Weight 120 lb. (54.43 kg.); complaint of dizziness immediately after meals, passing off in about 30 minutes. Not more than five liver injections had been given, at very irregular intervals, during the previous year. He was not enthusiastic about the injections, because he felt well without them. Diet consisted mainly of fish, meat, tripe, potatoes, milk puddings, bread, fruit, and milk; total calories about 2,000.

December, 1947.—Weight 116 lb. (52.61 kg.); dizziness immediately after meals persisted. A glucose-tolerance test gave a typical "lag storage" curve. Barium-meal examination showed no evidence of abnormal dilatation of the small bowel.

Recent Illness

The patient reported again in June, 1950, complaining of increasing tiredness and dyspnoea on exertion of six months' duration. His appetite was good, but a few minutes after each meal he suffered from a feeling of distension accompanied by light-headedness and a desire to lie down. These symptoms passed off in half an hour. For a few months he had noticed paraesthesiae of his finger-tips, but his tongue had not been painful. He had received no liver injections since the summer of 1947.

He was readmitted on June 13, having been at work until one week previously. He looked pale and thin, and his weight was 104 lb. (47.17 kg.). His tongue was normal in appearance, and the spleen was not palpable. The nervous system was normal.

Investigations.—Examination of the peripheral blood (Table I) revealed a severe macrocytic normochromic anaemia; the blood film contained macropolycytes and a few late megaloblasts, and appeared typical of Addisonian pernicious anaemia. There was a leucopenia, but the serum bilirubin was not raised. Bone-marrow smears showed megaloblastic erythropoiesis, and giant metamyelocytes were numerous. Barium-meal examination revealed no abnormality of the oesophagus or upper loops of the jejunum. The faeces were normal in appearance and contained no blood; fat comprised 12% (average of three specimens) of the dry substance of the stool. A fat-balance test was not done.

The daily food intake prior to readmission was estimated at 2,100 calories (protein 80 g., fat 85 g., carbohydrate 260 g.). He took three main meals, with milk drinks between; eggs, meat, tripe, fruit, potatoes, and fish were consumed regularly. Vitamins were considered to be adequate. The glucose-tolerance test again gave a "lag storage" curve.

Treatment.—An initial injection of 40 µg. of vitamin B₁₂ concentrate (prepared from *Streptomyces griseus*) was given on June 15. Within a few days he began to feel better. The reticulocyte response and rise of red cells in the first 15 days are shown in Table II. Further injections were given as detailed in Table I, and there was marked clinical improvement. The haemoglobin and red blood cells continued to rise for five weeks, but in the next six weeks there was no further increase despite frequent doses. The peak levels for haemoglobin and red blood cells were 10.8 g.% and 3.8 millions per c.mm. respectively. At this stage the bone marrow was considered to be normal, and there was no important degree of iron deficiency. The addition of folic acid (20 mg. a day) then raised the haemoglobin to 12.2 g.% and the red blood cell count to 4.6 millions per c.mm. in two weeks.

TABLE II.—*Rise of Red Blood Cells and Reticulocyte Response in the First 15 Days after a Single Injection of 40 µg. Vitamin B₁₂*

Day of Treatment	Hb (g.%)	R.B.C. (mill. per c.mm.)	Reticulocytes (%)
0	4.9	0.95	0.5
1	—	—	0.5
2	—	—	1.5
3	—	—	1.5
4	—	—	10.0
5	5.3	1.06	27.0
6	—	—	28.0
7	6.2	1.39	27.5
8	—	—	18.5
9	—	—	8.0
10	6.6	1.55	5.0
11	—	—	4.0
12	—	—	1.5
13	—	—	0.5
14	—	—	1.0
15	7.1	1.70	1.0

The patient was discharged from hospital on July 7 and returned to full employment a month later. On September 9 he felt extremely well and weighed 117 lb. (53.07 kg.). It is noteworthy that the "dumping syndrome" ceased when his general condition improved, and there has been no recurrence.

The effect of vitamin B₁₂ in maintenance treatment is being studied.

Discussion

It is interesting to consider the pathogenesis of the megaloblastic anaemia which occasionally follows total gastrectomy in the light of the classical theory of erythropoiesis. Meulengracht (1935) showed that, in the hog, the duodenum as well as the pyloric and cardiac regions of the stomach possessed anti-anaemic activity. In human beings, however, Castle, Townsend, and Heath (1930) found that duodenal secretion contained no intrinsic factor, and Fox and Castle (1942) produced strong evidence that only the body of the stomach is important. This is the site which shows diagnostic atrophic changes in patients dying of pernicious anaemia (Magnus and Ungley, 1938). Recently Landboe-Christensen and Plum (1948) have suggested that the pyloric area, in man, is a significant but minor source of intrinsic factor. Patients who have undergone total gastrectomy should therefore, theoretically, develop megaloblastic anaemia when their store of anti-anaemic principle is exhausted. The operation is, however, usually undertaken for malignant disease and the average survival time is short.

In 1929 only 10 cases were known to have lived for a year or longer (Finney and Rienhoff, 1929). This probably explains the clinical fact that macrocytic anaemia is relatively rarely encountered after total gastrectomy. Time is required for its development, and Bethell, Sturgis, Rundles, and Meyers (1945) considered that complete removal of the stomach is invariably followed by macrocytic anaemia provided that the patients live long enough. MacDonald, Ingelfinger, and Belding (1947) collected, from the literature and by correspondence, details of 53 patients who had survived for three or more years after operation. Twelve developed macrocytic anaemia and five anaemia of doubtful nature. Thirty-six did not develop this anaemia up to the time of death or review. Of the last group, seven received prophylactic liver therapy, 19 probably had liver but no accurate information was available, and 10 definitely had no liver. An exceptional case was stated to be alive and well 8½ years after operation even in the absence of prophylactic liver.

The time between operation and the first diagnosis of macrocytic anaemia in the cases previously recorded

varies from five months (Ungley, 1932) to eight years (Dennig, 1929; Hochrein, 1929; Weir, 1946); the average is approximately four and a half years. The case quoted by Hurst (1932) as having developed pernicious anaemia and subacute combined degeneration of the spinal cord 17 years after total gastrectomy was originally reported by Ellis (1925) to have undergone only partial gastrectomy. The length of the average latent period requires consideration, and in this respect the work of Schwartz and Legere (1944) is of interest. These authors found, in a study of 54 cases of Addisonian pernicious anaemia, that relapses occurred 2-38 months after stopping liver therapy. They pointed out that in individual cases the time of relapse was unpredictable and depended, apart from intercurrent disease, on the quantity of anti-anaemic factor stored, the consumption of food factors containing active principle, and the extent of deficiency of the intrinsic factor.

Goldhamer (1937) found that the lack of intrinsic factor in pernicious anaemia is not absolute but differs in degree in different patients. After total gastrectomy the intrinsic factor must, according to accepted theory, be completely absent, and yet the average period between operation and the development of macrocytic anaemia is considerably longer than the longest of the remissions noted by Schwartz and Legere (1944). The surgical patients may start off with a higher store of active principle than is carried by a treated case of pernicious anaemia, but it is difficult to conceive of preformed stores great enough to postpone the development of macrocytic anaemia for eight years. It is likely that other factors are involved, and the upset in vitamin B₁₂ metabolism following total gastrectomy may be less serious, at least in the beginning, than in Addisonian pernicious anaemia.

Recently investigations have been made into the presence in the intestinal wall of intrinsic factor as distinct from extrinsic and intrinsic factor together. The existence of intrinsic factor in the duodenal mucosa has been established (Bethell, Swendseid, Meyers, Neligh, and Richards, 1949), and it has been suggested that it may be present even lower in the intestine. This raises the question whether gastric atrophy is the primary lesion in Addisonian pernicious anaemia or only part of a widespread tissue defect. The whole problem of the metabolism of vitamin B₁₂, including its interaction with intrinsic factor and its relationship to the bacterial flora of the bowel, is receiving much attention at present (Dyke, Hind, Riding, and Shaw, 1950; Hall, 1950).

In all cases in which macrocytic anaemia occurs some years after extensive gastric resections there exists the possibility of coincidence. This requires serious consideration after partial gastrectomy, for of all the many patients who have been submitted to the operation relatively few have been reported to develop macrocytic anaemia, and coincidence probably cannot be absolutely ruled out in any single case. Statistical probability, however, makes coexistence very unlikely (Bloomfield and Pollard, 1935). The incidence of macrocytic anaemia in patients who have survived three or more years after total gastrectomy is so great as to exclude all possibility of coincidence.

The present case has several interesting features. No previous record has been found of macrocytic anaemia following gastrectomy performed for the effects of corrosive poisoning, but Alsted (1937) reported a case in which "pernicious anaemia" developed seven years after destruction of the gastric mucosa by nitric acid.

Two years after operation, and in the absence of symptoms, the red blood cell count was 3.51 millions per c.mm.; the anaemia was normochromic and macrocytic. Although erythropoiesis was normoblastic, many of the normoblasts were prematurely haemoglobinized. These changes were thought to represent an early stage of a "liver-factor" deficiency anaemia. It is rare for a patient with Addisonian pernicious anaemia to seek advice before the red blood cell count is considerably lower than 3.5 millions per c.mm., and information on the earliest marrow changes is scanty. Penati and Saita (1938) stated that megaloblasts first appear when the number of erythrocytes has fallen to 3.5 millions per c.mm. The findings in our case support the contention that macrocytosis is the earliest haematological abnormality in pernicious anaemia and is present before the onset of symptoms. The megaloblastic anaemia, diagnosed approximately three years after cessation of prophylactic liver therapy, appeared identical to Addisonian pernicious anaemia except for the absence of bilirubin-anaemia.

Treatment with vitamin B₁₂ produced striking clinical improvement, but the haematological response, though good, was less than expected. The peak of the reticulocyte rise (28%) was only 69% of that expected in Addisonian pernicious anaemia of the same severity from potent liver extract given intramuscularly (Isaacs and Friedman, 1938). It has been shown, however, that the reticulocyte response is not always a good measure of activity (Murphy, 1933; Fullerton, 1945); a better yardstick is the rate of increase of the red blood cells. The rise of the red blood cells in the first 15 days compared unfavourably with that expected in Addisonian pernicious anaemia, whether judged by the formula of Della Vida and Dyke (1942) for parenteral liver extract, or by that of Ungley (1949) for pure crystalline vitamin B₁₂ obtained from liver.

According to Ungley (1949) a single injection of 40 µg. of crystalline vitamin B₁₂, in a patient with Addisonian pernicious anaemia and initial red blood cell count of 0.95 million per c.mm., is expected to raise the count to 2.73 millions in 15 days. This compares with a figure of only 1.7 millions per c.mm. in our case. The biggest discrepancy, noted by Ungley (1949) in 63 cases, between the estimated and observed increase was 0.79 million per c.mm. Even if allowance has to be made for a slight difference in the therapeutic effect of the preparations used by Ungley and ourselves the evidence suggests that the megaloblastic anaemia which followed gastrectomy in this case was not identical with Addisonian pernicious anaemia.

Further support for this hypothesis is provided by the poor final effect of vitamin B₁₂ therapy. Ungley (1949) found, in 32 cases of Addisonian pernicious anaemia, that the maximum amount of vitamin B₁₂ required to raise the red cell count above 4.5 millions per c.mm. was 140 µg. In the present case a total of 320 µg., in divided dosage at weekly intervals, failed to increase the count above 3.8 millions per c.mm. After remaining around this level for six weeks a sharp rise followed the addition of folic acid. The inability of vitamin B₁₂ to bring about complete remission after its very beneficial initial effect was unexpected. Larger doses might have proved more successful.

The relationship between vitamin B₁₂ and folic acid is a much vexed question and has recently been reviewed by Girdwood (1950). One striking fact is that no satisfactory human case of megaloblastic anaemia has yet

been described which responded haemopoietically to vitamin B₁₂ after failing to respond to folic acid. The experimental work of Heinle, Welch, and Pritchard (1948) is of considerable interest. They produced megaloblastic anaemia in pigs by feeding a highly purified diet free of extrinsic factor and containing succinylsulphathiazole and a crude folic acid antagonist. This diet was calculated to produce a deficiency both of vitamin B₁₂ and of pteroylglutamic acid. A pig rendered anaemic by this method first responded incompletely to liver extract containing no folic acid, but two months later failed to respond to daily injections of liver extract; folic acid then produced an excellent effect. This is a sequence of events somewhat similar to that obtained in our patient. Those authors concluded, from this and other experiments, that in these experimental conditions neither the anti-pernicious-anaemia factor (regarded as identical with or similar to vitamin B₁₂) nor folic acid can function haemopoietically in the absence of the other, and that both substances are essential for the maturation of erythrocytes.

Israëls and Sharp (1950) suggested that the megaloblastic anaemias can be divided into two groups, depending on the effect of treatment. The first is typified by Addisonian pernicious anaemia, which responds well to parenteral or oral liver preparations, vitamin B₁₂, and folic acid. The second group includes "pernicious anaemia" of pregnancy, the megaloblastic anaemia of non-tropical sprue, and probably tropical sprue as well: these conditions react satisfactorily to folic acid but not to parenteral liver extracts or vitamin B₁₂, at least in doses suitable for the first group. The present case appears to fall between these two groups, for, although by no means refractory to vitamin B₁₂, the response was suboptimal compared with that expected in Addisonian pernicious anaemia. Folic acid is unnecessary at any stage in Addisonian pernicious anaemia treated with vitamin B₁₂ and usually essential from the outset in non-tropical sprue. In our case it was required only for "topping up" after vitamin B₁₂.

Final evaluation of the respective functions of folic acid and vitamin B₁₂ must await further work. Evidence collected from various clinical experiences, such as that described in this paper, may eventually help to solve the problem.

Summary

A case of megaloblastic anaemia following total gastrectomy performed for the effects of corrosive poisoning is described.

The pathogenesis of the anaemia is discussed in the light of the classical theory of erythropoiesis.

Striking clinical improvement followed vitamin B₁₂ therapy, but the haematological response was suboptimal compared with that expected in Addisonian pernicious anaemia. The addition of folic acid brought about complete haematological remission.

The effect of treatment suggested that this type of megaloblastic anaemia and Addisonian pernicious anaemia are not identical, although morphologically similar.

The relationship of the anaemia to other megaloblastic anaemias, as regards treatment with vitamin B₁₂ and folic acid, is discussed.

We wish to thank Professor C. F. W. Illingworth for permission to publish this case and Professor J. W. McNee for advice. The blood investigations in 1944 and 1946 were performed by Dr. Peter McKenzie, and we are indebted to him for permission to quote his results.

REFERENCES

- Alsted, G. (1937). *Lancet*, **1**, 76.
 Beebe, R. T., and Meneely, J. K., jun. (1949). *N.Y. St. J. Med.*, **49**, 2437.
 Bethell, F. H., Meyers, M. C., Andrews, G. A., Swendseid, M. E., Bird, O. D., and Brown, R. A. (1947). *J. Lab. clin. Med.*, **32**, 3.
 — Sturgis, C. C., Rundles, R. W., and Meyers, M. C. (1945). *Arch. intern. Med.*, **76**, 239.
 — Swendseid, M. E., Meyers, M. C., Neligh, R. B., and Richards, H. G. (1949). *Univ. Hosp. Bull., Ann. Arbor*, **15**, 49.
 Bloomfield, A. L., and Pollard, W. S. (1935). *J. clin. Invest.*, **14**, 321.
 Castle, W. B., Townsend, W. C., and Heath, C. W. (1930). *Amer. J. med. Sci.*, **180**, 305.
 Della Vida, B. L., and Dyke, S. C. (1942). *Lancet*, **2**, 275.
 Dennig, H. (1929). *Münch. med. Wschr.*, **76**, 633.
 Dyke, W. J. C., Hind, H. G., Riding, D., and Shaw, G. E. (1950). *Lancet*, **1**, 486.
 Ellis, A. (1925). *Proc. R. Soc. Med.*, **18**, Sect. Neur., 27.
 Finney, J. M. T., and Rienhoff, W. F., jun. (1929). *Arch. Surg., Chicago*, **18**, 140.
 Fox, H. J., and Castle, W. B. (1942). *Amer. J. med. Sci.*, **203**, 18.
 Fullerton, H. W. (1945). *Edinb. med. J.*, **52**, 412.
 Girdwood, R. H. (1950). *Ibid.*, **57**, 72.
 Go'dhamer, S. M. (1937). *Amer. J. med. Sci.*, **193**, 23.
 Hall, B. E. (1950). *British Medical Journal*, **2**, 585.
 Heinle, R. W., Welch, A. D., and Pritchard, J. A. (1948). *J. Lab. clin. Med.*, **33**, 1647.
 Hochrein, M. (1929). *Münch. med. Wschr.*, **76**, 1327.
 Hurst, A. F. (1932). *Quart. J. Med.*, n.s. **1**, 157.
 Isaacs, R., and Friedman, A. (1938). *Amer. J. med. Sci.*, **196**, 718.
 Israëls, M. C. G., and Sharp, J. (1950). *Lancet*, **1**, 752.
 Landboe-Christensen, E., and Plum, C. M. (1948). *Amer. J. med. Sci.*, **215**, 17.
 MacDonald, R. M., Ingelfinger, F. J., and Belding, H. W. (1947). *New Engl. J. Med.*, **237**, 887.
 Magnus, H. A., and Ungley, C. C. (1938). *Lancet*, **1**, 420.
 Meulengracht, E. (1935). *Ibid.*, **1**, 493.
 Morgans, M. E., Rimington, C., and Whittaker, N. (1947). *Ibid.*, **2**, 128.
 Murphy, W. P. (1933). *Arch. intern. Med.*, **52**, 829.
 Penati, F., and Saita, G. (1938). *Arch. Sci. méd.*, **65**, 790.
 Schwartz, S. O., and Legere, H. (1944). *J. Amer. med. Ass.*, **124**, 637.
 Ungley, C. C. (1932). *Lancet*, **2**, 1426.
 — (1949). *British Medical Journal*, **2**, 1370.
 Weir, J. F. (1946). *Surg. clin. N. Amer.*, **26**, 934.
 Wintrobe, M. M. (1946). *Clinical Haematology*, 2nd ed., p. 397. London.

HAEMOPOIETIC ACTIVITY OF VITAMINS B_{12c} AND B_{12d} IN PERNICIOUS ANAEMIA

BY

J. N. MARSHALL CHALMERS, M.D., M.R.C.P.
D.P.H.

(From the Haematology Department, St. George's Hospital and Medical School)

After the isolation of vitamin B₁₂ from liver (Lester Smith and Parker, 1948; Rickes *et al.*, 1948a), and the subsequent discovery that the vitamin could be obtained from botanical sources (Rickes *et al.*, 1948b), physico-chemical studies showed that there are other members of the vitamin B₁₂ group of factors. Vitamin B_{12a}, the reduction product of vitamin B₁₂ (Kaczka, Wolf, and Folkers, 1949), has been shown probably to be identical with vitamin B_{12b} (Brockman *et al.*, 1950; Anslow *et al.*, 1950), and crystalline vitamins B_{12c} and B_{12d}, derived from *Streptomyces griseus*, have been described (Lester Smith, 1950, 1951; Anslow *et al.*, 1950; Buchanan *et al.*, 1950). These factors appear to be chromatographically distinct from one another; in addition they have different properties in the microbiological assay (Lester Smith, 1951).

This paper records the primary haematological responses in pernicious anaemia to vitamin B_{12c} (nine cases) and vitamin B_{12d} (five cases).