VITAMIN B₁₂ AND FOLIC ACID IN MEGALOBlastic ANAEMIAS OF PREGNANCY AND THE PUERPERIUM

BY

C. C. UNGLEY, M.D., F.R.C.P.

Physician, Royal Victoria Infirmary, Newcastle-upon-Tyne

AND

R. B. THOMPSON, M.D., M.R.C.P.

Research Fellow in Medicine, King's College, Newcastle-upon-Tyne

Megaloblastic anaemia occurring in pregnancy and the puerperium is clearly distinct from Addisonian pernicious anaemia with its associated gastric atrophy and permanent loss of Castle's intrinsic factor. Trials with vitamin B₁₂ reported here emphasize that even the nature of the limiting haematopoietic factor may be different in the two disorders.

Haematological methods and dietary control have been described (Ungley, 1949). Several cases are excluded because of spontaneous remission, and only six will be presented. Five were classical examples of the so-called "pernicious anaemia of pregnancy"; the sixth had unusual features. All showed megaloblastic erythropoiesis on marrow puncture.

Case 1

Mrs. A., aged 34, was first seen in the fourth month of her third pregnancy. She then had hypochromic anaemia with a normoblastic marrow, and received transfusions and iron. At the eighth month she returned with severe swelling of the legs and vulva, and a marked anaemia, this time associated with a megaloblastic marrow. Except that she ate red meat only twice a week and disliked cabbage the diet had been fairly good. There was free HCl in the gastric juice. Red blood cells numbered 1,530,000 per c.mm.; haemoglobin, 30% (4.4 g. per 100 ml.); colour index, 0.98; M.C.V., 80 μ^3 ; M.C.H.C., 34.2%; M.C.H., 27.1 $\mu\mu\text{g}$. Plasma bilirubin was 0.64 mg. per 100 ml. (direct reaction negative).

A concentrate of Lester Smith's red pigments (not yet crystalline) was given parenterally in a dose colorimetrically equivalent to 65 μg . of vitamin B₁₂ (Fig. 1). There was no response, and after a few days the patient was transfused with blood of a group suitable for differential agglutination by the Ashby technique (Dr. W. Walker). Ten days after receiving vitamin B₁₂ her own red blood cells had fallen to 1,130,000 per c.mm. The marrow was still megaloblastic, and oedema had increased. She was now given 2.5 mg. of folic acid daily, alternately by mouth and by injection. After four days the marrow was normoblastic. A reticulocytosis of 26.6% followed, and the red blood cells increased to 2,240,000 per c.mm. in 10 days. When a yeast extract was substituted for folic acid there was no secondary reticulocytosis, and erythropoiesis continued at the same rate. The rapid rate of elimination of transfused red cells changed to normal after folic acid.

After delivering a healthy child the patient continued to improve, red cell counts reaching normal levels without further treatment, except a course of iron.

Case 2

Mrs. B., aged 24, twice had megaloblastic anaemia associated with pregnancy. The anaemia which followed the birth of her first child responded to an alcoholic extract of yeast. Free

HCl was present in the gastric juice. Throughout her second pregnancy she felt well, but by regular blood examination a recurrence of the anaemia was detected. At the eighth month the red blood cells numbered 3,470,000 per c.mm.; haemoglobin, 65% (9.6 g. per 100 ml.); colour index, 0.94; M.C.V., 96.0 μ^3 ; M.C.H.C., 29.2%; M.C.H., 27.7 $\mu\mu\text{g}$.; white blood cells, 8,900; the marrow was megaloblastic.

An injection of 80 μg . of vitamin B₁₂ did not produce a reticulocyte response, the marrow remained megaloblastic, and in nine days the red blood cells fell to 3,190,000 per c.mm. (Fig. 2). When 20 mg. of folic acid was given daily by mouth reticulocytes rose to 4.6% on the seventh day and the red blood cells increased to 3,760,000 in 17 days. After 28 days—nine days before parturition—the counts were unchanged. There was a slight fall to 3,360,000 after delivery, followed by a rapid rise to normal levels.

Case 3

Mrs. C., aged 32, had a routine examination at the sixth month of pregnancy which revealed an unduly low blood count; red blood cells, 2,410,000 per c.mm.; haemoglobin, 53% (7.8 g. per 100 ml.); colour index, 1.1; M.C.V., 103.7 μ^3 ; M.C.H.C., 31.4%; M.C.H., 32.5 $\mu\mu\text{g}$.; white blood cells, 6,200 per c.mm. Apart from pallor there were no signs of disease. Her appetite and diet had been excellent and she had been free from gastro-intestinal disorder. The tongue was well papillated, and free HCl was present in the gastric juice. A Price-Jones curve showed a mean diameter of 7.72 μ , standard deviation 0.77 μ , coefficient of variation 9.9%, megalocytosis 12%, microcytosis nil. The marrow was megaloblastic. Plasma bilirubin was normal (0.24 mg. per 100 ml.) and methaemalbumin was not detected (175 mm. depth). In 24 hours the excretion of ketosteroids was 5.1 mg. (Dr. F. K. Herbert). Professor Marrian, of the department of biochemistry, University of Edinburgh, kindly examined two 24-hour specimens of urine, with the following results: pregnanediol, 30.3 and 28.5 mg.; total oestrogen (estimated as oestradiol), 11.8 and 12.5 mg.

After a control period, 80 μg . of vitamin B₁₂ was injected intramuscularly (Fig. 3). There was no response and the marrow remained megaloblastic. A yeast extract also proved ineffective. Folic acid was then given daily, 2.5 mg. by intramuscular and oral routes alternately. Reticulocytes rose to a peak of 10.6% on the fifth day, and in 10 days the red blood cells increased from 1,760,000 to 2,200,000 per c.mm. When the dosage was increased to 15 mg. daily reticulocytes rose again slightly to 3.2% on the fifth day. After 10 days red blood cells numbered 2,860,000, but there was no further increase up to 20 days.

When later she received 120 ml. of an aqueous liver extract daily the red blood cells increased to 3,630,000 in 10 days, but there was no further increase in 16 days, and haemoglobin fell from 63% to 56% (8.3 g. per 100 ml.), with M.C.V. 83.6 μ^3 ,

M.C.H.C. 27.5%, M.C.H. 23.7 μg . On this account she was given ferrous sulphate, 1.2 g. daily. At the same time folic acid was restarted in doses of 20 mg. Two weeks later—six days before the child was born—the red blood cells numbered 3,650,000 per c.mm., with haemoglobin 67% (9.9 g. per 100 ml.). After delivery, with no change in treatment, recovery was rapid, and in 12 days the red blood cells numbered 4,420,000, with haemoglobin 82% (12.1 g. per 100 ml.). A Price-Jones curve at seven weeks gave normal values: mean diameter, 7.25 μ ; standard deviation, 0.63 μ ; coefficient of variation, 8.7%; megalocytosis, nil; microcytosis, 0.8%.

Case 4

Mrs. D., aged 25, was admitted to hospital with fever and anaemia two days after the delivery of her third child, which was healthy although 10 weeks premature.

Two weeks before delivery she took to bed because of diarrhoea, vomiting, and loss of weight, but from the second month she had had increasing weakness, soreness and ulceration of the mouth and throat, with pain on swallowing. She was pale and thin. The tongue, though normally papillated, showed several small white ulcers. A urinary infection was quickly controlled with hexamine. Gastric analysis at first showed achlorhydria, but 10 days later free HCl was present. Red blood cells numbered 1,460,000 per c.mm.; haemoglobin, 31% (4.6 g. per 100 ml.); M.C.V., 102.8 μ^3 ; M.C.H.C., 30.6%; M.C.H., 31.1 μg .; white blood cells, 15,100 per c.mm. The marrow was megaloblastic. Van den Bergh's test was negative and methaemalbumin was not detected (depth 160 mm.).

After transfusion and a control period the red blood cells numbered 2,220,000, and 80 μg . of vitamin B₁₂ was injected intramuscularly. Reticulocytes rose to 3.6% on the sixth day, but after 15 days the red cell count and marrow picture remained unchanged. During subsequent treatment with yeast

extract, folic acid, and marmite the red blood cells slowly increased to 4,370,000 per c.mm. There was a reticulocytosis of 4.4% on the fourth day of folic acid therapy (Fig. 4).

Case 5

Mrs. E., aged 40, gave a doubtful history of anaemia following the birth of her fifth child, but had otherwise been healthy. In the third month of her sixth pregnancy she lost her appetite,

ceased eating meat, eggs, and fish, and subsisted mainly on bread, milk, potatoes, fruit juice, and meat extract. In the fifth month she became listless and breathless, with swelling of the legs and, later, diarrhoea and headaches.

When admitted at eight months she was obese, pale, dyspnoeic, and moderately oedematous. Blood pressure was 170/90 mm. Hg, blood urea 43 mg. per 100 ml. Marked albuminuria persisted until after parturition. The tongue was smooth. The red blood cells numbered 980,000 per c.mm.; haemoglobin, 20% (3 g. per 100 ml.); M.C.V., 102 μ^3 ; M.C.H.C., 29.6%; M.C.H., 30.2 μg .; reticulocytes, 0.4%; white blood cells, 3,000 per c.mm. Blood films showed macrocytosis and the marrow was megaloblastic. Gastric analysis later showed free HCl. Plasma bilirubin was 0.48 mg. per 100 ml. Methaemalbumin was not detected.

The patient was transfused with cells from 560 ml. of blood, an equal volume of her own blood being removed simultaneously, using poly-

thene catheters (Fig. 5). At the same time 80 μg . of vitamin B₁₂ was given intramuscularly. After 38 hours her condition remained serious, and as a response to vitamin B₁₂ was unlikely she was given 30 mg. of folic acid intravenously. She was transfused then and again two days later because of epistaxis. Reticulocytes rose on the third day after folic acid, reaching a peak of 18.8% on the seventh day, with clinical improvement. On the fifth day after folic acid she

FIG. 1.—Case 1. Megaloblastic anaemia of pregnancy.

FIG. 2.—Case 2. Megaloblastic anaemia of pregnancy.

FIG. 4.—Case 4. Megaloblastic anaemia in the puerperium.

FIG. 3.—Case 3. Megaloblastic anaemia of pregnancy.

gave birth to a child, which died of cystic cerebral degeneration.

Her condition improved without further treatment until the 19th day, when the red blood cells numbered 2,650,000 per c.mm., with haemoglobin 52% (7.7 g. per 100 ml.). She was now given 20 mg. of folic acid daily by mouth, and after three weeks, two months, and four months the red blood cells numbered 4,310,000, 4,760,000, and 5,220,000 respectively. Treatment was stopped, and so far (two months) she has not relapsed.

Case 6

This patient, aged 26, was admitted four weeks after the birth of a full-term normal child. Iron was prescribed early in pregnancy for pallor, which in the terminal three months became more pronounced and was accompanied by swelling of the feet. The dietary history was satisfactory. Labour was normal, with no serious blood loss, but afterwards she became much worse, having dyspnoea and palpitation even in bed. Iron therapy brought no relief.

On admission she was extremely pale and slightly yellow. The tongue was smooth at the edges and clean, but never sore. The liver and spleen were just palpable. There was achlorhydria even after histamine and alcohol. Plasma bilirubin was 4.8 mg. per 100 ml. (direct reaction negative). Methaemalbumin was not detected. Thymol turbidity and thymol flocculation were normal. A fragility curve (after transfusion) showed a wide

range from 100% haemolysis at 0.34 to nil at 0.56% saline, but the mean corpuscular fragility was just within normal limits. The red blood cells numbered 1,060,000 per c.mm.; haemoglobin, 24% (3.6 g. per 100 ml.); reticulocytes, 4.6%; colour index, 1.13; cell volume, 11%; M.C.V., 103.8 μ^3 ; M.C.H., 33.5 $\mu\mu$; M.C.H.C., 32.3%; white blood cells, 2,700. A Price-Jones curve showed mean corpuscular diameter 7.66 μ , standard deviation 0.98 μ , coefficient of variation 12.8%, megalocytosis 12.8%, microcytosis 1.0%. The marrow was predominantly megaloblastic.

The patient was transfused with erythrocytes, which could be separated by differential agglutination (Dr. W. Walker). Afterwards there was a spontaneous reticulocytosis of 38.6%, with a transient rise of red blood cells (Fig. 6). Nevertheless the blood from this and two other transfusions was rapidly destroyed and the total red cell count fell to 820,000 per c.mm. The injection of 80 μ g. of vitamin B₁₂ had no effect on the blood picture or on the haemolysis, and the marrow remained megaloblastic.

The subsequent daily administration of 20 mg. of folic acid by mouth was followed by prolonged reticulocytosis, itself of doubtful significance because of the previous findings. After two weeks the rate of elimination of transfused red cells fell and subsequently became normal. The marrow was now normoblastic. The rise in the total red cell count flattened out at about 3,500,000, but normal levels were subsequently attained after prolonged treatment with refined liver extract ("examen" 10 ml. every two weeks for 10 weeks, supplemented latterly with iron). For the past three months the patient has remained well without treatment. The blood picture is normal and the liver and spleen are not palpable.

Comment

Case 1 received a concentrate of red pigments including vitamin B₁₂ and Lester Smith's slow-moving red components colorimetrically equivalent to 65 μ g. of vitamin B₁₂ (Ungley, 1948). The other five were given 80 μ g. of pure crystalline vitamin B₁₂, also prepared by Lester Smith from liver.

In Case 4 there was a slight reticulocytosis without increase of red blood cells. In Cases 5 and 6 the patients were so critically ill that folic acid had to be given within a few days of injecting vitamin B₁₂. The possibility of a delayed response to the latter cannot entirely be ruled

FIG. 5.—Case 5. Megaloblastic anaemia of pregnancy.

out, but is considered improbable. In Cases 1, 2, and 3 the results were clearly and completely negative. This indicates a lack of response to the injection of doses of vitamin B₁₂ considerably in excess of those needed to produce a response in Addisonian pernicious anaemia. All six patients subsequently responded to folic acid, in three cases to as little as 2.5 mg. daily.

In addition to these cases treated with vitamin B₁₂ another 21 patients (out of the series of 47 to be reported shortly) had parenteral liver therapy. Many showed a slight reticulocytosis, but in eight cases there was no increase of red blood cells, and in six the response was probably negative or poor. In the remaining seven patients apparent responses coincided with and may have

FIG. 6.—Case 6. An unusual case of megaloblastic anaemia in the puerperium.

been partly or entirely due to spontaneous remission. In no case was there a response to injections of crude liver extract after refined liver extract had failed. Yeast extracts were effective in some and failed in others. There were no failures with whole liver by mouth or with folic acid.

Bethell, Meyers, and Neligh (1948) and Day, Hall, and Pease (1949) each report failures to vitamin B₁₂ followed by response to folic acid in a single case of megaloblastic anaemia associated with pregnancy or the puerperium. Many others have reported failures to respond to parenteral liver therapy.

Since vitamin B₁₂ is clearly ineffective in these anaemias, theories based on deficiencies of Castle's intrinsic and extrinsic factors need revision. Some of our patients gave normal dietary histories, and there was nothing to suggest that the intake of foods containing folic acid was lower than in other pregnant women of similar social status who did not develop megaloblastic anaemia.

The only aetiological factor common to these anaemias was their relation to pregnancy or the puerperium. An abnormal pattern of excretion of hormones in the urine is reported by Day, Hall, and Pease (1949). In our Case 3 no abnormality could be detected in the excretion of ketosteroids, pregnanediol, or total oestrogens estimated as oestradiol. This does not rule out the possibility of some other abnormality in hormone production, and further investigations are contemplated.

In Case 1 the rapid elimination of transfused red cells fell to normal after folic acid. In Case 6 haemolysis was much more severe and reticulocyte counts were so high as to raise doubts about the diagnosis even though the marrow was megaloblastic. Vitamin B₁₂ had no effect on the blood and marrow picture, and excessive haemolysis continued. Folic acid had a dramatic effect: excessive haemolysis ceased, and the rate of elimination of transfused red blood cells fell to normal. Thereafter the patient's own red blood cells increased and eventually reached normal.

Full data regarding the survival of transfused red blood cells in this and other cases will be given in a later paper with Dr. W. Walker, when the interplay of toxic, haemolytic, and nutritional factors in various types of megaloblastic anaemia will be discussed.

Summary

The injection of vitamin B₁₂ in doses of 65 to 80 µg. was ineffective in six patients with megaloblastic anaemia associated with pregnancy or the puerperium, all of whom subsequently responded to folic acid, usually 2.5 mg. daily.

The aetiological significance of these findings is discussed.

We are indebted to the medical, nursing, technical, and clerical members of the staff for their co-operation, and to colleagues in the obstetrical units of the Princess Mary Maternity Hospital and Newcastle-upon-Tyne General Hospital for referring untreated cases of megaloblastic anaemia. We are also indebted to Dr. E. Lester Smith and his colleagues in the Research Division of Glaxo Laboratories Limited, Greenford, who prepared and supplied all the crystalline vitamin B₁₂ used in these investigations. In addition we wish to thank Glaxo Laboratories Limited for a research grant made to King's College, University of Durham, which provided for the Research Fellowship held by one of us (R. B. T.).

REFERENCES

- Bethell, F. H., Meyers, M. C., and Neligh, R. B. (1948). *J. Lab. clin. Med.*, **33**, 1477.
Day, L. A., Hall, B. E., and Pease, G. L. (1949). *Proc. Mayo Clin.*, **24**, 149.
Ungley, C. C. (1948). *British Medical Journal*, **2**, 154.
— (1949). *Ibid.*, **2**, 1370.

VITAMIN B₁₂ IN MACROCYTIC ANAEMIA OF PREGNANCY AND THE PUERPERIUM

BY

J. C. PATEL, M.D., B.Sc., Ph.D., M.R.C.P.

AND

B. R. KOCHER, M.B., B.S.

(From King Edward Memorial Hospital, Bombay, India)

It has been conclusively proved that vitamin B₁₂ is haemopoietically effective in Addisonian pernicious anaemia and subacute combined degeneration (West, 1948; Berk *et al.*, 1948; Ungley, 1948a; Spies *et al.*, 1949; Hall and Campbell, 1948a, 1948b), in nutritional macrocytic anaemia and sprue (Spies *et al.*, 1948a, 1948b, 1949), and in tropical macrocytic anaemia (Patel, 1948). But the reported results of treatment of macrocytic anaemia of pregnancy with vitamin B₁₂ have not been encouraging. Ungley (1948b) was unsuccessful with 65 µg. of vitamin B₁₂ in a case of megaloblastic anaemia of pregnancy which responded afterwards to 2.5 mg. of folic acid (pteroylglutamic acid) given daily. Bethell *et al.* (1948) treated one case of puerperal macrocytic anaemia with 1 µg. of vitamin B₁₂ for 10 days without any response, though it subsequently responded to 10 mg. of folic acid administered daily. Day *et al.* (1949) treated a case of macrocytic anaemia of pregnancy with a total of 27.5 µg. of vitamin B₁₂ without any response, though there was subsequent response to folic acid.

We here present the results of the treatment of five cases of macrocytic anaemia of pregnancy and the puerperium with vitamin B₁₂.

Method

The cases were admitted to the King Edward Memorial Hospital, Bombay. After obtaining the usual medical history and giving a thorough physical examination, the following laboratory investigations were carried out: red blood cell, haemoglobin, platelet, reticulocyte and leucocytic counts, cell volume and various indices, van den Bergh reaction, icterus index, total serum protein, gastric analysis with or without histamine stimulation, stool and urine examination, Wassermann or Kahn reaction, and bone-marrow smears. Reticulocyte counts were done daily. Red blood cell, haemoglobin, and cell-volume readings were obtained on alternate days after starting treatment. Vitamin B₁₂ was administered only after the blood count had become stationary or had decreased on two consecutive counts during the preliminary period of waiting. The sternal marrow was investigated before the institution of treatment and on the day following the reticulocyte peak. The patients were given hospital vegetarian diet (consisting

TABLE I.—Haemopoietic Response

Case No.	R.B.C. (millions c.mm.)		Haemoglobin (g.%)		Packed Cell Volume		Reticulocytes		W.B.C. (per c.mm.)	
	Initial	After 14 Days	Initial	After 14 Days	Initial	After 14 Days	Day of Peak	%	Initial	After 14 Days
1	0.72	1.50	3.50	5.00	10.5	18.0	5th	15.6	3,900	10,100
2	0.87	2.35	4.00	8.25	14.0	30.5	6th	17.4	3,500	2,600
3	1.00	2.91	2.00	7.25	11.0	27.5	5th	24.2	4,800	8,600
4	0.92	2.35	3.50	6.75	10.5	22.0	5th	27.3	6,700	6,200
5	0.96	2.03	3.50	6.50	12.0	22.0	8th	20.0	12,000	7,200