FOLIC ACID DEFICIENCY IN PREGNANCY

by

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A Thesis presented for the
Degree of Doctor of Medicine
in the University of Cape Town

July
1967

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ACKNOWLEDGEMENTS

This work was carried out in the Department of Pathology and Nuffield Departments of Obstetrics and of Clinical Medicine in the Radcliffe Infirmary. I wish to thank Dr A. H. T. Robb-Smith and Professors R. G. McFarlane, Chassor Moir and L. J. Witts for allowing me to make use of facilities in their Departments. Dr A. A. Sharp suggested the study and I am indebted to him for his guidance, encouragement and advice; also to Dr G. H. Spray who instructed me in the method used for folic acid assays. I am most grateful to Prof. L. J. Witts and Dr S. T. Callender for their helpful criticism of the study and to Mr G. J. Draper of the Unit of Biometry for his help with the statistical analysis.

Thanks are also due to Mrs Joan Barnett, Mrs Eileen Jackson, Miss Sheila Crouch, Sister Jean Nye and Mr D. L. Williams for their assistance. I am grateful to Mrs Dorothy Birtles for the typescript; to Miss M. C. McLarty for drawing the diagrams, to Dr Parry for the photography, and to the patients, whose co-operation made this study possible.

Finally I wish to thank my husband Dr R. Wright for his constant support and constructive criticism without which this study could not have been undertaken.

The study was financed by a grant from the Nuffield Committee for Advancement in Medicine. The folic acid was supplied by Lederle Laboratories and Folgemin by Riker Laboratories; the placebo iron tablets were made up by the Bayer Products Company and Sandoz Ltd.

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INTRODUCTION

It has been known for many years "that the blood state of healthy pregnancy is due to a large relative increase in the water of the plasma and that this condition does not constitute a true anaemia but is due to the progressive enlargement of the vascular area during pregnancy" (Willcocks, 1881). Inability to differentiate clearly the fall in haemoglobin (Hb) concentration which occurs in normal pregnancy from a true anaemia requiring investigation and correction has complicated our understanding of the causes of anaemia in pregnancy. Subsequent studies have proved that the volume of the blood is substantially increased, both major components being affected, and that the plasma increases in volume more than the red cells (Miller, Keith and Rowntree, 1915; Dieckmann and Wegner, 1934; Thomson, Hirsheimer, Gibson and Evans, 1938). As a result, there is a progressive fall in the Hb concentration during pregnancy, with a slight rise a few weeks before term (Elliott, 1944; Rath, Caton, Reid, Finch and Conroy, 1950; Benstead and Theobald, 1952).

The concept of a physiological anaemia of pregnancy has become widely accepted, but its existence has been questioned because of the observation that iron therapy can correct the fall in the Hb concentration and can even prevent its occurrence (Widdowson, 1939; Benstead and Theobald, 1952; Davis and Jennison, 1954; Fisher and Biggs, 1955). However, Witts (1962) considered that iron administration might have a stimulant action on the bone marrow of normal people causing a rise in the haemoglobin, and favours retention of the concept of physiological anaemia. Nevertheless he and others (Strauss and Castle, 1932; Davidson, Fullerton and Campbell, 1935; Boycott, 1936; Reid and MacKintosh, 1937; Elliott, 1944; Scott and Govan, 1949; Holly and Grund, 1959) have emphasized that iron deficiency is common during pregnancy. For example, in a number of surveys of pregnant women the incidence of a true iron-deficiency anaemia has varied from approximately one in 10 to as many as one in 3 pregnant women (Davidson et al.,

1935; Reid and MacKintosh, 1937; Scott and Govan, 1949; Lund, 1951; Doyle and McGrath, 1954).

Although iron deficiency is the common cause of anaemia in pregnant women it has been known for many years that an anaemia similar to Addisonian pernicious anaemia may develop during pregnancy or in the puerperium. The earliest description of this type of anaemia was given by Channing in 1842. He described the onset of the illness shortly after delivery in one of his patients in this way:— "A person, ordinarily in good health ... suddenly becomes pale, the surface of the body being waxy and bloodless; she is faint and fatigued." He commented that "on review of this case the most obvious cause which can be assigned to its phenomena is great loss of blood during or after labor," but this he carefully excluded. Further isolated case reports added little to the understanding of this condition which was then a fatal one (Lebert, 1854; Gusserow, 1871; Biermer, 1872). Ehrlich and Lazarus (1898) in their classic description of the blood in pernicious anaemia, noted that some cases occurred in association with pregnancy. In 1919 Osler, then Regius Professor in Medicine at Oxford, gave an account of a severe anaemia in pregnancy which could only be distinguished from a true Addisonian anaemia by its better prognosis and absence of recurrence. The cause of this was unknown and the condition was considered to be very rare. In the next 10 years there was a growing number of case reports from Europe and the United States (Esch, 1921; Alder, 1924; Rowland, 1924; Larrabee, 1925; Neale, 1927; Hoskin and Ceuriog-Cadle, 1927; Evans, 1929). Many of these were well substantiated cases of megaloblastic anaemia of pregnancy but as the bone marrow morphology was never examined and megaloblasts were not always found in the peripheral blood film, an alternative diagnosis could not be excluded in a number of these earlier cases.

Reports from India showed that the condition was much commoner there (McSwiney, 1927; Balfour, 1927; Wills and Mehta, 1929-1930; Mitra, 1931). Balfour (1927) described 150 cases encountered in Bombay in only 18 months; parasites, infection, poor nutrition and toxaemia were suggested as being causative factors but she concluded that none of these were

entirely satisfactory. Other theories about the aetiology of this condition considered at that time included the possibility that the anaemia was due to haemolysis (Larrabee, 1925) perhaps resulting from an haemolysin produced by the chorion (Rowland, 1924).

Soon after the introduction of liver therapy in the treatment of true pernicious anaemia (Minot and Murphy, 1926) the successful treatment of pernicious anaemia in pregnancy with liver was reported (Audebert and Fabre, 1928; Beckman, 1928; Brault, 1928; Vaidya, 1928; Evans, 1929; Peterson, Field and Morgan, 1930). Whitby (1932) recognised however that liver was not specific for the "megalocytic anaemia" of pregnancy as it was for true pernicious anaemia.

A series of experiments carried out by Castle and his colleagues, the first of which was reported in 1928, showed that Addisonian pernicious anaemia was due to a deficiency of the intrinsic factor normally present in human gastric juice. Together with Strauss he suggested that a temporary lack of the intrinsic factor, or a dietary deficiency of the extrinsic factor, was responsible for the pernicious type of anaemia in pregnancy (Strauss and Castle, 1933), although reduced gastric acid secretion was also implicated in the aetiology of hypochromic anaemia in pregnancy (Strauss, 1930; Strauss and Castle, 1933; Davies and Shelley, 1934; Boycott, 1936). Other reports indicated however that women with megaloblastic anaemia of pregnancy could have normal gastric acid secretion (Wills and Mehta, 1929-1930; Peterson et al., 1930) and in the first large series of cases reported in Britain (Stevenson, 1938) achlorhydria was present in only a small proportion. Subsequently it was confirmed that women with this condition had free hydrochloric acid in the gastric juice (Lescher, 1942; Miller and Studdert, 1942).

The value of marmite and other yeast extracts in the treatment of the macrocytic anaemia of pregnancy was first noted by Wills (1933). Certain other macrocytic anaemias, including the nutritional or tropical macrocytic anaemia, where there was free hydrochloric acid in the gastric juice were also known to respond to this therapy (Vaughan and Hunter, 1932; Ungley, 1933; Wills, 1934). Wills and colleagues produced a macrocytic anaemia

in Rhesus monkeys which resembled the tropical macrocytic anaemia in humans by feeding them a special diet and in 1937 reported the response of this anaemia to marmite, crude liver extract and other yeast products. There was no response however to the concentrated liver extract which had been developed by Cohn and his colleagues (1930) and shown to be successful in the treatment of pernicious anaemia (Ungley, Davidson and Wayne, 1936; Wilkinson, 1936).

This observation suggested that a second factor other than the extrinsic factor was necessary for normal erythropoiesis in humans and monkeys and was lacking in the diet of these patients under study. It was suggested that this second factor was lost in the preparation of a pure liver extract. It was not clear at that time which of the two factors was necessary for the treatment of the pernicious anaemia of pregnancy. A good response to yeast extract and crude liver or failure to respond to pure liver extract was observed by several workers (Miller and Studdert, 1942; Davidson, Davis and Innes, 1942; Fullerton, 1943).

Progress was made in 1941 when Mitchell, Snell and Williams extracted a substance from spinach which they called 'folic acid'. This substance was shown to be present in liver, yeast and other foods and proved to be a potent anti-anaemic factor. The synthesis by Angier and colleagues (1945) of a compound identical with the Lactobacillus casei factor isolated from liver, which they suggested should be known as pteroylglutamic acid, and the demonstration of its value in treating nutritional macrocytic anaemia in experimental animals, paved the way for its use in macrocytic anaemia in man. Early reports of its use in the treatment of the macrocytic anaemias including pernicious anaemia were favourable (Moore, Bierbaum, Welch and Wright, 1945; Spies, 1946; Wilkinson, Israëls and Fletcher, 1946) and for a time it was thought that folic acid was the missing anti-pernicious anaemia factor. As sufficient time elapsed to allow adequate follow-up of patients it became obvious that folic acid was not as good as liver extract in the treatment of pernicious anaemia (Meyer, 1947; Vilter, Vilter and Spies, 1947; Wilkinson, 1948), and reports of subacute combined degeneration of the cord being precipitated by treatment with folic

acid (Spies and Stone, 1947; Hall and Watkins, 1947; Wilkinson, 1948; Ross, Belding and Paegel, 1948) indicated that it might actually be harmful in such patients.

On the other hand some of the other macrocytic anaemias responded well to folic acid. Amongst these was pernicious anaemia of pregnancy and a number of reports of its successful treatment with folic acid appeared (Moore et al., 1945; Spies, 1946; Davidson, Girdwood and Clark, 1948).

At this time, working independently, Rickes and associates (1948) in America and Lester Smith (1948) in Britain isolated the active anti-pernicious anaemia factor which became known as vitamin B_{12}. Early reports of its value in pernicious anaemia and especially of its effectiveness in improving or preventing the neurological manifestations (Ungley, 1948; Spies, Suarez, Lopez, Milanes, Stone, Toca, Aramburu and Kartus, 1949; Ungley, 1949) proved that this latest substance was indeed the missing factor.

A further distinction between the macrocytic anaemia in pregnancy and true pernicious anaemia was made by the early demonstration that the former condition responded to pteroylglutamic acid but not to vitamin B_{12} (Bethel, Meyers and Neligh, 1948; Day, Hall and Pease, 1949; Ginsberg, Watson and Lichtman, 1950; Ungley and Thompson, 1950). The dose of vitamin B_{12} used in these reports was small; Moore, Lillie and Gatenby, (1955) used a larger dose (1000-5000 μg) and were able to produce a haematological response in a considerable proportion of their cases of megaloblastic anaemia during pregnancy.

However reports of the effective treatment of megaloblastic anaemia of pregnancy with folic acid continued (Goldenbergh and Wyatt, 1950; Thompson and Ungley, 1951; Israëls and Da Cunha, 1952; Scott, 1954) and a deficiency in folic acid became recognised as being the primary defect in most cases of megaloblastic anaemia in pregnancy.

In the next few years several series of cases of megaloblastic anaemia in pregnancy were reported (Thompson and Ungley, 1951; Clark, 1952; Israëls and Da Cunha, 1952; Scott, 1954; Lowenstein, Pick and Philpott, 1955; Moore et al., 1955; Girdwood, 1956; Tacchi, 1958; Forshaw,

Jones, Chisholm and McGinley, 1957; Aguëro and Layrisse, 1958; Giles and Shuttleworth, 1958). The diagnostic criteria remained the same as those used by Callender (1944) in her critical review — namely anaemia and the presence of megaloblasts in the bone marrow or peripheral blood — but the diagnosis was made much more frequently. Indeed in one study (Giles and Shuttleworth, 1958) the reported incidence was as high as 2.8% of all hospital deliveries.

With increasing awareness of the condition attempts were made to devise reliable tests with which to diagnose folic acid deficiency. One which received considerable attention was the measurement of the folic acid content of serum or whole blood by microbiological assay (Schweigert and Pearson, 1947; Toennies and Gallant, 1949; Toennies, Frank and Gallant, 1953; Nieweg, Faber, de Vreis and Kroese, 1954). Initially this was unsuccessful but once it was realised that the folic acid activity in human blood consisted of separate factors, one of which was destroyed by heat or storage, and that this labile factor could be protected by ascorbic acid (Toennies, Usdin and Phillips, 1956), reliable methods to estimate the serum folate using Lactobacillus casei as test organism were developed (Baker, Herbert, Frank, Pasher, Hutner, Wasserman and Sobotka, 1959; Waters and Mollin, 1961).

The clinical value of estimating the serum folate was soon established (Herbert, Baker, Frank, Pasher, Sobotka and Wasserman, 1960; Waters and Mollin, 1961; Cooper and Lowenstein, 1961; Hansen and Weinfeld, 1962), a positive correlation being found between the serum levels and other evidence of folic acid deficiency.

The folate content of the red cells or whole blood was found to be very much greater than the serum folate (Grossowicz, Aronovitch, Rachmilewitz, Izak, Sadovsky and Bercovici, 1960) but as it was not only reduced in patients with folic acid deficiency but also in some with pernicious anaemia (Nieweg et al., 1954; Hansen and Weinfeld, 1962) it was of little value in differentiating these conditions.

It was reported by Bakerman, Silverman and Daft in 1951, that the urine of folic acid deficient rats contained a substance which was later

identified as an intermediary in the breakdown of histidine to glutamic acid (Tabor, Silverman, Mehler, Daft and Bauer, 1953). This substance was identified as formiminoglutamic acid (Figlu) by Seegmiller, Silverman, Tabor and Mehler (1954) and was shown to be excreted in increased amounts in folic acid deficiency because an enzyme, tetrahydrofolic acid, a physiologically active form of folic acid, is necessary for its conversion to glutamic acid.

This observation formed the basis of a test for folic acid deficiency in man, the reliability of which was increased by a loading dose of histidine before urine collection (Luhby, Cooperman and Teller, 1959a). Initial results using this test were promising but it was found that increased excretion of Figlu occurred not only in folic acid deficiency but in vitamin B_{12} deficiency (Kohn, Mollin and Rosenbach, 1961; Zalusky and Herbert, 1961; Hansen and Weinfeld, 1962) and other conditions such as disseminated malignant disease (Kohn et al., 1961), sarcoidosis (Kohn et al., 1961) and liver disease (Carter, Schaffner and Heller, 1960; Kohn et al., 1961).

Several methods were used to detect Figlu:— enzymatic assay (Tabor and Wyngarden, 1958), a combined enzymatic and microbiological method (Silverman, Gardiner and Condit, 1958) and paper chromatography (Luhby, Cooperman and Teller, 1959b). Simplified methods using high voltage electrophoresis (Knowles, Prankerd and Westall, 1960) or low voltage electrophoresis (Kohn et al., 1961) were evolved, once the clinical application of the test was realised. The enzymatic microbiological method was the most sensitive and estimated both urocanic acid and Figlu. This was considered by Chanarin and Bennett (1962), who modified the method, to be an advantage because, in addition to Figlu, small amounts of urocanic acid may be excreted in folic acid deficiency.

In spite of its limitations the estimation of urinary excretion of Figlu was shown to be of value in the investigation of patients with suspected folic acid deficiency (Luhby et al., 1959a; Spray and Witts, 1959; Knowles et al., 1960; Kohn et al., 1961; Hibbard, 1962).

With the development of new methods of diagnosing folic acid deficiency a new concept of this condition in pregnancy arose. When these

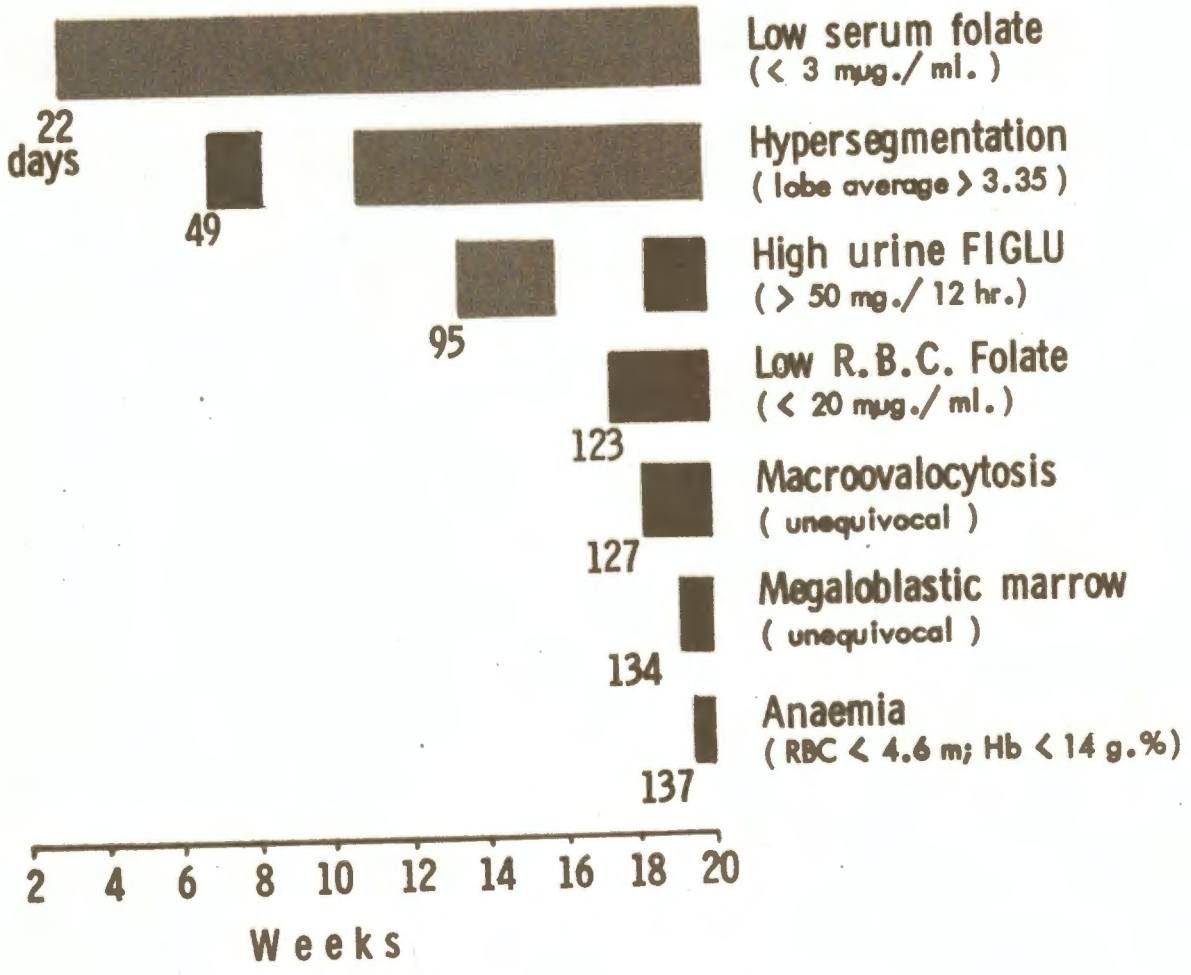

Fig. 1 Experimental nutritional folate deficiency in man: biochemical and haematological sequence of events.

tests were used to investigate anaemia in pregnancy considerable overlap between women with megaloblastic anaemia and other groups of pregnant women was observed. In 1959 Chanarin, MacGibbon, O'Sullivan and Mollin showed that the plasma clearance of intravenous folic acid was increased, not only in pregnant women with megaloblastic anaemia, but also in association with multiple pregnancy and in healthy pregnant women, and that the most rapid clearance was related to the period of marked foetal growth. Although their interpretation of this was questioned later (Girdwood and Delamore, 1961; Hansen and Klewesahl-Palm, 1963) this was thought to indicate a state of folic acid depletion. The serum folate levels in many healthy pregnant women were found to be as low as in patients with obvious folic acid deficiency (Baker, Frank, Pasher, Ziffer and Sobotka, 1960; Lowenstein, Hsieh, Brunton, De Leeuw and Cooper, 1962; Solomons, Lee, Wasserman and Malkin, 1962) and similarly this was interpreted to mean that folic acid deficiency without anaemia occurred commonly during pregnancy. The results of the Figlu excretion test in megaloblastic anaemia in pregnancy were initially promising (Luhby et al., 1959a; Hibbard, 1962; Lewis, Moore and Morris, 1962) but here too abnormal results were reported in pregnant women who were not anaemic.

The use of these tests provided evidence that degrees of folic acid deficiency existed, megaloblastic anaemia being a severe manifestation. This was confirmed by a close study of the development of folic acid deficiency in a man on a folic acid deficient diet (Herbert, 1962). The sequence of events are summarized in Fig. 1 (Herbert, 1962). This showed that the serum folate was a very sensitive index of folate deficiency and that other diagnostic tests in use were valuable.

Nevertheless application of these tests of folic acid deficiency to the study of pregnant women showed that the results were outside the normal range in a proportion of healthy women and the diagnosis of folic acid deficiency in pregnancy became confused. The value of these tests in the diagnosis of megaloblastic anaemia in pregnancy was unproven and the incidence and significance of folic acid deficiency in pregnancy difficult to assess.

SCOPE OF PRESENT STUDY

The present study began in January 1962 in the Department of Haematology, The Radcliffe Infirmary, and aimed to examine certain aspects of folic acid deficiency in pregnancy. Little was known about the frequency and relative importance of the different causes of anaemia in pregnancy in Oxford, although it was a common diagnostic problem. A special survey to examine these factors was undertaken and for a period of approximately 18 months every woman making her booking visit at one antenatal clinic for a hospital delivery was interviewed by the author and had a detailed examination of the blood carried out. The patients attending the clinic selected for study were referred for obstetrical reasons only and in no way differed from women attending any of the three other antenatal clinics held in this hospital. A total of 397 women were studied and subsequently followed during pregnancy. A proportion, who were found to be anaemic either at this first visit or at a later one, were further investigated to determine the cause of their anaemia. Considerable information about anaemia in pregnancy with special reference to the relative frequency of iron deficiency and folic acid deficiency was obtained by this pilot study and is presented in the first chapter.

While this survey was being carried out and for the following 18 months, a number of pregnant women were referred from other sources for the investigation of anaemia. As the initial survey aimed to determine the incidence and causes of anaemia in a relatively unselected group of pregnant women, those who were specially referred for investigation were not included in the pilot study described in Chapter I. All had one or more special investigations carried out personally by the author; these included bone marrow biopsy, serum folate and serum iron estimations and the estimation of urinary Figlu excretion.

In this way over a three-year period a total of 56 women with bone marrow evidence of folic acid deficiency was studied. The results of haematological investigations and a detailed assessments of other aspects of these women together with results obtained in two control groups of pregnant women are given in the second chapter.

In the course of this study the serum folate of 830 pregnant women was estimated by the author. These formed a miscellaneous group which included women who were anaemic as well as many unselected healthy women. Other information, both haematological and obstetrical, was available in most of these women and was correlated with their serum folate level. Factors examined included the Hb concentration, the mean corpuscular Hb concentration (MCHC), the serum iron, gestation period and certain maternal and foetal characteristics, and complications. The results are presented in Chapter III.

Information presented in the first three chapters indicated that iron deficiency was the common cause of anaemia in the population being studied. The incidence of florid megaloblastic anaemia was low yet a considerable number of women were anaemic in spite of being given iron. Results obtained using the Figlu excretion test and the estimation of the serum folate, provided evidence of folic acid deficiency both in association with anaemia which was not necessarily megaloblastic as well as in apparently healthy pregnancy. This raised the possibility that lesser degrees of folic acid deficiency might contribute to anaemia in pregnancy more commonly than the incidence of megaloblastic anaemia suggested. If this were so then a group of women treated with iron and folic acid could be expected to have (a) a greater rise in Hb concentration, and (b) a lower incidence of anaemia than a group given only iron and a controlled trial of iron and folic acid was designed to see if this did occur.

Since the amount of folic acid necessary to supply the requirements of a pregnant woman was not known two different doses were used and as it was felt that the value of routine oral iron needed re-assessing a group of women who were not given iron was included.

This trial was carried out by the author with some technical and secretarial help, and as a result it was possible to examine the effects of different doses of folic acid and iron on a total of 360 pregnant women. Treatment was started in the 28th week of pregnancy and was continued until delivery. The Hb concentration, MCHC, serum iron and serum folate levels were estimated immediately before treatment was begun and at least

once again before delivery. The effect of the different combinations of therapy on these parameters was noted and the results obtained will be presented in Chapter IV.

The significance of these findings, their relation to the work of others and their value in the management of anaemia during pregnancy are discussed in the final chapter.

I

A PILOT STUDY TO DETERMINE THE ROLE OF FOLIC ACID DEFICIENCY IN ANAEMIA IN PREGNANCY

INTRODUCTION

Prophylactic iron raises the haemoglobin concentration of most pregnant women (Benstead and Theobald, 1952; Fisher and Biggs, 1955; Verloop, Blockhuis and Bos, 1959a) and reduces the incidence of anaemia in pregnancy. It has been customary for a number of years to prescribe iron for all pregnant women attending the antenatal clinics at the Radcliffe Infirmary. Nevertheless anaemia during pregnancy has continued to be a diagnostic problem frequently encountered in the department of haematology in this hospital.

The role of folic acid deficiency in producing anaemia resistant to iron therapy during pregnancy is uncertain. With the development of new tests for the diagnosis of folic acid deficiency, it was realised that this condition occurs more frequently than was previously suspected. There appeared to be good reason for suspecting that a dual deficiency of iron and folic acid which could not be corrected by iron alone explained some iron resistant anaemias.

The study of a group of pregnant women attending the Nuffield Department of Obstetrics was undertaken to examine a number of aspects of anaemia during pregnancy. This was intended as a pilot study, the main aim of which was to determine the incidence, the severity and the causes of anaemia before delivery, and to examine the factors which were responsible for producing an anaemia which did not appear to respond to iron.

It is well recognized that changes in the plasma volume and red cell volume take place during pregnancy with resultant haemodilution and a fall in the haematocrit and haemoglobin concentration (Rath et al., 1950; Lund, 1951; Benstead and Theobald, 1952). Ideally any investigation into the causes of anaemia in pregnancy would take this hydraemia into

account. Although methods of measuring plasma or red cell volumes are available using radioactive isotopes or dyes such as Evans blue (T1824), practical, ethical and technical considerations preclude their use on a large scale in pregnancy. Because of the variable increase in the blood volume during pregnancy, the usual values of haemoglobin concentration accepted as normal in non pregnant women cannot be applied.

The haemoglobin levels found in normal pregnant women in the last trimester have differed considerably in a number of reported studies but in several the Hb concentration has been between 11 and 12.0 g/100 ml. (Lund, 1951; Ventura and Klopper, 1951; Benstead and Theobald, 1952; Darby et al., 1953; Sturgeon, 1959). It has been calculated that at its maximum an increased plasma volume could cause the haemoglobin to fall to as low as 10.0 g/100 ml (Lawrence, 1962) but undoubtedly many women with values above this will have a true deficiency anaemia, and for the purpose of this study therefore, anaemia during pregnancy was arbitrarily defined as a haemoglobin concentration below 11.0 g/100 ml.

MATERIALS AND METHODS

The antenatal clinic selected for study was one of four such clinics held in the Nuffield Department of Obstetrics. The clinic was always held between 2 p.m. and 4 p.m. on the same day each week so that the women included in this study were seldom fasting.

Women attending for their booking visit were interviewed by the author and social, medical and obstetrical details entered in a questionnaire (see Appendix I). It was ensured that all patients would receive adequate iron; if not already taking this when first seen, a prescription for ferrous gluconate 300 mg. to be taken three times a day was given immediately and regularly renewed. All patients were given a printed form on which to write details of their diet for one week (see Appendix I) and were asked to return this by post. The importance of taking iron regularly, of eating a well balanced diet and of regular attendance at the clinic was emphasized to every patient.

A venous sample of blood was always taken at the first visit. A clotted sample of approximately 10 ml was used for blood grouping and rhesus antibody estimation if indicated, and also for the Wassermann and Kahn reactions. A 5 ml sample was placed in a bottle containing the anticoagulant ethylenediamine tetra-acetic acid (EDTA) and a film was made immediately from a drop of blood obtained directly from the syringe. The Hb concentration, packed cell volume (PCV) and mean corpuscular haemoglobin concentration (MCHC), were estimated at the patient' s first visit and the peripheral blood film was always examined.

If the haemoglobin was 11 g/100 ml or more at the booking visit, subsequent estimations at later visits were usually performed on capillary blood. A Hb concentration of less than 11 g/100 ml noted on a capillary sample was always re-estimated on venous blood, at the same visit if possible, but often not until the patient was seen again. Patients whose Hb concentration was below 11 g/100 ml were investigated as fully as possible at their next visit.

Since it was ensured at the first visit that all women would be taking iron, many were no longer anaemic when next seen, and in some cases it could only be assumed that iron deficiency had caused the anaemia.

Antenatal care between the booking visit and the next visit to hospital eight weeks before delivery is usually carried out by the patient's general practitioner. Thereafter the patient will return to the antenatal clinic at 1 to 2 weekly intervals until she is admitted to hospital for delivery. Despite all possible precautions under certain circumstances contact with a patient was lost. Reasons for this include 1) continuing antenatal care by the patient's general practitioner until delivery; 2) change to another antenatal clinic; 3) change of name; 4) clinic defaulters; 5) emergency admission to hospital without notification of this. In the few patients not seen again until delivery, information about their subsequent course was obtained from hospital notes or from the general practitioner concerned.

It was intended that all patients would be seen soon after delivery and that a post-partum haemoglobin would be estimated before discharge.

However, a short stay in hospital after an uncomplicated confinement is common here, and it was soon obvious that a 24 to 48 hour stay in the postnatal wards resulted in patients leaving hospital before a haemoglobin estimation had been carried out. A red label measuring 5 x 7 cm with a perforated edge so that it could be detached was therefore attached to the front of the patient's notes requesting immediate notification of her admission to hospital. Again, although this arrangement worked in the majority of cases, for several reasons a number of women were discharged from hospital before notification of their admission had been received.

METHODS

Details of laboratory techniques used in this study are presented in Appendix II.

RESULTS

The number of women interviewed and followed until delivery was 397; an additional 39 patients were originally booked for a hospital delivery and therefore interviewed but had their confinement elsewhere. The 397 patients in whom hospital delivery took place are divided into two main groups: group I, those whose Hb concentration never fell below 11 g/100 ml and group II, those in whom the haemoglobin fell below 11 g/100 ml at least once before delivery. Table 1 shows the number of women in each group.

TABLE 1

To Show the Incidence of Anaemia in 397 Women in the Pilot Study

Group I	Hb concentration 11 g/100 ml or more	No. of women 273	
Group II	Hb concentration below 11 g/100 ml		
	a) One capillary estimation only	31	124
	b) Noted at booking visit	60	
	c) Developed subsequently	33	
	TOTAL	397	

From Table 1 it is apparent that nearly one third of pregnant women booked to have a hospital delivery and attending the antenatal clinic selected for study was found to have a Hb concentration below 11 g/100 ml at least once during pregnancy.

GROUP 1. Hb CONCENTRATION 11 g/100 ml OR MORE

In 273 women the haemoglobin did not fall below 11 g/100 ml before delivery. The Hb concentration at the patients' booking visit and again within the 4 weeks preceding delivery is shown in Table 2. In 16 women the haemoglobin concentration 4 days after delivery is used, since the last antepartum estimation was made before the 36th week of pregnancy.

TABLE 2

To Show the Hb Concentration at Booking Visit and Near Term in 273 Women who did not Develop Anaemia Before Delivery

	Hb concentration, g/100 ml			
	11-11.9	12-12.9	13 & over	Total
At booking visit	114	113	46	273
Near term	111	107	55	273

Evidence of iron deficiency was found in 38 of these 273 women although the Hb concentration never fell below 11 g/100 ml. With one exception the MCHC was estimated in every woman in this group and in 32 women it was less than 30%. The peripheral film was examined in 250 women and evidence of iron deficiency was noted in 12. Both parameters of iron deficiency were found in 6 women. Despite the fact that a small proportion of these 273 non-anaemic women were iron deficient they form a valuable control group and information obtained from studying them will be used in the following chapter to assess the significance of certain associations and complications of anaemia during pregnancy.

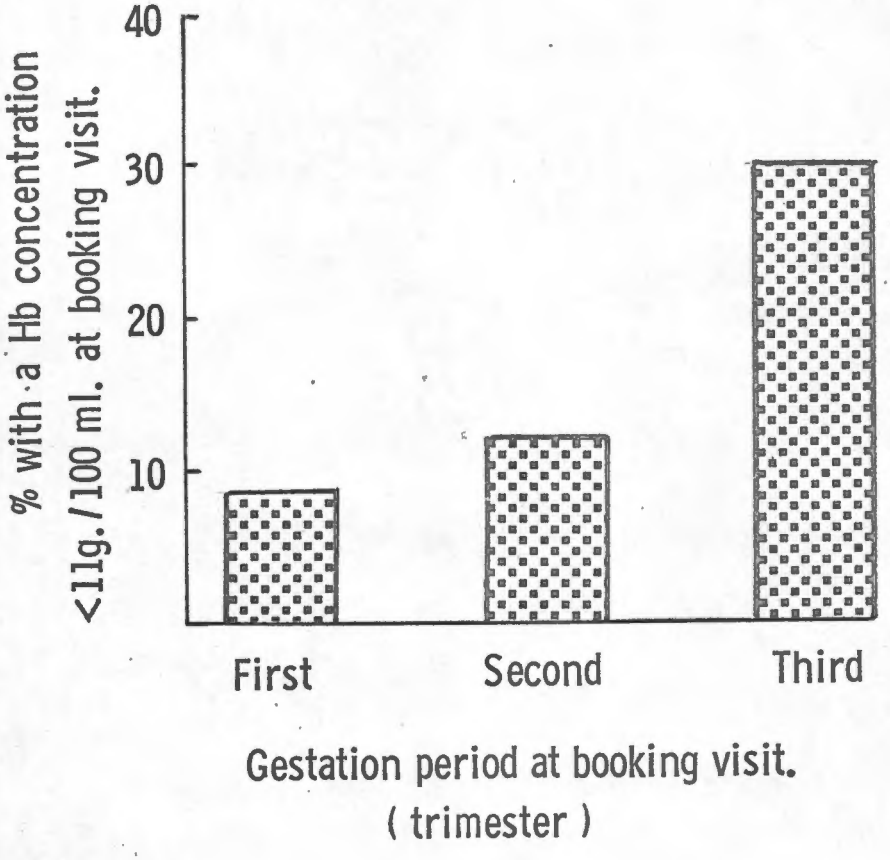

Fig. 2 To show that the incidence of a Hb concentration below 11g./100 ml. at first hospital visit increases with each trimester.

GROUP II. a) Hb CONCENTRATION BELOW 11 g/100 ml ON ONE CAPILLARY READING ONLY

In 31 women a capillary Hb concentration of less than 11 g/100 ml was found on one occasion only. The significance of this could not be assessed as the haemoglobin had risen by the next visit. It is possible that these patients may have developed a transitory anaemia which was corrected by continued administration of iron. A temporary greater increase in the plasma volume than the red cell mass, may also have accounted for this transitory fall in the Hb concentration. Technical error is a third possibility since sampling error in estimating the Hb concentration is a recognized difficulty (Biggs and Allington, 1951). A considerable variation in the results of repeated haemoglobin estimations was noted frequently while this survey was being carried out and a haemoglobin concentration below 11 g/100 ml was always confirmed by repeat estimation before a diagnosis of anaemia was made. This was not always possible however and in these 31 patients a check estimation was delayed until the patient's next visit when the haemoglobin was found to be normal.

As the significance of this temporary fall in the haemoglobin concentration could not be determined, no other results in this group will be presented.

GROUP II. b) Hb CONCENTRATION BELOW 11 g/100 ml ON A VENOUS SAMPLE

In 93 women it was confirmed that the Hb concentration was less than 11 g/100 ml before delivery, and in all, the haemoglobin was estimated in duplicate on a venous sample.

Sixty patients (i.e. 15% of the total) were found to be anaemic when first attending the antenatal clinic. Women presenting late in pregnancy for their first visit were more likely to be anaemic than those who presented early (Table 3; Fig. 2). The majority of women attended the antenatal clinic before the 28th week of pregnancy. Approximately one in nine was anaemic at that stage compared with nearly one in three who presented in the last 12 weeks of pregnancy. Since none of these patients

presenting late had been referred because of anaemia it would appear that the association of anaemia with late booking is probably partly due to the fact that many had only recently attended their general practitioner for the first time and had therefore received little iron. Thirty-four of these 60 women had already started taking iron before coming to hospital, but in 21 the duration of therapy was less than one month.

TABLE 3

To Show Increasing Incidence of a Hb Concentration of Less than 11 g/100 ml with Late Booking

		No. of women with Hb conc. < 11 g/100 ml	11 g/100 ml or more	Total
Gestation period at booking visit	1st trimester	6 (8.8%)	62	68
	2nd trimester	31 (12.2%)	222	253
	3rd trimester	23 (30.3%)	53	76

Antenatal anaemia which actually developed after the booking visit was noted in 33 of the 397 women on survey, i.e. in approximately 8%. Because antenatal care between the hospital booking visit and the last 8 to 10 weeks of pregnancy was usually carried out by the general practitioner it was not possible to determine when anaemia had developed. All but four were found to be anaemic when they came to the antenatal clinic for the second time which was usually between the 30th and 32nd weeks. On average the booking visit had taken place 12 weeks before this and in 18 women the Hb concentration at that stage, although normal by definition, was nevertheless borderline (11.0 to 11.5 g/100 ml). The mean fall in the haemoglobin concentration which occurred in these 33 women between the booking visit and the subsequent visit when anaemia was diagnosed was 1.1 g/100 ml; all were taking iron when anaemia developed although 5 patients admitted taking it irregularly. The average duration of therapy was approximately 17 weeks and almost all had had at least one month's treatment.

The haemoglobin concentration of these 93 women at the time anaemia

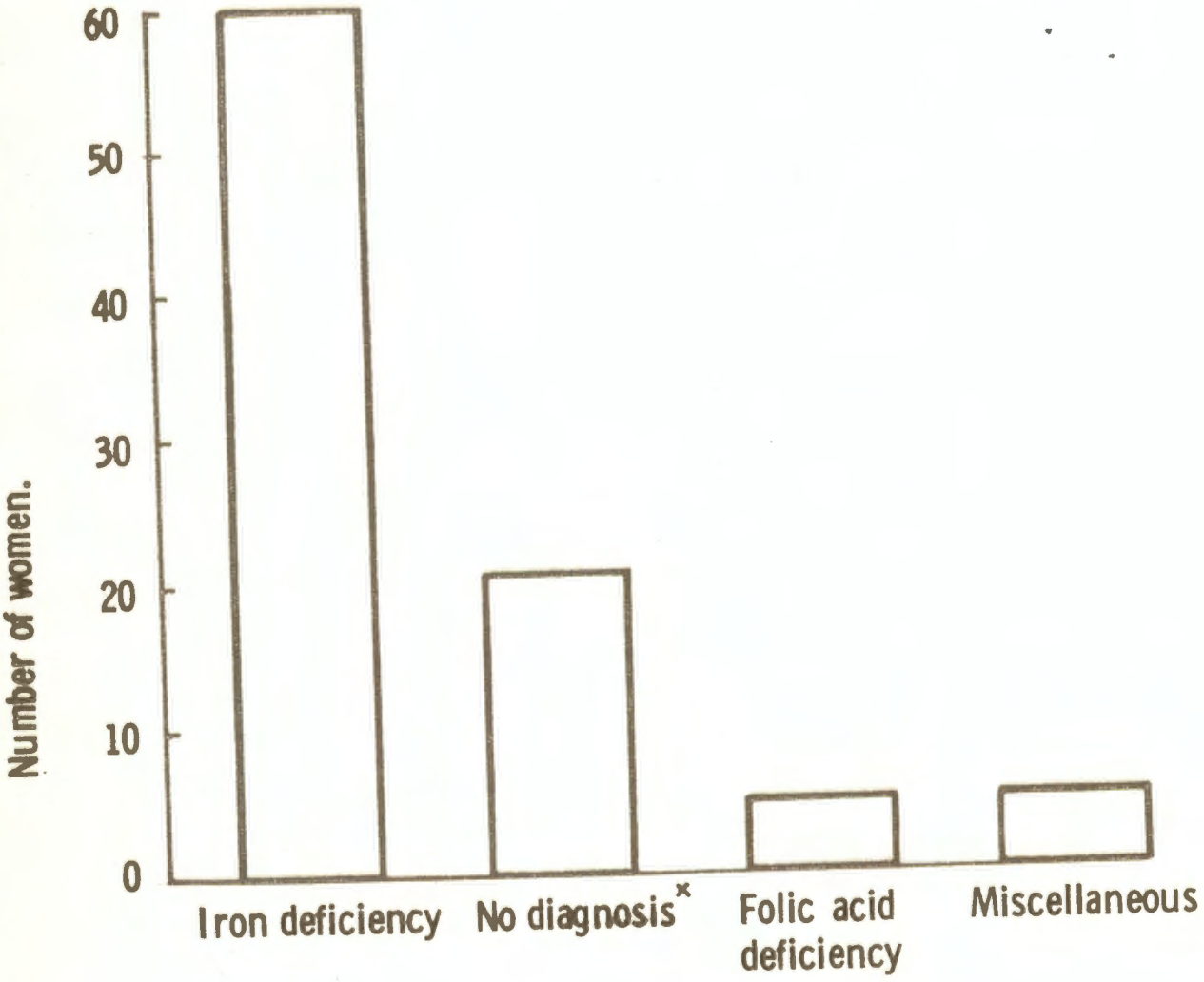

Fig. 3 To show the cause of anaemia in 93 women with a Hb concentration below 11 g./ml. during pregnancy.

(*Hb = 11 g./100 ml. or more at delivery)

was diagnosed is shown in Table 4. It is apparent that in the majority anaemia was mild and was corrected before delivery.

TABLE 4

Haemoglobin Concentration in 93 Women who Developed Anaemia before delivery, a) at diagnosis, and b) near term

	Hb concentration, g/100 ml					Total
	< 9.0	9-9.9	10-10.9	11-11.9	12.0 >	
At diagnosis	2	16	75	-	-	93
Near term	2	5	22	39	19	87*

*Not available on six patients.

The reason for the development of anaemia and results of investigations in these 93 women are summarized in Fig. 3 and are presented in Table 5.

TABLE 5

Cause of Anaemia in 93 Women with a Haemoglobin Below 11 g/100 ml Before Delivery

Diagnosis	Total	Criteria for diagnosis			
Iron deficiency	60	Hypochromia of red cells 32 (92)*	MCHC < 30% 38 (92)	Serum iron <60 µg/100ml 16 (50)	Absent bone marrow iron 20 (27)
Folic acid deficiency	6	Megaloblastic or transitional erythropoiesis (combined with iron deficiency in 4)			
Miscellaneous	3	1. Thalassaemia 1. Sickle cell anaemia 1. Pulmonary tuberculosis			
No diagnosis, ? Iron deficiency ? Hydraemia	21	No evidence of iron deficiency. Hb rose to 11 g/100 ml or more during iron therapy			
Inadequate follow-up	3				
TOTAL	93				

*Number in brackets indicates total number of women tested.

CAUSE OF ANAEMIA IN 93 WOMEN IN PILOT STUDY

Iron deficiency: In 60 patients there was definite evidence of iron deficiency as judged by hypochromia of red cells in the peripheral blood film, a low MCHC or serum iron, or evidence of iron deficiency on examination of the bone marrow film. It is apparent that an abnormal MCHC and abnormal film did not necessarily occur together and it is obvious also that estimation of the serum iron is not a reliable investigation in pregnant women who are taking iron. Although all 60 women shown in Table 5 had other evidence of iron deficiency, a normal serum iron was noted in the majority.

Folic acid deficiency: Only 6 women had bone marrow evidence of folic acid deficiency and in 4 this was combined with iron deficiency. The serum folate was estimated in 53 of these 93 patients and in 17 it was less than 2.1 mμg/ml, the lower limits of normal for healthy non-pregnant controls.

No definite diagnosis: In 21 of the 93 patients, although it was confirmed that the haemoglobin was below 11 g/100 ml on at least one occasion, it rose above this level usually before, but occasionally immediately after, delivery without evidence of iron or folic acid deficiency having been detected. These patients were not fully investigated in that a bone marrow biopsy was not done, and as they were all receiving iron therapy it is possible that some of them were iron deficient. Certainly in 6 women whose anaemia was corrected by their second visit early in the 3rd trimester this seemed likely. No patients in this group were given folic acid so that the rise in haemoglobin cannot be attributed to correction of folic acid deficiency. In two patients a rapid rise in the haemoglobin (± 1 g/100 ml per week) followed treatment with intramuscular iron. The remainder were treated with oral iron only. When the Hb concentration rose near term (11 women) or immediately after delivery (2 women) the possibility that the previously low reading might have been the result of hydraemia was considered. Since blood volume studies were not undertaken in these patients this could not be confirmed.

RESPONSE TO TREATMENT

All 93 patients were treated with oral iron and in 8 patients intramuscular iron was given in addition. Seven patients were treated with folic acid and 2 patients were treated by blood transfusion before delivery.

Table 4 shows that in the majority of patients the Hb concentration had risen to 11 g/100 ml or more near term. Included amongst those who responded were 2 patients with megaloblastic anaemia treated with folic acid and iron. Six women, found to be anaemic at their booking visit, did not reattend the antenatal clinic for follow-up either because they refused to do so or because they delivered prematurely.

There were thus 29 patients whose last haemoglobin before delivery was less than 11 g/100 ml (for details see Appendix III). Fourteen of these had a bone marrow biopsy; in 4 women erythropoiesis was megaloblastic or transitional and in the remainder was normoblastic. Of the 15 who did not have a marrow biopsy, 11 had a serum folate estimation and in only 3 was this less than 2.1 mμg/ml. The majority (17 out of 29) had evidence of iron deficiency only, yet anaemia persisted in spite of treatment with iron. A possible explanation for this was noted in a number of these 17 women — inadequate intake of iron was admitted by 6; anaemia was diagnosed late in pregnancy in 1, and genito-urinary tract infection was present in 3.

Nevertheless the possibility that a dual deficiency of iron and folic acid might be a factor in producing the anaemia was considered. Although 3 women, who did not have a bone marrow biopsy, had an abnormal serum folate in addition to evidence of iron deficiency, it seemed unlikely that folic acid deficiency was responsible for their anaemia since all 3 had a normal haemoglobin soon after delivery without being given folic acid therapy. Similarly, in 2 of the 4 patients who had neither a bone marrow biopsy nor serum folate estimation the haemoglobin rose post partum. In fact in the group as a whole including those with an iron-deficiency anaemia, a rise in the Hb concentration a few days after delivery occurred frequently, being noted in 15 of the 29 women.

These findings suggest that folic acid deficiency was unlikely to be

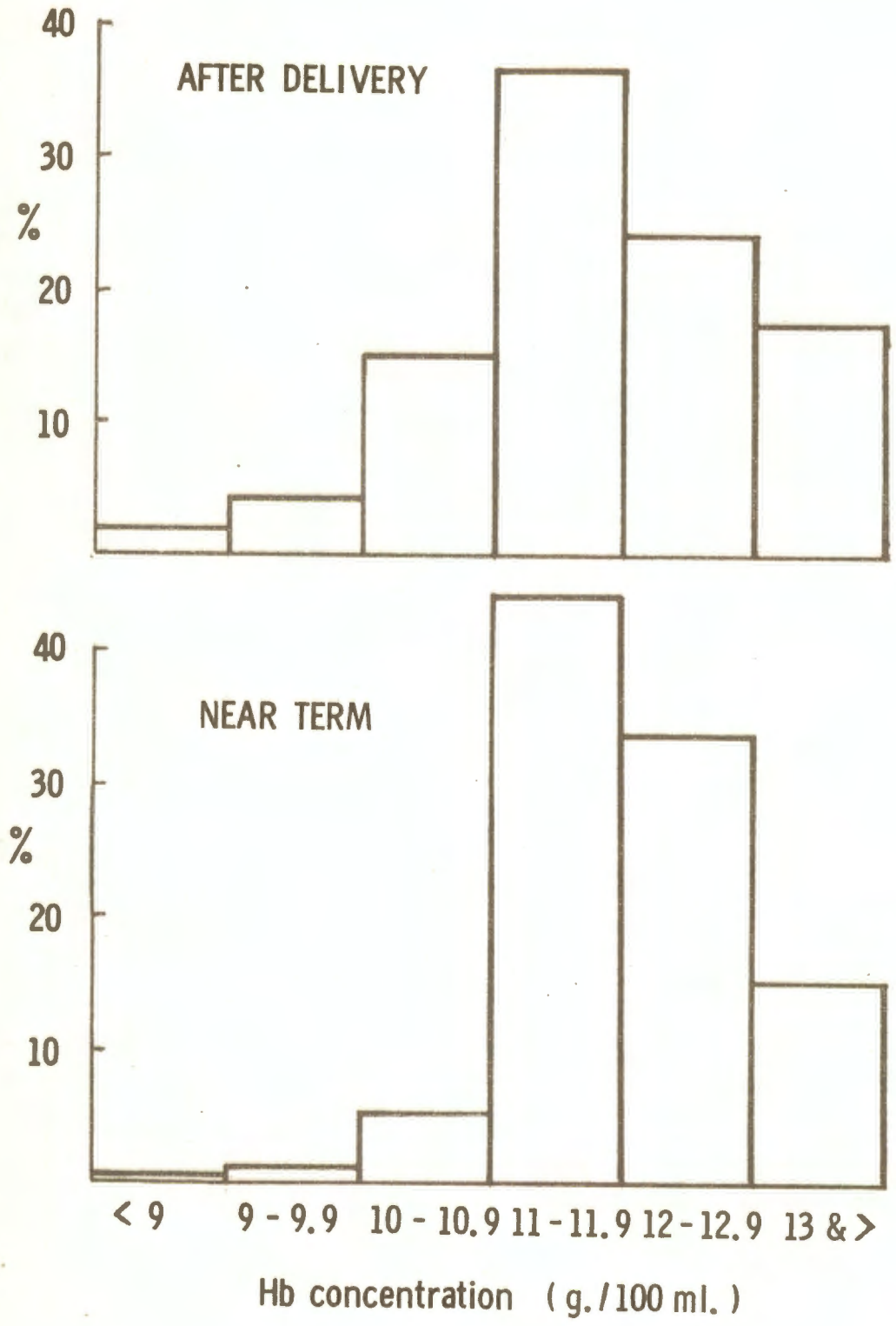

Fig. 4 To show Hb concentration near term and after delivery in 397 women in pilot study.

a significant factor in the aetiology of the anaemia in the majority of patients who failed to respond to oral iron.

ANAEMIA AFTER DELIVERY

It is intended that every woman delivering in the Radcliffe Infirmary should have a haemoglobin estimation before discharge. This is performed on capillary blood by a technician who visits the postnatal wards every day. It was necessary to rely on this method for postnatal haemoglobin estimation in the 397 women being studied since the amount of work involved in doing this personally could not be undertaken.

Although precautions were taken to ensure that all 397 women were seen after delivery approximately 14% were discharged without having a postnatal haemoglobin estimation. The main reason for this is the practice in the Radcliffe Infirmary of early discharge 24 to 48 hours after a normal delivery. The Hb concentration in the 342 women in whom it was estimated is shown in Fig. 4. Although a Hb concentration of less than 11 g/100 ml was common after delivery, anaemia was seldom marked and in only 22 women (i.e. approximately 6%) was the haemoglobin less than 10 g/100 ml (see Table 6).

TABLE 6

The Hb Concentration in 74 Women whose Haemoglobin was Less than 11 g/100 ml After Delivery

	Haemoglobin concentration, g/100 ml				Total
	< 9.0	9-9.9	10-10.9	Blood transfusion, Hb presumed to be < 10 g/100 ml	
Number of women	7	12	52	3	74

In 40 of these 74 women the haemoglobin fell below 11 g/100 ml for the first time after delivery; the remainder were patients in group II and had been anaemic during pregnancy. In the latter group post partum anaemia appeared to be a continuation of anaemia during pregnancy since

less than one-quarter had achieved a Hb concentration of 12 g/100 ml before delivery. Indeed in 14 women anaemia had not been corrected at term.

In 15 women the Hb concentration after delivery was less than 11 g/100 ml on one capillary haemoglobin estimation only and the diagnosis of anaemia was not confirmed. It is possible therefore that the incidence of anaemia after delivery has been overestimated. Although post-partum anaemia was usually mild, other complications of pregnancy occurred frequently in this group (Table 7).

TABLE 7

To Show the Incidence of the Complications of Pregnancy in 74 Women with a Hb Below 11 g/100 ml Post Partum

	Group I Hb concentration 11 g/100 ml or more during pregnancy	Group II Hb concentration < 11 g/100 ml during pregnancy	
		(a)	(b)
Infection	8	2	9
Ante-partum haemorrhage	3	-	2
Post-partum haemorrhage	11	-	5
Delivered by LSCS	4	-	4
None	23	2	16
TOTAL	40	4	30

The incidence of these complications was no greater in group II patients than the previously normal patients in group I and it is unlikely therefore that they are the consequence of anaemia. The frequency of these complications was particularly striking in the 22 women whose Hb concentration was below 10 g/100 ml, no less than 17 women having one or more of these complications. Only 5 women with a haemoglobin below 10 g/100 ml after delivery had no other complication; 3 of these were iron deficient, one had a transitional megaloblastic anaemia during

pregnancy and one with a normochromic, normocytic blood film was not investigated further.

Adequate explanation for anaemia was obtained in most of the 22 women with a Hb concentration below 10 g/100 ml and no attempt was made to assess the incidence of megaloblastic anaemia in this group. The evidence of others indicates that megaloblastic erythropoiesis is not uncommon both after ante-partum and post-partum haemorrhage but this was not investigated in the present study. Only 2 patients had bone marrow biopsy after delivery because folic acid deficiency was suspected and both were found to have normoblastic erythropoiesis.

COMMENT

In retrospect it would appear that the scope of this preliminary investigation was too great. One person alone was unable to carry out as detailed a study as this was intended to be. Incomplete investigation of women with anaemia and failure to estimate the haemoglobin after delivery could have been avoided in a number of cases had this study been the work of a team. Nevertheless the purpose of this pilot study was achieved. Much general information was obtained about anaemia during pregnancy in the community studied and this will now be briefly discussed.

It was confirmed that anaemia during pregnancy is a common condition; nearly one-quarter of the 397 women studied had a Hb concentration of less than 11 g/100 ml before delivery. The diagnosis was made in slightly more than half of these women at their booking visit, and the more advanced the pregnancy the greater was the likelihood that anaemia was present. The commonest cause of anaemia was iron deficiency and definite evidence of this was obtained in approximately 15% of all women studied.

Folic acid deficiency was uncommon, and bone marrow evidence of this was obtained in 6 patients only. In 2 others with normoblastic erythropoiesis a deficiency of folic acid was considered likely and there was an apparent haematological response to folic acid but the diagnosis

was not proved. The incidence therefore in this series is approximately 1.5% to 2% of unselected women attending the hospital ante-natal clinics. It is possible that with more intensive investigation, particularly of women with mild anaemia and those becoming anaemic after delivery, the diagnosis of folic acid deficiency might have been made more often. A mild degree of this condition may be missed but certainly frank megaloblastic anaemia was rare; more than half the cases diagnosed in this survey were combined with iron deficiency.

There are several possible reasons why megaloblastic anaemia during pregnancy was not common in the 397 women studied. It will be remembered that only booked patients were included in this survey and that emergency admissions and patients referred because of anaemia were excluded. Other relevant factors include the good economic circumstances and good nutritional state of most of our patients, the dietary instruction given to each woman at her first visit, and the hospital practice of prescribing prophylactic iron.

Certain observations made during this study suggested that a deficiency of folic acid might occur more often than the low incidence of megaloblastic anaemia indicated. The serum folate was estimated at least once in 186 women in this study and was below the lower limits of the normal range in 42. Nearly one-third of the anaemic patients in whom it was estimated had levels below 2.1 mμg/ml. Furthermore anaemia developed in a number of women in spite of their being prescribed oral iron. Although the majority did eventually attain a normal haemoglobin without other treatment there remained some who appeared to be resistant to iron therapy. The evidence to suggest that folic acid deficiency was a significant factor in producing an iron-resistant anaemia has been considered and although there appeared to be little direct evidence to support this theory it cannot be entirely ruled out since serial bone marrow biopsies were not performed.

Inadequate iron intake, underlying disease and late presentation, explained anaemia at term in a number of cases, but there remained a proportion in whom the cause was not obvious. In addition to undiagnosed

folic acid deficiency, the possibility of haemodilution producing a low Hb concentration must be considered. Certainly the return to normal haemoglobin levels near term or immediately after delivery noted in several women was suggestive of this but since blood volume studies were not carried out this could only be surmised.

This study has shown clearly the difficulty of determining with certainty the underlying cause of anaemia during pregnancy. Iron deficiency was the commonest cause of anaemia and presented no difficulty in diagnosis when marked. However, it is evident that milder degrees of iron deficiency are not easy to detect. Hypochromia of the red cells and a MCHC of less than 30% are late signs of iron deficiency and may be absent when the bone marrow iron stores are depleted. Estimation of the serum iron is not a reliable diagnostic test in pregnant women for two reasons: firstly recent intake of iron may result in a normal serum iron level even in women who are iron deficient and conversely because the serum iron falls in normal pregnancy (Fay, Cartwright and Wintrobe, 1949; Verloop, Blokhuis and Bos, 1959a; Morgan, 1961) low values are of less significance. Unless bone marrow biopsy is carried out therefore and the iron stores examined, mild iron deficiency in pregnancy may be difficult to diagnose.

It is obvious that only limited conclusions can be drawn from an apparent response to iron therapy in anaemia in pregnancy since the effect of other factors in addition to iron deficiency has to be taken into consideration. While it is difficult, even with intensive investigation, to determine the relative importance of these factors in the individual case, study of the therapeutic response of a group of pregnant women with simultaneous observations made on a matched untreated control group might allow for easier interpretation of results. Interest in the possibility that unsuspected folic acid deficiency was responsible for a poor response to iron, and that anaemia during pregnancy might be more effectively treated and prevented by iron and folic acid, led to a controlled trial to answer this question. The practical value of this is considerable and the gathering of information from which this hypothesis arose, fully established the value of the pilot study.

II

MEGALOBLASTIC ANAEMIA OF PREGNANCY AND THE PUERPERIUM

INTRODUCTION

As was shown in the previous chapter only 6 of the 397 women included in the pilot study were found to have bone marrow evidence of folic acid deficiency. It was pointed out that because the author was known to have a special interest in anaemia in pregnancy a number of anaemic patients who had their confinement at the Radcliffe Infirmary during the three-year period of the study were referred for investigation. Fifty additional patients with evidence of folic acid deficiency as judged by the bone marrow appearance (46 patients) or the presence of megaloblasts in the peripheral blood (4 patients) were obtained in this way, making a total of 56 patients.

The diagnosis of folic acid deficiency during pregnancy is based on the morphological appearances of the bone marrow and peripheral blood film, but there is considerable variation in the criteria adopted. For example, Ball and Giles (1964) accept as their minimum criteria for diagnosis patients with a haemoglobin concentration below 10 g/100 ml who have transitional megaloblastic change in at least 10% of the erythroblasts, whereas Lowenstein, Brunton and Hsieh (1966) regard the presence of giant metamyelocytes in bone marrow films as being indicative of megaloblastic change even in the absence of anaemia.

In the present study the diagnosis of folic acid deficiency has been made in a few instances solely on the basis of typical megaloblasts in the peripheral blood film, but in the majority megaloblastic or transitional change in the bone marrow as defined below was considered essential for the diagnosis.

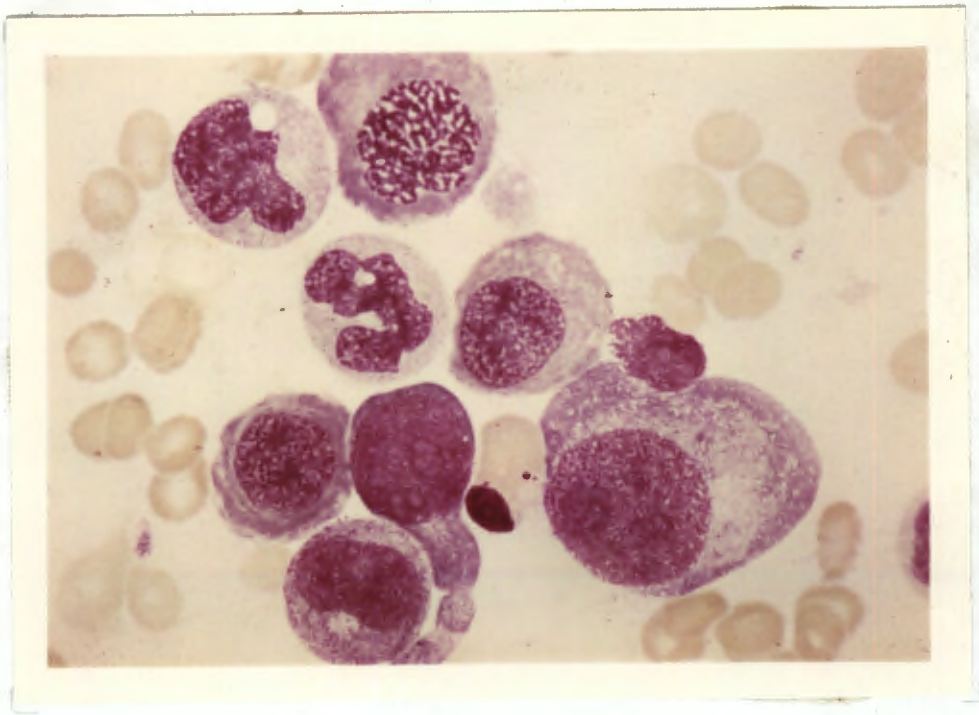

Fig. 5: Megaloblastic erythropoiesis.

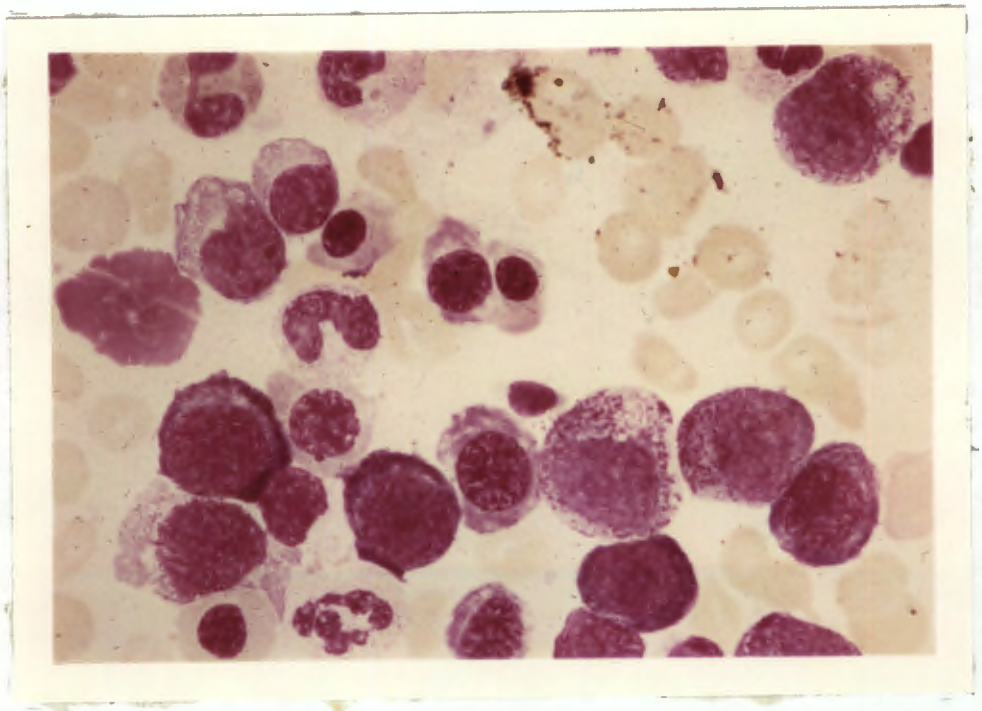

Fig. 6a: Transitional megaloblastic erythropoiesis.

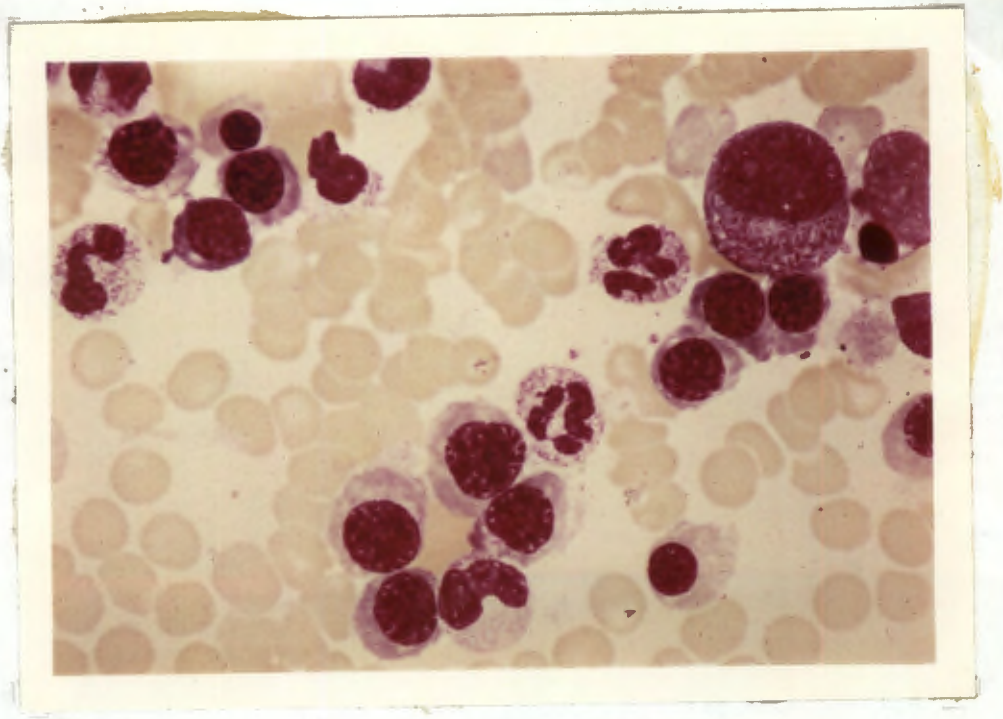

Fig. 6b. Transitional megaloblastic erythropoiesis.

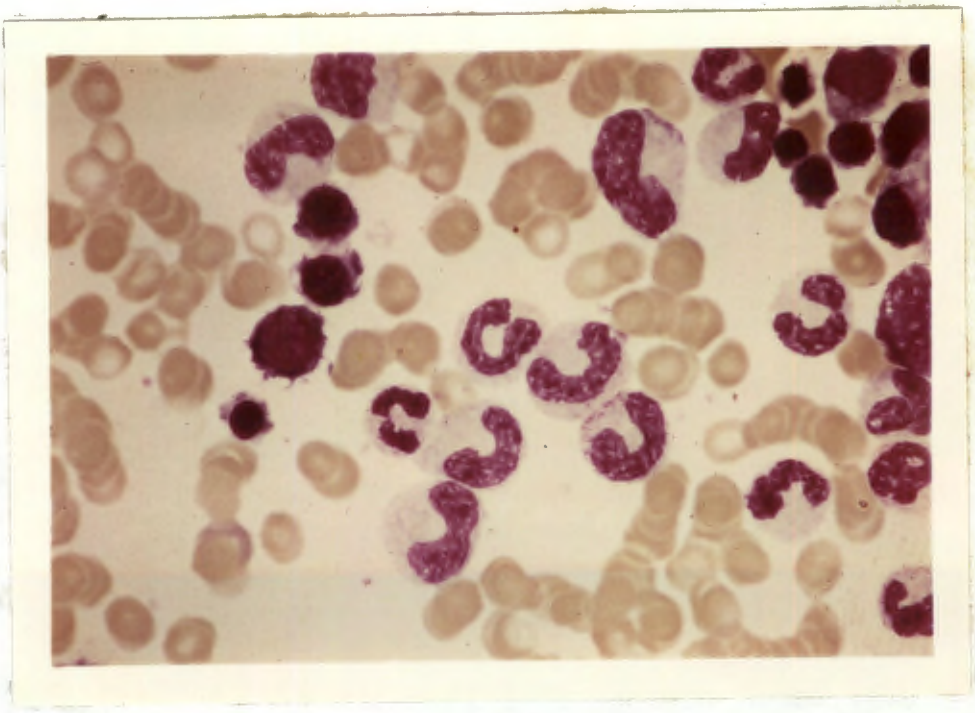

Fig. 7: Normoblastic erythropoiesis and giant metamyelocytes in patient taking folic acid.

BONE MARROW APPEARANCE IN MEGALOBLASTIC ANAEMIA

MEGALOBLASTIC ERYTHROPOIESIS

The classical megaloblast may be easily recognised by its size and typical nuclear appearance (Fig. 5). Howell-Jolly bodies, which may be multiple, are common and the associated abnormalities in the white cell series — giant metamyelocytes and increased hypersegmentation of the polymorphonuclear leucocytes — are invariably present. In the present series erythropoiesis was usually both megaloblastic and normoblastic; in only a few cases did megaloblastic change predominate. In nearly half of the cases erythropoiesis was mainly normoblastic and only some erythroblasts showed typical megaloblastic features.

TRANSITIONAL ERYTHROPOIESIS

When classical megaloblasts were not seen in the bone marrow smears, but definite abnormalities of erythropoiesis together with giant metamyelocytes were observed, the marrow was described as "transitional", a term first used by Israëls in 1951 (see Fig. 6). The following abnormalities of erythropoiesis have been taken to indicate transitional megaloblastic change:

i) Size: Cells which are larger than normoblastic erythrocytes at the same stage of development, although seldom as big as true megaloblasts.

ii) Nuclear appearance: A nuclear pattern which is coarser than that of the classical megaloblast, the fine chromatin strands having thickened and the nucleus condensed, but the appearance is not that of a normoblast with its very much coarser chromatin.

iii) Premature haemoglobinisation: that is early haemoglobinisation of a cell which by the appearance of its nucleus would seem to be more primitive.

In the present study no marrow was considered to be transitional on the evidence of minimal red cell changes only. The abnormalities of erythropoiesis were always accompanied by associated abnormalities in

the white cells.

NORMOBLASTIC ERYTHROPOIESIS

When erythropoiesis is entirely normoblastic it is not usually accompanied by bone marrow features suggesting folic acid deficiency. Although Howell Jolly bodies were occasionally seen, these were rarely multiple and myelopoiesis was usually normal. In a few otherwise normal marrows, however, an occasional giant metamyelocyte has been observed. Fig. 7 shows a typical example in a patient with iron-deficiency anaemia who had a serum folate of 33 mμg/ml as a result of taking folic acid for 8 weeks. It is considered therefore that a few giant metamyelocytes in normoblastic bone marrow smears are of little diagnostic value. No distinction has been made between normoblastic marrows with and without giant metamyelocytes in the present study, although in some series macrogranulocytosis has been accepted as an adequate criterion for diagnosing "megaloblastic change" (Dawson, 1962; Lowenstein et al., 1966).

The bone marrow during pregnancy is often very cellular and occasionally a number of macronormoblasts may be present. These cells are larger than normoblasts of the same maturity, although not usually as large as megaloblasts, but the nuclear structure is typically normoblastic. In a few marrows these cells were quite numerous and such marrows have been described as macronormoblastic. This is considered a normal variation and is of no diagnostic significance.

At the time of referral for investigation the bone marrow films were scanned independently by two observers and erythropoiesis classified as megaloblastic, transitional or normoblastic. At the end of the three-year period of study the marrow films were reclassified "blind" into the same categories. Where the two observers differed in their classification, or the later classification differed from the initial one, the opinion of a third haematologist was sought before making a diagnosis.

Although the number of cases available for study was relatively small by comparison with some series, it was felt that a detailed analysis of

our own material would be of value because a number of important issues are still controversial. In an attempt to examine this condition more precisely we have subdivided patients in this study into those whose bone marrow showed megaloblastic erythropoiesis and those with transitional megaloblastic changes. These two groups of patients with folic acid deficiency have been compared with two control groups consisting of (a) patients with anaemia whose bone marrow film showed normoblastic erythropoiesis, and (b) the 273 women referred to in the previous chapter whose haemoglobin never fell below 11.0 g/100 ml throughout pregnancy.

The four different groups and the number of pregnant women in each are shown in Table 8. Full details of these 120 women are given in Appendix I.

TABLE 8

To Show the Number of Pregnant Women with Folic Acid Deficiency and Controls

	Diagnosis	No. of cases	Total
Folic acid deficiency	Megaloblastic anaemia	33	56
	Transitional megaloblastic anaemia	23	
Control groups	Normoblastic anaemia	64	337
	Non-anaemic pregnant women	273	

Results of the study of the 56 women with folic acid deficiency will be presented in this chapter and where possible have been compared with those in the control groups. The results will be dealt with under two main headings:

i) The clinical features of megaloblastic anaemia.

ii) The laboratory diagnosis of megaloblastic anaemia.

In addition to bone marrow biopsy most of the anaemic women had one or more special investigations carried out by the author. It was not possible to measure the serum folate in patients seen in the earlier part of the study since it was some months before the technique was established. Figlu excretion on the other hand was not estimated in the latter

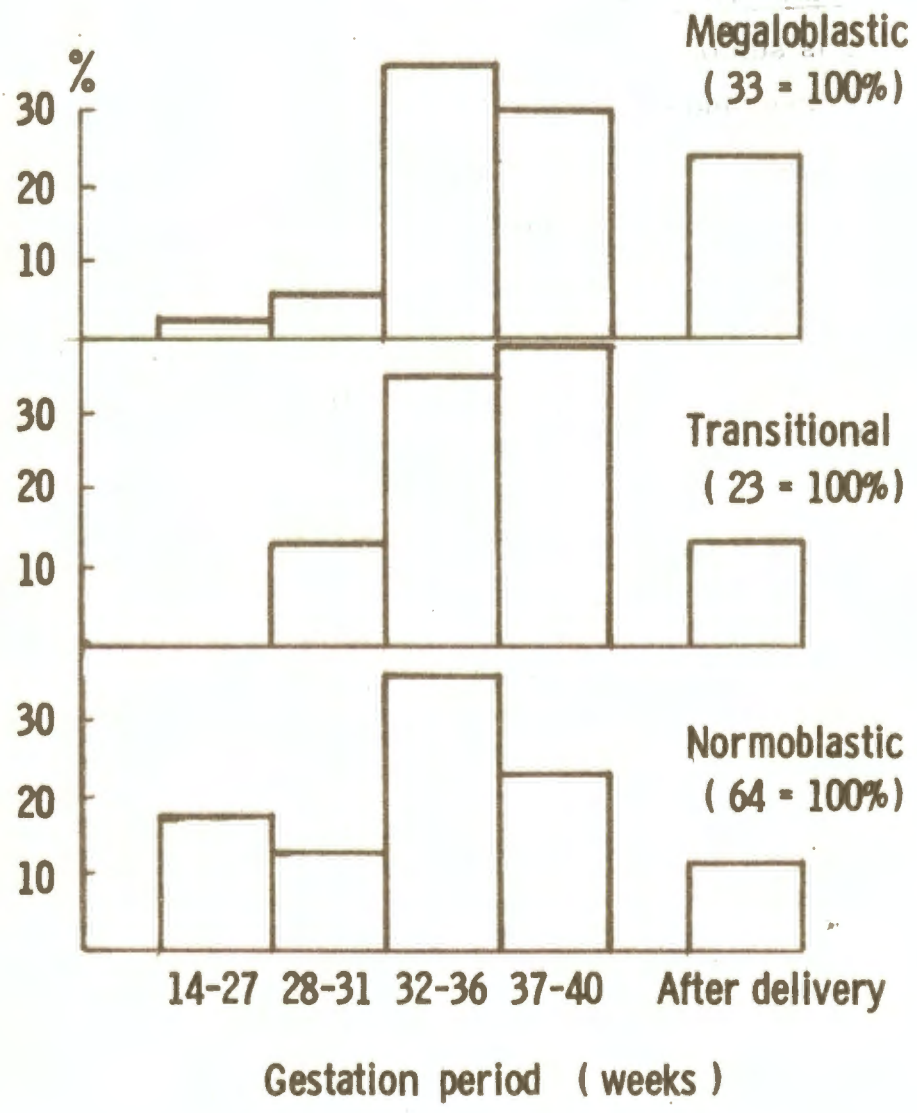

Fig. 8 Relates gestation period at diagnosis to erythropoiesis in 120 anaemic pregnant women.

part of the study. Once the value of this latter investigation had been assessed it was discontinued.

CLINICAL FEATURES

PRESENTATION OF MEGALOBLASTIC ANAEMIA

Symptoms and signs: The diagnosis of megaloblastic anaemia in pregnancy is seldom made on clinical grounds. Symptoms which may be present include anorexia, lassitude, weakness, dyspnoea, sore tongue, swelling of the feet and nausea and vomiting in late pregnancy. Signs of anaemia may be noted and glossitis, hepato-splenomegaly and pyrexia observed in severe cases. With the introduction of routine ante-natal care, however, this condition may be diagnosed in the laboratory without the patient being aware that she is ill.

In the present study the symptoms of anaemia, malaise and gastro-intestinal disturbances were more commonly associated with marked anaemia. Of 17 patients whose haemoglobin concentration lay below 9 g/100 ml, 8 complained of one or more of these symptoms, but most of the others had no complaints. Of the 16 with milder anaemia only 6 had symptoms which might be relevant, and the remainder were asymptomatic.

Oedema was noted in 12 women and in half of these the haemoglobin was less than 9.0 g/100 ml. Fever was not common and was observed in only 1 patient at the time of diagnosis. This patient (R.I. No. 43975) was suffering from idiopathic steatorrhoea which presented as a megaloblastic anaemia in pregnancy; other abnormal physical signs, which included angular stomatitis and glossitis, were present. These were rarely observed in the remaining women.

Gestation period: The stage of pregnancy at which megaloblastic anaemia was diagnosed is contrasted with that of anaemic women with transitional and normoblastic erythropoiesis in Fig. 8. Biopsy was seldom performed early in pregnancy and often was not performed on women who were obviously iron deficient; therefore no conclusion as to the relative frequency of normoblastic and megaloblastic erythropoiesis

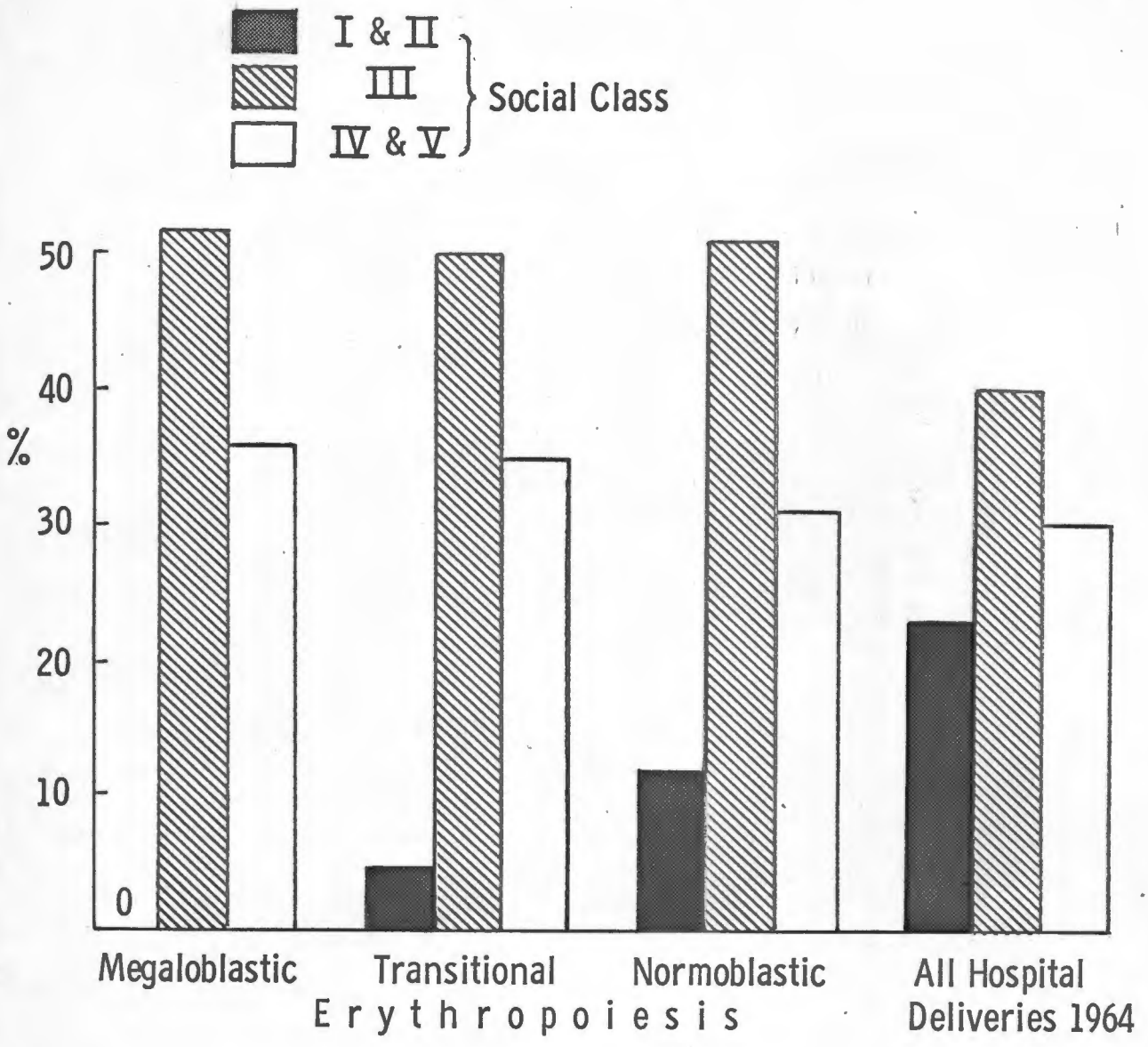

Fig. 9 Relates social class to erythropoiesis in 120 anaemic pregnant women. The social class of all women delivered in the Radcliffe Infirmary in 1964 is shown for comparison. (Armed forces excluded)

can be made. The increasing frequency of megaloblastic and transitional erythropoiesis in the last 8 weeks of pregnancy and the puerperium is apparent. Only the one patient already referred to with idiopathic steatorrhoea presented with a megaloblastic anaemia before the 28th week of pregnancy. In 12 it was diagnosed in the puerperium.

Only 2 of the 12 women in whom a megaloblastic or transitional megaloblastic anaemia was diagnosed in the puerperium were known to have a normal haemoglobin before delivery. Just over half were known to be anaemic before delivery but had not been investigated and three others had had no antenatal supervision. On the other hand all but one of the patients who developed a normoblastic anaemia in the puerperium had a normal haemoglobin before delivery. Thus although the result in this series confirmed that megaloblastic anaemia commonly presents in the puerperium the evidence suggests that with earlier investigation a number of cases would be diagnosed before delivery.

SOCIO-ECONOMIC CIRCUMSTANCES

None of the patients with megaloblastic anaemia appeared to be in obvious economic difficulty; only 2 were unmarried but both were supported by their parents. Only one of the anaemic pregnant women shown in Table 9 had a husband who was out of work and this was due to ill health.

The social class of 33 women with megaloblastic anaemia was assessed on their husbands' occupations (in the case of the 2 single women, on their own) and the results, shown in Table 9 and Fig. 9, showed a deviation from the overall social class distribution of women delivering in the Radcliffe Infirmary.

No woman who developed frank megaloblastic anaemia during pregnancy belonged to social classes I and II, and proportionately more women in this group belonged to social class V than in any of the others. Results in women with normoblastic anaemia were intermediate between those of anaemic women with megaloblastic and transitional erythropoiesis and the healthy controls. The number of anaemic women belonging

TABLE 9

Compares the Social Class Distribution of Women with Megaloblastic Anaemia and the Controls

Social class	Anaemic women with Megaloblastic erythropoiesis		Transitional erythropoiesis		Normoblastic erythropoiesis		All women delivered in R.I. during 1964	
I	-	(0%)	1	(5%)	4	(7%)	223	(11%)
II	-	(0%)	-	(0%)	3	(5%)	240	(12%)
III	17	(51%)	10	(50%)	28	(51%)	778	(40%)
IV	7	(21%)	5	(25%)	12	(22%)	409	(21%)
V	5	(15%)	2	(10%)	5	(9%)	183	(9%)
Armed Forces*	4	(12%)	2	(10%)	3	(5%)	132	(7%)
TOTAL	33	(100%)	20	(100%)	55	(100%)	1965	(100%)

*Excluded from statistical analysis.

to social classes I and II was small, but no significant difference was found in the social class distribution of women with folic acid deficiency and those with a normoblastic anaemia ($\chi^2 = 3.291$, $n = 1$, $p > 0.05$). However when the social class distribution of all women delivering in the Radcliffe Infirmary in a year was compared with (a) the social class distribution of women who were folic acid deficient there was a significant difference ($\chi^2 = 13.197$, $n = 2$, $p < 0.01$) which was not noted when it was compared with (b) the social class distribution of women with normoblastic anaemia ($\chi^2 = 4.359$, $n = 2$, $p > 0.05$).

AGE

The age distribution in anaemic women and the non-anaemic controls is compared in Table 10.

Apart from a tendency for megaloblastic and transitional megaloblastic anaemia to be less common in younger women, there was little difference between the three groups. The age distribution of women with normoblastic anaemia and non-anaemic patients was similar.

More women with megaloblastic anaemia were aged 30 years and

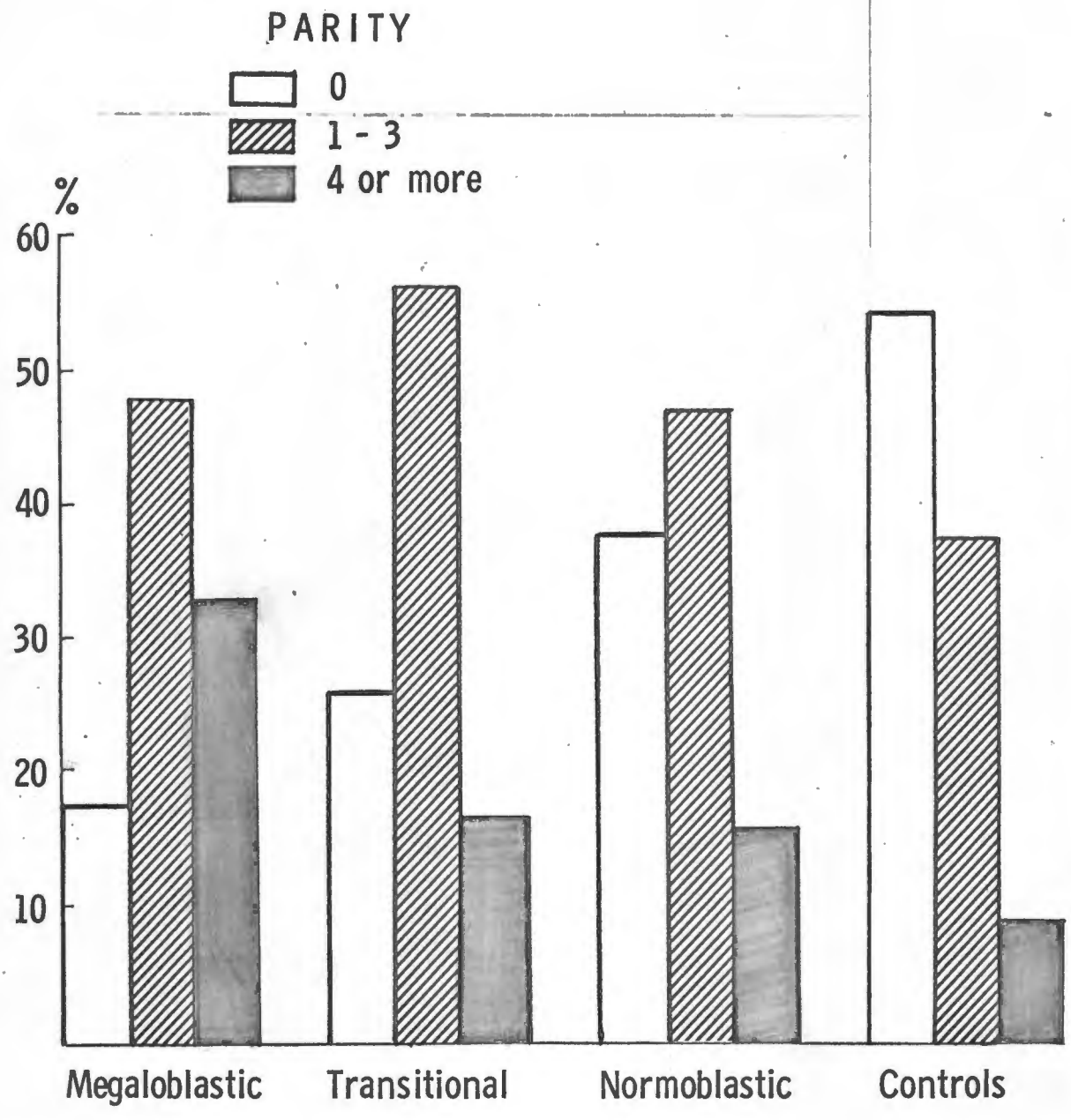

Fig. 10 Relates parity to erythropoiesis in 120 anaemic pregnant women. Parity in a control group of 273 pregnant women is shown for comparison.

TABLE 10

Compares the Age Distribution of Women with Megaloblastic Anaemia and the Controls

Age in years	Anaemic women with Megaloblastic erythropoiesis		Transitional erythropoiesis		Normoblastic erythropoiesis		Non-anaemic controls	
19 & under	2	(6%)	2	(9%)	9	(14%)	35	(13%)
20-29	16	(49%)	16	(70%)	36	(56%)	148	(54%)
30-39	12	(36%)	3	(13%)	18	(28%)	82	(30%)
40 & over	3	(9%)	2	(9%)	1	(2%)	8	(3%)
TOTAL	33	(100%)	23	(100%)	64	(100%)	273	(100%)

over but this was offset by fewer women in this age group with transitional changes. Taken together these results show that there is no tendency for older women to develop folic acid deficiency.

PARITY

The parity of anaemic women with megaloblastic, transitional and normoblastic erythropoiesis and non-anaemic controls are compared in Table 11 and in Fig. 10.

TABLE 11

Compares the Parity of Women with Megaloblastic Anaemia and the Controls

No. of previous viable pregnancies	Anaemic women with Megaloblastic erythropoiesis		Transitional erythropoiesis		Normoblastic erythropoiesis		Non-anaemic controls	
Nil	6	(18%)	6	(26%)	24	(37.5%)	147	(54%)
1-3	16	(48%)	13	(56.5%)	30	(47%)	101	(37%)
4 & over	11	(33%)	4	(17%)	10	(16%)	25	(9%)
TOTAL	33	(100%)	23	(100%)	64	(100%)	273	(100%)

Fewer women with frank megaloblastic anaemia were in their first pregnancy and proportionately more were in their fifth or subsequent

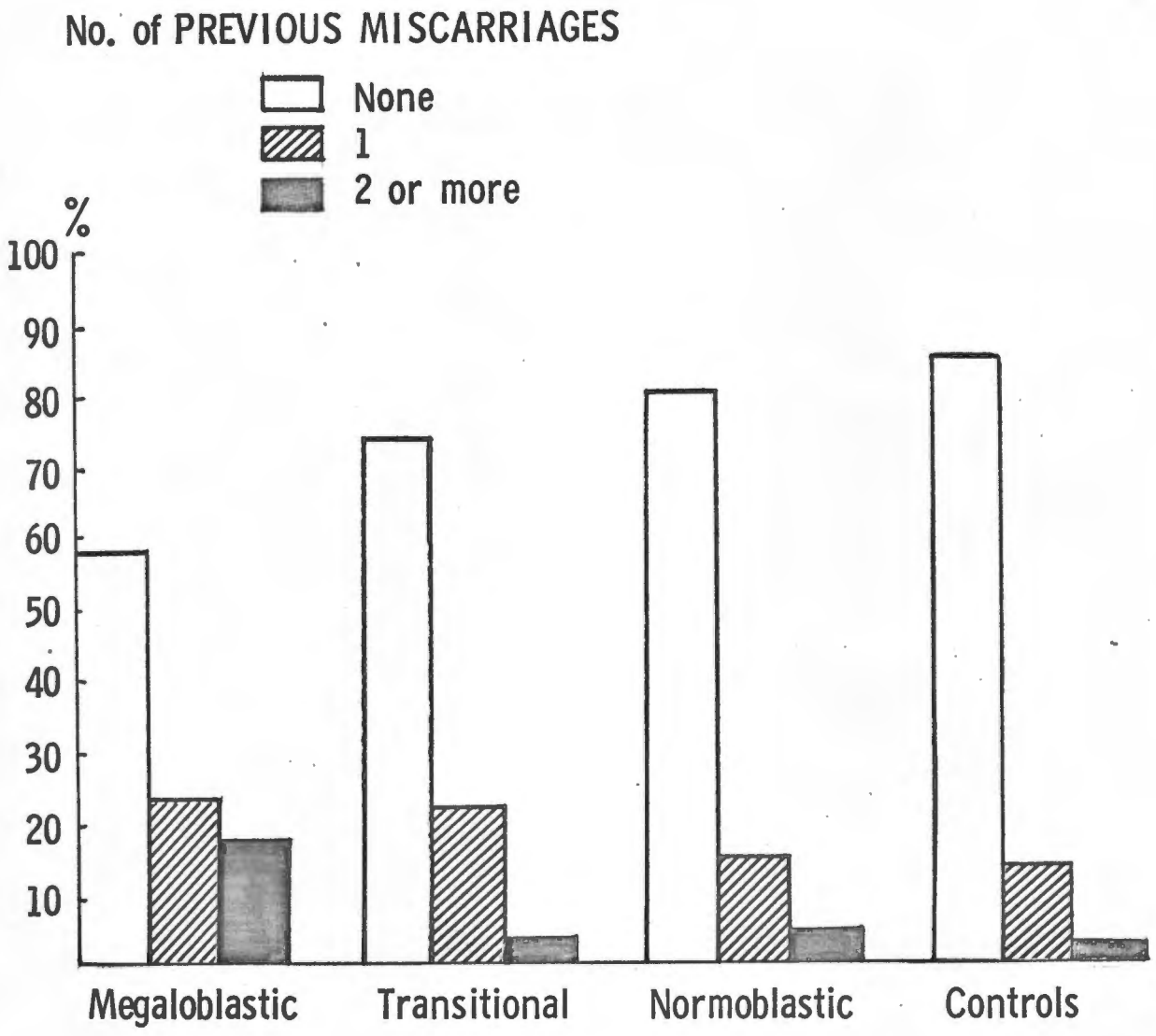

Fig. 11 Relates a history of previous miscarriage to erythropoiesis in 120 anaemic pregnant women. Results in a control group of 273 pregnant women are shown for comparison.

pregnancy than was observed in the other three groups. Results in the normoblastic group were intermediate between the group with folic acid deficiency and the healthy controls. There was no statistical difference when the parity of women with transitional and megaloblastic erythropoiesis was compared with the parity of (a) women with normoblastic anaemia ($\chi^2 = 4.503$, $n = 2$, $P > 0.05$), but a highly significant difference when compared with (b) the non-anaemic controls ($\chi^2 = 24.555$, $n = 2$, $P < 0.001$). The difference in parity between the women with normoblastic anaemia and the healthy controls was also significant ($\chi^2 = 6.118$, $n = 2$, $P < 0.05$).

The incidence of a history of previous miscarriages in women with megaloblastic anaemia and three other groups is shown in Table 12 and Fig. 11 .

TABLE 12

To Show the Incidence of a History of Previous Miscarriages in Women with Megaloblastic Anaemia and the Controls

No. of previous miscarriages	Anaemic women with						Non-anaemic controls	
	Megaloblastic erythropoiesis		Transitional erythropoiesis		Normoblastic erythropoiesis			
None	19	(58%)	17	(74%)	51	(80%)	228	(84%)
1	8	(24%)	5	(22%)	10	(15%)	39	(14%)
2 or more	6	(18%)	1	(4%)	3	(5%)	6	(2%)
TOTAL	33	(100%)	23	(100%)	64	(100%)	273	(100%)

Women with frankly megaloblastic anaemia were more likely to have had a previous miscarriage than the women with normoblastic anaemia and the non-anaemic controls. When results in women with megaloblastic and transitional erythropoiesis are considered together and compared with results in the latter group the difference is significant ($\chi^2 = 10.840$, $n = 1$, $P < 0.001$) but is not significant when compared with the group with a normoblastic anaemia ($\chi^2 = 3.554$, $n = 1$, $P > 0.05$). Results in the two control groups were similar ($\chi^2 = 0.533$, $n = 1$, $P > 0.05$).

TABLE 13

To Show the Incidence of Maternal Complications in Women with Megaloblastic Anaemia and the Controls

Maternal complications	Anaemic women with						Non-anaemic controls	
	Megaloblastic erythropoiesis		Transitional erythropoiesis		Normoblastic erythropoiesis			
Infection	10	(30%)	4	(17%)	15	(23%)	40	(15%)
Ante-partum haemorrhage	3	(9%)	2	(9%)	5	(8%)	7	(3%)
Post-partum haemorrhage	4	(12%)	0	(0%)	6	(9%)	13	(5%)
Pre-eclamptic toxaemia	8	(24%)	5	(22%)	9	(14%)	68	(25%)
None	15	(45%)	13	(57%)	39	(61%)	156	(57%)
TOTAL	33		23		64		273	

DIET

A detailed dietary history (Appendix I) was taken from 225 pregnant women, 15 of whom had megaloblastic anaemia. An approximate estimate of the folic acid content was made based on the number of meat meals per week and the frequency with which dairy products, green vegetables, salads and fresh fruit were consumed. This indicated that many women were having a reasonable intake of food containing folic acid, but as 50 to 100% of the folic acid content of foods may be destroyed in cooking (McCance and Widdowson, 1960) it is difficult to be certain how much of the folic acid was available. Although some had little uncooked source of folic acid, most of the women with megaloblastic anaemia whose diet was analysed appeared to be having a diet which was similar to that of other women attending the ante-natal clinics.

BLOOD GROUP

The blood group of 31 of the 33 women with megaloblastic anaemia was known. Blood group A (18 women) was found more commonly than blood group O (11 women). The blood group of 2 women with transitional marrow changes was known and in 11 it was group O and in 7 it was group A. The numbers are too small to submit to statistical analysis but are in keeping with the observation that there is an increase in blood group A in women with a frankly megaloblastic anaemia during pregnancy.

MATERNAL COMPLICATIONS

The incidence of certain complications of pregnancy found in the three groups of anaemic women, and non-anaemic pregnant controls, is summarized in Table 13.

Infection: The incidence of infection either during pregnancy or after delivery was greatest in women who developed a megaloblastic anaemia. In 6 of the 10 women with megaloblastic anaemia and infection shown in Table 13 a genito-urinary tract infection was diagnosed; one had a gastro-intestinal infection and 2 (both diagnosed ante-partum) had a persistent pyrexia after delivery

for which no cause could be found. The incidence of infection in women with normoblastic anaemia was only slightly less, however, (23%) and indeed the combined incidence amongst the two groups with bone marrow evidence of folic acid deficiency was almost the same (25%) as amongst the normoblastic group.

A significant difference was noted however when the incidence of infection in all the anaemic women was compared with the incidence in the 273 non-anaemic controls ($\chi^2 = 5.213$, $n = 1$, $P < 0.05$).

Ante-partum haemorrhage: There was no increase in the frequency of ante-partum haemorrhage in women with frank megaloblastic anaemia compared with those in whom erythropoiesis was transitional or normoblastic. However the incidence in women who were never anaemic during pregnancy was less. It is obvious that if blood loss were severe enough to produce anaemia before delivery then by definition women in whom this occurred would not be included in a non-anaemic group.

It is thus important to know how many of the anaemic women who developed ante-partum haemorrhage were anaemic prior to blood loss. Only four of the 10 women were known to be anaemic before haemorrhage took place. In 3 others anaemia was not present at the time of the ante-partum haemorrhage but developed some time after; in only one of these did anaemia appear to be directly related to blood loss. The remaining 3 women were admitted to hospital because of haemorrhage and only then were found to be anaemic; one of the 3 was known to have a haemoglobin concentration of 11.1 g/100 ml one week previously but in the other 2 no previous haemoglobin estimation had been carried out.

The proportion of all anaemic women in whom ante-partum haemorrhage took place was small and in only 4 was anaemia known to be present before haemorrhage occurred. Although this possibility could not be ruled out in 2 other women the infrequency with which anaemic women developed an ante-partum haemorrhage makes it unlikely that folic acid or iron deficiency had played a role in producing ante-partum haemorrhage.

Post-partum haemorrhage: Post-partum haemorrhage occurred most frequently in women with megaloblastic anaemia. In 3 of the 4 women

in this group anaemia was diagnosed after post-partum haemorrhage had occurred although all three were known to be anaemic at term, suggesting that a pre-existing maternal deficiency existed and the extra strain produced by blood loss precipitated frank megaloblastosis.

No woman with transitional erythropoiesis had a post-partum haemorrhage. There were 6 women with normoblastic anaemia who had a post-partum haemorrhage; one of these (R.I. No. 41760) became anaemic only after she had lost one pint of blood at delivery. The 5 others had been anaemic during pregnancy.

Thus 9 out of the 10 women who developed post-partum haemorrhage were known to be anaemic before delivery.

The overall incidence of post-partum haemorrhage in women who were anaemic before delivery is 8.4% compared with 4.7% in women with a Hb concentration of 11 g/100 ml or more during pregnancy, a difference which is not significant ($\chi^2 = 2.837$, $n = 1$, $P > 0.05$).

Pre-eclamptic toxaemia: Toxaemia of pregnancy is diagnosed in the Nuffield Department of Obstetrics, when at least two of the three following criteria are present:—

1) a minimum blood pressure of 130 mm Hg systolic or 90 mm Hg diastolic on one certified occasion;
2) oedema;
3) albuminuria.

There was little difference in the incidence of pre-eclamptic toxaemia in the four groups of women shown in Table 13. The incidence varied from 14% to 25%, being lowest in women with normoblastic anaemia and greatest in non-anaemic controls.

FOETAL COMPLICATIONS

Only the foetal complications reputedly associated with maternal folic acid deficiency have been included in Table 14. Other complications such as neonatal jaundice, asphyxia and infection did occur but unless otherwise stated the babies were all in good condition at the time of their discharge from hospital.

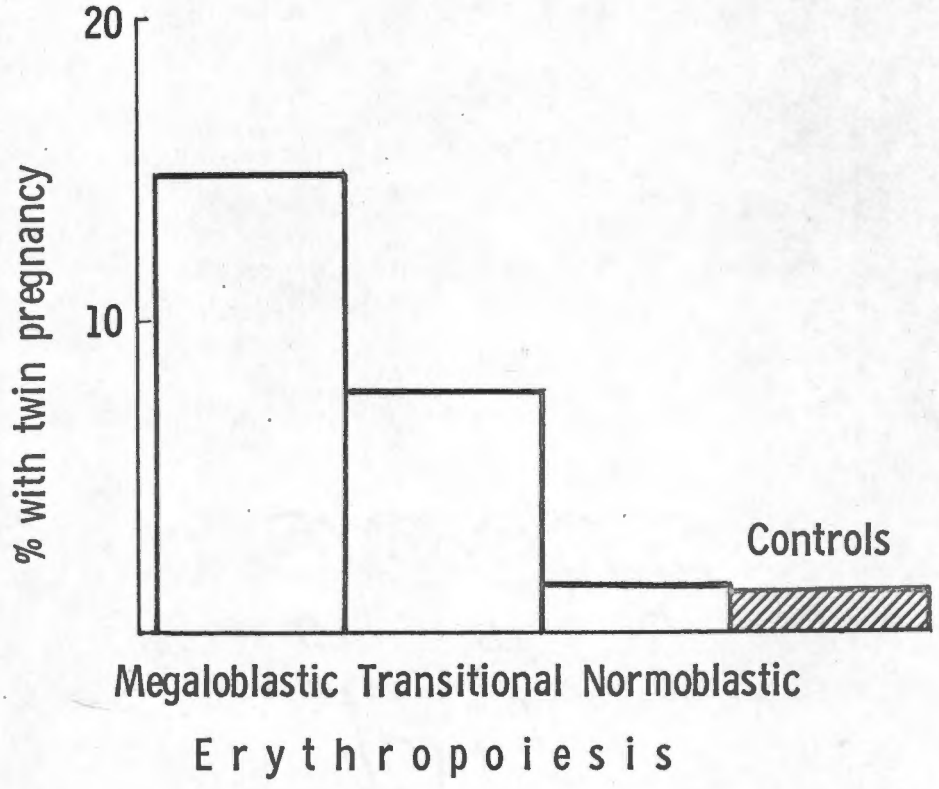

Fig. 12 Relates the incidence of a twin pregnancy to erythropoiesis in 120 anaemic pregnant women. Results in a control group of 273 pregnant women are shown for comparison.

TABLE 14

To Show the Incidence of Twins and Certain Foetal Complications in Women with Megaloblastic Anaemia and the Controls

Foetal complications	Anaemic women with			Non-anaemic controls
	Megaloblastic erythropoiesis	Transitional erythropoiesis	Normoblastic erythropoiesis	
Twins*	5 (2)	2 (2)	1 (1)	4 (2)
Prematurity	1	2	2	11
Stillbirth	1	-	2	6
Congenital abnormality	-	2	4	12
None	26 79%	17 74%	55 86%	238 87%
TOTAL	33	23	64	273

*Number of babies weighing less than $5\frac{1}{2}$ lb in brackets.

Twins: There was a marked increase in the incidence of twin pregnancies in women with megaloblastic anaemia (Table 14). The observation that a multiple pregnancy, and the greater requirement for folic acid which accompany it, increase the risk of the mother developing megaloblastic anaemia is well supported in this table. A multiple pregnancy would appear to be an important aetiological factor in producing megaloblastic anaemia.

The number of women with transitional erythropoiesis was small but here too the incidence of twins was apparently increased (8.7%) although not as frequently as in the frankly megaloblastic group (15.2%).

The proportion of anaemic women with normoblastic erythropoiesis and non-anaemic controls who gave birth to twins is similar (1.6% and 1.5% respectively).

Prematurity and birth weight: There was no evidence to suggest that babies born to mothers with megaloblastic anaemia were more likely to be premature by weight. Two of the 10 twins weighed less than $5\frac{1}{2}$ lb, but these are considered separately. The risk of prematurity in women with megaloblastic anaemia (± 3%) approximated that of women with normoblastic anaemia. If anaemic women with megaloblastic and transitional erythropoiesis are considered together the combined incidence

of prematurity is only slightly above this (5%), being close to that found in non-anaemic controls (4%).

The birth weights of infants born to women with anaemia of pregnancy are summarized in Table 15.

TABLE 15

Birth Weights of Babies Born to Anaemic Women

Birth weight of infants born to anaemic mothers	Anaemic women with		
	Megaloblastic erythropoiesis	Transitional erythropoiesis	Normoblastic erythropoiesis
< 5½ lb	3 (2)*	5 (3)	5 (1)
5 lb 8 oz – 6 lb 15 oz	11 (7)	7 (1)	20 (1)
7–8 lb	11 (1)	5	19
> 8 lb	13 34%	8 32%	19 (29%
Not known	--	-	2†
TOTAL	38	25	65

*Number of twins in brackets.
†Two infants – normal; birth weight not recorded.

When comparing the weights of babies born to mothers who had megaloblastic anaemia with that of babies born to mothers with transitional or normoblastic erythropoiesis, due allowance must be made for the increased incidence of twins in the former group. If twins weighing less than 7 lb are excluded then there is no tendency for women with transitional and megaloblastic anaemia to have small babies. The proportion of mothers having infants weighing more than 8 lb was similar in the three groups of anaemic women shown in Table 15.

Stillbirth: There was no increase in the number of stillborn infants born to mothers with megaloblastic anaemia. The incidence in women with megaloblastic and normoblastic anaemia was the same (± 3%) and there was little difference when compared with non-anaemic controls.

Congenital abnormalities: None of the 33 women with megaloblastic anaemia gave birth to a child with any abnormality

detectable at birth. All babies born in the Radcliffe Infirmary are fully examined before being discharged.

The incidence in the three other groups including the non-anaemic controls were all a little above the reported incidence of congenital abnormalities in a general population. Although a number of abnormalities listed here were serious (for example exomphalos, oesophageal atresia, hydrocephalus), minor ones (for example accessory digits, deformity of the external auricle) have all been included.

TREATMENT

Ideally a period of observation without therapy should precede treatment to allow for the basic characteristics of the anaemia to be established. In pregnancy, factors such as recent intensive iron therapy, a dual deficiency of iron and folic acid, impaired absorption of folic acid, a spontaneous rise in the Hb concentration near term or after delivery and underlying infection may complicate the interpretation of a response to therapy.

In the present study the treatment of anaemic pregnant women was undertaken by the obstetrician in charge and was often considered a matter of some urgency. In the majority of women with megaloblastic anaemia several forms of therapy were given simultaneously or in quick succession, and it was not possible to determine which particular treatment had corrected the anaemia. Seventeen women were given a blood transfusion in addition to haematinics and in a further 4, iron and folic acid were begun at the same time. In 9 women folic acid was the only treatment and the usual dose used was 15 mg daily by mouth; 3 of the 4 women treated in this way before delivery responded well, the fourth only partially. Although it was not proved, 4 of the 5 women treated after delivery appeared to respond well to treatment with folic acid.

Three women were treated with vitamin B_{12} (100 μg daily by intramuscular injection), but it was not possible to draw any conclusions about their response to this therapy because other treatment was given. One patient (R.E. No. 38269) had a reticulocyte count of 10% 4 days after

the injections were started but was then given a blood transfusion.

It is obvious from the foregoing account that in the present study the treatment of women with megaloblastic anaemia was directed at achieving a normal Hb concentration as soon as possible. Little information was obtained about the responsiveness of this condition to folic acid and vitamin B_{12}.

THE DIAGNOSIS OF FOLIC ACID DEFICIENCY IN PREGNANCY

As indicated earlier, the criteria used for the diagnosis of folic acid deficiency has been a morphological one based on the bone marrow appearance or the presence of unequivocal megaloblasts in the peripheral blood. Because of the strict criteria adopted a group of patients with undoubted folic acid deficiency was available and so the value of a number of other laboratory tests in the diagnosis of folic acid deficiency could be assessed. In each instance the patients with megaloblastic anaemia have been divided into those with frank megaloblastic erythropoiesis and those with transitional megaloblastic change, and the results compared with the control patients showing normoblastic erythropoiesis, and the group of 273 patients who had a haemoglobin level greater than 11 g/100 ml throughout pregnancy.

The following investigations were used in this study: the Hb concentration, MCHC, examination of the peripheral blood and bone marrow films, estimation of the serum iron, serum folate, serum vitamin B_{12} and urinary Figlu excretion. The last four tests were not performed on every patient but a large enough number of women was studied to allow the relative value of these investigations in the diagnosis of megaloblastic and transitional anaemia to be assessed and to allow the relationship of iron deficiency and megaloblastic anaemia in pregnancy to be examined.

The results will be discussed under the following headings:—

1) Laboratory diagnosis of megaloblastic anaemia.
2) The association of megaloblastic anaemia and iron deficiency.

LABORATORY DIAGNOSIS OF MEGALOBLASTIC ANAEMIA

HAEMOGLOBIN AND MCHC

Table 16 shows that the patients with megaloblastic or transitional erythropoiesis were more likely to have severe anaemia than those who had normoblastic marrows. The numbers in each group with a Hb concentration below 7.0 g/100 ml are too small for statistical analysis; however, the number of women with a haemoglobin of less than 9.0 g/100 ml with megaloblastic and transitional erythropoiesis is significantly greater than those with normoblastic erythropoiesis ($\chi^2 = 6.122$; $n = 1$; $P < 0.05$).

There was no direct relationship between the severity of megaloblastic change in the bone marrow and degree of anaemia. Four women with a haemoglobin of 11.0 g/100 ml or more at the time of bone marrow biopsy had morphological evidence suggesting folic acid deficiency. All 4 had been anaemic shortly before but by the time of investigation the haemoglobin had risen on iron therapy. The probable explanation is that the deficiency had been a dual one and that, although anaemia had been partly corrected by iron, the megaloblastic change persisted.

All but one of the 5 women with a Hb concentration below 7 g/100 ml and megaloblastic anaemia shown in Table 16 had marked megaloblastic

TABLE 16

To Show Hb Concentration at Time of Bone Marrow Biopsy in Relation to Erythropoiesis

Hb concentration, g/100 ml	Anaemic women with		
	Megaloblastic erythropoiesis	Transitional erythropoiesis	Normoblastic erythropoiesis
< 7	5	2	4
7-7.9	2 } 50%	1 } 56%	4 } 31%
8-8.9	10	10	12
9-9.9	8	5	21
10-10.9	6	3	18
11.0 ≥	2	2	5
TOTAL	33	23	64

change. Nevertheless milder anaemia (a Hb concentration of 9 g/100 ml or more) was also associated with a frankly megaloblastic marrow and it was concluded that the degree of anaemia and severity of megaloblastic change are not directly related.

The MCHC was of little help in differentiating a megaloblastic from a normoblastic anaemia, since it was normal in approximately the same proportion of the three groups of anaemic women. On the other hand nearly one-third of the women with a megaloblastic anaemia had an MCHC below 30%, indicating that a dual deficiency was present.

MORPHOLOGY OF THE PERIPHERAL BLOOD

In Table 17 the appearance of the peripheral blood film is related to erythropoiesis.

TABLE 17

Relates the Appearance of the Peripheral Blood Film to Erythropoiesis

Peripheral blood film	Anaemic women with		
	Megaloblastic erythropoiesis	Transitional erythropoiesis	Normoblastic erythropoiesis
Macrocytosis and/or hyperseg.	15	4	4
Hypochromia	4	4	37
Both	12	11	10
Normal*	1	3	13
TOTAL	33†[1]	23†[1]	64

*Anisopoikilocytosis only.
†Report not available.

The films, which were examined without knowledge of the clinical state or the bone marrow appearances, were assessed for evidence of folate deficiency based on the presence of macrocytosis and/or hypersegmentation of the polymorphonuclear leucocytes, and for evidence of iron deficiency based on the presence of hypochromia and microcytosis. Hypersegmentation of the leucocytes was considered to be present if

several cells showed 5 or more lobed nuclei but a formal count was not made. It can be seen that the presence of macrocytosis and hypersegmentation of polymorphs usually indicated megaloblastic or transitional erythropoiesis but was occasionally found in patients with normoblastic marrows. On the other hand, megaloblastic or transitional changes in the bone marrow were observed in a few patients although there were no features suggestive of this in the peripheral blood. It is of interest that more than half the patients with megaloblastic or transitional erythropoiesis showed evidence of iron deficiency in the peripheral blood film, confirming that a dual deficiency is frequently present.

SPECIAL INVESTIGATIONS USED TO DETECT MEGALOBLASTIC ANAEMIA

The special investigations used to detect megaloblastic anaemia in the pregnant women studied were the estimation of (1) the serum folate, (2) serum vitamin B_{12}, and (3) the urinary excretion of Figlu. For practical reasons it was not possible to carry out the three investigations in all the anaemic women in this study.

In addition to the 64 women shown in Table 8 with a normoblastic anaemia, a further group of pregnant women with normoblastic erythropoiesis was studied. In 20 of these, bone marrow films were available for review at the end of the 3-year period of study, but clinical features and other laboratory data have not been included in previous tables for the following reasons:—

1) Thirteen were non-anaemic volunteers in the last trimester of pregnancy who were willing to co-operate in this study;
2) Six were anaemic women who delivered elsewhere and could not be traced;
3) One patient was investigated for anaemia in two consecutive pregnancies and details of one have already been included.

Since the three diagnostic tests to be described were performed on a considerable proportion of these patients, the results of their investigations are given in the next five tables in this chapter, the number of healthy controls in each group being shown in brackets. Details of these

20 additional patients whose results are to be presented are given in Appendix V.

Table 18 summarizes the results of investigation by means of the three diagnostic tests in a total of 140 women during pregnancy.

TABLE 18

To Show the Relationship of the Serum Folate, Serum Vitamin B_{12} Levels and Figlu Excretion to Erythropoiesis

		Anaemic women with			Total	
		Megaloblastic erythropoiesis	Transitional erythropoiesis	Normoblastic erythropoiesis		
Serum folate, mμg/ml	< 2.1	13	8	5 (1)*	26	78
	2.1 & more	9	2	41 (10)	52	
Serum vit. B_{12} μμg/ml	< 100	7	2	2 -	11	109
	100-149	7	6	13 (2)	26	
	150 & more	15	11	46 (9)	72	
Figlu excretion	Increased	9	4	6 (2)	19	94
	Normal	11	12	52 (11)	75	

*Non-anaemic controls in brackets.

THE SERUM FOLATE

This was related to erythropoiesis in 78 women, of whom 32 had bone marrow evidence of folic acid deficiency. The results ranged from 0.2 mμg/ml to 4.5 mμg/ml in women with frank megaloblastic anaemia and the mean value in this group was 1.8 mμg/ml. It is surprising to note that more than one-third of the women had levels within the normal range. In women with transitional erythropoiesis results ranged from 0.6 mμg/ml to 6.6 mμg/ml and most of the women in this group had values below 2.1 mμg/ml.

The serum folate of women with normoblastic erythropoiesis ranged from 1.0 mμg/ml to 10.4 mμg/ml with a mean of 4.1 mμg/ml. One in 9 women with normoblastic erythropoiesis had a serum folate below the lower limits of normal for non-pregnant controls.

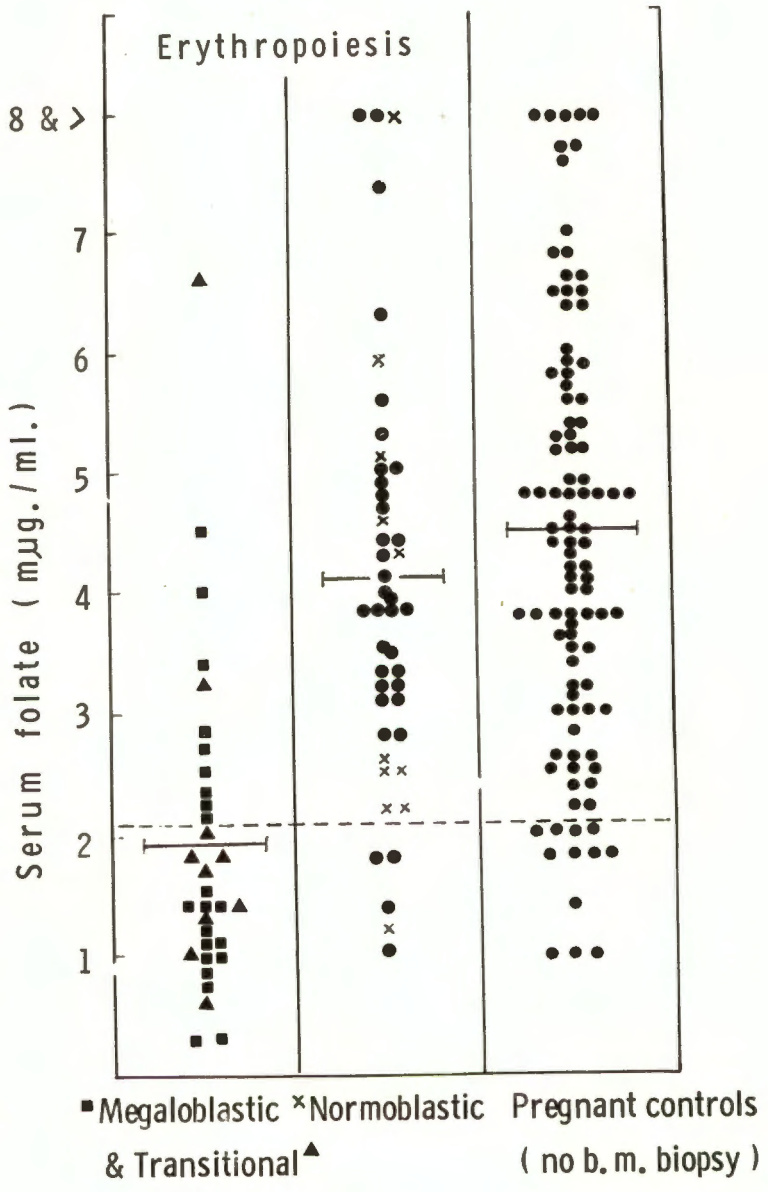

Fig. 13 Relates serum folate levels to erythropoiesis, and gives results obtained in a control group of pregnant women.†

(† Hb concentration 12g./100 ml. or >, gestation 28 weeks or >)

× Healthy pregnant volunteers with normoblastic erythropoiesis.

The detailed results of the serum folate levels and their relationship to the bone marrow morphology in these 78 women are shown in Fig. 13. 66% of women with megaloblastic and transitional erythropoiesis had low values compared with only 11% of women with normoblastic erythropoiesis. If a serum folate of 2.1 mμg/ml is used as a dividing line to distinguish patients with megaloblastic or transitional erythropoiesis from those with normoblastic erythropoiesis, then in nearly 80% of anaemic women the correct diagnosis would have been made. Values below 1.0 mμg/ml were only found in women with megaloblastic erythropoiesis but such a low value is obviously of limited use in diagnosis since the majority of women in this group had levels above this.

THE SERUM VITAMIN B_{12}

One hundred and nine women in whom bone marrow biopsy was performed had a serum vitamin B_{12} estimation. Just under half the women with megaloblastic erythropoiesis and approximately 42% with transitional erythropoiesis had levels below 150 μμg/ml. Approximately one-quarter of women with normoblastic erythropoiesis and anaemia, and a similar proportion of non-anaemic women with normoblastic erythropoiesis, had B_{12} levels of this order. When results in women with megaloblastic and transitional erythropoiesis were compared with those in women with a normoblastic anaemia, a significant difference was observed ($\chi^2 = 5.269$, $n = 1$, $P < 0.05$).

Although values between 100 and 150 μμg/ml are regarded as indicating possible vitamin B_{12} deficiency in non-pregnant subjects, those in whom a diagnosis of pernicious anaemia is made usually have levels below 100 μμg/ml. In order to assess the significance of serum vitamin B_{12} levels in anaemia during pregnancy the serum vitamin B_{12} of 74 women in the last trimester of pregnancy with a Hb concentration of 12 g/100 ml or more was estimated. The results ranged from 45 to 800 μμg/ml with a mean of 193.4 μμg/ml; 8% had values below 100 μμg/ml while approximately one-third had values below 150 μμg/ml. Results in these 74 women, together with results in all women in whom bone marrow biopsy

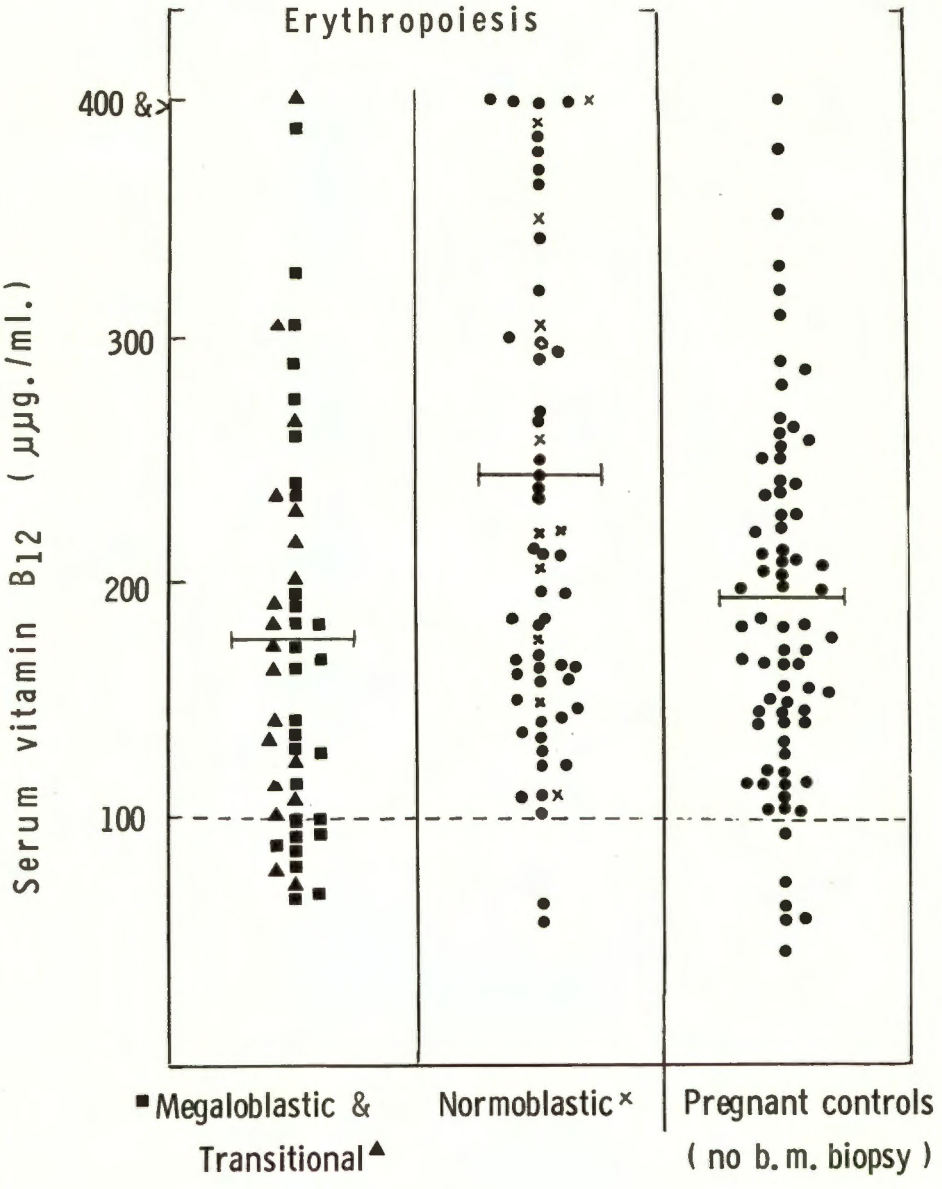

Fig.14 Relates serum vitamin B_{12} levels to erythropoiesis and gives results obtained in a control group.†

(† Hb concentration 12g./100 ml. or > , gestation 28 weeks or >)

(× Healthy pregnant volunteers with normoblastic erythropoiesis)

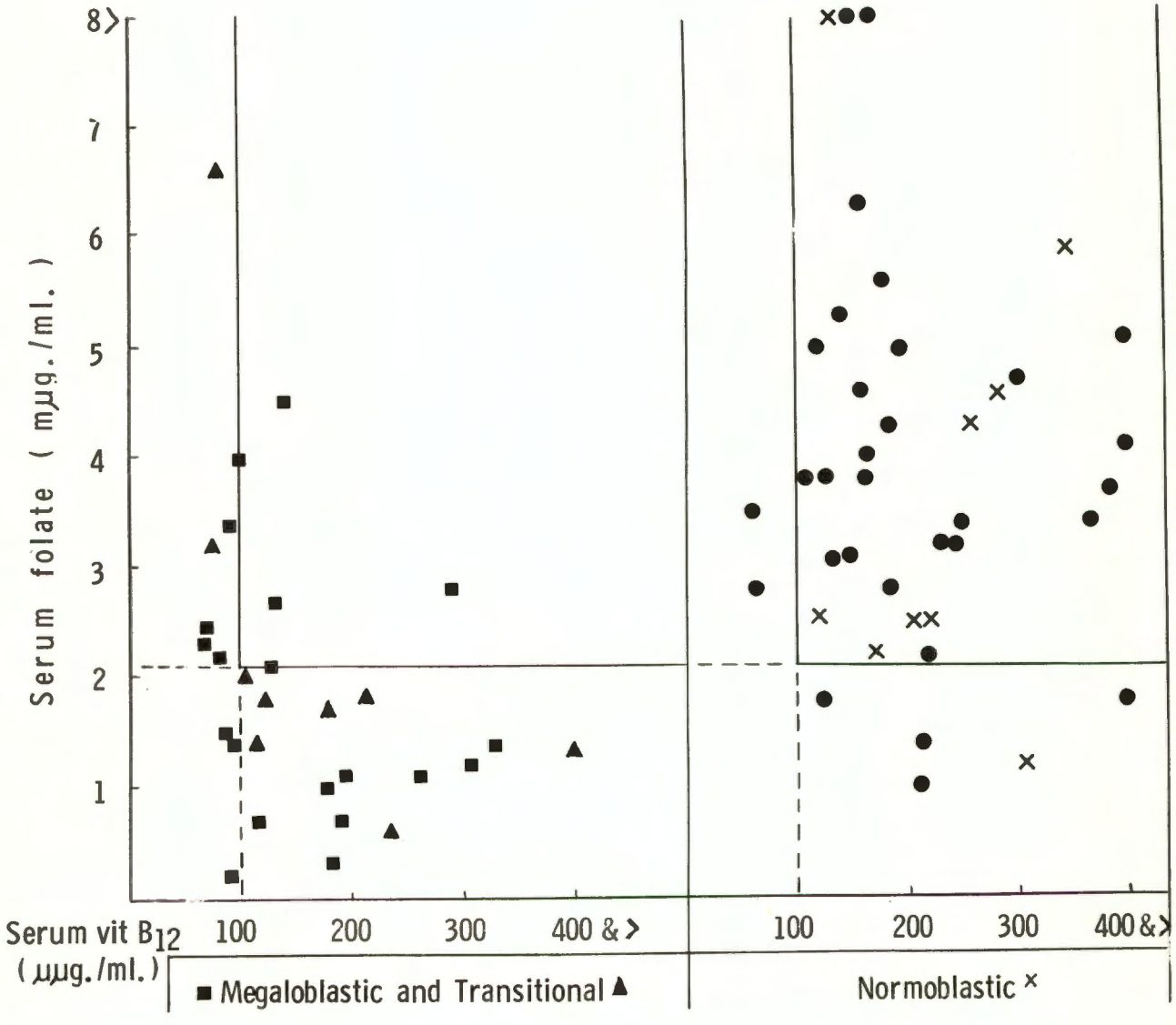

Fig. 15 To show relationship of serum folate & vitamin B_{12} levels to erythropoiesis.

× Healthy pregnant volunteers with normoblastic erythropoiesis.

was carried out, are plotted in Fig. 14.

From the results obtained in non-anaemic pregnant women it appears that a serum vitamin B_{12} level between 100 and 150 μμg/ml. in the later stages of pregnancy is of little significance in anaemia in pregnancy. Indeed the proportion of women with values between 100 and 150 μμg/ml was very similar in the four groups. The differences observed in vitamin B_{12} levels between the groups are therefore accounted for by the larger number of women with megaloblastic or transitional erythropoiesis who have levels below 100 μμg/ml.

Relationship of serum folate and vitamin B_{12} levels to erythropoiesis: Sixty-nine of the 78 women in whom serum folate and bone marrow studies were carried out also had a serum vitamin B_{12} estimation. The serum folate and vitamin B_{12} results are related to erythropoiesis in Table 19 and Fig. 15.

TABLE 19

To Show Correlation Between Serum Folate and Serum Vitamin B_{12} Levels and Erythropoiesis in 69 Women

	Serum vit. B_{12}, μμg/ml	Anaemic women with			Total
		Megaloblastic erythropoiesis	Transitional erythropoiesis	Normoblastic erythropoiesis	
Serum folate < 2.1 mμg/ml	< 100	3	-	-	3
	100 or more	8	7	5 (1)*	20
Serum folate 2.1 mμg/ml or more	< 100	4	2	2	8
	100 or more	5	-	33 (10)	38

*Non-anaemic controls in brackets.

Only 3 women had a low folate and low vitamin B_{12} level and in each case erythropoiesis was megaloblastic. In the majority of women (38) both the serum folate and serum vitamin B_{12} were normal and of these only a few (13%) had megaloblastic or transitional erythropoiesis. Three-quarters

of the remaining 28 women in whom either the serum folate or the serum vitamin B_{12} level was reduced, had megaloblastic or transitional erythropoiesis.

Results in Table 19 indicated that in two-thirds of women with megaloblastic or transitional erythropoiesis and a low serum vitamin B_{12} the serum folate was normal. The number of patients studied was small, however, and the results must be treated with some reservation. From results obtained in a larger number of women presented in Table 18 it appears that 18.8% of women with megaloblastic or transitional erythropoiesis have serum vitamin B_{12} levels below 100 μμg/ml. By combining results in these two tables it can be calculated that approximately 12.5% of women with megaloblastic anaemia will have a normal serum folate and an abnormal serum vitamin B_{12} level.

Thus there is evidence of vitamin B_{12} deficiency rather than folic acid deficiency in a proportion of cases of megaloblastic anaemia associated with pregnancy. It has been shown also that a serum vitamin B_{12} below 100 μμg/ml is of some diagnostic significance in this condition. Table 20 summarizes and compares the value of an abnormal serum folate and vitamin B_{12} in detecting megaloblastic anaemia during pregnancy.

TABLE 20

Compares (a) the Frequency and (b) the Diagnostic Value of a Low Serum Folate and Low Serum Vitamin B_{12} in 69 Women in whom both Tests were Carried out Simultaneously

	Serum folate, < 2.1 mμg/ml	Serum vitamin B_{12} < 100 μg/ml
Number of results	23	11
Number with megaloblastic or transitional erythropoiesis	18 (78.0%)	9 (81.8%)

Although the number of observations is small, Table 20 shows that in the present study a low serum vitamin B_{12} was as accurate an index of megaloblastic erythropoiesis as a low serum folate but occurred

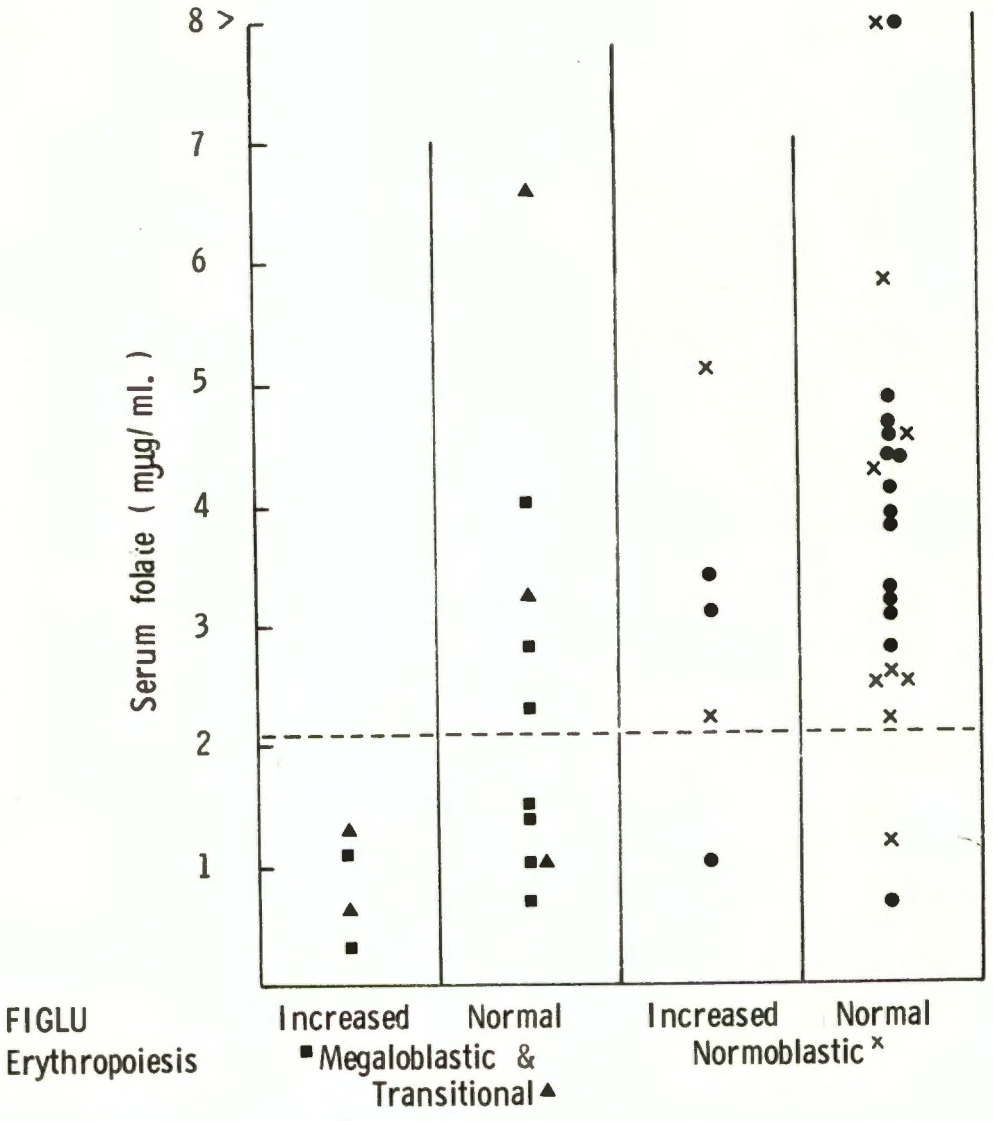

Fig. 16 Relates FIGLU excretion to serum folate levels & erythropoiesis.

× Healthy pregnant volunteers with normoblastic erythropoiesis.

less frequently.

EXCRETION OF URINARY FIGLU

The amount of Figlu excreted in the urine after a loading dose of 15 g of histidine was estimated in 75 of the 140 women shown in Table 18 Patients had fasted overnight and were starved for the first hour of the test; fluids were restricted to a certain extent throughout.

Women who excreted increased amounts of Figlu were more likely to have megaloblastic anaemia ($\chi^2 = 9.144$, $n = 1$, $P < 0.01$), morphological evidence of this being noted in 68% of patients with increased excretion. Normal Figlu excretion was of less diagnostic value and was noted in more than half the women with megaloblastic anaemia and even more commonly in women with transitional erythropoiesis. Nearly 40% of the women with normal Figlu excretion had bone marrow evidence of folic acid deficiency. Thus increased Figlu was a useful sign in the investigation of anaemia during pregnancy but the test as a whole was not a reliable one.

In a proportion of women in whom Figlu excretion was estimated, serum folate and serum vitamin B_{12} levels were available and the relationship of these three tests will now be examined.

(i) Figlu excretion and the serum folate: In Table 21 and Fig.16 results in 40 women in whom Figlu excretion and the serum folate were simultaneously estimated are presented and are related to erythropoiesis.

TABLE 21

To Show Relationship of Serum Folate and Figlu Excretion to Erythropoiesis

	Serum folate, mμg/ml	Anaemic women with Megaloblastic erythropoiesis	Transitional erythropoiesis	Normoblastic erythropoiesis	Total
Normal Figlu excretion	< 2.1	4	1	1	6
	2.1 or more	3	2	20 (8)*	25
Increased Figlu excretion	< 2.1	2	2	1	5
	2.1 or more	0	0	4 (2)	4

*Non-anaemic controls in brackets.

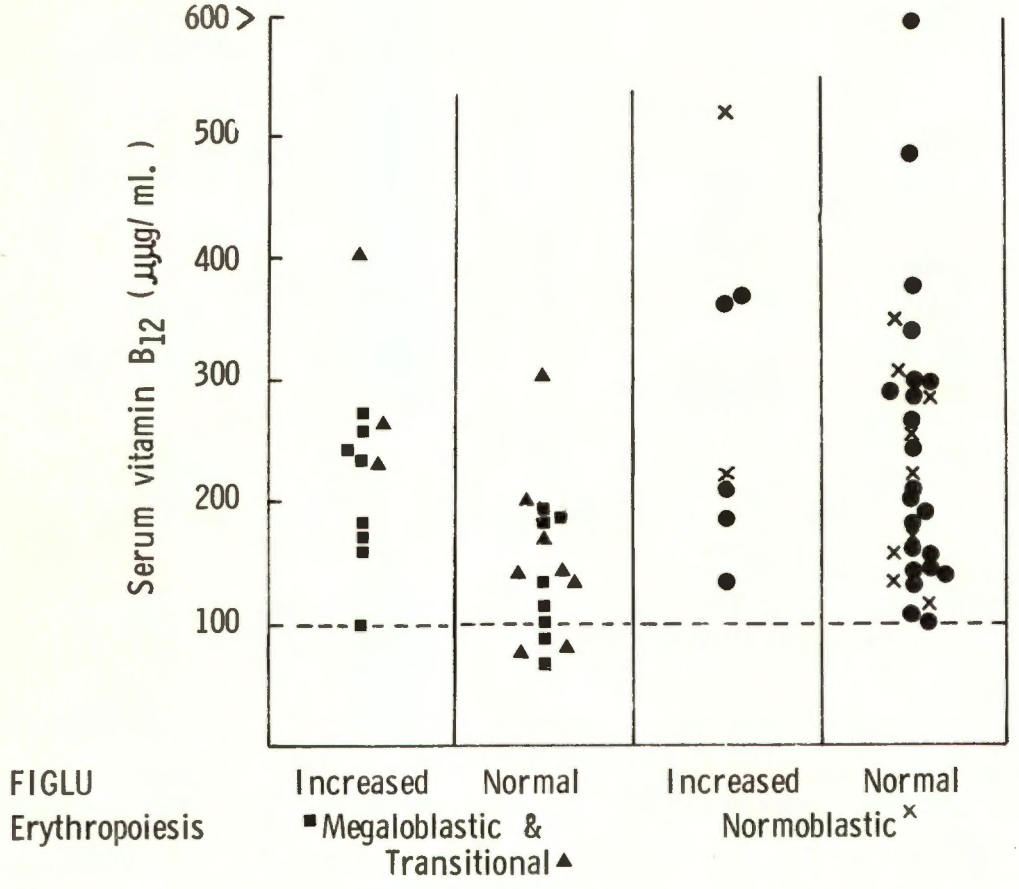

Fig. 17 Relates FIGLU excretion to serum vitamin B_{12} levels & erythropoiesis.

× Healthy pregnant volunteers with normoblastic erythropoiesis.

The majority of women in whom Figlu excretion was normal had a normal serum folate. However less than half the women with low serum folate levels had increased Figlu excretion. Results in this table show that the serum folate is a better test to use in the investigation of anaemia during pregnancy since it correctly indicated the type of erythropoiesis in 33 cases whereas Figlu excretion was correct in 25.

(ii) Figlu excretion and the serum vitamin B_{12}: Figlu excretion and serum vitamin B_{12} levels were known in 66 women and are related to erythropoiesis in Table 22 and Fig. 17.

TABLE 22

To Show Relationship of Serum Vitamin B_{12} and Figlu Excretion to Erythropoiesis

	Serum vit. B_{12} µµg/ml	Anaemic women with Megaloblastic erythropoiesis	Transitional erythropoiesis	Normoblastic erythropoiesis	Total
Normal Figlu	< 100	2	2	0	4
	100-149	3	3	7 (2)*	13
	150 or more	4	4	24 (7)	32
Increased Figlu excretion	< 100	0	0	0	0
	100-149	1	0	1	2
	150 or more	8	3	5 (2)	16

*Non-anaemic controls in brackets.

A reduced serum vitamin B_{12} level occurred more commonly in association with a normal Figlu excretion. This was marked when erythropoiesis was megaloblastic or transitional and when results in these two groups were considered together and compared with results in the normoblastic group, the difference was significant ($\chi^2 = 5.465$, $n = 1$, $P < 0.05$). All four women with vitamin B_{12} levels in the range usually associated with pernicious anaemia had normal Figlu excretion and megaloblastic or transitional erythropoiesis. A similar result was obtained in one woman with normoblastic erythropoiesis but was invalidated when it was learnt that she had

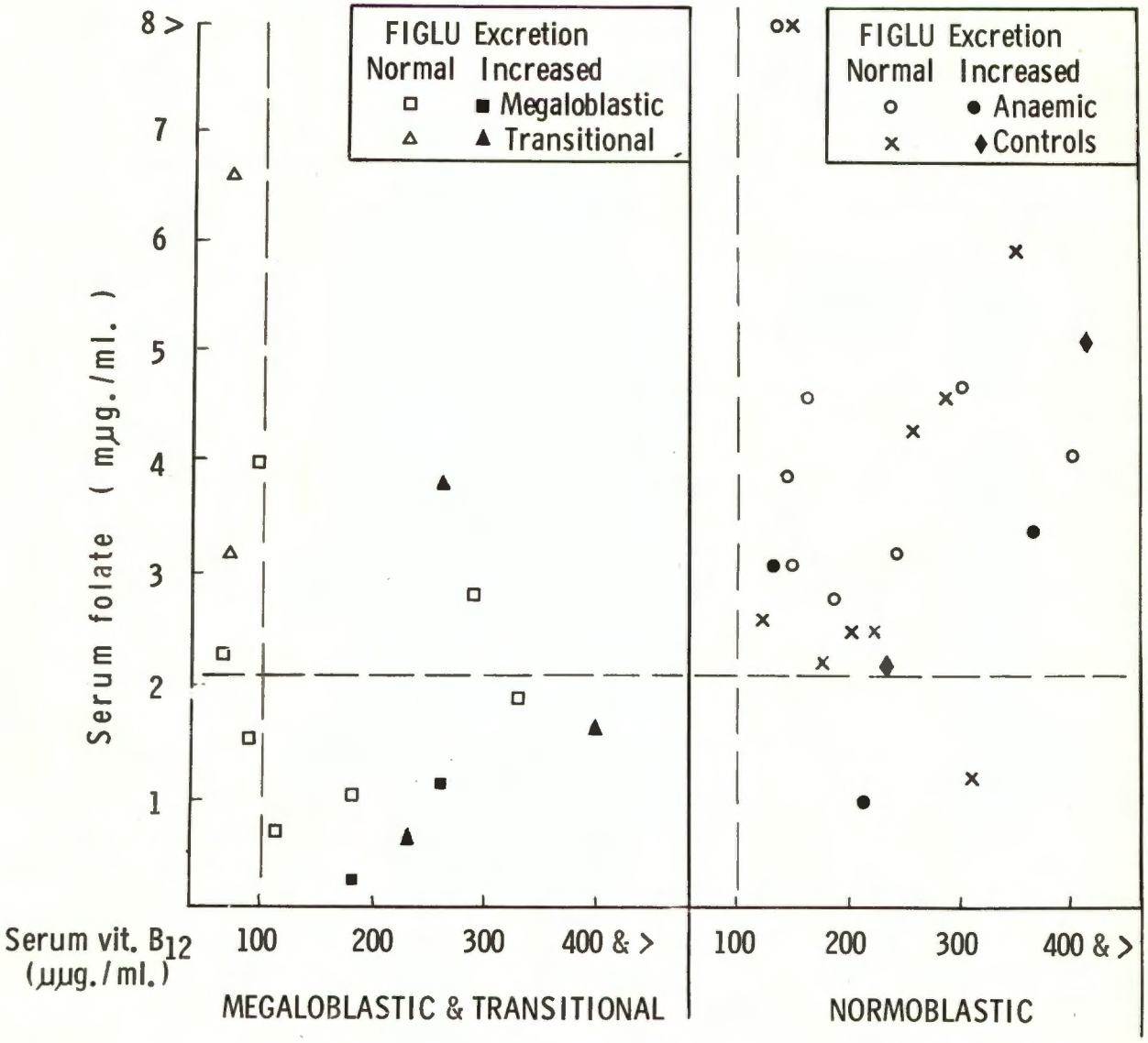

Fig.18 To show the relationship of serum folate & vitamin B_{12} levels, FIGLU excretion and erythropoiesis.

been treated with folic acid before bone marrow biopsy was performed.

(iii) Figlu excretion and the serum folate and vitamin B_{12} levels: Simultaneous estimation of the serum folate and vitamin B_{12} was carried out in 36 women who had a Figlu excretion test. Detailed results in the 36 women are presented in Fig. 18.

There were 14 women with bone marrow evidence of folic acid deficiency. Increased Figlu excretion was associated with an abnormal serum folate and a normal serum vitamin B_{12} level in 4 women and normal Figlu excretion was associated with a normal serum folate and reduced serum vitamin B_{12} levels in 4 others. Results in the remaining women with bone marrow evidence of folic acid deficiency were all to some extent contradictory, although some abnormality associated with megaloblastic change was present in all but one.

More than half the women with normoblastic erythropoiesis had normal Figlu excretion, a normal serum folate and a serum vitamin B_{12} level of 150 $\mu\mu$g/ml or more; 4 more had similar results except that the vitamin B_{12} level was equivocal. In the remaining women at least one parameter, increased excretion of Figlu being most frequent, was indicative of megaloblastic change.

It is concluded that these tests have a definite but limited value in the diagnosis of folic acid deficiency in pregnancy. With one exception the diagnosis of megaloblastic or transitional anaemia would have been made in every case by the use of all three tests but in a proportion of women with normoblastic anaemia one or more of these tests was abnormal.

THE ASSOCIATION OF MEGALOBLASTIC ANAEMIA AND IRON DEFICIENCY

The three tests used to assess the relationship of folic acid and iron deficiency were the MCHC, serum iron and the bone marrow iron.

THE MCHC

The MCHC results obtained in the 120 anaemic women are presented in Table 23.

TABLE 23

Shows the MCHC in Relation to Erythropoiesis

MCHC	Anaemic women with		
	Megaloblastic erythropoiesis	Transitional erythropoiesis	Normoblastic erythropoiesis
< 30%	10	9	23
30-30.9	5	2	18
31-31.9	2	4	9
32% or more	3	3	7
None	13	5	7
TOTAL	33	23	64

On the basis of a MCHC of less than 30% it would appear that approximately half the women with megaloblastic and transitional erythropoiesis were iron deficient. However levels above this are compatible with the diagnosis of iron deficiency and more than half the women with a normoblastic anaemia and evidence of iron deficiency had values of 30% or more.

Although a number of reports indicate that the MCHC does not alter during pregnancy (Rath et al., 1950; Lund, 1951) in the present study MCHC values below 32% were noted commonly in pregnant women with a normal Hb concentration. For example in 100 women all about 28 weeks pregnant with a Hb concentration of 11 g/100 ml or more, the MCHC was less than 32% in 18 and below 31% in 2; in none was it less than 30%. Whether MCHC values below 32% in these women were due to iron deficiency or technical error was not determined but the frequency with which they occurred reduced the diagnostic value of the MCHC and for this reason a lower limit of normal than that found in healthy non-pregnant controls was used. It is recognised however that considerable overlap exists and that MCHC levels between 30% and 32% may be associated with iron deficiency.

THE SERUM IRON CONCENTRATION

The serum iron results in the 120 anaemic women in this study are

presented in Table 24.

TABLE 24

Shows the Serum Iron Level Related to Erythropoiesis

Serum iron, µg/100 ml	Anaemic women with		
	Megaloblastic erythropoiesis	Transitional erythropoiesis	Normoblastic erythropoiesis
< 60	3	5	26
60-149	7	6	17
150 or more	9	4	6
TOTAL	19	15	49

A low serum iron was not common in women with megaloblastic anaemia. It is likely that this was partly due to previous iron treatment. Twenty-seven of the 33 women with this condition had been taking iron before the diagnosis was made; duration of therapy was not known in every case but 14 women had been taking oral iron for at least 4 weeks. Four had been given parenteral iron prior to bone marrow biopsy.

BONE MARROW IRON STORES

Bone marrow films of 45 women with megaloblastic and transitional anaemia were stained for iron and the results are given in Table 25.

TABLE 25

Relates Bone Marrow Iron Stores to Erythropoiesis

Erythropoiesis	Bone marrow iron						Total
	Absent	Normal		Increased			
	0	1	2	3	4	5	
Megaloblastic	7	0	6	10	2	0	25
Transitional	10	3	4	3	0	0	20
Normoblastic	40	4	1	0	1	0	46

While absent iron stores were noted in a number of women with megaloblastic and transitional erythropoiesis, the majority had normal or

increased iron in their bone marrow. Increased bone marrow iron occurred more commonly in women with unequivocal megaloblastic change whereas absent iron stores were noted frequently when erythropoiesis was transitional.

Thus although the peripheral blood film, MCHC and serum iron levels indicated that iron deficiency was present in 19 (58%) of the women with frank megaloblastic anaemia and 18 (78%) with transitional megaloblastic anaemia, examination of the bone marrow suggested that a considerable proportion had adequate or increased iron stores. Accumulation of bone marrow iron is observed in pernicious anaemia and is presumed to result from the arrest of erythropoiesis associated with megaloblastic erythropoiesis. These observations therefore are not contradictory and their significance will be discussed in detail later.

COMMENT

This study has shown that in certain respects women with folic acid deficiency differ from a control group which is not apparently deficient; these differences were always more striking when erythropoiesis was megaloblastic rather than transitional. In particular, there was a significant difference in social class distribution, parity and the incidence of a previous abortion when women with bone marrow evidence of folic acid deficiency and non-anaemic controls were compared. Results in women with normoblastic anaemia were usually intermediate between the megaloblastic and non-anaemic groups. There were no significant differences when the above factors were compared in the megaloblastic and normoblastic groups and the only significant difference when the normoblastic and non-anaemic control groups were compared, was in their parity. An increased number of twin pregnancies and a preponderance of blood group A were noted in women with frank megaloblastic anaemia but in each case the numbers were too small for statistical analysis.

All other features examined, and these include foetal and maternal complications, reputedly associated with folic acid deficiency, were shown

to be no more common in women with bone marrow evidence of folic acid deficiency than in the two control groups.

Two complications of pregnancy, infection and haemorrhage, were noted more frequently in the anaemic women but occurred as often in women who were folate deficient as in those with iron deficiency. As will be shown later, the evidence suggests that infection and haemorrhage are factors which contribute to anaemia and are not the consequence of it.

The results obtained in this study do not support the argument that anaemia due to folic acid deficiency is more harmful during pregnancy than at any other time. In contrast to some other series, the present study was carried out in a well-nourished community where socio-economic conditions are good. It is possible that certain reported complications such as ante-partum haemorrhage and foetal abnormalities (Gatenby and Lillie, 1960; Hourihane, Doyle and Drury, 1960; Hibbard, 1964) may arise because of associated conditions and not because of the folic acid deficiency per se.

In this study the diagnosis of folic acid deficiency was a morphological one based on finding classical or transitional megaloblasts in the bone marrow or peripheral blood films. Other abnormalities in the peripheral film, such as macrocytosis and hypersegmentation in particular, were usually reliable indications that bone marrow changes of folic acid deficiency were present, although this was not always the case in this and other series (Hansen, 1964; Chanarin, Rothman and Berry, 1965). It is important, however, to recognise that the absence of these changes does not rule out the diagnosis of megaloblastic anaemia. Iron deficiency commonly co-existed with folic acid deficiency and occasionally changes in the peripheral film were those of iron deficiency only.

The serum folate, serum vitamin B_{12} and urinary Figlu excretion all proved to be of some value in the diagnosis of megaloblastic erythropoiesis, but each had its limitations and no single test was specific enough for it to be used as a substitute for bone marrow biopsy. Estimation of the serum folate was the most useful test but it was our experience, like that of other workers (Ball and Giles, 1964) that in pregnant women abnormal

values were not confined to those with other evidence of folic acid deficiency. A low serum folate therefore cannot alone be considered evidence of such a deficiency. The diagnostic value of this test has been further reduced by finding normal serum folate levels in women with megaloblastic anaemia.

Despite the initial technical difficulties in establishing a method of folic acid assay which would give reasonably reproducible results, low levels of the serum folate using the Lactobacillus casei method of microbiological assay are very closely correlated with other evidence of folic acid deficiency when this is not associated with pregnancy (Mollin and Hoffbrand, 1965). This has also been the experience in our laboratory (Spray, 1964) so that the lack of correlation found in pregnancy is not likely to be due to faulty technique. Other factors peculiar to pregnancy such as hydraemia or a rapid uptake and utilisation because of the demands of the foetus might account for this and will be discussed later, but it is possible that other techniques might be more valuable in pregnancy. For example, the red cell or whole blood folate is a more accurate index of the body stores of folic acid than the serum folate and has been shown to correlate well with other evidence of folic acid deficiency (Hoffbrand, Newcombe and Mollin, 1966). Hansen (1964) considered the red cell folate to be the best biochemical test of folate deficiency in pregnancy, but Lowenstein et al. (1966) found that it correlated less well with "megaloblastic change" than the serum levels. It is likely that when minimal change in the bone marrow is the criterion for the diagnosis of megaloblastic anaemia, the serum folate only will be reduced. Different degrees of megaloblastic change in the bone marrow could explain apparently dissimilar results using the serum and red cell folate levels. In the investigation of anaemia in pregnancy estimation of serum and red cell folate simultaneously should give more information than either test alone.

Results similar to those in the present study were obtained by Ball and Giles (1964), the serum folate being normal in 43% of their patients with megaloblastic anaemia in pregnancy. By direct estimation of the labile factor in the serum of these women, by means of simultaneous assay

of duplicate samples, one with the other without ascorbic acid, Ball and Giles reduced the incidence of normal values in women with megaloblastic anaemia in pregnancy to 4%. To date no other reports have been published which confirm their findings but their evidence suggests this could be an important modification. Although Chanarin, Rothman, Ardeman and Berry (1965) questioned the sensitivity and reproducibility of folic acid assay when values as low as 1.0 mμg/ml are being estimated, it has been the experience in our laboratories that serum folate levels of this order are reproducible, not only on repeat assay but on samples of serum taken from the same patient on separate occasions.

Neither the red cell folate nor labile factor was estimated during the present study. Both investigations appear worthy of further assessment.

The serum vitamin B_{12} level was seldom within the range found in pernicious anaemia in pregnant women in the present study. When this occurred and erythropoiesis was examined megaloblastic change was usually present. However 8% of a group of healthy pregnant controls with a normal Hb concentration in whom bone marrow biopsy was not performed had a serum vitamin B_{12} level below 100 μμg/ml. Nevertheless when used in the investigation of anaemic pregnant women, levels of this order were of diagnostic significance, and as Table 20 (page 49) shows were as reliable a pointer to the diagnosis of megaloblastic and transitional anaemia in pregnancy as a low serum folate, but were found less commonly. Serum vitamin B_{12} levels between 100 and 150 μμg/ml appeared to be of little significance and were found in the same proportion of anaemic women, irrespective of erythropoiesis, and of normal controls. The significance of low levels of serum vitamin B_{12} in relation to folic acid and B_{12} metabolism in pregnancy will be discussed in detail later.

Low voltage electrophoresis was used in the present study to estimate the urinary excretion of Figlu. This is a simple and reliable method but results obtained indicated that this test was of limited value in the investigation of anaemia during pregnancy. Agreement was poor in megaloblastic anaemia where normal excretion occurred in more than half of the cases. Similar results have been reported by a number of other workers

(Chanarin, Rothman and Watson-Williams, 1963; Chanarin, 1964; Karthigaini, Gnanasundaram and Baker, 1964; Hansen, 1966) but in two large studies of anaemic pregnant women, the Figlu excretion correlated very well with the bone marrow morphology (Hibbard, 1964; Scott and Sommerville, 1965).

The reason for these differences is not clear. It does not appear to be due to the different methods used to measure the Figlu excretion. Hansen (1966) compared high voltage electrophoresis with enzymatic assay and found very close agreement. Hibbard used high voltage whereas Scott and Sommerville used low voltage electrophoresis. High voltage electrophoresis is more sensitive and has the added advantage that it separates the Figlu and glutamic acid spots, but it would appear that the sensitivity of the low voltage method is adequate. Hibbard (1964), using high voltage electrophoresis, estimated Figlu excretion in a group of women with megaloblastic anaemia in pregnancy and found that all excreted more than 40 μg/ml. With care, low voltage electrophoresis can detect concentrations of Figlu as small as 20 μg/ml. It is concluded therefore that although some methods are more sensitive than others in practice this is of little importance.

It is doubtful whether simultaneous estimation of urocanic acid, an earlier product in the breakdown of histidine to glutamic acid, is likely to add to the value of this investigation (Chanarin, 1964; Mollin and Hoffbrand, 1965) and in the present study was not attempted.

Altered metabolism of histidine during pregnancy has been shown to be an important factor in making Figlu excretion unreliable after a loading dose of histidine, but this does not explain the differing assessment of its value when studied in apparently comparable groups of women. Estimation of Figlu excretion without histidine loading (Lewis, Moore and Morris, 1962) appears to be a more physiological approach but here too there was considerable overlap between normal and anaemic women.

An interesting association between normal Figlu excretion and low levels of serum vitamin B_{12} when erythropoiesis is megaloblastic or transitional was observed. The significance of this in relation to the metabolism

of folic acid and vitamin B_{12} in pregnancy will be discussed in detail later.

Whatever the reasons for the poor correlation of Figlu excretion and megaloblastic and transitional megaloblastic anaemia in pregnancy, the serum folate was the more valuable investigation. In the course of this three-year study the serum folate was estimated in a large number of pregnant women, both healthy and anaemic. It has been possible by the use of a record linkage scheme in operation here in Oxford to add to haematological information already available, further information about these women. All results have been correlated and resulting information will be presented in the following chapter.

III

THE RELATIONSHIP OF THE SERUM FOLATE DURING PREGNANCY AND OTHER FACTORS

INTRODUCTION

Results in the previous chapter have shown that estimation of the serum folate is worthwhile in the investigation of anaemia during pregnancy. Although low values were not diagnostic of megaloblastic erythropoiesis, a good correlation between a low serum folate and erythropoiesis was obtained. Results in this study, and in a number of other series however have shown that abnormal values are not confined to megaloblastic anaemia. When a dietary deficiency of folic acid is developing a fall in the serum folate occurs early and precedes the development of megaloblastic change in the bone marrow (Herbert, 1962). Although the relationship between the serum folate and folic acid deficiency in pregnancy may be more complex than it is in a simple dietary deficiency, and the significance of normal values in the presence of megaloblastic anaemia difficult to explain, it is likely that in pregnancy also the serum folate is a useful index of folic acid deficiency.

Since the serum folate is more readily examined than the bone marrow, results can easily be obtained in large numbers of patients, and in the present study more than 800 women had their serum folate measured on one or more occasions by the author. All these results have been related to certain features of pregnancy to see if any significant associations were present. The particular factors examined include gestation period, Hb concentration, parameters of iron deficiency and serum vitamin B_{12} levels, social class, parity, and various maternal and foetal complications. The results will be described in the present chapter.

MATERIAL AND METHODS

The serum folate of 830 pregnant women was assayed by the author using L. casei as test organism. A detailed description of the method used has been published by Spray (1964) and is included in Appendix III. The serum folate in healthy non-pregnant controls studied by him ranged from 2.1 mμg/ml to 28.0 mμg/ml with a mean of 7.8 mμg/ml.

The majority of the pregnant women whose serum folate was estimated were unselected and were attending the ante-natal clinic of the Nuffield Department of Obstetrics at the Radcliffe Infirmary for routine ante-natal care. A small proportion consisted of anaemic women who had been referred for investigation, and of these, 32 had bone marrow changes suggesting folic acid deficiency. Results in these 32 women have been excluded from Tables 26 to 29 and Figs. 18 to 20 inclusive since the purpose of correlating the serum folate with the Hb concentration and with gestation period was to see if they were related in the absence of a manifest deficiency of folic acid. Results in these women are included in the other tables and figures in this chapter. With the exception of women in whom the excretion of urinary Figlu was being estimated, patients were not fasting at the time of venepuncture. A careful check on the intake of any vitamin preparation which might contain folic acid was made and any results in which this was a possibility have been excluded.

CHOICE OF A SERUM FOLATE LEVEL TO ALLOW FOR ANALYSIS OF RESULTS

The number of women in whom definitely abnormal values (i.e. below 2.1 mμg/ml) were obtained was often too small for adequate analysis and it therefore became necessary to choose another serum folate level to divide low values from "normal" values. It was decided to compare values lying below a representative mean serum folate with those lying above it. As will be shown in the first part of the chapter, mean values varied from 3.8 to 5.3 mμg/ml depending on the gestation period and haemoglobin at the time of assay. When serum folate results of the anaemic and non-anaemic women in the last trimester of pregnancy shown in Fig. 21 were combined and the mean calculated, a mean value of 4.0

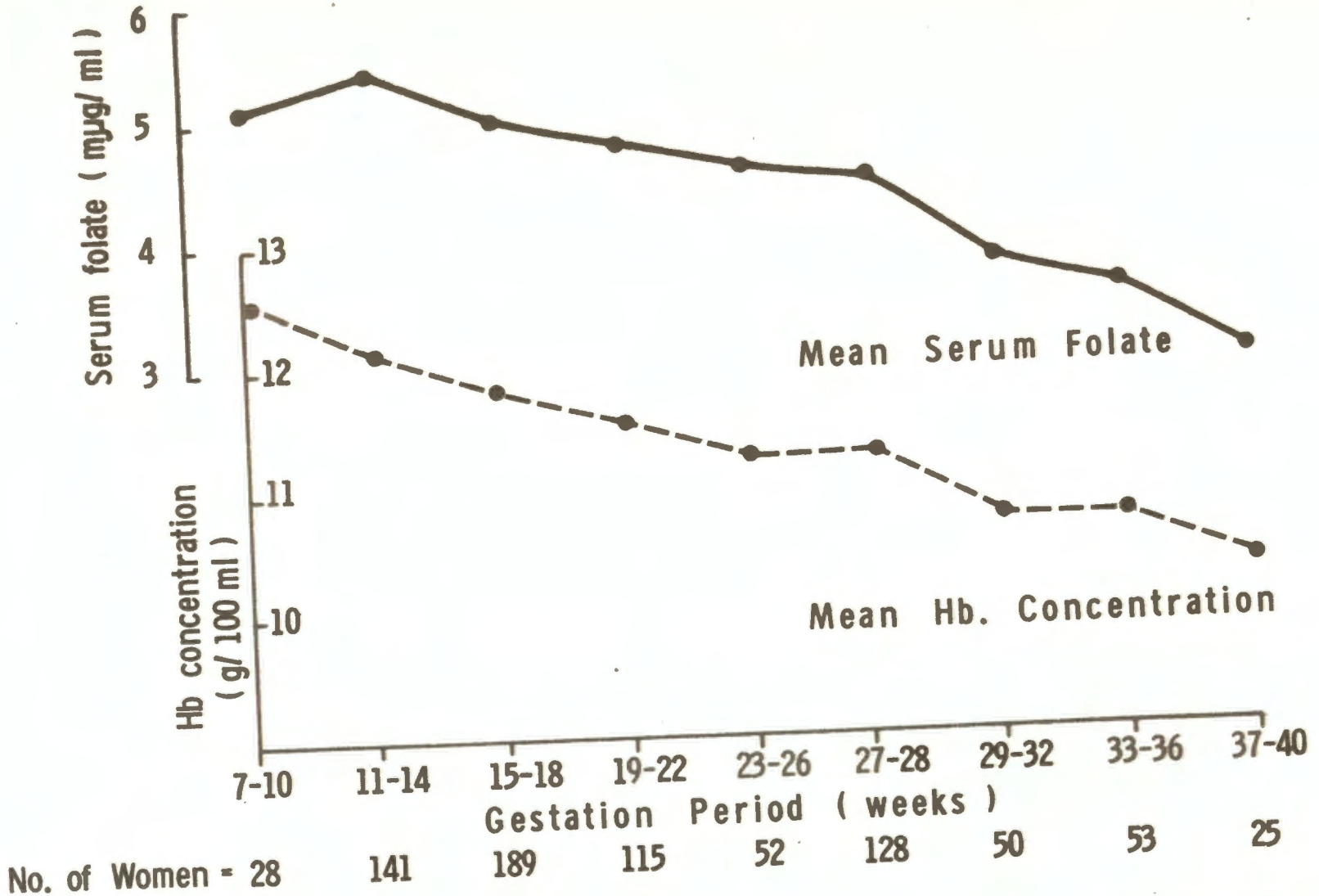

Fig. 19 Relates the mean serum folate and mean Hb concentration with gestation period at initial assay.

mμg/ml was obtained. To simplify the analysis of results it was decided to use this mean figure as an arbitrary dividing line for all gestation periods. In practice this proved to be satisfactory since even with subdivision there remained sufficient values to enable statistical comparisons to be made.

RESULTS

The serum folate of 382 women whose Hb concentration at the time of venepuncture was at least 12 g/100 ml has been used to establish the mean value and range in healthy pregnant women. The serum folate levels ranged from 1.0 mμg/ml to 13.0 mμg/ml, the mean being 4.9 mμg/ml; 8.4% had levels below 2.1 mμg/ml, the lower limit of normal in non-pregnant controls.

Results in all women studied during pregnancy, with the exception of those known to have a megaloblastic or transitional megaloblastic anaemia, are shown in Fig. 19. Only one value for each patient has been included here, that obtained at the first assay being used. Results have been grouped according to gestation period at the time of venepuncture and the mean Hb concentration at that stage has been calculated and is plotted below the mean serum folate levels.

The fall in the serum folate as pregnancy progresses is obvious and grows more marked after the 28th week. The results shown in Fig. 19 suggest that there may be two principal reasons for this. Firstly, it is possible that the serum folate and gestation period are directly related and that a fall in the serum folate is associated with advancing pregnancy per se. However, the simultaneous fall in the Hb concentration due in part to an increased number of low haemoglobin values, suggests alternatively that reduced serum folate levels are related directly to the Hb concentration. Women, whose results are shown in Fig.19, were not all unselected. Although those known to have bone marrow evidence of folic acid deficiency have been excluded, a number had been referred for blood tests because of anaemia and since bone marrow biopsy was not always

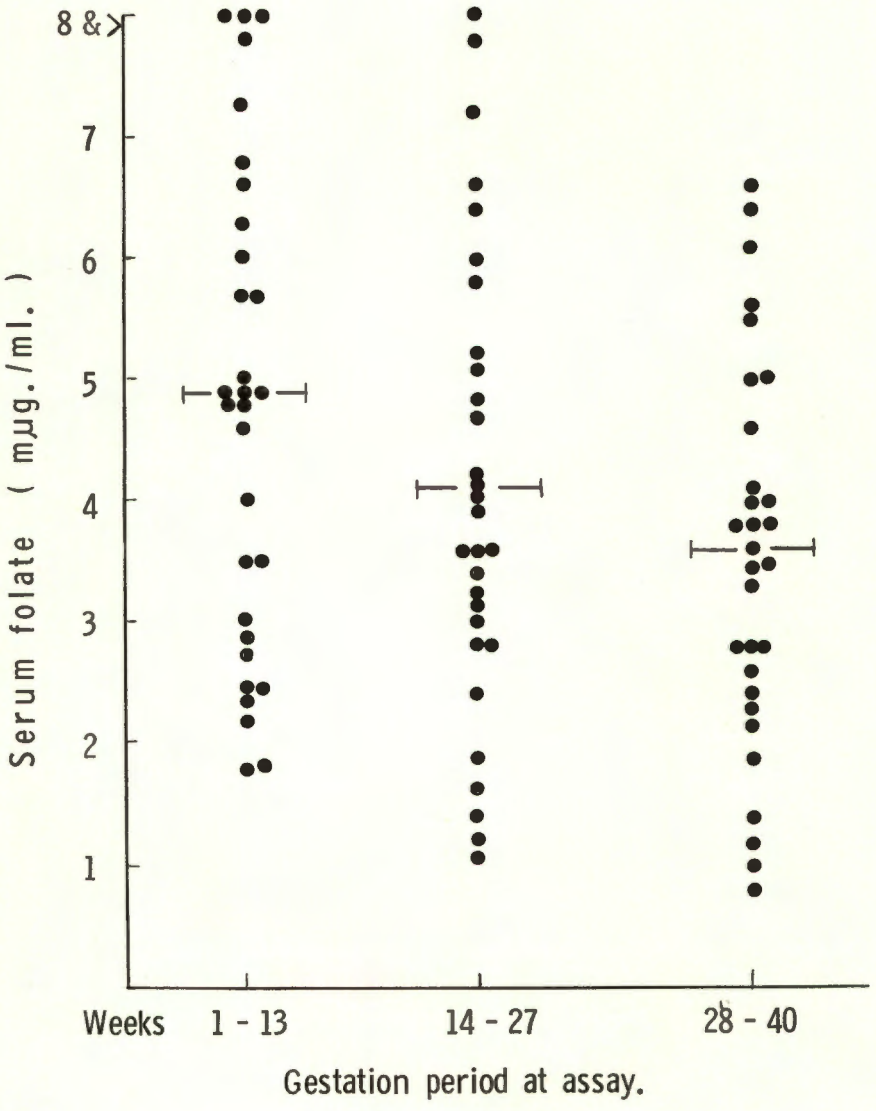

Fig. 20 Shows serum folate levels in 30 women in whom serial estimations were made in each trimester of pregnancy.

performed, some undiagnosed cases of megaloblastic anaemia may have been included.

The relationship of these two factors — gestation period and the Hb concentration — to the serum folate will now be examined separately.

THE RELATIONSHIP OF THE SERUM FOLATE TO GESTATION PERIOD

Serial observations in each trimester of pregnancy were obtained in 30 pregnant women. All participated in a therapeutic trial to be described in Chapter IV and had taken little or no iron before the 28th week of pregnancy; none were examined in the first place because of anaemia and are therefore a random sample of women attending the Radcliffe Infirmary antenatal clinics. The results presented in Fig.20 confirm that there is a fall in the serum folate with each succeeding trimester. However, there was some individual variation in this and, in approximately one-third, the serum folate actually increased or remained unchanged as pregnancy progressed.

The fall in the mean serum folate is considerably less than that noted previously, however, suggesting that the more marked fall in the last few weeks of pregnancy noted in Fig.19 was at least partly due to the inclusion of a number of patients with undiagnosed megaloblastic anaemia. A small number of women with a Hb concentration below 11 g/100 ml in the last 12 weeks of pregnancy have been included in Fig. 20 , however, and again it is possible that these results could have been influenced by this factor.

Undiagnosed megaloblastic erythropoiesis must be rare in women with a normal Hb concentration and therefore the serum folate of 382 women whose haemoglobin was 12 g/100 ml or more at assay has been related to stage of pregnancy. The results are presented in Table 26 and in Fig.21 and have been grouped together into trimesters because the numbers are unevenly distributed over shorter periods. The results are similar to those shown in Fig.20 and, once again, a fall in the mean, and an increase in the number of women with low values, takes place as pregnancy advances.

THE RELATIONSHIP OF THE SERUM FOLATE AND THE HAEMOGLOBIN

Although the preceding results have indicated that the serum folate and gestation period are related, it is apparent that when the additional factor of a low haemoglobin is also present the fall in the serum folate is likely to be greater. While both the serum folate and the haemoglobin might have fallen as a result of folic acid deficiency, the possibility that both have been affected by a third factor such as hydraemia must be examined. This has been done in two ways. Firstly the serum folate levels in anaemic women at all stages of pregnancy have been compared with results in a non-anaemic group to see if the difference between anaemic and non-anaemic groups is present throughout pregnancy, and secondly, the serum folate levels have been related directly to the Hb concentration in a group of women with the same gestation period at a time when folic acid deficiency would be unlikely to be associated with a significant degree of anaemia.

Results in Fig.21 and Tables 26 and 27 show that the mean serum folate in anaemic women and non-anaemic women in the first two trimesters of pregnancy did not differ. In the last trimester not only is the mean serum folate lower in the anaemic women but the proportion with values below the lower limits of normal for non-pregnant controls is increased although this difference is not significant. However when the number of values lying below the mean serum folate for women in the third trimester, 4.0 mμg/ml, is compared in the anaemic and non-anaemic groups, a significant difference is noted (Table 28). Thus there is no correlation between the haemoglobin and the serum folate in the first two trimesters but a significant association present in the later stages of pregnancy.

That this association exists only in the latter weeks of pregnancy is shown in Table 29 where the Hb concentration is related to the serum folate results in an unselected group of women all approximately 28 weeks pregnant at the time of assay. These results show that there is no significant difference when the proportion of reduced serum folate values (< 4 mμg/ml) associated with a low Hb concentration (< 11 g/100 ml) and a normal Hb concentration are compared ($\chi^2 = 1.176$; $n = 1$, $P > 0.05$).

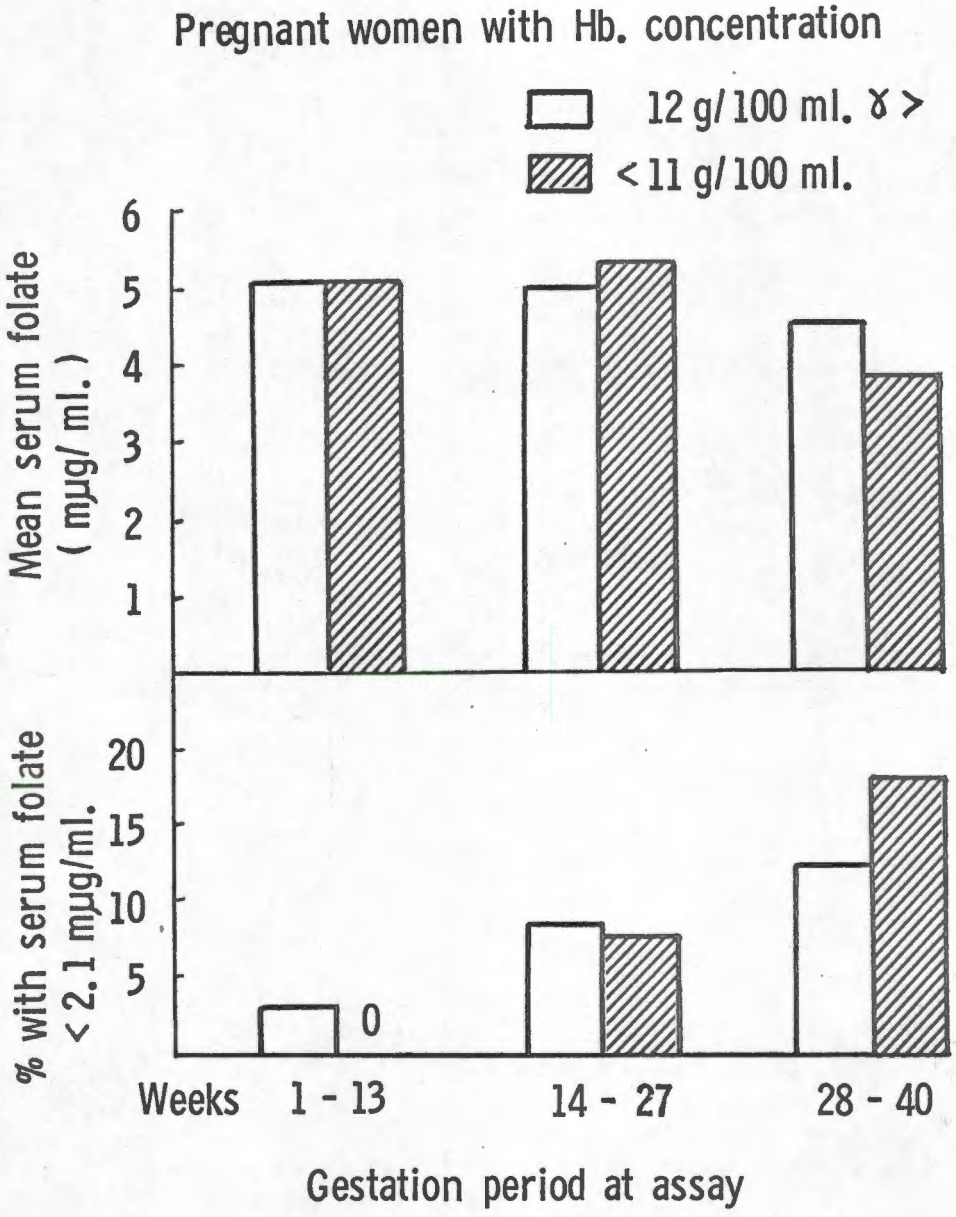

Fig. 21 Relates the serum folate levels in normal and anaemic pregnant women to gestation period.

TABLE 26

To Show Relationship of Serum Folate to Gestation Period in 382 Women With a Hb Concentration of 12 g/100 ml or More

Gestation period, weeks	Number of readings	Serum folate, mμg/ml			
		< 2.1 mμg/ml	< 4 mμg/ml	Mean	Range
1-13	72	2 (2.8%)	26 (36.1%)	5.1	1.3-12.4
14-27	208	18 (8.6%)	81 (38.9%)	5.0	1.0-13.0
28-40	102	12 (11.8%)	43 (42.2%)	4.5	1.0-11.6

TABLE 27

To Show Serum Folate Related to Stage of Pregnancy in 270 Pregnant Women with a Hb Concentration Below 11 g/100 ml

Gestation period, weeks	Number of readings	Serum folate, mμg/ml			
		< 2.1 mμg/ml	< 4 mμg/ml	Mean	Range
1-13	14	- (0%)	5 (35.7%)	5.1	2.8-8.5
14-27	80	6 (7.5%)	27 (33.7%)	5.3	1.1-13.6
28-40	176	32 (18%)	104 (59.1%)	3.8	0.4-12.4

TABLE 28

To Compare Incidence of Low Serum Folate Levels in Relation to the Hb Concentration in the Third Trimester

Hb concentration g/100ml	Number of readings	Serum folate, mμg/ml			
		< 2.1 mμg/ml		< 4 mμg/ml	
< 11	176	32 (18%)	$\chi^2 = 1.996$ n = 1	104 (59.1%)	$\chi^2 = 7.493$ n = 1
⩾ 12	102	12 (11.8%)	P > 0.05 Not significant	43 (42.2%)	P < 0.01 * Significant

It is concluded therefore that there is no direct association between the Hb concentration and the serum folate early in pregnancy but there appears to be some relationship at a stage of pregnancy when a deficiency of folic acid might be a factor in producing anaemia.

TABLE 29

To show the Relationship of the Serum Folate and Hb Concentration in 363 Unselected Women All About 28 Weeks Pregnant

Serum folate mμg/ml	Hb concentration, g/100 ml				Total
	< 10.0	10-10.9	11-11.9	⩾12	
< 1.0	-	-	-	-	-
1-2.0	- } 59%	10 } 52%	14 } 47%	8 } 48%	32 } 49%
2.1-3.9	13	37	61	36	147
⩾4.0	9	43	84	48	184
TOTAL	22	90	159	92	363

SERUM FOLATE LEVELS AND IRON DEFICIENCY

In the course of the therapeutic trial to be described in Chapter IV the Hb concentration, MCHC, serum folate and serum iron were estimated frequently in a large sample of pregnant women. These tests were usually performed at the booking visit early in pregnancy, again at 28 weeks gestation, and finally on one or more occasions in the weeks preceding delivery. In this way many results were obtained and it has been possible to examine the relationship of the serum folate to the MCHC and serum iron in a large number of pregnant women.

Serum folate and the MCHC: The serum folate and MCHC were both estimated simultaneously several times during pregnancy. When the MCHC did not fluctuate from normal (32% or more) to abnormal (less than 30%) throughout pregnancy only the first result has been included. However, it was not uncommon for the MCHC to be normal at one stage of pregnancy and abnormal at another, and if this occurred, the serum folate associated with a normal MCHC as well as the serum folate associated with an abnormal MCHC were both included once in the appropriate section. Thus two different sets of results in one patient could be used.

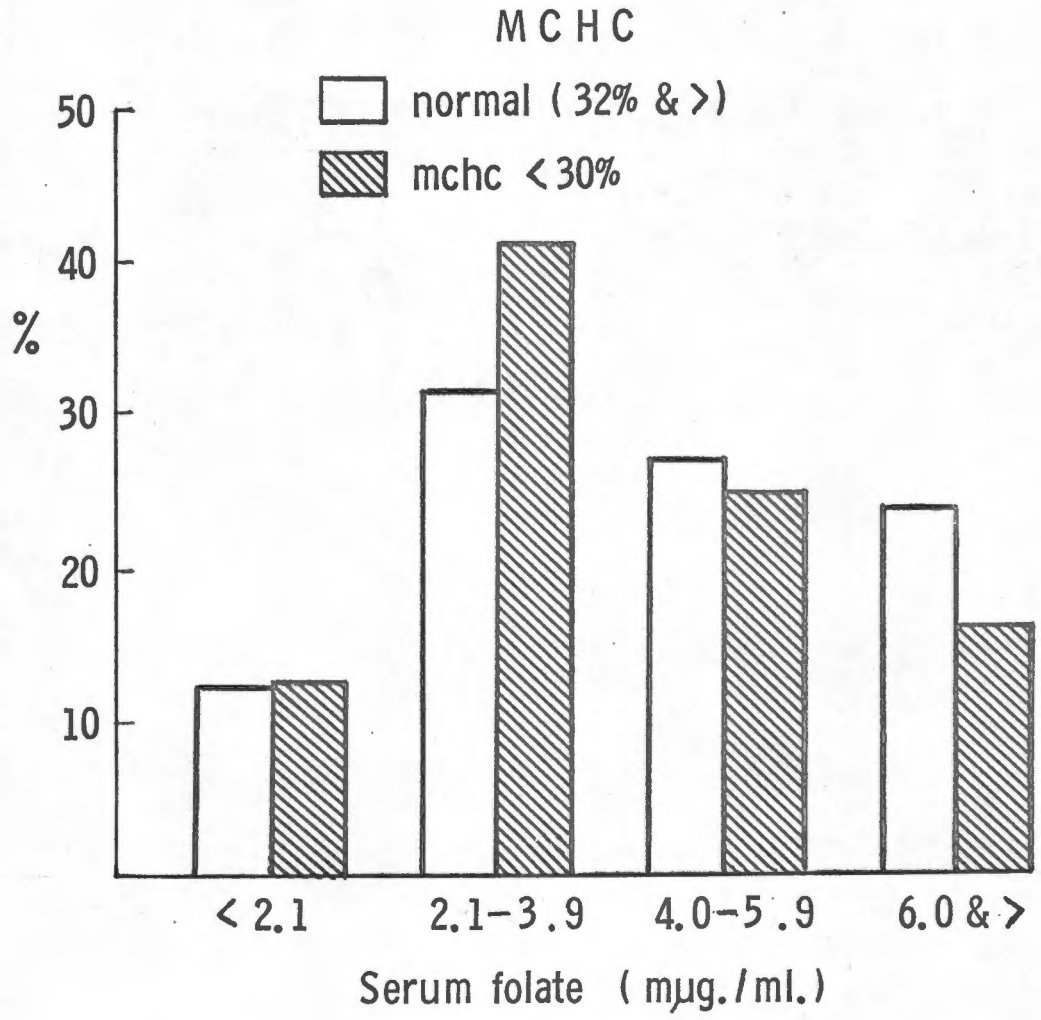

Fig. 22 Examines the relationship between :-

a. the serum folate and a normal MCHC (556 results)
and b. the serum folate and a low MCHC (112 results)

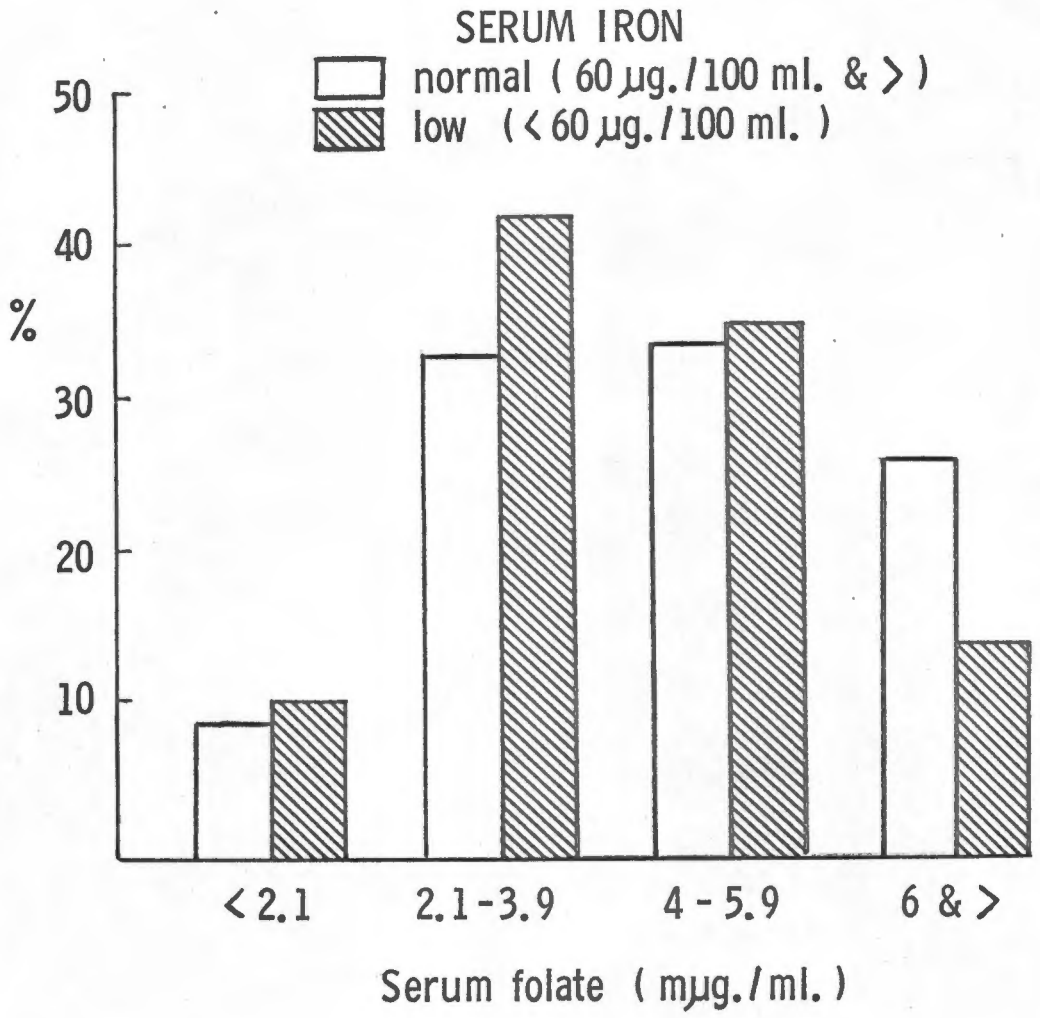

Fig. 23 Examines the relationship between :

a. the serum folate and normal serum iron (502 results)
and b. the serum folate and a low serum iron (302 results)

In many women the serum folate was associated with an MCHC between 30% and 31.9%; these results have not been included here since the significance of MCHC values in that range is questionable.

The total number of MCHC and serum folate values which were related was 668 and the results are presented in Fig. 22 and are briefly summarised in the following table:

TABLE 30

To Show Serum Folate Related to MCHC

MCHC	Serum folate, mμg/ml			Significance of the difference
	< 4	⩾ 4	Total	
< 30%	60 (53.6%)	52	112	$\chi^2 = 3.396$ n = 1 P > 0.05 Not significant
⩾ 32%	245 (44.1%)	311	556	

Although there is a tendency for low serum levels to be more common when the MCHC was abnormal this was not significant.

Serum folate and serum iron levels: As with MCHC results, several pairs of serum iron and serum folate results were obtained in the same patient. If the serum iron remained normal (60 μg/100 ml or more) only the first pair of results has been used and similarly, if the serum iron remained low, only one result relating the serum folate and serum iron has been included. However, a considerable number of women had both a normal and abnormal serum iron at different stages of pregnancy and under those circumstances, two sets of results from the one patient have been used. A total of 804 sets of paired serum iron and serum folate results have been obtained, and the relationship of the serum folate to a normal and low serum iron level has been examined (Fig.23) and is summarised in Table 31.

A serum folate below 4 mμg/ml occurred more frequently when the serum iron was low than when it was normal and the difference is significant. While it is recognised that a fall in the serum iron during pregnancy is not diagnostic of iron deficiency, the significant association between

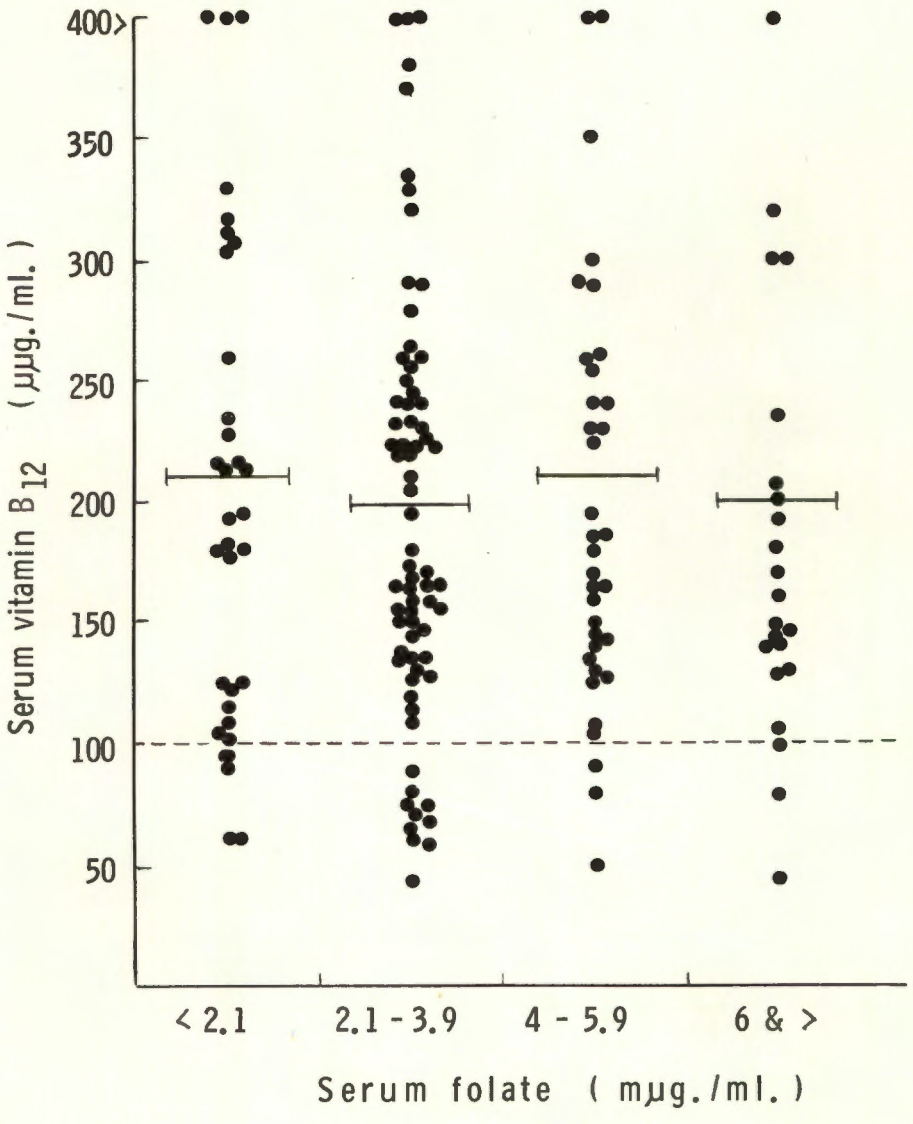

Fig.24 Relates serum folate to serum vitamin B_{12} levels.

TABLE 31

To Show Serum Folate Related to Serum Iron

Serum iron, µg/100 ml	Serum folate, mµg/ml			Significance of the difference
	< 4	⩾ 4	Total	
< 60	157 (52.0%)	145	302	$\chi^2 = 8.470$ n = 1
⩾ 60	208 (41.4%)	294	502	P < 0.01 Significant

reduced serum folate and serum iron levels would appear to indicate that a common factor has resulted in their fall.

SERUM FOLATE AND SERUM VITAMIN B_{12} LEVELS

Results in the previous chapter have shown that in megaloblastic anaemia of pregnancy there is a reduction in the serum vitamin B_{12} level and an increase in the number of values in the range usually associated with a deficiency of vitamin B_{12}. Similarly abnormal serum folate levels occurred more frequently in the megaloblastic group and although both abnormalities were noted independently of each other, their association in megaloblastic anaemia of pregnancy suggested that the serum vitamin B_{12} and serum folate levels might be related.

In addition to the 69 women shown in Fig.19 (Chapter II, page 48) in whom the bone marrow morphology was examined, simultaneous assay of the serum folate and serum vitamin B_{12} was made on a further 90 pregnant women who did not have a bone marrow biopsy. The results in all 159 women are presented in Fig. 24.

The mean serum vitamin B_{12} and range of values do not alter with different serum folate levels and it is obvious that there is no direct relationship between the serum folate and serum vitamin B_{12} levels during pregnancy.

The Hb concentration at the time of assay was known in the 159 women shown in Fig. 24 and is related to the serum vitamin B_{12} level in Table 32.

Results shown in this table indicate that the serum vitamin B_{12} is not

TABLE 32

Relates the Serum Vitamin B_{12} and Hb Concentration in 159 Women in whom both the Serum Folate and the Vitamin B_{12} were Estimated

Hb concentration, g/100 ml	Number of results	Serum vit. B_{12}, μμg/ml < 100		< 150		Mean serum vitamin B_{12} μμg/ml	
< 9.0	26	4	15%	12	46%	182.6	206.4
9-9.9	49	5	10%	13	27%	236.9	
10-10.9	23	4	17%	11	48%	168.3	
11-11.9	19	0	0%	5	26%	234.7	198.9
⩾12	42	6	14%	15	36%	182.7	
TOTAL	159	19	12%	56	35%		

related to the Hb concentration. There is no association between a fall in the haemoglobin and low vitamin B_{12} levels.

THE RELATIONSHIP OF THE SERUM FOLATE AND CLINICAL FEATURES OF MEGALOBLASTIC ANAEMIA IN PREGNANCY

In Oxford a record linkage study is in progress which aims at linking the medical records of patients from their birth until death. As part of the scheme a survey of the maternity services in this district has been carried out by Dr Michael Hobbs and details of all patients delivering in the Oxford area were entered on an 80 column punch card. In 742 of the 830 women in whom folate assay was performed, it was possible to match up this card with another bearing the hospital number, serum folate and gestation period at assay. A standard IBM sorting machine was used to relate the serum folate to other factors, and by this means the relationship of the serum folate and a number of possible complications or associations of megaloblastic anaemia in pregnancy was examined.

Two factors were taken into consideration when grouping values together and assessing the significance of an alteration in their distribution.

i. **Gestation period at assay.** It was a possibility that a reduced serum folate might be related to a particular factor at one stage of pregnancy only. It was necessary therefore to sub-divide results obtained throughout pregnancy according to gestation period at assay. When the number of values in each week of pregnancy were examined and grouped, they fell into three distinct groups which were of reasonable size; these three different gestation periods were (a) the early booking visit; (b) about the 28th week; and (c) the latter weeks of pregnancy. Results obtained at these three gestation periods have been related separately to the different factors.

A booking visit before the 19th week of pregnancy was made by 385 women and all were interviewed by the author and had blood taken for routine tests and folic acid assay.

After the 19th week only a small number of women were seen at the ante-natal clinic until the 28th week, when a total of 350 women attended the Radcliffe Infirmary for special interview and blood test. This visit was made to receive iron and folic acid tablets.

Visits to the ante-natal clinic in the latter weeks of pregnancy usually begin at the 32nd week. Some time between the 32nd week and term, 231 women, none of whom were given folic acid, had blood taken for assay. A considerable number of women had their serum folate estimated after treatment with folic acid was begun at 28 weeks but none of these later results have been included in this section.

ii. **Multiple values.** In 380 women the serum folate was estimated on more than one occasion and repeat readings have only been included in tables when these fell into different gestation period groupings. If two or more values per patient were available within a given gestation period, only the initial one was included. For example serum from a patient (R.I. No. 42869) was taken for assay at the 18th, 28th, 35th and 39th weeks of pregnancy. The value at 18 weeks and at 28 weeks each fell into the first two gestation grouping; only the value at 35 weeks was included in the third grouping, the value at 39 weeks being excluded. This was necessary to avoid bias resulting from repeat folate estimation because an abnormal

result had been obtained.

In the tables which follow therefore, results have been grouped and are presented in that form. The numbers of women in each gestation period are constant throughout; 385 in the period before the 19th week, 350 at the 28th week and 231 from the 32nd week onward, but in a varying number in each table, the specific information sought was lacking.

Once again, a serum folate of 4.0 mμg/ml has been used as a dividing line to allow statistical analysis to be carried out. In the tables which follow, only values lying below 4.0 mμg/ml and the total in each group have been shown. Full details of these results are given in Appendix VI.

Serum folate related to social class: As before, this has been assessed on the husband's occupation. A miscellaneous group of women whose husbands' occupations were classified separately (for example students, armed forces) or not stated, is not included in Table 33.

TABLE 33

Relates the Serum Folate to Social Class

Serum folate mμg/ml	Gestation period: Before 19th week			At 28th week			After 32nd week		
	Social class I & II	III	IV & V	Social class I & II	III	IV & V	Social class I & II	III	IV & V
< 4	25 (40%)	52 (39%)	35 (35%)	23 (43%)	63 (47%)	52 (54%)	16 (57%)	52 (55%)	40 (62.5%)
⩾ 4	38	83	65	30	71	44	12	43	24
TOTAL	63	135	100	53	134	96	28	95	64
Comment	No difference			$\chi^2 = 1.900$; n = 2 P > 0.05 Not significant			No difference		

There was no correlation between social class and the serum folate. The absence of social classes I and II noted in women with megaloblastic anaemia was not supported by a similar observation in women with a reduced serum folate. A trend was apparent at 28 weeks but was not significant.

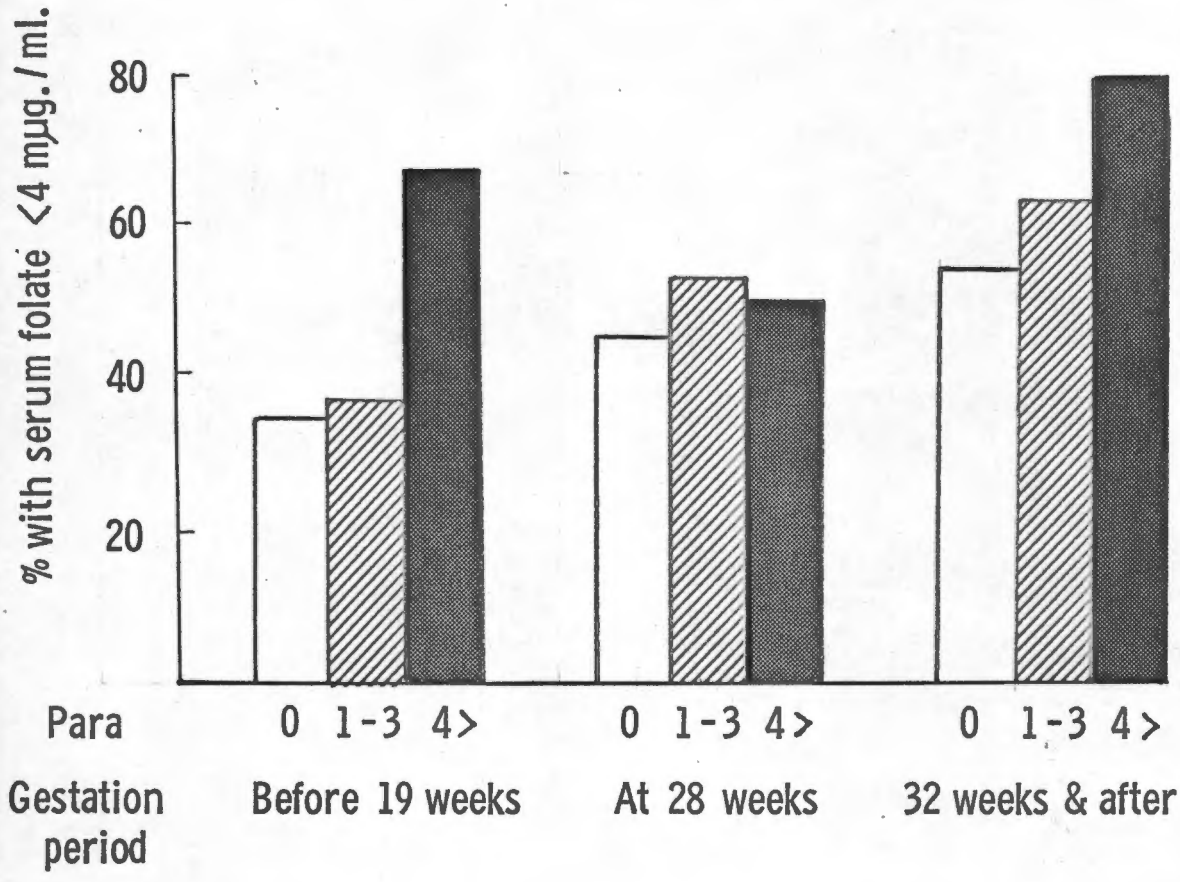

Fig. 25 Examines the relationship between parity and serum folate levels at different gestation periods.

Serum folate related to parity: Serum folate results are related to folate in Table 34 and Fig. 25.

TABLE 34

Relates Parity to the Serum Folate

Serum folate, mμg/ml	Gestation period: Before 19th week — Parity 0	1-3	⩾4	At 28th week — Parity 0	1-3	⩾4	From 32nd week — Parity 0	1-3	⩾4
< 4	64 (35%)	59 (37%)	16 (67%)	81 (44%)	76 (53%)	10 (50%)	50 (54%)	66 (62%)	22 (79%)
⩾4	120	101	8	101	68	10	43	40	6
TOTAL	184	160	24	182	144	20	93	106	28
Comment	$\chi^2 = 9.278$; n = 2 P < 0.001 * significant			No difference			$\chi^2 = 5.737$; n = 2 P > 0.05 Not significant		

A reduced serum folate early in pregnancy occurred more frequently in women in their fifth or subsequent pregnancy and supports the observation that parity and megaloblastic anaemia in pregnancy are related. At 28 weeks no such correlation was obtained. In the last 8 weeks a trend towards results in early pregnancy was noted but was not significant. When direct comparison between the number of low values in primiparous women and women in their fifth or subsequent pregnancy was made, however, the difference was significant ($\chi^2 = 5.496$; n = 1; P < 0.05).

Serum folate related to a history of a previous abortion: A past history of an abortion or stillbirth was related to the serum folate and the results are presented in Table 35.

The serum folate differed in women with a past history of abortion or stillbirth from those with no such history. Both in early and late pregnancy there was a tendency for a reduced serum folate to be associated with a positive history but neither association was significant. The serum folate was estimated in 6 women (between 12 and 18 weeks pregnant at the time) whose pregnancy later ended in abortion, although at the time of assay there was no indication of this. The results in these 6 women were all

TABLE 35

Relates a History of Previous Abortion or Stillbirth to the Serum Folate

Serum folate mμg/ml	Gestation period: Before 19th week		At 28th week		From 32nd week	
	Previous misc. or SB		Previous misc. or SB		Previous misc or SB	
	No	Yes	No	Yes	No	Yes
< 4	100 (35%)	30 (47%)	141 (47%)	23 (50%)	104 (57%)	31 (72%)
⩾ 4	190	34	157	23	78	12
TOTAL	290	64	298	46	182	43
Comment	$\chi^2 = 3.063$ n = 1 P > 0.05 Not significant		No difference		$\chi^2 = 3.2392$ n = 1 P > 0.05 Not significant	

above the lower limits of normal (6.0, 5.1, 5.0, 4.7, 4.4, 2.5 mμg/ml). In 2 others assay was performed after bleeding had commenced and in both the serum folate was normal (5.1 and 4.4 mμg/ml).

Serum folate levels related to ante-partum haemorrhage: No evidence that ante-partum haemorrhage was a complication of megaloblastic anaemia was obtained in this series. The serum folate was known in 21 women who subsequently had an ante-partum haemorrhage. A total of 35 results was obtained because assay was carried out on 2 or more occasions in 13 patients. For comparison the percentage of all women with a serum folate below 4 mμg/ml in each of the three gestation periods is shown at the side. The results are presented in Table 36.

The number of cases is small, particularly in the latter weeks of pregnancy, so only limited conclusions can be drawn. The results however show that the serum folate was not reduced in the 21 women who later had an ante-partum haemorrhage. Folate assay was performed on all these 21 women before there was any indication of haemorrhage, which means that the effect of blood loss on the folic acid stores has been eliminated. In

TABLE 36

Serum Folate Results in 21 Women who Subsequently Had an Ante-partum Haemorrhage

Serum folate mμg/ml	Gestation period: Before 19th week		At 28th week		From 32nd week	
	APH	All results in this group < 4.0 mμg/ml	APH	All results in this group < 4.0 mμg/ml	APH	All results in this group < 4.0 mμg/ml
0-1.9	3		2		0	
2-2.9	- 45%	37%	3 33%	48%	1 17%	59%
3-3.9	2		1		0	
4-5.9	2		8		3	
⩾6	4		4		2	
TOTAL	11		18		6	

some other series investigation of the folic acid status has been carried out after haemorrhage had occurred, making the interpretation of results difficult.

The incidence of ante-partum haemorrhage in this study of 742 women was 2.8%, and approximates that of other series (Hourihane et al., 1960; Hibbard and Hibbard, 1963).

Serum folate levels in twin pregnancy: Fifteen twin pregnancies occurred among the women whose serum folate was estimated and a total of 18 results was obtained. Five results before the 19th week of pregnancy were normal (11.3, 10.7, 8.6, 6.4, 3.0 mμg/ml), only one value being less than 4 mμg/ml. Only 2 values were obtained at 28 weeks (4.7 and 3.5 mμg/ml). There were 8 results in the last weeks of pregnancy (7.2, 6.3, 4.9, 3.8, 2.6, 2.3, 1.3 and 1.1 mμg/ml), 5 of which were below 4 mμg/ml. Three results obtained post-partum were reduced (2.2, 2.0 and 1.4 mμg/ml).

In this small group of women with twin pregnancy, early serum levels were normal. Later in pregnancy low folate levels were common, but 5 of the women, 3 of whom had abnormal serum folate levels, had a megaloblastic anaemia. It is therefore in part a selected group from which only

limited conclusions can be drawn.

Serum folate related to prematurity and birthweight: The relationship of birthweight and the serum folate was examined. Two possible effects might have been noted: folic acid deficiency in the mother could have limited foetal growth, alternatively marked foetal growth could have caused a reduction in the maternal folic acid stores. The results obtained by correlating birthweight and the serum folate are presented in Table 37.

TABLE 37

To Show the Relationship of Birthweight to the Serum Folate

Serum folate, mμg/ml	Gestation period: Before 19th week — Birthweight			At 28th week — Birthweight			From 32nd week — Birthweight		
	$< 5\frac{1}{2}$	$5\frac{1}{2}$-8	> 8	$< 5\frac{1}{2}$	$5\frac{1}{2}$-8	> 8	$< 5\frac{1}{2}$	$5\frac{1}{2}$-8	>8
< 4	9 (36%)	98 (40%)	31 (34%)	8 (35%)	115 (48%)	42 (49%)	6 (55%)	90 (61%)	37 (53%)
⩾ 4	16	150	61	15	124	43	5	57	33
TOTAL	25	248	92	23	239	85	11	147	70
Comment	No difference			$\chi^2 = 1.651$; n = 2; P > 0.05 Not significant			No difference		

There was no evidence that prematurity by weight was associated with a low maternal serum folate. There was no evidence either that larger babies were born to mothers with reduced levels although a tendency towards this was noted in the results obtained at 28 weeks.

Serum folate related to congenital abnormalities: Twenty-three babies with congenital malformations were born to mothers included in this study, the overall incidence of congenital malformations being therefore 3.1 % (23 out of 742). Ten of these were severe (a major abnormality of the central nervous system in 3, abnormality of the gastro-intestinal tract in 4, fibro-elastosis cordis in 2, cleft palate and hare lip

in 1) and 13 were relatively minor (abnormalities of hands, feet or ears in 7, abnormalities of the genito-urinary tract in 4, others 2). In every case blood for assay was taken before delivery and in two-thirds more than one value was available. The total number of serum folate results was 46 and details of the abnormalities and folate levels have been given in full in Appendix VI.

Two of the 10 women whose children had severe deformities and 3 of the 13 women withless affected infants, had definitely abnormal values.

The serum folate in 16 of the 23 women was estimated before the 28th week of pregnancy and was below 4.0 mμg/ml in 6 (37.5%) and less than 2.1 mμg/ml in 2. Fourteen values were available at 28 weeks; 8 of these were below 4.0 mμg/ml but not one was less than 3.0 mμg/ml.

In the last few weeks of pregnancy only 13 values were obtained in 9 women; using first readings only, 5 had values below 4.0 mμg/ml, 3 of which were definitely abnormal.

Although folate assay was only carried out on a small number of women later giving birth to an abnormal baby, no evidence was obtained that a low serum folate was associated with this complication. Thus the serum folate followed a normal distribution in the early stages of pregnancy when folic acid deficiency could have had a teratogenic effect. At 28 weeks results were also normal and it was only in the last few weeks of pregnancy that a low serum folate was common.

A SEASONAL VARIATION IN SERUM FOLATE LEVELS

Following the severe winter of 1962-1963 it was noted that there was a sharp increase in the number of low serum folate values. The mean value calculated for May 1963 was 2.3 mμg/ml and 22 of the 43 values at all stages of pregnancy were definitely abnormal. This observation prompted the following table in which the seasonal variation is calculated over a 2-year period.

There is considerable variation from month to month in the number of women with low values. When grouped to form seasons, no clear pattern emerges. It must be concluded that a winter restriction of vegetable source

TABLE 38

To Show Serum Folate Related to Season in Pregnant Women (All at 28 Weeks Gestation)

Serum folate, mμg/ml	SEASON											
	Spring			Summer			Autumn			Winter		
	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
< 4	12	21	18	20	9	9	10	9	8	17	16	12
	(53%)			(48%)			(41%)			(45%)		
⩾ 4	15	18	13	14	15	12	19	7	13	13	15	28
TOTAL	27	39	31	34	24	21	29	16	21	30	31	40

of folic acid might have occurred after the severe freeze early in 1963, but if so this was an isolated observation not repeated in the following year.

COMMENT

The serum folate has been studied in a large number of pregnant women and possible factors which might be associated with an alteration in it examined. A number of observations made in this study confirm the results of other workers.

Not only was the mean serum folate during pregnancy found to be lower than in non-pregnant normal controls but abnormal values usually only found in patients with clinical folic acid deficiency were not uncommon during apparently normal pregnancy. The frequency with which this occurred increased with advancing pregnancy. In a group of women whose Hb concentration was 12 g/100 ml or more, a small but steady fall was noted in the mean serum folate, calculated for the three trimesters of pregnancy, all three mean values being lower than the mean for non-pregnant controls (7.8 mμg/ml). It is apparent, however, that the greatest fall below the non-pregnant mean has already taken place in the first trimester, before a true deficiency of folic acid would be present. This fall was associated

with an increasing number of women with abnormal values which in non-anaemic women in the last trimester was nearly 12%. Similar results have been obtained by other workers (Solomons et al., 1962; Ball and Giles, 1964; Chanarin, Rothman and Berry, 1965; Metz, Festenstein and Welch, 1965) although the amount by which the mean serum folate falls varies considerably.

In the present study the mean serum folate in the first two trimesters of pregnancy was the same in anaemic and non-anaemic women. In the last trimester, however, when the Hb concentration was below 11 g/100 ml the mean serum folate fell more steeply and a significantly greater number of women had reduced levels. The association between low serum folate levels and a low Hb concentration was therefore only present at a time when developing folic acid deficiency and anaemia might be expected to present together. There was no evidence of a direct relationship between a low haemoglobin and low serum folate levels in the earlier stages of pregnancy and even at the 28th week no such correlation was obtained. This suggests that folic acid deficiency may be contributing to the anaemia in a proportion of women in the last few weeks of pregnancy.

In addition to the direct correlation between the haemoglobin and serum folate a significant association between low serum iron and low serum folate levels was also obtained but the effect of gestation period on this relationship was not examined. Thus in the present study evidence was obtained that the serum folate, Hb concentration and serum iron are all related in some way during pregnancy.

The interpretation of these results presents some difficulty. One explanation suggested by the association of the serum folate and serum iron levels is that haemodilution has produced a fall in both these factors. Since haemodilution is a continuous process during most of pregnancy, however, the lack of correlation between low folate levels and the haemoglobin in the first two trimesters of pregnancy argues against this possibility. For the same reason it appears unlikely that iron deficiency resulted in a fall in the serum folate level since, as was shown in Chapter I, many women with a Hb concentration below 11 g/100 ml were iron deficient.

Although in the present study it was shown that no direct relationship between the serum folate and serum vitamin B_{12} levels was present, both altered in a similar manner during pregnancy. However it is known that the body stores of vitamin B_{12} are not depleted within a few months and therefore the fall in the serum vitamin B_{12} and levels in the range associated with pernicious anaemia, noted in healthy pregnant women cannot indicate a true deficiency of vitamin B_{12}. Folic acid stores on the other hand may be exhausted quite rapidly but it should not be assumed because of this that a low serum folate necessarily indicates folic acid deficiency.

There is no consistent correlation between the serum folate and other tests of folic acid deficiency, such as the folic acid clearance and Figlu excretion, in pregnancy (Lowenstein et al., 1962; Mollin and Hoffbrand, 1965). This suggests that there is an alteration of folic acid metabolism in pregnancy and despite the relationship between a low serum folate and a low haemoglobin noted in the last few weeks of pregnancy, a low serum folate cannot be equated with folic acid deficiency.

Although the serum folate was related to a number of clinical features of megaloblastic anaemia in pregnancy the only positive correlation obtained was between the serum folate and parity.

It was interesting to note that parity and folic acid levels only correlated early in pregnancy but this is not surprising as it is likely that other factors associated with a fall in the serum folate are present at the later stages of pregnancy. This observation, together with the increased parity noted in women with megaloblastic anaemia confirm that foetal demands, are a strain on the maternal folic acid metabolism. An increased incidence of a low serum folate both before the 19th week and again late in pregnancy was noted in women with a positive history of a previous miscarriage but was not significant. Using increased Figlu excretion as an index of folic acid deficiency, Hibbard (1964) noted an association between this test and a previous miscarriage but this too was not significant. The serum folate of 6 women whose pregnancy ended in abortion was normal.

Although the numbers are small this observation is of interest because blood for assay was taken before abortion had started. In two reported series where low serum folate levels were associated with spontaneous abortion, blood was taken for folate estimation after the onset of bleeding (Martin and Davis, 1964; Martin, Harper and Kelso, 1965) and it is possible the haemopoietic stimulus resulting from haemorrhage might have had an effect on the serum folate levels.

Similarly the significance of a normal distribution of serum folate levels in 21 women who had an ante-partum haemorrhage is noteworthy because there was no sign of pending haemorrhage at the time of assay. There is evidence that a disturbance of folic acid metabolism is present after ante-partum haemorrhage, especially abruptio placentae (Coyle and Geoghegan, 1962; Hibbard, 1964) but this would not appear to be a factor in producing the haemorrhage since in most reported series of megaloblastic anaemia the incidence of ante-partum haemorrhage is normal.

Study of the serum folate and its relationship to foetal complications confirmed the lack of association with folic acid deficiency noted in the preceding chapter. Reduced serum folate levels were not associated with a tendency to prematurity or smaller babies; in fact a trend the other way was noted at 28 weeks but was not significant. This observation confirms that of others (Giles, 1966; Varadi, Abbott and Elwis, 1966) but is contrary to that of Hibbard and Hibbard (1963). Using Figlu excretion as an index of defective folic acid metabolism, these authors noted an increase in prematurity and reduced birthweight in pregnant women with folic acid deficiency. In a later study an increased excretion of Figlu was noted in a significant number of mothers recently delivered of an abnormal foetus (Hibbard and Smithells, 1965) and the authors suggested that a deficiency of folic acid might be important in producing these abnormalities. This has not been confirmed in the present study as both the incidence of foetal abnormalities amongst babies born to mothers with megaloblastic anaemia and the distribution of serum folate levels before the 28th week of pregnancy in 16 women who later gave birth to an infant with a congenital malformation, were normal.

This detailed analysis has shown that a number of factors may influence the serum folate during pregnancy, and these have been briefly commented upon. There remained however a proportion of pregnant women in whom no explanation of a reduced folate was found.

One way to assess the significance of an abnormal serum folate is to observe the effect of treatment with folic acid. This might be expected to produce an improvement in the haematological state of anaemic pregnant women if a subclinical deficiency of folic acid was common. It might also prevent the development of anaemia in women already taking iron if subclinical folic acid deficiency was contributing to it. To answer these questions and to investigate the amount of folic acid necessary to maintain a normal serum folate during pregnancy a therapeutic trial was designed and is presented in the following chapter.

IV

A CONTROLLED TRIAL OF IRON AND FOLIC ACID IN PREGNANCY

INTRODUCTION

It was shown in Chapter I that iron-deficiency anaemia occurs frequently in women attending the Radcliffe Infirmary ante-natal clinics. Despite treatment with iron a proportion of anaemic women did not attain a haemoglobin concentration of 11 g/100 ml before delivery. Furthermore anaemia actually developed in 33 of the 397 women on survey while taking prophylactic iron. Although in a number of patients there was an explanation for this, in others there was no obvious cause. Two reasons for an apparently iron-resistant anaemia were considered. In a few women the haemoglobin concentration rose soon after delivery and hydraemia of pregnancy was considered likely in such patients. Theother possibility was that an undiagnosed folic acid deficiency was present and that either alone or combined with iron deficiency was causing a persistent anaemia.

While the low incidence of megaloblastic anaemia obtained in the survey indicated that severe folic acid deficiency was not common, other evidence of folic acid deficiency was obtained more often. A serum folate of less than 2.1 mμg was found in nearly one-quarter of the women investigated in the pilot study. Some of those tested were selected because of a mild anaemia, and probably this accounted for the high incidence of low values in this group. In women known to have normoblastic erythropoiesis the proportion with a low serum folic acid was less (nearly 12%). The incidence of increased Figlu excretion in women with normoblastic anaemia was also of this order. There was thus suggestive evidence that folic acid deficiency occurred more commonly than the 1.8% incidence of megaloblastic anaemia indicated. The results of these tests for folic acid deficiency suggested that the proportion of anaemic women with folic acid deficiency might in fact be 12% or more.

A controlled therapeutic trial using iron and folic acid was designed

to see if folic acid deficiency was a factor which had an appreciable effect on the Hb concentration during pregnancy in the absence of a frankly megaloblastic anaemia. Since the requirements for folic acid during pregnancy were not known a dose of folic acid, which was known to be adequate, was used in one group and a smaller and more physiological dose was used in another.

The aims of this trial were therefore to see if folic acid was able:

1) to raise the Hb concentration of a group of unselected pregnant women;
2) to produce a greater rise in the Hb concentration in women who were already anaemic; and
3) to prevent the development of anaemia before delivery.

Iron was not given to all women in this trial because results obtained in the pilot study had indicated that in a proportion of pregnant women, iron treatment both therapeutic and prophylactic was not entirely effective. It was considered that comparison of an iron-treated group and a group given no iron might help in the assessment of the value of iron therapy.

Because of the design adopted in the trial it was possible to observe the effect of therapy with iron and folic acid on hypersegmentation of the neutrophil leucocytes, and at the same time to examine the relationship of hypersegmentation and the serum folate.

DESIGN OF THE TRIAL

METHOD OF SELECTION

Women attending the ante-natal clinic at their first visit which usually took place before the 20th week of pregnancy were asked to participate in a clinical trial to investigate the best method of preventing anaemia during pregnancy.

If already receiving iron they were asked to stop taking their tablets. To ensure adequate comparison between the groups no patient was included in the trial if she had taken iron after the 20th week of pregnancy. At this stage any patient who had a haemoglobin concentration below 11 g/100 ml as well as a serum iron below 60 μg/100 ml was not included in the trial

and was treated immediately with iron.

The others were asked to re-attend shortly before the 28th week of pregnancy and were interviewed and had a sample of blood taken. Before allocating patients at random to one of six treatments and giving them numbered bottles containing their tablets the Hb concentration was estimated. All those with a haemoglobin of 10.2 g/100 ml (70%) or more were then admitted to trial. If the Hb concentration was less than this the serum iron was estimated before treatment was begun. Women whose serum iron was normal were admitted to trial, their tablets being posted to them a few days later. However, those with a low haemoglobin and a low serum iron could not for ethical reasons be admitted to a blind trial and were either given ferrous gluconate or admitted to another trial in which all patients received iron. In all, there were 50 patients who were not included in the trial because iron-deficiency anaemia was either present at their booking visit or had developed by the 28th week of pregnancy. All were treated immediately with iron and subsequently followed up; only one developed a megaloblastic anaemia.

TREATMENT GROUPS

Various combinations of ferrous gluconate and folic acid with appropriate placebo tablets were used as shown in Table 39.

Half the patients were treated with ferrous gluconate 5 gr (300 mg) 3 times daily and half with a placebo tablet indistinguishable from the ferrous gluconate tablet. In addition to the iron or placebo tablets, patients were given one capsule daily containing a low dose of folic acid (500 μg) or a high dose of folic acid (5 mg) or a folic acid placebo. One in six patients therefore received neither iron nor folic acid. Bottles containing the tablets had been numbered by random selection at source and the code was not known while the patients were still on trial.

INVESTIGATIONS DURING THE COURSE OF THE TRIAL

The capillary haemoglobin was checked before delivery usually at fortnightly intervals and finally early in the puerperium. At 28 weeks, and

TABLE 39

Results of Investigations in the 6 Groups Before the Start of Treatment

Iron therapy	Placebo			Ferrous gluconate		
Dose of folic acid	Placebo	500 µg	5 mg	Placebo	500 µg	5 mg
Number of patients	60	58	59	60	61	62
Mean Hb, g/100 ml	11.62	11.58	11.55	11.42	11.35	11.49
Overall mean Hb		11.59			11.42	
Number with Hb < 11 g/100ml	15	13	11	17	14	18
Mean serum iron, µg/100ml	72	80	76	72	77	78
Number with serum iron < 60 µg/100 ml	22	14	13	21	20	16
Mean serum folate mµg/ml	4.3	4.5	5.1	4.4	4.8	4.6
Number with serum folate <2.1 mµg/ml	5	4	5	5	3	2

again at least once before delivery, a venous sample of blood was taken and the haemoglobin, haematocrit, MCHC, serum iron and serum folate were estimated. It was intended that every patient should have at least one venous sample of blood examined after treatment had been started and before delivery. Several days before this visit was due, a letter was sent asking the patient to stop taking her iron and vitamin tablets 24 hours before coming to hospital. A film was examined if the haemoglobin fell below 11.0 g/100 ml. Treatment was considered to have failed in the haemoglobin fell below 10g/100 ml and the patient was withdrawn from trial. Unless the anaemia was characteristic of iron deficiency further investigation including bone marrow biopsy and a serum vitamin B_{12} estimation were carried out and then the necessary corrective treatment was instituted.

RESULTS

360 women were admitted to the trial and Table39 gives the number of patients in each treatment group.

HAEMATOLOGICAL FINDINGS AT THE START OF TREATMENT

Table39 records the mean Hb concentration, serum iron and serum folate, as well as the incidence of a low haemoglobin and low serum levels of iron and folate at 28 weeks. As judged by these parameters randomisation appears to have resulted in approximately comparable groups.

Hb CONCENTRATION AT THE END OF THE TRIAL

The results in the 360 women after therapy are shown in Table 40 Where it was necessary to give additional therapy, the last haemoglobin before this was started is recorded. The mean Hb concentration in the three groups of women treated with iron rose by 0.95 g/100 ml and differs significantly from that of the three groups treated with placebo iron, where the mean Hb concentration fell by 0.41 g/100 ml (difference of the mean increase in haemoglobin = 1.36 g; overall standard error (s.e.) = 0.075; $P < 0.001$).

TABLE 40

Results of Investigations in the 6 Groups after Treatment

Iron therapy	Placebo			Ferrous gluconate		
Dose of folic acid	Placebo	500 µg	5 mg	Placebo	500 µg	5 mg
Mean Hb, g/100 ml	11.23	11.03	11.27	12.38* (5)	12.21* (3)	12.53
Overall mean Hb		11.18			12.37	
Mean serum iron, µg/100 ml	60	57	67	86	89	102
Number with serum iron < 60 µg/100 ml	37	34	26	6	11	10
Mean serum folate mµg/ml	4.0	14.6	30.1	4.3	13.6	28.2
Number with serum folate <2.1 mµg/ml	9	-	-	6	-	-

*Iron tablets not taken. The number of patients who did not take iron is indicated next to the asterisk.

The use of folic acid did not have the same beneficial effect. There was no difference between the mean haemoglobin of the groups treated with folic acid (either in the high or low dosage) and that of the groups treated with placebo folic acid. Iron and 5 mg folic acid together produced a rise in the Hb concentration of 1.04 g/100 ml compared with a rise in Hb of 0.96 g/100 ml in the group treated with iron alone. This was not significant (difference of the mean increase in haemoglobin = 0.08 g; overall s.e. = 0.14; $P > 0.05$).

Although the incidence of a Hb concentration below 11 g/100 ml was not altered by folic acid therapy, there was a tendency both in the women given no iron and the iron-supplemented women for increasing dosage of folic acid to be associated with an increasing frequency of a Hb concentration of 12 g/100 ml or more (see Table 41).

TABLE 41

To Show Details of Hb Concentration in 360 Women at End of Trial

Iron therapy	Placebo			Ferrous gluconate		
Dose of folic acid	Placebo	500 µg	5 mg	Placebo	500 µg	5 mg
Hb concentration g/100ml ⩾13	3	4	2	12	11	21
12-12.9	7 } 16.7%	7 } 19%	15 } 28.8%	25 } 61.7%	31 } 69%	24 } 72.6%
11-11.9	30	21	23	20	14	15
10-10.9	15	15	12	3	4	2
9- 9.9	4	11	6	-	1	-
<9	1	-	1	-	-	-
TOTAL	60	58	59	60	61	62

This was not significant however ($\chi^2 = 3.564$; $n = 2$; $P > 0.05$) and may have been partly due to the failure of 5 patients in the group given placebo folic acid to take their iron tablets.

RESPONSE TO THERAPY IN ANAEMIC WOMEN

Amongst these 360 women were 88 who had a Hb concentration of less

TABLE 42

Haemoglobin Concentration at Term Related to Treatment in 88 Women whose Hb was less than 11.0 g per 100 ml Before Therapy

Iron therapy	Placebo			Ferrous gluconate		
Dose of folic acid	Placebo	500 µg	5 mg	Placebo	500 µg	5 mg
13 g/100 ml and over	-	-	-	3	1	3
11-12.9 g/100 ml	5	1	3	11	10*[1]	13
10-10.9 g/100 ml	6	5	4	3	2*[1]	2
< 10.0 g/100 ml	4	7	4	-	1	-
Total number of patients	15	13	11	17	14	18

*Iron tablets not taken.

than 11.0 g/100 ml when 28 weeks pregnant. If the response to therapy of these 88 is considered separately (Table 42) it can be seen that even in mildly anaemic women the empirical use of folic acid does not bring about any improvement in the haemoglobin.

As judged by the response to iron therapy it would seem that most were iron deficient although this was not always obvious at the time.

The Hb concentration in 9 women who were not given iron also rose above 11 g/100 ml and this was independent of treatment with folic acid. Four of these had a considerable rise in the Hb concentration (1.1, 1.1, 1.4 and 1.7 µg/100 ml); one had evidence of iron deficiency but this was minimal (serum iron of 54 µg/100 ml). The rise in the Hb concentration in the 5 others was less than 1 g/100 ml (0.4, 0.6, 0.6, 0.7 and 0.8 g/100 ml) and signs of iron deficiency were noted in 3 (serum iron < 40 g/100 ml in 3 and hypochromia of the red cells in 2). It seems likely that those without evidence of iron deficiency and a substantial rise in the Hb concentration near term had an apparent anaemia caused by haemodilution, since it is well recognised that the Hb concentration may fall progressively during pregnancy as a result of haemodilution but rise before term. It is apparent from the rising Hb concentration of those with signs of iron deficiency that iron-deficiency anaemia does not necessarily progress during pregnancy.

DEVELOPMENT OF ANAEMIA DURING THE TRIAL

The number of patients who developed an ante-partum Hb concentration of less than 11 g/100 ml after treatment had begun is shown in Table 43. Thirty-five patients not treated with iron became anaemic before delivery and the dose of folic acid made no difference to the number of anaemic patients in each group. Only one patient taking iron became anaemic but her haemoglobin only fell from 11.1 g/100 ml to 10.9 g/100 ml.

Twenty-eight women were removed from trial and given other treatment. The usual indication was a Hb concentration below 10 g/100 ml and evidence of iron deficiency but 3 patients with a Hb concentration above this were treated with iron for other reasons. Results in these 28 patients are summarized in Table 44.

TABLE 43

The Hb Concentration in 37 Women who Developed Ante-Partum Anaemia after 28 Weeks

	Iron therapy					
	Placebo			Ferrous gluconate		
Dosage of folic acid	Placebo	500 µg	5 mg	Placebo	500 µg	5 mg
10-10.9 g/100 ml	9	10	8	-	2*[1]	-
< 10.0 g/100 ml	1	4	3	-	-	-
Total no. of patients	10	14	11	-	2	-

*Iron tablets not taken.

TABLE 44

Haematological Details of 28 Women Removed from Trial Before Delivery

Iron therapy	Dosage of folic acid	Number of patients	Hb concentration g/100 ml		Evidence of iron deficiency			Bone marrow biopsy
			< 10.0	10.0-10.9	Peripheral blood film	MCHC < 30%	Serum iron <60 µg/100 ml	
PLACEBO IRON	Placebo	6	5	1	4 (6)*	0 (5)	6 (6)	3†
	500 µg	12	11	1	6 (11)	4 (12)	7 (12)	7
	5 mg	8	7	1	3 (8)	1 (8)	8 (8)	3
IRON	Placebo	1	1	-	0 (1)	0 (1)	1	1
	500 µg	1	1	-	1 (1)	0 (1)	1	-
	5 mg	-	-					-

*Number in whom test performed shown in brackets.
†One patient in this group with megaloblastic anaemia; remainder all normoblastic.

The serum folate was estimated in 27 of these women; only one patient had an abnormal level and she had a transitional megaloblastic marrow. The results once again show the value of iron therapy in preventing anaemia and the lack of effect of folic acid. These results confirm that a MCHC below 30% is a late sign of iron deficiency.

THE SERUM FOLATE AND SERUM IRON LEVEL RELATED TO THERAPY

The effectiveness of folic acid in raising the serum folate of the 345 patients in whom it was measured can be seen in Table 40. The mean serum folate of patients treated with the 500 μg dose was more than three times that of those given placebo folic acid and the increase was even greater in those treated with 5 mg. No patients given folic acid had serum levels below 2.1 mμg/ml.

As one would expect the mean serum iron was higher in the iron-supplemented group (Table 40). (The serum iron level of patients who could not take their iron tablets was not included here.) Folic acid therapy does not appear to have a marked effect on the serum iron of the treated patients; and the reverse also appears to be true. It is interesting to note, however, that the greatest rise in the serum iron level occurred in those patients treated with iron and 5 mg folic acid.

ERYTHROPOIESIS AND SERUM VITAMIN B_{12} LEVELS RELATED TO THERAPY

Bone marrow biopsy was carried out on 29 patients who became anaemic during the trial. Only 2 were found to have megaloblastic erythropoiesis; neither had taken folic acid and both had a serum folate of less than 2.1 mμg/ml. The serum folate ranged from 2.0 mμg/ml to 44.8 mμg/ml in the others. Megaloblastic anaemia presented before delivery in one; in the other it was diagnosed in the puerperium.

Nine of the 29 women on whom a bone marrow biopsy was performed had a serum vitamin B_{12} level below 150 μμg/ml but in none was it below 100 μμg/ml; one of the 9 had a megaloblastic anaemia. In the groups treated with folic acid there were fewer patients with low levels and the mean serum vitamin B_{12} level was higher than in the other groups, but the numbers in each group are too small for accurate analysis.

HYPERSEGMENTATION OF POLYMORPHONUCLEAR LEUCOCYTES RELATED TO THERAPY

Hypersegmentation of the nuclei of polymorphonuclear leucocytes may be assessed either by the "lobe average" (Herbert, 1964) or a count

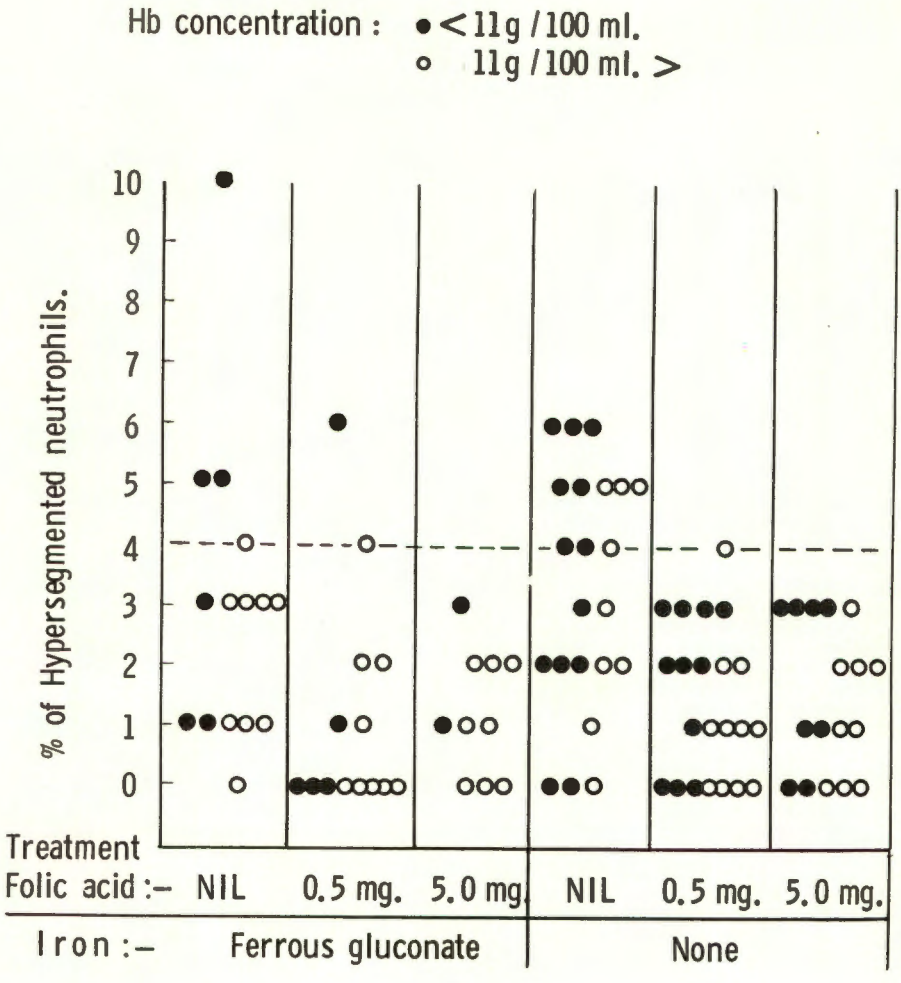

Fig. 26 To show the effect of treatment with iron and folic acid on the incidence of hypersegmentation.

of leucocytes showing five or more lobes. The latter is less time consuming and has been adopted in the present study. The significance of hypersegmentation in relation to this clinical trial was assessed after the trial was completed. Peripheral blood films from 100 patients were selected so as to include both anaemic and non-anaemic patients in the different treatment groups. The microscopic examination was carried out "blind" without a knowledge of the clinical state, treatment or other laboratory data. Repeat examination of films showed a high level of reproducibility of counts. The films were examined by one observer with a high power (400 x) oil emersion objective; 100 leucocytes were counted and the number showing five or more lobes was recorded. The lobes were regarded as being discreet provided they were joined only by a thin filament of nuclear material.

The results are shown in Fig. 26 and Table 45.

TABLE 45

To Show the Relationship of the Neutrophil Lobe Count to Treatment Group in 100 Pregnant Women

Iron therapy	Dosage of folic acid	Number of hypersegmented neutrophils per 100 cells		Total
		< 4%	4% or more	
Placebo	Placebo	11	11	22
	500 μg	21	1	22
	5 mg	17	0	17
Ferrous gluconate	Placebo	11	4	15
	500 μg	12	2	14
	5 mg	10	0	10

This shows that patients who were not given folic acid were much more likely to have 4% or more hypersegmented leucocytes than those receiving folic acid supplements ($\chi^2 = 20.216$; $n = 1$; $P < 0.001$). Not one of 27 patients taking 5 mg folic acid daily showed 4% or more hypersegmented neutrophils. Included amongst these were a number who had an iron-deficiency anaemia. A few women given 500 μg daily had increased

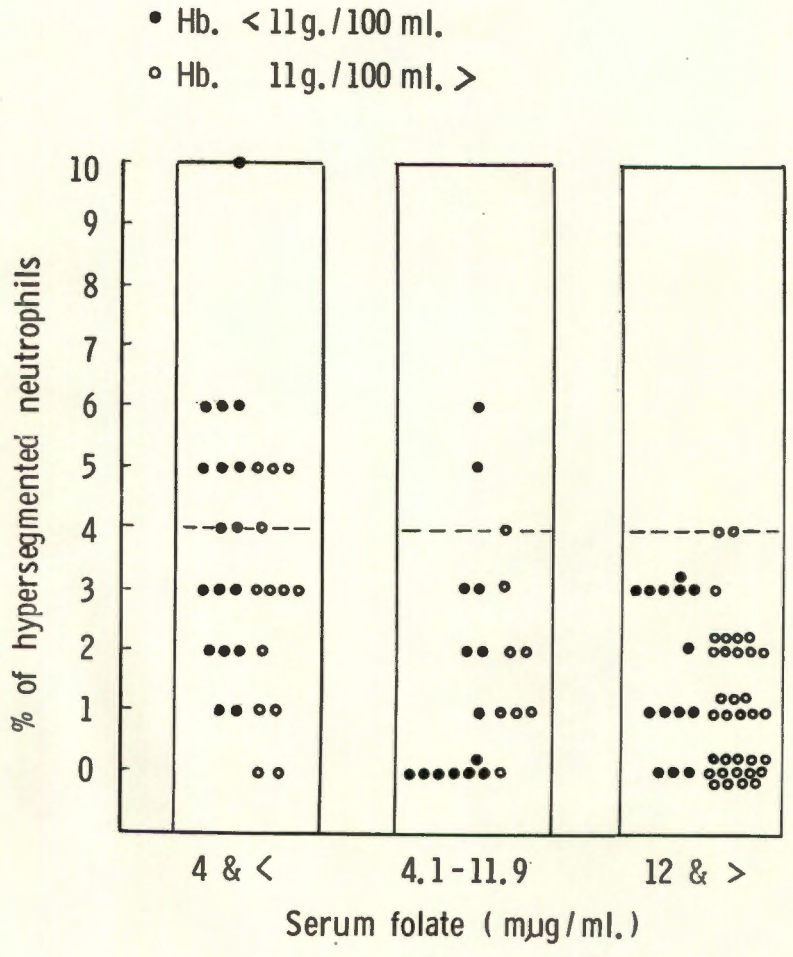

Fig. 27 Relates serum folate levels to hypersegmentation.

hypersegmentation; those given no folic acid commonly had 4% or more hypersegmented neutrophils, the highest incidence being noted in those given neither iron nor folic acid supplements.

Amongst the unsupplemented women hypersegmentation was only slightly more common in anaemic women when compared with those who were not anaemic. This is in keeping with iron deficiency being the cause of the anaemia but not the main factor in producing hypersegmentation.

The serum folate was estimated at the time the films were made and is related to the incidence of hypersegmentation in Table 46 and Fig. 27.

TABLE 46

To Show the Relationship of the Neutrophil Lobe Counts to the Serum Folic Acid of 100 Women Included in the Trial

Serum folate mμg/ml	Number of hypersegmented neutrophils per 100 cells			Total
	Less than 4%	More than 4%		
4 and below	17	13	43%	30
4.1 - 11.9	19	3	13.6%	22
12 and over	46	2	4.3%	48

These results show clearly that the serum folate and hypersegmentation are related ($\chi^2 = 18.635$; $n = 1$; $P < 0.001$). The incidence of hypersegmentation increases as the serum folate level falls. Nearly half the women with a serum folate of 4 mμg/ml or less had increased hypersegmentation whereas almost all the women with a high serum folate (12 mμg and over) had normal counts. Nevertheless some overlap was present and a number of women with an abnormally low folic acid did not have increased counts.

COMMENT

The high prevalence of iron deficiency observed in the initial pilot study was confirmed by this therapeutic trial. Fifty women originally interviewed and willing to participate in the trial, could not be included

because iron-deficiency anaemia had developed by the 28th week of pregnancy. A considerable number of the women admitted to the trial at 28 weeks developed evidence of iron-deficiency anaemia in the 12 weeks that followed, and almost all had not been taking iron. Anaemia was usually mild but in 25 patients it was necessary to institute active treatment before delivery to correct a progressive iron-deficiency anaemia.

The use of prophylactic folic acid made no difference to the haemoglobin level of the treated women. Not only was the mean Hb concentration the same in the supplemented and unsupplemented groups but anaemia developed as frequently in those who were treated with folic acid as in those given a folic acid placebo. Furthermore anaemia already noted before the start of treatment was not more effectively treated by the addition of folic acid. It was thus obvious that while the use of iron was of considerable value the addition of either 0.5 mg or 5 mg folic acid daily conferred no additional benefit.

The incidence of megaloblastic anaemia was low. No patient taking either the 0.5 mg or 5 mg folic acid was found to have a megaloblastic anaemia although the bone marrow of 19 anaemic women taking folic acid was studied. It thus appears likely that both doses of folic acid are adequate to prevent this condition. Further confirmation of this is obtained from the very marked rise in the serum folate as a result of taking folic acid. The serum folate levels, even with the smaller dose, ranged widely but were never less than 2.1 mμg/ml; values between 2.1 and 4.0 mμg/ml (inclusive) were noted in five women but approximately half of the women treated with folic acid had a serum folate of 12 mμg/ml or more. It was apparent that the folic acid was well absorbed and that even the smaller dose was greater than the requirements of most pregnant women.

The effect of folic acid therapy on the nuclear segmentation of the neutrophil leucocytes was studied and the marked reduction of hypersegmentation in women given folic acid confirmed that this sign is mainly related to folic acid deficiency. This effect of folic acid therapy is considered to be more significant than its effect on the serum folate. Whereas a reduced serum folate is the earliest sign of folic acid deficiency and

develops before there is depletion of the body stores, an alteration in the nuclear formation of the leucocytes suggests that a degree of tissue deficiency of folic acid is present. Although hypersegmentation correlated well with a low serum folate, a normal count was noted in 9 women with values below 2.1 mμg/ml.

The patient with 10% hypersegmented neutrophils (Fig.26) had a megaloblastic anaemia. Hypersegmentation was obvious without a formal count and was accompanied by other changes in the peripheral blood, anaemia and a serum folate below 2.1mμg/ml. The degree of hypersegmentation noted in the majority of women in Fig. 26 (4%, 5%) was not apparent unless a count was performed, In patients not given folic acid, hypersegmentation was noted more frequently when the haemoglobin was below 11 g/100 ml but this association was not significant ($\chi^2 = 2.3686$; $n = 1$; $P > 0.05$). However since 7 of the 18 women showing hypersegmentation were not anaemic it is apparent that this sign may not necessarily be of clinical significance.

Similar results have been obtained in a number of other trials. It has been shown that prophylactic folic acid raises both the serum folate (Metz et al., 1965; Willoughby and Jewell, 1966) and the whole blood folate levels (Vanier and Tyas, 1966), reduces the incidence of hypersegmentation (Chanarin et al., 1965; Willoughby and Jewell, 1966) and can largely prevent abnormal Figlu excretion (Metz et al., 1965).

Results obtained in the trial carried out by Chanarin and his colleagues suggested to them that iron deficiency might predispose to the development of megaloblastic anaemia. No evidence to support this hypothesis was obtained in the trial described here. Although it was confirmed that iron therapy alone reduced the incidence of hypersegmentation, this was not statistically significant ($\chi^2 = 1.6163$; $P > 0.05$). The fall in the mean serum folate and the number with low values were the same in the group given no treatment and in those given iron alone. Iron-deficiency anaemia was common but the incidence of megaloblastic anaemia in the groups not given folic acid supplements was no higher than that noted in the pilot study when all women were taking iron from an early stage in

pregnancy. Although bone marrow biopsy was performed more often in the trial described by Chanarin and his colleagues, the fact that in the present study treatment with folic acid did not produce any improvement in anaemia not brought about by iron alone, indicates that megaloblastic anaemia was not being missed in a significant number of our patients.

Since folic acid alone was unable to prevent the fall in the haemoglobin, the progressive fall which occurs with advancing pregnancy must be due to iron deficiency and to haemodilution. When patients were supplemented with iron, the fall in the Hb concentration was largely prevented and this would suggest that iron deficiency was either being prevented or corrected. The significance of the rise in the Hb concentration which occurs when iron is taken requires consideration however. This might indicate a true iron deficiency due to insufficient maternal stores and increased foetal utilisation but an alternative explanation which has been suggested is that iron therapy produces non-specific stimulation of the bone marrow which counters the effect of a physiological hydraemia in lowering the haemoglobin.

It is questionable that iron can produce a rise in the Hb concentration of the healthy non-pregnant individual. Two reports present evidence to support the concept that iron can raise the Hb concentration in the absence of iron deficiency. The first (Fowler and Barer, 1941) has been criticised on the grounds that the control "non-anaemic" groups were probably iron deficient (Undritz, 1964); and the second only showed an average difference in Hb concentration of 0.2 g/100 ml between supplemented and unsupplemented groups of healthy men and women (Garry, Sloan, Weir and Wishart, 1954).

Widdowson and McCance (1936) showed that this occurred in women but not in men and Beutler, Larsh and Gurney (1960) found in a study of non-anaemic women that only those with reduced iron stores had a rise in haemoglobin after iron therapy. Verloop, Blokhuis and Bos (1959b) found no significant rise in Hb concentration in a small group of healthy men and women treated with iron, and similar results were obtained by De Leeuw, Lowenstein and Hsieh (1964).

Paintin, Thomson and Hytten (1966) suggested that an alteration in iron metabolism in pregnancy was the explanation for their finding that large iron supplements (115 mg daily) produced a rise in the Hb concentration whereas a small dose (12 mg daily) did not, but such a conclusion is invalid if the small dose is inadequate to meet the requirements of the pregnant women studied (Callender, 1967).

Although most women in the present trial had a rise in haemoglobin while taking iron, in a number of women this did not take place and similar observations have been made by others (Fisher and Biggs, 1955; Edgar and Rise, 1956; Kerr and Davidson, 1958). It is apparent therefore that iron does not always raise the Hb concentration even during pregnancy.

Thus the concept that iron will raise the Hb concentration in the absence of iron deficiency is not proven and it seems likely that the effect of iron in raising the Hb concentration of women in this trial by nearly one gram in the space of 8 weeks is, for the most part, due to the correction of iron deficiency. Although the haemoglobin fell in some women in the iron-supplemented groups presumably due to hydraemia, it rarely fell below 11 g/100 ml and thus hydraemia appeared to be of little importance in producing anaemia in pregnancy in the presence of adequate iron intake. In the present study 50 women developed an iron-deficiency anaemia at or before the 28th week of pregnancy preventing their inclusion in the trial. Response to iron was good and only one developed a megaloblastic anaemia. This provides additional evidence that iron deficiency was most important in producing anaemia in pregnancy in this area and that folic acid deficiency was only rarely responsible.

DISCUSSION

INTRODUCTION

The present study has shown that anaemia in pregnancy is still a common problem, even in an area such as Oxford where socio-economic conditions are good. A survey of 397 women attending an ante-natal clinic in the Radcliffe Infirmary indicated that anaemia was present in 60 at their booking visit and investigation showed that the majority of these women were iron deficient. Many women were already taking iron tablets when first seen and the rest were all given a prescription for iron at their booking visit. In a further 33 the haemoglobin dropped below this level before delivery despite apparently adequate iron therapy. Although most eventually responded to iron, achieving a Hb concentration of 11 g/100 ml near delivery, in 29 women anaemia was still present at term . Bone marrow biopsy was performed in 28 women in this pilot study but in only 6 was there morphological evidence of folic acid deficiency.

The results indicate that iron deficiency is the major cause of anaemia in pregnancy in this hospital and anaemia due to folic acid deficiency as manifested by megaloblastic change in the bone marrow occurs much less frequently. However, it was considered possible that lesser degrees of folic acid deficiency might be more common and might be a factor in the development of anaemia. There were two main reasons for suspecting this possibility. Firstly, as indicated above, anaemia persisted or developed in some patients despite iron therapy. Secondly, laboratory tests of folic acid deficiency, such as the serum folate and urinary Figlu excretion, were more often abnormal in pregnant women than in healthy non-pregnant controls. In order to examine the significance of these observations the diagnostic value of laboratory tests of folic acid deficiency in pregnancy was assessed by applying them to the investigation of groups of pregnant

women and comparing results obtained in women with megaloblastic or transitional erythropoiesis with results in those known or presumed to have normoblastic erythropoiesis. It was shown that increased excretion of Figlu and a low serum folate were present in just over 10% of women with normoblastic anaemia and a similar proportion of normal pregnant women without anaemia. The diagnostic value of the serum folate was further assessed by relating it to a number of clinical features of megaloblastic anaemia in pregnancy and a significant association between a reduced serum folate and parity was obtained. The serum folate correlated reasonably well with the bone marrow appearance and was shown to be a useful test in the investigation of anaemia in pregnancy.

Study of laboratory tests used in the diagnosis of folic acid deficiency confirmed that not one was sufficiently reliable to be considered diagnostic of megaloblastic anaemia in pregnancy. Although the serum folate was some guide to the bone marrow appearances, it was not possible to determine if low values found in women who did not have megaloblastic anaemia, indicated an actual deficiency of folic acid. It was considered that a practical way to assess the prevalence and significance of folic acid deficiency was to treat comparable groups of pregnant women, both anaemic and non-anaemic, with folic acid and observe the effect of therapy on the Hb concentration, serum folate and hypersegmentation of the neutrophil polymorphs.

A total of 360 pregnant women was given iron or a placebo, together with either folic acid in two different dosages or a placebo, and the results compared. Treatment with folic acid did not produce a rise in the Hb concentration of anaemic women nor did it prevent anaemia, whereas iron therapy clearly did so. A rise in the serum folate was noted in the women given folic acid but this occurred in almost all the supplemented women and was so great (especially in those given 5 mg daily) that it could not have been regulated by a need for folic acid. The effect of folic acid supplements on hypersegmentation of the neutrophils was examined, and it was shown that although the 0.5 mg and 5 mg dose of folic acid did not entirely prevent hypersegmentation, there was a highly significant

difference when the number of hypersegmented neutrophils in the peripheral blood film of women who were given folic acid was compared with the numbers in an unsupplemented group. There was also a significant association between the serum folate and the presence of hypersegmentation. Thus although prophylactic folic acid had no effect on the Hb concentration it was able in most women to prevent both the fall in the serum folate and hypersegmentation of the neutrophils. It was concluded therefore that, although there was evidence of folic acid deficiency in a considerable proportion of the women studied, this was of no importance in producing the mild anaemia commonly noted in late pregnancy since treatment with folic acid had no effect on the Hb concentration.

It is necessary however to determine if the evidence of folic acid deficiency obtained during pregnancy can be accepted as indicating that a true deficiency exists. In the non-pregnant state it has been shown that a fall in the serum folate, hypersegmentation and increased Figlu excretion are sensitive and reliable signs of folic acid deficiency (Herbert, 1962). A similar study has not been made in pregnancy and here the sequence of events may not be so clearcut for a number of reasons. It has been shown that the metabolism of histidine is altered during pregnancy and therefore the Figlu excretion test is not a reliable one in pregnant women. Haemodilution, as a result of the blood volume changes in pregnancy, affects parameters such as the Hb concentration, serum folate and serum iron, and therefore these too become less reliable investigations during pregnancy.

The folic acid status of the mother represents a balance between the maternal intake and stores on the one hand and the maternal and foetal utilisation on the other. As this is a dynamic process, it is likely that the balance will sometimes be disturbed during pregnancy, with a fall in the serum folate and perhaps an increase in Figlu excretion as temporary phenomena, without significant depletion of the maternal or foetal stores of folic acid and without an effect on the maternal Hb concentration. Thus it is essential to observe some structural alteration in the blood which could only be related to a deficiency of folic acid or vitamin B_{12} before

folic acid deficiency in pregnancy can be diagnosed with certainty. Hypersegmentation of the nuclei of the neutrophil leucocytes, morphological changes in the white cells in the bone marrow and finally the development of megaloblastic erythropoiesis, are all examples of cellular changes which are produced by nutritional folate deficiency and the specificity of each of these abnormalities for folic acid deficiency during pregnancy will now be discussed.

1) **Hypersegmentation of the nuclei of the neutrophil leucocyte**

Herbert showed that hypersegmentation is an early sign of folate deficiency which developes some time before the liver stores are depleted. Thus hypersegmentation is a valuable diagnostic sign (Herbert, 1964; Mollin and Hoffbrand, 1965) and to a certain extent this is also true during pregnancy (Chanarin et al., 1965; Scott and Sommerville, 1965; Willoughby and Jewell, 1966; Varadi et al., 1966). However several studies of anaemic pregnant women show that considerable overlap exists. Hypersegmentation was absent in 5 of 15 women with megaloblastic anaemia studied by Chanarin and colleagues and in 7 of 17 women with hypersegmentation the bone marrow morphology was normal. Varadi confirmed that hypersegmentation might be absent when erythropoiesis was megaloblastic but when hypersegmentation was present found that erythropoiesis was always megaloblastic. The experience of Lowenstein et al. (1966) has been different, only 37% of pregnant women with abnormalities of erythropoiesis and/or leucopoiesis had associated changes in the peripheral blood.

Experience in the value of hypersegmentation in the present study was limited. Neutrophil lobe counts were not carried out on patients with megaloblastic anaemia. As a result of the therapeutic trial it was possible to relate the neutrophil lobe count to the dosage of folic acid and the serum folate, and it was shown that treatment with folic acid was able, for the most part, to prevent hypersegmentation. Furthermore a good correlation between the serum folate and the presence of hypersegmentation was noted. This confirms that hypersegmentation is related to folic

acid metabolism but in view of the fact that 40% of the women in the clinical trial who were not supplemented with folic acid showed hypersegmentation, it must be concluded that this sign occurs so frequently in late pregnancy as to be of doubtful significance. Furthermore, since hypersegmentation may be absent in some women with megaloblastic anaemia during pregnancy, it cannot be used as the basis for the diagnosis of folic acid deficiency.

2) White cell changes in the bone marrow

In several series (Dawson, 1962; Lowenstein et al., 1966; Varadi et al., 1966) white cell changes in the bone marrow have been considered diagnostic of folic acid deficiency and the diagnosis of megaloblastic anaemia has been made on this evidence alone. In all three series however evidence to support this contention is not convincing.

Dawson (1962) observed signs of iron deficiency in the majority of 33 anaemic pregnant women with giant metamyelocytes and normoblastic erythropoiesis. He noted that 5 patients responded to folic acid alone but apart from this there were no important differences between these 33 women and a normoblastic group with normal leucopoiesis.

Lowenstein et al. (1966) diagnosed "megaloblastic anaemia" in 77 pregnant women, 70% of whom had bone marrow changes limited to the white cells. Anaemia was caused by iron deficiency in the majority and responded satisfactorily to adequate iron therapy. Only 15 of the 77 women were shown to respond to folic acid. Although the serum folate was low in 80% of these 77 women compared with 33% of 228 women with normoblastic anaemia, in another study by the same group (Lowenstein, Brunton, Cooper, Milad and Hsieh, 1963), 57% of 130 non-anaemic pregnant women had a low serum folate. Their evidence suggests that these white cell changes are likely to be due to folate deficiency in a proportion of cases, but again cannot be considered diagnostic of folate deficiency.

In 34 women with normoblastic erythropoiesis and white cell changes (Varadi et al., 1966) other evidence of folate deficiency was limited and

only 3 had a low serum folate.

Nevertheless, in spite of their variable association with other signs of folic acid deficiency, when giant metamyelocytes are seen in bone marrow smears of anaemic pregnant women, it is difficult to prove that there is not a minor degree of folic acid deficiency present. However, these same white cell changes have been noted commonly in non-anaemic women in the last few weeks of pregnancy (Lowenstein et al., 1963; Roberts, Waters and Mollin, 1961). Hansen (1966) commented that in his experience myeloid changes in the bone marrow smears of pregnant women with a normal Hb concentration did not correlate with any other parameter of folic acid deficiency. Furthermore he has reported occasional giant forms in just over one third of 112 non-pregnant patients with iron-deficiency anaemia responding to iron alone (Hansen, 1964). In the present study, giant metamyelocytes were noted in the films of an anaemic pregnant woman whose serum folate was 33 $\mu g/ml$ as a result of taking folic acid for 10 weeks prior to bone marrow biopsy. The effect of folic acid on the frequency of these white cell changes has been examined (Lowenstein et al., 1963) and was shown to reduce but not prevent their occurrence. Thus it must be concluded that, as is the case with hyper-segmentation of neutrophils in the peripheral blood, although giant metamyelocytes are related to folic acid metabolism, their occurrence in pregnant women is not diagnostic of folic acid deficiency. It should be noted however that white cell changes are invariably present when erythropoiesis is megaloblastic.

3) **Red cell changes in the bone marrow**

Megaloblastic erythropoiesis and the development of anaemia are the very last events to take place when nutritional folate deficiency develops. Prophylaxis with an adequate dosage of folic acid will prevent their occurrence in pregnancy (Lowenstein, Pick and Philpott, 1955; Giles and Burton, 1960). It is generally accepted that megaloblastic erythropoiesis, as defined in Chapter II, is diagnostic of folate or vitamin B_{12} deficiency. Since most cases of megaloblastic anaemia during pregnancy diagnosed in

temperate climates are corrected by folic acid, for practical purposes megaloblastic erythropoiesis in pregnancy may be considered to be diagnostic of folate deficiency.

It is apparent, however, that megaloblastic change in the marrow may be present with minimal or no anaemia. Two cases in the present series with megaloblastic erythropoiesis had a normal Hb concentration at the time of biopsy but both had been anaemic shortly before. Megaloblastic erythropoiesis without anaemia has been observed in several series. For example, unexpected megaloblastic erythropoiesis has been discovered occasionally when bone marrow biopsy was performed for such indications as macrocytosis or hypersegmentation without anaemia (McKenzie and Abbott, 1960), accidental haemorrhage without anaemia (Hourihane et al., 1960), and when the incidence of megaloblastic change in the bone marrow of normal controls was being assessed in the course of a therapeutic trial during pregnancy (Chanarin et al., 1965). Exceptionally the reported incidence of "megaloblastic change" in non-anaemic women has been as high as 18% (Thambu and Llewellyn-Jones, 1966). However, this suggests that a different interpretation of the morphology of the pregnant marrow has been used to that accepted in most studies.

As was shown in Chapter II, the bone marrow morphology during pregnancy differs from that of the non-pregnant state and interpretation of the marrow may be difficult. In the present study no marrow was termed megaloblastic unless definite abnormalities of erythropoiesis were seen. Distinction was made between transitional and megaloblastic erythropoiesis, because there is some evidence that the latter indicates a more severe degree of folic acid deficiency (Dawson, 1962), but this was not confirmed in the present study; the severity of anaemia did not differ in the two groups and although increased Figlu excretion was noted more commonly in the frankly megaloblastic group, the serum folate was more often abnormal in the transitional group.

This assessment of the frequency and significance of the cellular abnormalities associated with folic acid or vitamin B_{12} deficiency in pregnancy, indicates the difficulty in making a confident diagnosis of

folic acid deficiency in a pregnant woman. Since megaloblastic anaemia is the undoubted clinical condition associated with a deficiency of either of these vitamins, it seems reasonable to adopt the presence of megaloblastic change in the bone marrow as the criterion for the diagnosis of folic acid deficiency in pregnancy. It is recognised that other abnormalities, such as changes in the peripheral blood, low serum folate and increased Figlu excretion, may all indicate the presence of folate deficiency but it is considered that none is diagnostic of such a deficiency.

Adopting this criterion, a detailed study of folic acid deficiency in pregnancy was made by examination of a group of pregnant women in whom megaloblastic anaemia had developed. This indicated that the clinical significance of folic acid deficiency in pregnancy depended on the associated anaemia. No adverse effect on the mother or her child that would not also result from iron-deficiency anaemia was observed.

The definition, incidence and significance of folic acid deficiency in other series has differed, however, and the whole question of folic acid deficiency in pregnancy, whether mild or severe, has taken on a greater significance in view of the reports that such a deficiency might be responsible for congenital abnormalities in the foetus or accidental haemorrhage in the mother. If this were so, then the case for the prophylactic administration of folic acid routinely in pregnancy becomes a strong one. It is necessary therefore, to discuss the results obtained in the present study and those reported in the literature before the importance of this condition and the necessity to prevent it can be assessed.

The particular features of folic acid deficiency in pregnancy which will be considered are:

1) The laboratory diagnosis of megaloblastic anaemia;
2) The relationship of folic acid deficiency to iron and vitamin B_{12} deficiency;
3) Its incidence and clinical significance to the mother and foetus;
4) The indications for the administration of prophylactic folic acid

and the schedule to be adopted;

and 5) The aetiology of folic acid deficiency in pregnancy.

DIAGNOSIS OF MEGALOBLASTIC ANAEMIA IN PREGNANCY

The symptoms associated with megaloblastic anaemia in pregnancy are variable and are mainly related to the severity of the accompanying anaemia. Even then, more than half the patients in the present study with a Hb concentration of less than 9 g/100 ml were asymptomatic and it is obvious that the diagnosis of this condition is made in the laboratory. The value of haematological and biochemical investigations in indicating that erythropoiesis was megaloblastic will now be discussed.

THE Hb CONCENTRATION

The results in the present study confirmed that megaloblastic anaemia of pregnancy may be associated with a considerable degree of anaemia. Only 11 of the 120 anaemic women reported in Chapter II had a Hb concentration below 7 g/100 ml and nearly two-thirds of these had transitional or megaloblastic erythropoiesis. The number of cases in this series was small but similar results were obtained by Giles (1966) who studied a total of 1,004 cases of anaemia in pregnancy and showed that nearly two-thirds of women with a Hb concentration below 50% (7.3 g/100 ml) had a megaloblastic bone marrow. Giles observed that the marrow was almost invariably megaloblastic when the presenting haemoglobin was less than 5.2 g/100 ml; in the present series of 120 anaemic women, only one had a Hb concentration below 5.0 g/100 ml and she had a frankly megaloblastic marrow.

Other tests for folic acid deficiency which were used were more often abnormal when the anaemia was marked, but there was considerable overlap. Increased Figlu excretion occurred more commonly in megaloblastic anaemia when the Hb concentration was below 9 g/100 ml and there was a tendency for the serum folate to be low more frequently when the Hb concentration was below 9 g/100 ml. There was however no direct correlation either between the Hb concentration and the serum folate or

between the Hb concentration and the serum vitamin B_{12}.

In the present study the degree of anaemia did not appear to be important in determining whether erythropoiesis was megaloblastic or transitional. This has been the experience of Giles (1966) who, although noting a slightly lower mean Hb concentration in the megaloblastic group, commented on the variable correlation between the severity of anaemia and the severity of megaloblastic change. Other factors contributing to anaemia, particularly iron deficiency, infection and haemorrhage are obviously important, and when women who had bled were excluded from a series of 112 cases of megaloblastic anaemia, Dawson (1962) also noted little difference in the mean haemoglobin associated with transitional and frankly megaloblastic anaemia. The evidence indicates, therefore, that the degree of anaemia and severity of megaloblastic change are not directly related.

THE PERIPHERAL BLOOD PICTURE

The peripheral blood film was found to be of considerable diagnostic value in the present study. In the majority of cases of megaloblastic anaemia the diagnosis was indicated by the red cell and/or white cell morphology. It was not common to find a megaloblastic marrow in the absence of these changes although in a small proportion this occurred. Macrocytosis and hypersegmentation were rarely associated with normoblastic erythropoiesis although this also was noted. Signs of iron deficiency were commonly present in the peripheral blood film of women with megaloblastic anaemia. It is recognised that coexistent iron deficiency may prevent the morphological changes of folate deficiency in the marrow and peripheral blood (Herbert, 1965; Rachmilewitz, 1965); even when megaloblastic anaemia has developed after parenteral treatment of associated iron-deficiency anaemia signs of folic acid deficiency may not develop in the peripheral film (Scott and Sommerville, 1965).

A normal peripheral film has been described in several series (Chanarin, Rothman, Ardeman and Berry, 1965; Giles, 1966; Lowenstein et al., 1966), and in Herbert's study mild megaloblastic change in the

marrow was noted weeks before a change in the red cells took place. Since folic acid is incorporated in the red cell at the erythroblast stage and does not leave it until the cell dies, the circulating red cells will not reflect the folic acid deficiency for some time. However, severe megaloblastic erythropoiesis has also been described in a number of pregnant women with normal red cell appearances (Callender, 1944; Chanarin and Davey, 1964), the mechanism suggested here being that megaloblastic change was precipitated acutely, usually by infection or blood loss.

Megaloblastic anaemia during pregnancy was diagnosed by Willoughby and Jewell (1966) when two or more features suggestive of folic acid deficiency were found in the peripheral blood. These included hypersegmentation and a number of abnormalities in the buffy coat. Buffy coat smears have their value as a screening test for megaloblastic anaemia (Goodall, 1957; Rannie and McTaggart, 1961; Scott and Somerville, 1965). For certain diagnosis, however, it is usually necessary to perform marrow biopsy, since nucleated red cells with megaloblastic or transitional features are rarely seen in the peripheral blood; for example, they were found in buffy coat smears in only 7 of the 31 cases of megaloblastic anaemia studied by Scott and Somerville (1965). In the preliminary stage of this present study buffy coat smears were made on all anaemic patients but were discontinued when it became obvious that they were not a substitute for bone marrow biopsy. They are of value however when it is not possible or convenient to perform a bone marrow biopsy.

FIGLU EXCRETION

In the present study estimation of Figlu excretion was disappointing as a diagnostic test for megaloblastic anaemia, since more than half the women with unequivocal megaloblastic change excreted normal amounts. This has already been discussed in Chapter II.

The altered metabolism of histidine in pregnancy is the likely explanation of normal Figlu excretion in some women with megaloblastic anaemia in pregnancy. Direct measurement of the amount of histidine in

the blood and urine after a loading dose indicated that not only was there an altered absorption of histidine with a flat prolonged curve, but that there was also an increased urinary excretion. Histidinuria in pregnancy reported in 1923 by Honda, is due to increased glomerular filtration and reduced tubular reabsorption (Page, Glendening, Dignam and Harper, 1954). As a result it is suggested that less of the loading dose of histidine is available for breakdown to Figlu so that the test becomes less reliable in pregnancy.

The reason for increased Figlu excretion in normal pregnant women is not clear. It occurs early in pregnancy when the drain on the maternal stores of folic acid must be minimal and diminishes as pregnancy progresses (Berry, Booth, Chanarin and Rothman, 1963; Hansen, 1964; Metz et al., 1965). In the first trimester 23% (Metz et al., 1965) to 36% (Berry et al., 1963) of women excreted increased amounts of Figlu. While agreeing that Figlu excretion is maximal early in pregnancy, Hansen (1964) considered that by setting the upper limits of normal at 17 μmoles/h Berry et al. had overestimated the incidence of abnormal excretion of Figlu; the excretion of Figlu in 14% of Hansen's non-pregnant controls was greater than this.

A sharp increase of Figlu excretion in the puerperium was noted in all three series. The return to the increased levels noted in early pregnancy might be due to the fact that histidine is no longer being metabolised to meet the foetal requirements but on the other hand other evidence of folic acid deficiency is frequently noted after delivery — both megaloblastic anaemia and low serum folate levels being relatively common then, so Figlu excretion may be increased for the same reason. Metz et al. (1965) showed that although folic acid supplements did not alter the pattern of Figlu excretion in pregnancy the number of women with abnormal excretion was significantly reduced.

It is obvious therefore that several factors influence the excretion of Figlu during pregnancy. These are:—

a) Increased urinary loss and altered absorption of histidine;

b) Increasing utilisation of histidine for protein synthesis;

c) Developing folic acid deficiency in the latter weeks of pregnancy and

the puerperium.

The interaction of these factors varies in different women. False positive as well as false negative results mean that in practice this test is not reliable in the diagnosis of megaloblastic anaemia during pregnancy.

THE SERUM FOLATE

A low serum folate cannot be considered diagnostic of folic acid deficiency during pregnancy. The evidence presented in Chapter III has shown that the serum folate falls throughout pregnancy and healthy pregnant women may have low levels. This suggests that a rapid uptake and utilisation of folic acid occurs in normal pregnancy and as a result, there is an altered response to tests of folic acid function.

However estimation of the serum folate proved to be a useful test in the investigation of anaemia during pregnancy. In approximately 80% of 78 anaemic pregnant women the serum folate accurately reflected the bone marrow morphology. Values of less than 1 mμg/ml were rare; when the bone marrow was studied in such cases there was always evidence of megaloblastic change. Values between 1 mμg/ml and 2.1 mμg/ml were found more often in women with megaloblastic erythropoiesis but were less specific, being noted in approximately 11% of women with normoblastic anaemia.

Nevertheless results in megaloblastic anaemia were disappointing. When results in women with megaloblastic and transitional megaloblastic erythropoiesis were considered together, a normal serum folate was found in one woman in three who had bone marrow evidence suggesting folic acid deficiency.

As Table 47 shows these results are similar to those reported by others and are not due to faulty technique. Many minor variations in technique have been described resulting in a widely differing range of values; for example in normal subjects in different laboratories the mean serum folate has ranged from 4.6 to 15.8 mμg/ml (Mollin and Hoffbrand, 1965). The variable results in megaloblastic anaemia during pregnancy indicate the complexities of folic acid metabolism in this condition.

TABLE 47

To show Incidence of Low Serum Folate Levels in Pregnant Women with Megaloblastic Change in the Bone marrow

Year	Reference	Number of cases	% with abnormal serum folate
1962	Lowenstein et al.	40	78%
1962	Waters and Mollin	32	84%
1964	Chanarin	38	79%
1964	Kershaw and Girdwood	11	100%
1966	Varadi et al.	59	34%
1966	Giles	100	57%
1966	Willoughby and Jewell	52	85%
1967	Lawrence and Klipstein	23	83%
	Present study	32	66%

There are a number of possible reasons for a normal serum folate in megaloblastic anaemia in pregnancy but there is no satisfactory explanation for this finding. In a small number of cases the serum vitamin B_{12} level is reduced and the serum folate normal and the significance of this will be discussed later. Another explanation is suggested by the observation of Ball and Giles (1964) that in some women with megaloblastic anaemia the total serum folate was normal because of an increase in the stable factor; the labile active factor was always reduced. The reason for this is not known. Recent intake of folic acid either in a compound vitamin preparation or in food is another possible explanation for a normal serum folate and normal vitamin B_{12} levels in pregnant women with megaloblastic anaemia. Many women take vitamin preparations during pregnancy and a small dose of folic acid can produce a rise in the serum folate although it may not prevent megaloblastic anaemia.

It is recognised that blood samples should be taken from fasting patients but in practice this is not always done. In the present study five patients with a normal serum folate and megaloblastic or transitional megaloblastic anaemia were fasting at the time of venepuncture.

A marked rise in the serum folate after a meal of chicken or beef liver (Herbert, 1964) and a haematological response to lettuce (Baumslag and

Metz, 1964) have been reported. Six vegans with vitamin B_{12} deficiency were shown to have much higher serum folate levels than 103 patients with vitamin B_{12} deficiency due to other causes, suggesting that a high dietary intake of folic acid might have raised the serum levels (Waters and Mollin, 1962). It is questionable if an ordinary meal can raise the serum folate (Herbert, 1964; Mollin and Hoffbrand, 1965) although a significant difference in the mean serum folate after eating was noted in a group of healthy controls studied by Ramo Rao, Lagerlöf, Einhorn and Reizenstein (1963).

Since Herbert noted slight bone marrow changes within days of the fall in the serum folate, it is possible that minimal megaloblastic change might antedate the fall in the serum folate. This seems inadequate to explain the normal serum folate levels found in cases of severe megaloblastic anaemia, however, and there is no satisfactory explanation for this finding.

THE SERUM VITAMIN B_{12}

The serum vitamin B_{12} was shown to be abnormally low in a proportion of women with megaloblastic anaemia in pregnancy and in some the serum folate was normal. In the present study the serum vitamin B_{12} level was within the range associated with pernicious anaemia in nearly one-quarter of the patients with frank megaloblastic anaemia. This was commoner than was noted in other anaemic pregnant women and although this finding was not specific, a low vitamin B_{12} was considered to be of some diagnostic value. The relationship of folic acid and vitamin B_{12} deficiency will be discussed in detail in the following section.

RELATIONSHIP OF FOLIC ACID DEFICIENCY AND VITAMIN B_{12} DEFICIENCY

The results presented in Chapter II showed that a serum vitamin B_{12} level in the range associated with Addisonian pernicious anaemia occurred in 24% of pregnant women with a definitely megaloblastic anaemia. Simultaneous assay of the serum folate and vitamin B_{12} levels was not

carried out in many patients but results indicated that just over half the pregnant women with a megaloblastic anaemia and low levels of vitamin B_{12} had a normal serum folate; the overall incidence of a low vitamin B_{12} and normal serum folate in this condition is thus approximately 12%.

There are few other series of megaloblastic anaemia in pregnancy in which serum folate and serum vitamin B_{12} levels are simultaneously available; in one series of 77 cases (Lowenstein et al., 1966) approximately 10% had a normal serum folate and abnormal vitamin B_{12} levels. Hansen (1964) reported normal folate levels and low serum B_{12} levels in 3 of 26 patients with megaloblastic anaemia during pregnancy, one of whom had a true Addisonian pernicious anaemia. In another series (Lawrence and Klipstein, 1967) a normal serum folate was noted in 4 women and 3 of these had a low serum vitamin B_{12} level.

However the serum vitamin B_{12} level alone has been frequently estimated in groups of pregnant women with megaloblastic anaemia and results in some of the reported series are presented in Table 48.

TABLE 48

To Show Serum Vitamin B_{12} Levels in Different Series of Megaloblastic Anaemia in Pregnancy (all grades)

Year		Number of cases	Number with a low serum vitamin B_{12}
1954	Mollin and Ross	31	2
1956	Girdwood	15	0
1957	Izak et al.	11	11
1960	Layrisse et al.	15	9
1962	Metz, Brandt and Stevens	44	2
1964	Ball and Giles	± 100	±30
1964	Hansen	13	1
1966	Lowenstein et al.	77	25%
1966	Varadi et al.	177	8
1966	Chanarin	22	7
1967	Lawrence and Klipstein	24	17
	Present study	48	9
	TOTAL	577	115 (20%)

It can be seen that low serum vitamin B_{12} levels are found in approximately 20% of women with megaloblastic anaemia of pregnancy which is considerably higher than the percentage of normal pregnant women who have abnormal values (8% in the present study). Hansen (1964) noted reduced B_{12} levels in about 40% of his patients in the third trimester but if the diagnostic limits for healthy pregnant women were applied then only 5.4% had abnormal values.

Thus in megaloblastic anaemia of pregnancy the incidence of abnormal serum vitamin B_{12} levels is considerably increased. The reason for this is unknown; in temperate climates this cannot be the result of a nutritional deficiency. The daily requirement of vitamin B_{12} are small (0.1 µg/d Sullivan and Herbert, 1962) and with the exception of strict vegetarians there have only been isolated case reports (Harrison, Booth and Mollin, 1956) of nutritional vitamin B_{12} deficiency. Nutritional folic acid deficiency results in megaloblastic anaemia within $4\frac{1}{2}$ months (Herbert, 1962), but megaloblastic anaemia due to vitamin B_{12} deficiency following total gastrectomy takes at least four years to develop (Girdwood, 1962).

In megaloblastic anaemias due to a deficiency of folic acid a low serum B_{12} level is found frequently. In such conditions the absorption of vitamin B_{12} is often abnormal; for example of 73 patients with idiopathic steatorrhoea 44 had abnormal B_{12} absorption (Girdwood, 1962). Nevertheless low serum vitamin B_{12} levels are observed in folate deficiency even when the absorption of vitamin B_{12} is normal (Chanarin and Bennett, 1962b; Mollin, Waters and Harriss, 1962; Persson and Hansen, 1963) as it has been shown to be in megaloblastic anaemia of pregnancy (Badenoch, Callender, Evans, Turnbull and Witts, 1955; Layrisse et al., 1960; Metz et al., 1962).

Lawrence and Klipstein (1967) found subnormal vitamin B_{12} levels in 17 of 24 cases when estimated by microbiological assay using L. leichmannii as test organism. When 11 of the 17 sera with low values were retested by charcoal assay, the serum B_{12} was now found to be normal in 9. This suggested to the authors that an inhibitor in the serum of some pregnant women prevented the growth of L. leichmannii. The

binding capacity of the serum proteins for vitamin B_{12} was increased both in normal pregnancy and pregnancy complicated by a megaloblastic anaemia and there was no altered pattern similar to that found in a true deficiency of vitamin B_{12}. The authors concluded that the low vitamin B_{12} levels probably did not reflect a deficiency state in pregnancy.

In the present study, the significant association between normal Figlu excretion and reduced serum vitamin B_{12} levels (less than 150 $\mu\mu$g/ml observed in megaloblastic anaemia in pregnancy suggested that a disturbance in the metabolism of vitamin B_{12} might be a primary factor in some cases of megaloblastic anaemia in pregnancy. One other report indicates that normal Figlu excretion in megaloblastic anaemia of pregnancy was associated with an abnormal serum vitamin B_{12} level but exact details were not given. In a series of 27 cases reported from India (Karthigaini et al., 1964) the mean serum vitamin B_{12} was 78 $\mu\mu$g/ml (10 women having levels below 80 $\mu\mu$g/ml) and normal Figlu excretion was present in 20. In another series normal Figlu excretion was common but the serum vitamin B_{12} levels were normal (Chanarin, Rothman and Watson-Williams, 1963). The observation made in the present study is interesting but would have to be confirmed in a larger number of cases before its significance can be assessed.

Most of the available evidence indicates that a true deficiency of vitamin B_{12} is rarely the cause of megaloblastic anaemia during pregnancy. Low serum vitamin B_{12} levels are common in other megaloblastic anaemias due to a primary deficiency of folic acid. As has been shown, these are usually reversible and in megaloblastic anaemias due to folic acid deficiency unassociated with pregnancy treatment with folic acid produces a rise in the serum vitamin B_{12} levels (Narayanan, Shenoy and Ramasarma, 1956; Mollin et al., 1962). Similarly when megaloblastic anaemia of pregnancy is treated with folic acid low serum vitamin B_{12} levels return to normal (Ball and Giles, 1964). Conversely too, treatment with vitamin B_{12} raises tne serum folate level. The dose of folic acid used in the treatment of megaloblastic anaemia in pregnancy is usually large enough to produce a response even in patients with pernicious anaemia. Reports

of the successful response of megaloblastic anaemia of pregnancy to vitamin B_{12} therapy have likewise usually been to large doses (Moore et al., 1955, Adams, 1956; Killander, 1958) although Nieweg (1952) reported a good response to a dose of vitamin B_{12} as small as 45 µg. Since it is now recognised that both primary B_{12} and primary folic acid deficiency will respond to large doses of either vitamin, diagnostic doses of these vitamins should be used initially in any doubtful case. It should be possible to determine by this means whether a primary defect in vitamin B_{12} metabolism ever causes megaloblastic anaemia in pregnancy apart from the rare patient with latent or manifest Addisonian pernicious anaemia.

There is no satisfactory explanation for the low serum vitamin B_{12} levels found in healthy pregnant women. It seems unlikely that it is a simple effect of haemodilution since they did not correlate with the haemoglobin (Ball and Giles, 1964; present study). Again as the present study and others have shown (Ball and Giles, 1964; Metz et al., 1965) low serum vitamin B_{12} levels do not correlate with low serum folate levels. The serum vitamin B_{12} level is not influenced by a number of factors which are known to affect the serum folate, and oral supplementation with vitamin B_{12} does not keep the vitamin B_{12} level from falling as pregnancy progresses (Metz et al., 1965). The total circulating serum vitamin B_{12} in normal pregnancy does not alter (Lowenstein et al., 1962), there is a prompt return to normal soon after delivery (Metz et al., 1965) and it is unlikely that normal pregnancy produces an appreciable drain on the maternal stores (Ross and Mollin, 1957) although foetal levels are higher than maternal levels at birth (Boger, Wright and Bayne, 1957; Baker, Ziffer, Pasher and Sobotka, 1958).

The evidence indicates that low vitamin B_{12} levels in normal pregnant women are not the result of a deficiency of vitamin B_{12}. It seems likely that they are produced by some alteration in the metabolism of vitamin B_{12} which is physiological during pregnancy. In megaloblastic anaemia in pregnancy, however, low serum vitamin B_{12} levels appear to be significant and for the most part are related to the metabolism of folic acid. A

few cases similar to one in the present study (R.I. no. 40489) in which failure to respond to folic acid was followed by correction of the anaemia by vitamin B_{12} have been described (Tasker, 1954) and it is possible that in such patients, a disturbance of vitamin B_{12} metabolism is the primary abnormality. Although it rarely happens, true Addisonian pernicious anaemia has presented as a megaloblastic anaemia during pregnancy (Hansen, 1964) and this possibility should be considered in patients with a megaloblastic anaemia in pregnancy which does not respond to folic acid or one which is associated with abnormal serum vitamin B_{12} and normal serum folate levels.

RELATIONSHIP OF FOLIC ACID DEFICIENCY AND IRON DEFICIENCY

In the present study iron deficiency occurred commonly in association with megaloblastic anaemia, evidence of iron deficiency being found in 58% of women with florid megaloblastic and 78% of those with transitional megaloblastic anaemia. The marrow iron stores of 25 women with megaloblastic change were examined and iron was present in 18, markedly increased amounts being noted in 12. These observations are not contradictory, however. It is known that iron accumulates in the marrow of patients with pernicious anaemia (Rath and Finch, 1948; Herbert, 1959) and this is true too of megaloblastic anaemia in pregnancy (Layrisse et al., 1960; Dawson, 1962; Hansen, 1964). Similarly the serum iron level may be raised, falling once treatment with folic acid is begun (Scott, 1957; Giles, 1966). The accumulation of iron in the serum and bone marrow of such patients is presumed to result from the arrest of erythropoiesis associated with vitamin B_{12} or folic acid deficiency.

Results presented in Chapter II showed that evidence of iron deficiency both in the blood and bone marrow was found more commonly in women with transitional than those with frank megaloblastic change. The frequent association of transitional megaloblastic erythropoiesis and iron deficiency has been confirmed in several other studies of anaemic pregnant women. In one series of 17 cases with megaloblastic anaemia iron deficiency was

common in those with intermediate megaloblastic change but rare in those with frankly megaloblastic erythropoiesis (Layrisse et al., 1960). A correlation between different grades of bone marrow(change and bone marrow iron stores (lesser changes being associated with the most marked reduction in marrow iron) was observed by Dawson (1962), and similarly Giles (1966) noted that evidence of accompanying iron deficiency was present more often when marrow changes of folic acid deficiency were less severe.

Transitional megaloblastic changes have been described in a number of other conditions, including nutritional macrocytic anaemia (Trowell, 1942), sprue (Davidson, Girdwood and Innes, 1947; Dacie and White, 1947; Cooke, Frazer, Peeney, Sammons and Thompson, 1948) and nutritional anaemia of infancy (Zuelzer, Newhall and Hutaff, 1947), all conditions in which a dual deficiency of iron and folic acid is common. Fudenberg and Estren (1958) suggested that defective iron metabolism might hinder the full morphological development of megaloblastic erythropoiesis resulting in a transitional picture. Tasker (1959) considered that co-existing iron deficiency could prevent the development of megaloblastic change in patients with severe folic acid deficiency. He reported a series of 25 patients with a nutritional folic acid deficiency and a normoblastic anaemia, who developed grossly megaloblastic marrows after iron therapy and required folic acid to correct their anaemia. Many similar patients with normoblastic erythropoiesis and a poor response to iron, did not develop a megaloblastic picture and the likely explanation is that in some patients folic acid stores were just adequate to maintain normoblastic erythropoiesis until iron therapy stimulated new red cell production.

Nevertheless, a considerable overlap exists and unequivocably megaloblastic erythropoiesis and iron deficiency may occur together. Thus, although there is evidence that iron deficiency may limit the full morphological expression of folic acid deficiency, it is obvious that it does not invariably do so.

The suggestion that iron deficiency might actually predispose to the development of folic acid deficiency in pregnancy was based on results of

a therapeutic trial in pregnancy conducted by Chanarin et al. (1965). Signs of folic acid deficiency as shown by an increased incidence of megaloblastic anaemia and of neutrophil hypersegmentation, and a greater fall in the serum folate, were more marked in women given no iron than in an iron-supplemented group. Neither the proportion who developed a megaloblastic anaemia nor the fall in the serum folate was significant and in fact the mean serum folate values at 35 weeks and in the puerperium were lower in the iron-supplemented groups; thus their evidence rests largely on the significant increase in hypersegmentation in women not taking iron. In the clinical trial described in Chapter IV, no difference in the serum folate levels of the iron-supplemented and iron-free groups was obtained and there was no significant difference in the amount of hypersegmentation. The incidence of megaloblastic anaemia was low in both groups and thus no evidence to support this hypothesis was obtained in the present study.

Some experimental evidence supports Chanarin's hypothesis. In an experiment on young rats given different diets, Vitale, Streiff and Hellerstein (1965) showed that an enzyme, glutamate formimino transferase, necessary for the transfer of the formimino group from Figlu to tetrahydrofolic acid, was reduced in the liver of the iron-deficient but not of the control animals and concluded that iron deficiency may result in a defect in folate metabolism. They suggested this might explain the increased Figlu excretion reported in association with iron deficiency (Chanarin, Bennett and Berry, 1962; Vitale et al., 1965). Since increased Figlu excretion is not common in iron deficiency and is produced by a number of other factors, this explanation must be considered with reserve until further study allows the significance of this experimental work to be assessed.

The relationship of iron and folic acid deficiency in pregnancy is of fundamental importance. If iron deficiency predisposed to folic acid deficiency then in a group of pregnant women in whom iron deficiency was common, a deficiency of folic acid would be diagnosed relatively frequently also. As was shown in Chapter I, however, this was not the

case; iron-deficiency anaemia occurred in nearly one-quarter of the women attending the Radcliffe Infirmary ante-natal clinic selected for study but the incidence of overt folic acid deficiency was less than 2%. In a large series reported by Giles (1966) megaloblastic anaemia and low serum folate levels were found to be commoner among 620 pregnant women taking iron supplements than among 254 who were not and in a recent study Willoughby and Jewell (1966) did not show a significant difference in the incidence of a "macrocytic anaemia" in women not supplemented with iron compared with those who were taking iron and the mean serum folate level was the same in the two groups.

It is apparent that there is clinical and laboratory evidence that iron deficiency and folic acid deficiency commonly occur together both in pregnancy and other conditions. At present there is insufficient evidence to suggest that either condition predisposes to the other.

INCIDENCE OF FOLIC ACID DEFICIENCY IN PREGNANCY

In the present study, a survey of women attending one ante-natal clinic indicated that megaloblastic anaemia occurred in between 1.5% and 2% of patients. It is difficult to determine how representative women in the present study are of all pregnant women in this area, since only 51% of women have a booked delivery in hospital (Hobbs, 1967). A further 1.2% are admitted for delivery as emergencies and the remainder either deliver at home or in a general practitioner unit. As the reasons for a hospital delivery include grand multiparity and a previous or present complication of pregnancy, it is likely that, if anything, megaloblastic anaemia would occur in the population as a whole less frequently than the 1.5% to 2% incidence calculated in this study.

In earlier reports the estimated incidence was between 0.2% and 0.5% of hospital confinements (Davidson et al., 1942; Scott, 1954; Cowan, 1957; Forshaw et al., 1957) but a planned study specifically aimed at assessing the incidence of this condition (Giles and Shuttleworth, 1958) indicated that megaloblastic anaemia occurred in 2.8% of all hospital

confinements.

Since then other laboratory investigations of value in the diagnosis of folic acid deficiency have become widely used, and with the growing awareness of this condition, the reported incidence of megaloblastic anaemia has increased (Table 49). It is apparent that this varies considerably in different parts of the world and the reasons for this will be discussed.

TABLE 49

To Show the Reported Incidence of Megaloblastic Anaemia in Pregnancy in some Recently Reported Series

Country	Year	Author	No. of cases	Incidence	
				% of hospital cases	% of anaemic pregnant women
United Kingdom and Ireland	1960	McKenzie & Abbott	73	3.6	
	1960	Gatenby & Lillie	100	0.42	
	1960	Hourihane et al.	95	4.2	
	1961	Ainley	116	1.09	
	1962	Dawson, More & Aird (group III & IV)	36	4.3	
	1964	Hibbard	7	4.2	
	1965	Chanarin et al., selected groups: iron supplemented		9.7	
		not iron supplemented		16.6	
Other countries					
S.America	1960	Layrisse et al.	38	-	3.8
Sweden	1964	Hansen	20	0.087	7.7
Malaysia	1966	Thambu & Llewellyn-Jones	373	-	35
Canada	1966	Lowenstein et al.	63	2.35	23
India	1966	Krishna Menon, Sengupta & Ramaswamy	69	-	17.2
S.Africa	1966	Metz	-	12	

Several factors influence the reported incidence of megaloblastic anaemia in pregnancy. These are:

a) the diagnostic criteria;

b) the indications for bone marrow biopsy; and

c) the local conditions of the population under study.

THE DIAGNOSTIC CRITERIA

The lowest incidence shown in Table 49 is that reported by Hansen from Gothenburg, Sweden. The diagnostic criteria used by him were strict; patients investigated had an iron-resistant anaemia, megaloblastic erythropoiesis and increased iron in the bone marrow or serum. Lowenstein et al. (1966) found an incidence of "megaloblastic" anaemia in pregnancy of 2.35% but nearly three-quarters of his cases had only white cell changes. In their study nearly one-quarter of all anaemic pregnant women had "megaloblastic" change compared with 7.7% of all anaemic women studied by Hansen. Hansen noted, however, that if minor morphological changes had been used as a basis for the diagnosis of megaloblastic anaemia, then in half of the anaemic pregnant women in his study the diagnosis would have been made.

These two examples illustrate the effect that different diagnostic criteria have on the estimated frequency of this condition. Since the interpretations of bone marrow smears is subjective, it will always be difficult to make a valid comparison of the frequency of megaloblastic anaemia in pregnancy in different centres. However this would be facilitated if the diagnostic criteria used were carefully stated, and if the term "megaloblastic anaemia" was avoided unless this was unquestionably present.

INDICATION FOR BONE MARROW BIOPSY

The frequency with which bone marrow biopsy is carried out also has a direct bearing on the frequency with which megaloblastic anaemia is detected. This has been shown recently when interest in two particular aspects of this condition has stimulated a number of workers to perform a

bone marrow biopsy on women who would otherwise not have been investigated.

The reported association of abruptio placentae and megaloblastic anaemia has been intensively studied, and bone marrow biopsy performed on women who were not necessarily anaemic (Hourihane et al., 1960; Coyle and Geoghegan, 1962; Hibbard and Hibbard, 1963; Krishna Menon et al., 1966; Thambu and Llewellyn-Jones, 1966). Confirmation of this association was not necessarily obtained, but with the possible exception of the report from India where all the women were anaemic, megaloblastic erythropoiesis has been found in patients who but for an accidental haemorrhage would not have been investigated.

During a trial of prophylactic folic acid by Chanarin and his associates (1965), bone marrow biopsy was carried out on a large number of pregnant women including some without anaemia and the exceptionally high incidence of megaloblastic erythropoiesis obtained as a result of this intensive investigation is shown in Table 49.

These two examples illustrate that unless a special study of anaemia in pregnancy is being undertaken assessment of the incidence of megaloblastic anaemia based on the number of women who present with a megaloblastic anaemia will underestimate the true incidence. As has been shown in the foregoing account and confirmed in the present study, pregnant women may feel well yet have a megaloblastic anaemia. It is likely that with modern ante-natal care few severe cases will be missed but it is apparent that milder ones may pass unnoticed.

REGIONAL DIFFERENCES

These may be most important in determining the frequency of anaemia during pregnancy in different countries. In tropical and subtropical countries malnutrition, poverty and ignorance are commonly found in a considerable proportion of the community. Other factors such as religious dietary restrictions, parasitic diseases and the haemoglobinopathies may be present and will increase the frequency and severity of anaemia. Karthigaini et al. (1964) studied 50 consecutive women, half of whom

were vegetarian, attending an ante-natal clinic in South India; 27 had a megaloblastic anaemia and the overall incidence of megaloblastic anaemia must be high.

In parts of Africa, malnutrition, malaria and the haemoglobinopathies are all common and as a result anaemia during pregnancy occurs frequently; Fullerton and Watson-Williams (1962) reported that 50% of all women attending their ante-natal clinic had a haemolytic anaemia and of those with haemoglobin SC disease, approximately 30% had megaloblastic erythropoiesis. Metz (1966), reporting on African patients attending a Johannesburg hospital, noted that approximately 12% had a frank megaloblastic anaemia and a further 13% had lesser changes. This incidence is as high as that seen in tropical Africa although malaria, the haemoglobinopathies and tropical sprue are rarely seen in the patients he was studying. Anaemia in his patients was due to prolonged lactation and marked dietary deficiency.

There are regional differences in the incidence of megaloblastic anaemia in pregnancy in Britain although these are much less striking. Differences in diet, parity and socio-economic circumstances might account for this. For example almost all patients attending a Liverpool hospital belonged to social classes IV and V, 20% were grand multiparous, 25% had unemployed husbands and dietary standards were generally poor (Hibbard, 1964). In such a community 4.2% of pregnant women developed a megaloblastic anaemia, an incidence which is comparable to that noted in Manchester, Dublin and Sunderland. The lower incidence noted in the present study may have been partly due to the better socio-economic status of patients attending the Radcliffe Infirmary.

THE SIGNIFICANCE OF FOLIC ACID DEFICIENCY IN PREGNANCY

It is clearly important to know if folic acid deficiency has a harmful effect on the mother or foetus. If so the argument for giving folic acid prophylactically to pregnant women, either routinely or to selected groups who appear to be at special risk, would be strengthened. Before

this can be fully assessed it is necessary to determine the effects of folic acid deficiency in pregnancy.

EFFECTS OF FOLIC ACID DEFICIENCY ON THE MOTHER

There was no evidence in this series that any of the maternal complications of pregnancy were more likely to occur in women as a result of folic acid deficiency.

Infection: It was shown in Chapter II that infection occurred more frequently in anaemic women (23%) than in the pregnant control groups (15%). The incidence of infection in women with bone marrow evidence of folic acid deficiency was, however, the same as in those with normoblastic anaemia. It is considered that infection plays a part in the production of anaemia rather than that anaemic predisposes to infection. The evidence for this will be discussed later.

Haemorrhage: Haemorrhage at delivery was similarly shown to be more frequent in the 120 anaemic women (9%) than the controls (5%) but not more so in women with folic acid deficiency than in those with iron deficiency.

It is recognised that post-partum haemorrhage may precipitate megaloblastic anaemia (Badenoch et al., 1955; Dawson, 1962; Giles, 1966) and the role of haemorrhage in producing megaloblastic anaemia in the puerperium has been discussed in Chapter II. There is no evidence that folic acid deficiency has predisposed to the development of post-partum haemorrhage; there was no increase in the incidence of this complication in several series of cases of megaloblastic anaemia when compared either with a group with normoblastic anaemia or with normal controls. Recently an association between thrombocytopenia and "serious haemorrhage at delivery" in untreated megaloblastic anaemia in pregnancy was reported (Lawrence and Klipstein, 1967). Although it is known that thrombocytopaenia may occur in megaloblastic anaemia of pregnancy (Callender, 1944; Layrisse et al., 1960; Pritchard, 1962) its association with haemorrhage at delivery has received little attention.

The association of folic acid deficiency with ante-partum haemorrhage

and in particular haemorrhage occurring from a normally placed placenta (abruptio placentae) has been examined in a number of recent publications. In the present series the incidence of ante-partum haemorrhage was increased in anaemic women but was the same in the megaloblastic, transitional and normoblastic groups. Results in some of the larger studies of megaloblastic anaemia in pregnancy are given in Table 50.

TABLE 50

To Show the Frequency of Ante-partum and Post-partum Haemorrhage in Several Large Studies of Megaloblastic Anaemia in Pregnancy

Year	References	No. of cases	Number with ante-partum haemorrhage	Number with post-partum haemorrhage
1958	Forshaw	33	None	2
1960	Gatenby and Lillie	100	2	2
1960	Hourihane et al.	95	13 (2.5%)†	*
1960	McKenzie and Abbott	68	4 (2.7%)	*
1961	Ainley	116	6% (3.3%)	3.4%
1964	Hibbard	163	13 (1.3%)	*
1966	Giles	335	15 (5%)	4%
1966	Krishna Menon et al.	69	1	*
1966	Varadi et al.	186	11 (3.9%)	*
1967	Present series	56	5 (3%)	4

†Incidence in control groups given in brackets. *Not stated.

A normal incidence or insignificant increase is noted in most series; in two series however an increased incidence was noted. The first report to indicate an association between accidental haemorrhage and megaloblastic anaemia was that of Hourihane et al. (1960). The incidence of 14% is the highest yet reported but the authors comment that it became their practice to investigate patients with ante-partum haemorrhage and since two of their cases had a Hb concentration greater than 12 g, selection of patients may have contributed to the increased incidence in their series. Another factor which might increase the incidence is the effect of loss of blood on folic acid stores which were possibly only just adequate before haemorrhage occurred. Hibbard (1964), considering reasons for the association of abruptio placentae and folic acid deficiency,

examined this possibility by means of the Figlu excretion test and showed that increased excretion was much greater in abruptio placentae when compared with other causes of haemorrhage, such as placenta praevia; he therefore considered this factor unimportant, but nevertheless the possibility remains.

In the present study the serum folate was estimated in 21 women who subsequently had an ante-partum haemorrhage before there was any sign of this and a normal distribution was noted. Giles (1966) found a normal serum folate in 19 out of 21 cases of abruptio placentae after the diagnosis was made, and similarly Krishna Menon et al. (1966) found normal serum levels in most cases of accidental haemorrhage they investigated (97 out of 101 patients). These results make the constant association of increased Figlu excretion and abruptio placentae (72 out of 73 cases) reported by Hibbard and Hibbard (1963) difficult to explain.

Bone marrow examination of women after ante-partum haemorrhage by Coyle and Geoghegan (1962) showed megaloblastic change in 45% of 77 women. Hibbard (1964) subsequently showed an incidence of 34% in 165 patients with all types of ante-partum haemorrhage which increased to 64% when the 73 women with abruptio placentae were examined separately. No other haematological details were given in the affected women in either series and since the term used is "megaloblastic erythropoiesis" rather than "megaloblastic anaemia" presumably they were not all anaemic. Several recent reports do not confirm these findings. Krishna Menon et al. (1966) found a normal incidence of megaloblastic erythropoiesis (10.7%) in 112 women with accidental haemorrhage and anaemia and Thambu and Llewellyn-Jones (1966) reported a similar incidence of megaloblastic anaemia (± 21%) in their controls and in 82 cases of abruptio placentae.

The interpretation of bone marrow morphology particularly after haemorrhage is not easy and differences in interpretation might explain some of the contradictory reports of the association of ante-partum haemorrhage and megaloblastic erythropoiesis. However, increased Figlu excretion is an objective sign and its strong correlation with

abruptio placentae reported by Hibbard and Hibbard (1963) must be considered as evidence of a disturbance in folic acid metabolism in this condition. Nevertheless in most series of megaloblastic anaemia in pregnancy the incidence of ante-partum haemorrhage is not significantly increased and therefore it is most unlikely that folic acid deficiency has caused the haemorrhage.

Toxaemia of pregnancy: The incidence of toxaemia of pregnancy amongst the megaloblastic and transitional megaloblastic groups in the present study was no greater than in the normal subjects. This is in agreement with the normal incidence of toxaemia of pregnancy reported in two large groups of women with megaloblastic anaemia of pregnancy (Ainley, 1961; Giles, 1966).

In other series however an increased incidence has been noted (Gatenby and Lillie, 1960; McKenzie and Abbott, 1960).

Oedema is frequently noted in women with megaloblastic anaemia of pregnancy; for example Thompson and Ungley (1951), Tacchi (1958) and Gatenby and Lillie (1960) all noted oedema in over half their patients with this condition, and obviously therefore a diagnosis of toxaemia may be made relatively easily in women with megaloblastic anaemia of pregnancy. Since diagnostic criteria are not usually stated in reported studies it is not possible to decide if this association is true or only apparent, and there is no definite evidence to indicate that folate deficiency predisposes to toxaemia of pregnancy.

Abortion: A significant correlation between megaloblastic anaemia and a history of previous miscarriage was obtained in the present series. There was a tendency also for low serum folate levels to be associated with a history of previous miscarriage but this was not significant. A similar association, which was not statistically significant (Hibbard, 1964), was found in a large series of pregnant women with increased Figlu excretion in a community characterised by poverty, low social class, grand multiparity and unemployment. It seems likely that a number of common factors are present, one of which might be responsible for producing both folic acid deficiency and abortion, especially recurrent

abortion.

Increased Figlu excretion was found in a significant number of women (22%) whose pregnancy had recently ended in abortion (Hibbard, 1964) and was more striking (41%) in women who had two or more consecutive miscarriages. Although the increased incidence of Figlu excretion, after abortion had taken place, may have been partly due to a temporary deficiency of folic acid precipitated by blood loss the greater incidence of increased Figlu excretion in the group with recurrent abortion indicates that a difference exists between the two groups. This might be related to folic acid deficiency but there is no evidence that this deficiency antedated and was responsible for the abortion.

In the present study the serum folate was estimated in 7 women whose pregnancy ended later in abortion, and was normal in all. The number was small but the importance of serum folate estimations in this small group lies in the fact that blood was taken and assayed before abortion had started. In two reports of an association between low serum folate and spontaneous abortion, the serum folate estimation was made after the onset of bleeding. Abnormal serum folate levels were noted in 25% of 150 women with spontaneous abortion consecutively studied by Martin et al. (1965). Within 24 hours of admission, the Hb concentration of 42% of these women had fallen below 10.5 g/100 ml which suggests that blood loss was considerable and as a result an increased demand for folic acid would be present. No serum folate results in a control group, for example in a group of women with induced abortion, were given for comparison. The same authors treated 17 women, with a history of at least two consecutive previous miscarriages, with 15 mg folic acid daily by mouth in a subsequent pregnancy with a successful outcome in at least 15, but this was not a controlled trial and the significance of this is difficult to assess. In another study Martin and Davis (1964) found that serum folate levels were significantly lower in a group of women with threatened abortion than in normal controls but that the levels were higher than the controls when the abortion was inevitable. It is not clear whether abortion had already occurred when blood for assay was

taken. It is hard to accept their explanation that the high levels after delivery were due to a slackening of demand for folic acid, if it is being suggested at the same time that folic acid deficiency was important in the aetiology of the abortion. The increased incidence of megaloblastic anaemia in the puerperium indicates that there is no immediate reduction in the folic acid requirements of the mother. Martin, Davis and Hähnel (1963) noted an association between low serum folic acid levels and reduced urinary oestrogen excretion. Since the latter has been noted in unsuccessful pregnancy this suggested that folic acid deficiency might result in an impaired production of ovarian hormones. This clearly requires further study.

There is experimental evidence that folic acid deficiency and abortion might be related. Aminopterin, a powerful folic acid antagonist, has been used to procure therapeutic abortions in experimental animals and man (Thiersch, 1952). Nelson, Asling and Evans (1952) observed that if folic acid deficiency is induced early in pregnancy, abortion and resorption of the rat foetus will occur. In the light of these observations it is tempting to consider the association of evidence of folic acid deficiency and abortion in man as a causal one.

As has been shown most evidence suggestive of folic acid deficiency has been obtained once abortion had commenced. There is no evidence that folic acid deficiency was present at an earlier stage in pregnancy, and therefore no evidence that maternal folic acid deficiency results in abortion of the human foetus, but the association requires further study.

EFFECTS ON THE FOETUS

An increased incidence of twins was noted in pregnancies associated with a megaloblastic anaemia in the present series. No other association or even trend was found when the frequency of other complications, such as prematurity, stillbirths and congenital abnormalities, was examined.

Twins: The incidence of twins in the present series of 33 cases of frank megaloblastic anaemia was 15% compared with less than 2% in

the control groups. The incidence of twins in many other series has been increased (Tacchi, 1958; Chanarin et al., 1959; Gatenby and Lillie, 1960; Hourihane et al., 1960; McKenzie and Abbott, 1960; Ainley, 1961; Giles, 1966) and Chanarin et al. (1959) showed that a megaloblastic anaemia developed 8 times more frequently in a twin pregnancy than a single pregnancy. McKenzie and Abbott (1960) calculated that megaloblastic erythropoiesis was present in 24% of twin pregnancies, and similarly a high incidence was found by Giles (1966) in anaemic women with multiple pregnancy. Hibbard (1964) confirmed these findings and in a study of 74 patients with a multiple pregnancy found megaloblastic erythropoiesis in 27% and increased Figlu excretion in 51%.

Other tests of folic acid function may be disturbed in multiple pregnancy. In Chapter III it was shown that a low serum folate is found in the latter weeks of a multiple pregnancy but not in the early stages. Some of these patients were anaemic so that this was in part a selected group but Ball and Giles (1964) studied 12 normal pregnant women with multiple pregnancy and showed that the mean serum folate was significantly lowered. The whole blood folate may also be low (Hansen, 1964). Chanarin and colleagues (1959) found that the plasma clearance of injected folic acid in normal twin pregnancy could be as rapid as in pregnancy complicated by megaloblastic anaemia.

There is thus considerable evidence that a multiple pregnancy results in an increased strain on the maternal stores of folic acid. It is apparent that if this cannot be met by increased dietary intake, overt deficiency is likely to develop in a significant proportion of cases.

Prematurity and birth weight: The normal incidence of prematurity and normal distribution of birth weights noted in Chapter II in women with megaloblastic anaemia was also noted when these complications were related to maternal serum folic acid levels (Chapter III). Varadi et al. (1966) were similarly unable to show any deviation from the normal birth weight in babies born to 214 women with megaloblastic anaemia and Giles (1966) noted no increase in the number of premature infants in 335 pregnancies. In several series an association

between megaloblastic anaemia and prematurity has been reported (Tacchi, 1958; Hourihane et al., 1960; Gatenby and Lillie, 1960) but this was partly due to the number of twin pregnancies. Other factors such as poor socio-economic circumstances and high parity, are associated with prematurity (Peel, 1963) and this too may contribute to this association.

Stillbirths: The incidence of a stillbirth was the same in megaloblastic and normoblastic anaemia, and when all anaemic women are considered together 2.5% had a stillborn baby compared with 2.2% of the non-anaemic controls. There is thus no evidence in the present study that anaemia in pregnancy is related to stillbirths. However an increased number of stillborn infants has been noted in some series (Hourihane et al., 1960; Giles, 1966) but has not been observed in others (Forshaw, 1958; Ainley, 1961; Varadi et al., 1966). Hibbard (1964) showed a significant association between increased Figlu excretion and a previous history of prematurity, previous perinatal mortality and previous unsuccessful pregnancy, and considered that defective folic acid metabolism produces an unsatisfactory foetal-maternal relationship. Results in the present study confirmed that women with megaloblastic anaemia were more likely to have had a previous miscarriage, particularly on more than one occasion, but as has been pointed out previously evidence of folic acid deficiency in association with complications of pregnancy is insufficient proof of a direct causal relationship between these conditions.

Foetal abnormalities: No correlation between either megaloblastic anaemia or low serum folate levels and abnormal infants was obtained in this study. With the exception of one report (5 abnormal babies in a small series of 17 patients, Fraser and Watt, 1964), the incidence of abnormal infants born to women with megaloblastic anaemia is not increased. In a number of the larger series in which details are given (Table 51) the incidence of abnormal infants is 2.5%, which approximates to the incidence of 17.3/1,000 live births reported by McKeown and Record (1960) in a population survey.

TABLE 51

To Show Incidence of Congenital Abnormalities in Reported Series of Megaloblastic Anaemia

Year	Reference	Cases of megaloblastic anaemia	Number of abnormal infants
1958	Forshaw	33	1
1960	Layrisse et al.	38	1
1960	Gatenby and Lillie	100	1
1960	Hourihane et al.	95	1
1961	Ainley	116	1
1964	Fraser and Watt	17	5
1966	Giles	335	8
	Present study	56	2
		790	20 (2.5%)

A study of Figlu excretion in 98 mothers who had just given birth to infants with severe malformations was carried out by Hibbard and Smithells (1965). Comparison with 54 matched controls showed an increased Figlu excretion to be 5 times more common in the former group (62% compared with 17%). The observations of Hibbard and Smithells are most interesting and suggest that some metabolic defect is present in these women which may be significant.

There is no evidence however that such a defect was present at the time of formation of the affected foetal organs. The lack of correlation between florid megaloblastic anaemia and congenital abnormalities similarly does not disprove that folic acid deficiency early in pregnancy could produce foetal abnormalities. This has been well documented in experimental animals (Nelson, 1960) and in a number of women given aminopterin to produce abortion (Thiersch, 1952). This form of folic acid deficiency is very difficult however to the usual type seen in pregnancy. The latter is a disorder of late pregnancy and the puerperium and it seems most unlikely that it plays any part in producing congenital abnormalities.

It is concluded that apart from possible maternal ill health due to the development of anaemia, there is no convincing evidence that folic acid deficiency has a

harmful effect either on the mother or her infant. It is likely that reported complications of this condition in a number of series are in fact associations.

PROPHYLACTIC FOLIC ACID IN PREGNANCY

Iron deficiency is common and as a result the Hb concentration of a group of women given iron supplements will be increased. As shown in the present study, no such benefit results from the use of folic acid supplements. On the basis of these results subclinical folic acid deficiency does not seem to contribute to anaemia in pregnancy nor does it appear to have any harmful effects on the mother or the foetus. The benefit from prophylactic folic acid will therefore be limited to the prevention of megaloblastic anaemia, the incidence of which is approximately 2-4% of hospital deliveries.

As pointed out in a recent editorial (Brit.med.J., 1966) the prophylactic administration of folic acid has now been given a thorough trial, but several aspects of this practice require consideration. These are

a) Is prophylactic folic acid effective?
b) Does it have any harmful effects?
c) What dose of folic acid should be used and when should it be started?
d) Is routine prophylaxis with folic acid worthwhile or should its use be restricted to patients at special risk?

These will now be discussed.

THE EFFECTIVENESS OF PROPHYLACTIC FOLIC ACID

Large doses of folic acid have been used in several centres with considerable success. Lowenstein et al. (1955) reported that since introducing a daily supplement of 3 mg folic acid and 4.5 μg of vitamin B_{12}, megaloblastic anaemia had disappeared from the hospital wards and antenatal clinics. Giles and Burton (1960), using 15 mg dose daily from the 29th week of pregnancy, did not find any cases of megaloblastic anaemia in the supplemented groups; nevertheless 25 cases (either emergency admissions or cases referred by the family doctor) were diagnosed during

that period, indicating that nearly one-quarter of cases of megaloblastic anaemia may not be prevented by the hospital administration of folic acid.

Dawson et al. (1962) used one dose of 15 mg weekly from the 28th week of pregnancy and found this regime only partially successful. The incidence of "megaloblastic change" was 8% compared with 10% in the control groups but only one treated patient (0.1% of total) had florid megaloblastic erythropoiesis; this patient had taken only 2 doses of folic acid 11 weeks previously, so was not in fact adequately treated.

In the trial described in Chapter IV none of the patients supplemented with folic acid from 28 weeks onwards whether at a dose of 5 mg daily or 0.5 mg daily developed megaloblastic anaemia, but as this was only seen in a few patients in the control groups no definite conclusion can be drawn.

It is apparent that if several milligrams of folic acid are taken daily megaloblastic anaemia in pregnancy will not develop. However, a number of patients presenting with megaloblastic anaemia will have had no ante-natal care (Giles and Burton, 1960; Pritchard, 1962; present study). Such cases may form a significant proportion of the total, as in Pritchard's series, in a community where ante-natal care is not sought until late pregnancy. For this reason routine prophylaxis will not entirely prevent this condition but the incidence will be considerably reduced.

THE HARMFUL EFFECTS OF ROUTINE PROPHYLAXIS

The possible danger of giving several milligrams of folic acid daily to large numbers of pregnant women has led to studies to determine the minimal effective dose capable of preventing megaloblastic anaemia. The inclusion of as small a dose as 0.4 mg folic acid in vitamin preparations may have serious consequences if taken by patients with undiagnosed pernicious anaemia. A dose of this order will, for a time, produce a haematological response in such patients but will not prevent, and can even make worse, the neurological disturbances of pernicious anaemia (Baldwin and Dalessio, 1961; Hansen and Weinfeld, 1962).

The likelihood of a pregnant women having undiagnosed pernicious anaemia is remote but a few cases have been reported (Hansen, 1964). Nevertheless this is the main argument against the routine use of large doses of folic acid. Other conditions presenting as a megaloblastic anaemia in pregnancy might also be masked for a time by routine administration of folic acid. The malabsorption syndrome occurs more commonly in pregnant women than pernicious anaemia but has received less comment probably because delay in diagnosis may be less disastrous. Subacute bacterial endocarditis (Giles, 1966), gastric carcinoma and Hodgkins disease (Gatenby and Lillie, 1960) are examples of other conditions which presented as a megaloblastic anaemia in pregnancy; these examples show that an underlying disease is present in a proportion of cases of megaloblastic anaemia, those presenting before the 28th week of pregnancy being particularly suspect, and lend support to the argument that each case of megaloblastic anaemia during pregnancy should be investigated and followed up with care.

SCHEDULE OF PROPHYLACTIC FOLIC ACID

Pregnancy results in an increased requirement for folic acid. The normal dietary requirements (50 μg/day) are increased and a daily dose of folic acid of 150 μg has proved to be inadequate to prevent megaloblastic anaemia in pregnancy (Dawson, 1966; Willoughby and Jewell, 1966). A controlled trial to test the effectiveness of 150 μg of folic acid in preventing folate deficiency was carried out by the author in two groups of anaemic pregnant women and the results are given in full in Appendix VII. In this trial one group received iron alone and the other group iron plus 150 μg of folic acid. There was no significant difference in the Hb concentration of the two groups. Although the group given 150 μg folic acid daily had a significantly higher mean serum folate, one patient developed megaloblastic anaemia in each group.

The effective safe prophylactic dose of folic acid therefore should be greater than 150 μg but less than 400 μg folic acid daily if the neurological sequelae of pernicious anaemia are to be avoided. Willoughby and Jewell (1966), using three different doses of folic acid, found that

a daily dose of 300 μg folic acid prevented megaloblastic anaemia and maintained a normal post-partum serum folate in 84% of their patients.

As a result of their findings, Willoughby and Jewell considered that 300 μg folic acid was close to the daily requirements in late pregnancy. Experience obtained in the present study confirms this. The use of 500 μg daily from the 28th week of pregnancy resulted in serum folate levels above the normal range in a proportion of patients and appeared to be in excess of many patients' requirements; however 150 μg daily from the 28th week of pregnancy proved inadequate.

It is possible that a smaller dose of folic acid given at an earlier stage of pregnancy will be effective. From a trial of prophylactic folic acid reported by Rybo (1966) it appears that a normal red cell folate is maintained by 100 μg daily begun approximately at the 20th week of pregnancy; nevertheless even at this earlier stage a dose in excess of 200 μg and probably less than 500 μg was necessary to maintain normal serum levels. Since the dietary intake of folic acid varies in different communities, the results obtained in different trials of prophylactic folic acid may vary. The minimal effective dose in one population might be more than adequate for another and will largely depend on dietary habits. Nevertheless a recommended dose must be shown to be effective and at the same time harmless for all groups; 300 μg daily appears to be such a dose. Since megaloblastic anaemia is occasionally diagnosed earlier than the 28th week, if prophylactic folic acid is used it should probably be given for the latter half of pregnancy.

INDICATIONS FOR PROPHYLACTIC FOLIC ACID

Prophylactic folic acid prevents megaloblastic anaemia of pregnancy. In a well-nourished population such as that found in the present study and others (Metz et al., 1965) it does not produce any rise in the haemoglobin, although in a poorer community this may occur (Giles and Burton, 1960). The benefit to be expected from prophylactic folic acid therefore depends on the frequency with which megaloblastic anaemia occurs in a particular community. As has been shown, there is no advantage to the

mother or foetus in correcting a folic acid deficiency which is diagnosed solely on tests of folic acid function since there is no evidence that this is attended by any ill effects.

It is evident that certain pregnant women are at risk and the following factors are thought to be of especial importance:

a) high parity; results in the present study have shown that increased parity is an important factor in the development of megaloblastic anaemia in pregnancy;
b) a multiple pregnancy; the risk of increased foetal requirements leading to depletion of the maternal stores is considerable;
c) a past history of megaloblastic anaemia during pregnancy; this condition may recur in subsequent pregnancies (Callender, 1944; Thompson and Ungley, 1957; Ainley, 1961; Giles, 1966; Varadi et al., 1966)
d) epilepsy; it has been shown that epileptic women taking anticonvulsant drugs have an increased liability to develop megaloblastic anaemia during pregnancy (Gatenby, 1960).

Under any of these circumstances the use of prophylactic folic acid would appear to be strongly indicated.

One of the chief problems is to decide whether these indications for the use of prophylactic folic acid which are present in a minority of pregnant women justify its routine use.

In a community such as Oxford it is considered that routine prophylactic folic acid is of little benefit. The emphasis should be on dietary instruction and adequate iron intake and this will reduce the incidence of anaemia as effectively as giving iron and folic acid together. It is suggested also that tablets containing iron and folic acid are likely to be less effective than separate preparations. Intolerance to iron was not uncommon but intolerance to folic acid was encountered only once. It is likely that patients in whom iron-deficiency anaemia develops because of inadequate iron intake, will not be protected from folic acid deficiency if the iron and folic acid are contained in one tablet.

In a community where increased multiparity, poor social and economic conditions and dietary deficiency are frequently present prophylactic folic

acid should be given to all pregnant women, being prescribed both by hospital clinics and family doctors.

AETIOLOGY OF FOLIC ACID DEFICIENCY IN PREGNANCY

In the majority of women who develop megaloblastic anaemia during pregnancy there is evidence of a disturbance in folic acid metabolism. For this reason, in the present study the terms have been used interchangeably. For the most part therefore the aetiology of megaloblastic anaemia and folic acid deficiency during pregnancy is the same and will be discussed in this way.

The factors producing a deficiency of folic acid fall under three main headings:

a) Nutritional deficiency of folic acid;

b) Impaired absorption of folic acid;

c) Faulty utilisation of folic acid.

DEFICIENCY OF FOLIC ACID

In the present study a detailed dietary history was obtained in nearly half the women with unequivocal megaloblastic change; most appeared to be having an adequate diet, and a similar observation was reported by Ainley (1961). However a temporary dietary deficiency due to anorexia, vomiting or diarrhoea may occur in pregnancy and ante-dated the diagnosis of a megaloblastic anaemia in a few patients in this series as in others (Davidson et al., 1942; Callender, 1944; Thompson and Ungley, 1951; Forshaw, 1958; Tacchi, 1958; Giles, 1966).

Nevertheless there is considerable evidence that a nutritional deficiency of folic acid is important in producing megaloblastic anaemia in pregnancy. In the tropics and subtropics a nutritional macrocytic anaemia which responds to folic acid is common (Spies, 1946; Goodall, Goodall and Banerjee, 1948) and is indistinguishable from the megaloblastic anaemia occurring in pregnancy, which is also associated with markedly deficient diets (Kothari and Bhende, 1949; Das Gupta, 1954).

Most reports of a nutritional deficiency of folic acid in Britain have

been in elderly, infirm or psychiatrically maladjusted people (Gough, Read, McCarthy and Waters, 1963; Forshaw, Moorhouse and Harwood, 1964; Varadi and Elwis, 1964). Megaloblastic anaemia due to a simple dietary deficiency of folic acid without an additional precipitating factor is rare (Girdwood, 1966) and it is surprising therefore that in a recent dietary survey of a random group of 150 ante-natal patients it was estimated that approximately 60% had a diet containing less than 50 μg folic acid per day. Our knowledge of the folic acid content of various foodstuffs is incomplete (McCance and Widdowson, 1960) and possibly this may prove to be an over-estimation.

Several reports from nontropical areas have emphasised that dietary deficiency was present in women with megaloblastic anaemia in pregnancy, the proportion varying from one-third to three-quarters of the cases (Callender, 1944; Thompson and Ungley, 1951; Forshaw, 1958; Tacchi, 1958; Layrisse et al., 1960; Lowenstein et al., 1962; Hansen, 1964; Giles, 1966) and it must be concluded that nutritional deficiency of folic acid is commonly responsible for this condition.

In some cases, the deficiency of folic acid may develop because of the presence of an additional factor which has strained the maternal stores of folic acid. The best example of this is a multiple pregnancy and in the present series of women with megaloblastic anaemia, as in many others (Thompson and Ungley, 1951; Tacchi, 1958; Gatenby and Lillie, 1960; McKenzie and Abbott, 1960; Giles, 1966) the incidence of twins was increased.

It appears also that frequent childbearing predisposes to the development of folic acid deficiency. In the present study and others (Thompson and Ungley, 1951; Layrisse et al., 1960; McKenzie and Abbott, 1960; Hibbard, 1964) megaloblastic anaemia was noted more often in multiparous women and it was shown (Fig. 25) that in the early weeks of pregnancy the serum folate was significantly lower in women in their fifth or subsequent pregnancy. Prolonged lactation is a feature of the presentation of megaloblastic anaemia in the South African Bantu (Metz, 1966) and presumably the output of folic acid in

breast milk, estimated by Hansen (1964) to be about 4 μg/day, results in maternal deficiency.

Other haematological disorders, and in particular the haemolytic anaemias, are associated with an increased demand for folic acid and their association with megaloblastic anaemia in pregnant women are reported (Drury and Geoghegan, 1957; Kohler, Meynell and Cooke, 1960; Fullerton and Watson-Williams, 1962; Giles, 1966). Such conditions are rare however. Haemorrhage at delivery (Badenoch et al., 1955; Hourihane et al., 1960; Dawson, 1962) and increased erythropoiesis which results after an iron-deficiency anaemia has been treated (Scott, 1954; Tacchi, 1958; Ainley, 1961) are two instances where haemopoietic activity precipitates a folic acid deficiency.

There is considerable evidence therefore that a deficiency of folic acid, either dietary or because a relative deficiency has developed, plays an important role in producing megaloblastic anaemia in pregnancy. There are many reported instances, however, where this condition has developed in women in good health and taking a nutritious diet (Callender, 1944; Girdwood, 1956; Ainley, 1961; Giles, 1966; present study) so that it is necessary to consider other aetiological factors.

IMPAIRED ABSORPTION OF FOLIC ACID

Steatorrhoea has been noted in a few women with megaloblastic anaemia during pregnancy (Davidson et al., 1948; Tuck and Whittaker, 1950; Thompson and Ungley, 1951; Nieweg, van Buchem and Kroese, 1952; Davis and Brown, 1953; Lawrence and Klipstein, 1967). Giles (1966) found that in 22% of 83 women with megaloblastic anaemia the faecal fat output was above the upper limits of normal. However, normal fat absorption has been noted by others (Badenoch et al., 1955; Moore et al., 1955; Lowenstein et al., 1955; Adams, 1956). In the present series only one patient was shown to have malabsorption but a formal study was not made.

Malabsorption of folic acid was noted by Chanarin et al. (1959) in a group of pregnant women, and particularly in those with a megaloblastic

anaemia. Similarly Giles (1966) and Layrisse et al. (1960) noted impaired absorption in a proportion of their cases of megaloblastic anaemia. Girdwood and Delamore (1961) confirmed that there was an abnormality of folic acid metabolism in pregnancy but questioned the practical value of the test because of the difficulty in interpreting the absorption of folic acid in pregnancy.

Even after delivery, however, there is evidence of a disturbance of folic acid metabolism. In a follow-up study (Hansen and Klewesahl-Palm, 1963) of 19 women who had previously had a megaloblastic anaemia of pregnancy, 17 had abnormal results to tests of folic acid function. Similarly Giles (1966) noted that malabsorption of folic acid was still present years after megaloblastic anaemia in pregnancy was diagnosed, and both these authors considered that this indicated that constitutional factors were the main cause of megaloblastic anaemia in pregnancy, pregnancy being merely a precipitating factor. Layrisse et al. (1960) also found persistent malabsorption in some of their patients at follow-up. Clearly this is a variable finding and in two other reports of folic acid absorption in cases of megaloblastic anaemia studied after delivery, this was shown to be normal (Chanarin et al., 1959; Stevens and Metz, 1964).

There is at present insufficient evidence to assess the relative importance of malabsorption of folic acid as an aetiological factor in producing megaloblastic anaemia in pregnancy. Both Hansen and Giles have applied strict criteria for the diagnosis of the condition and it is possible that malabsorption may be more significant in the aetiology of the more severe cases.

FAULTY UTILIZATION OF FOLIC ACID

Large doses of folic acid or vitamin B_{12} have usually been used to treat megaloblastic anaemia in pregnancy and response to a dose of folic acid of 5 mg or more is usually good (Forshaw, 1958; Tacchi, 1958; Giles and Shuttleworth, 1958; Gatenby and Lillie, 1960). A small, more

"physiological" dose of folic acid was reported by Marshall and Jandl (1960) to be of value in the investigation of patients with megaloblastic anaemia not associated with pregnancy. Anaemia caused by a folic acid deficiency responded to the dose used (0.4 mg folic acid daily by intramuscular injection) whereas anaemia caused by vitamin B_{12} deficiency did not. Subsequently it was shown that a smaller dose (0.1 mg folic acid daily) is more satisfactory (Zalusky and Herbert, 1961b; Hansen and Weinfeld, 1962; Izak, Rachmilewitz, Zan and Grossowicz, 1963).

It has been thought that much larger doses of folic acid are needed to treat megaloblastic anaemia in pregnancy than megaloblastic anaemias due to folic acid deficiency unassociated with pregnancy, and because of this the concept that malutilisation of folic acid is present in pregnancy has arisen.

There is however little objective evidence to support this since there are few detailed reports of the treatment of megaloblastic anaemia in pregnancy in which a small dose of folic acid was used. Hansen (1964) noted a good response to a daily intramuscular injection of 0.2 mg folic acid in 3 women with this condition and Lowenstein et al. (1966) treated 2 cases successfully with the same dose given by mouth. A large dose (0.4-0.8 mg daily) was used in other women in Lowenstein's study and most responded. Alperin, Hutchinson and Levin (1966) observed that more than 0.2 mg folic acid daily before delivery by mouth was necessary although both this dose and 0.1 mg folic acid were effective post-partum. Both Pritchard (1962) and Willoughby and Jewell (1966) considered that 0.4 mg might not be sufficient. In contrast to these reports, Chanarin (1966b)observed that a reticulocyte response would be obtained to a dose of folic acid as small as 25 μg daily in almost all cases of megaloblastic anaemia in pregnancy.

Since the requirements of folic acid are increased during pregnancy it is to be expected that the therapeutic dose of folic acid in megaloblastic aaaemia in pregnancy would be greater than that used in other megaloblastic anaemias due to folate deficiency. It was shown that in nutritional

macrocytic anaemia a daily dose of 0.5 mg folic acid by mouth produced a haematological response but was insufficient to restore the whole blood folate (Izak et al., 1963) and it is apparent from this that although evidence of a response to folic acid is obtained with micro-doses of folic acid, much larger doses are needed to correct the deficiency. Consideration of the available information summarized in the previous paragraph, indicates that if allowance is made for foetal requirements the effective dose of folic acid in megaloblastic anaemia in pregnancy is not very different from that required in other megaloblastic anaemias.

Exceptions do occur, however (Badenoch et al., 1955; Lowenstein et al., 1966) and in such cases it is necessary to ensure that there is no malabsorption of folic acid, that erythropoiesis is unquestionably megaloblastic, that no complicating factor such as infection, iron deficiency or haemolysis is present and finally that there is no vitamin B_{12} deficiency. Any of these factors may prevent a complete response to folic acid and it is necessary to assess critically the reasons for an apparent failure to respond to treatment before considering the case to be resistant to folic acid.

Other evidence supporting the theory of a metabolic block in megaloblastic anaemia in pregnancy is inconclusive. The clinical observations and biochemical and microbiological data of Vilter, Horrigan, Mueller, Jarrold, Vilter, Hawkins and Seaman (1950) suggested that a metabolic defect somewhere in the chain reactions leading to the formation of folinic acid coenzyme was present in this condition.

Using folinic acid, the active enzyme derived from folic acid, Scott (1957) observed that it was not necessary to continue treatment once a satisfactory reticulocyte response had been obtained, and suggested that folinic acid acted as a catalyst at some stage of erythropoiesis. However the continued response after stopping treatment has also been observed when folic acid was used (Pritchard, 1962) and is not therefore characteristic of folinic acid.

Ball and Giles (1964) observed that a relative increase in the stable metabolically inactive folic acid factor occurred in a small number of

women with megaloblastic anaemia of pregnancy; the labile metabolically active factor was reduced in all. Their results have not yet been confirmed but could be explained by a failure to convert from the inactive to active form of folic acid.

Other evidence suggests, however (Grzesiukowicz, Jennison and Gowenlock, 1965; Cowan, Hoffbrand and Mollin, 1966) that an inhibitor in the plasma depresses the serum folate levels. Grzesiukowicz et al. (1965) observed that a number of pregnant women with megaloblastic anaemia had low levels of the plasma factor responsible for releasing folate activity from the precursors which are set free from red cells when they die, and thought this might be due to inhibition of the plasma factor. Cowan et al. (1966) demonstrated that the addition of whole serum from a group of patients with folic acid deficiency, including megaloblastic anaemia in pregnancy, to a known concentration of folic acid reduced the expected growth of L. casei. This suggested an inhibiting factor in the serum of such patients but since this was also present in the serum of some normal pregnant women the significance of this observation is not certain.

Two other causes for the development of megaloblastic anaemia in pregnancy have been reported, and it is suggested that both may operate by interfering with the metabolism of folic acid. Gatenby (1960) drew attention to the frequency with which megaloblastic anaemia developed in epileptic women and discussed the role of barbiturate therapy in its causation. The chemical structure of these drugs and folic acid is similar and it is thought that they interfere with folic acid metabolism. In four women in whom a genito-urinary tract infection was associated with the acute onset of megaloblastic anaemia, it was suggested (Chanarin and Davey, 1964) that a toxin or antagonist produced by bacteria might have caused malutilisation of folic acid. The association of infection with megaloblastic anaemia in pregnancy is well recognised (Davidson et al., 1942; Callender, 1944; Gatenby and Lillie, 1960) and was confirmed in the present series. However the incidence of infection did not differ in women with bone-marrow evidence of folic acid deficiency and

those with a normoblastic anaemia. Similar results were obtained by Giles and Brown (1962) who showed clearly that anaemia developed as a result of infection of the urinary tract, but this was commonly hypochromic and rarely megaloblastic. The mechanism of the production of anaemia by infection is not clear, but the authors considered that in their cases diminished output of erythropoietin was unlikely.

Until further evidence is obtained it is not possible to assess the role of malutilisation of folic acid in producing megaloblastic anaemia in pregnancy. Carefully controlled trials in a large number of cases using small intramuscular doses of folic acid should be able to prove whether malutilisation of folic acid is important. The necessity for taking other factors into consideration when assessing the response of anaemia in pregnancy to treatment has been mentioned and conclusions should not be drawn from results obtained in a few cases.

In conclusion, it would appear that there is no single aetiological factor in the production of a megaloblastic anaemia in pregnancy. A temporary deficiency of folic acid, either dietary or because of increased maternal requirements due to some additional factor appears to be most important. In a proportion of cases, malabsorption of folic acid, possibly associated with a constitutional predisposition to this, is present. Although defective utilisation of folic acid has not been excluded there is as yet no conclusive evidence of this.

THE IMPORTANCE OF FOLIC ACID DEFICIENCY IN PREGNANCY

The present study has indicated that the problem of anaemia in pregnancy is mainly related to iron deficiency. The low incidence of megaloblastic anaemia and failure of prophylactic folic acid therapy to affect a rise in the Hb concentration indicates that folic acid deficiency is relatively unimportant compared with iron deficiency in the causation of anaemia in pregnancy. Furthermore from results obtained in the therapeutic trial it can be inferred that in the present study folic acid deficiency played only a small part in an apparently iron-resistant anaemia. Since

the dispensing of iron tablets combined with close supervision of the iron intake reduced the incidence of anaemia at term to approximately 5%, it is clear that a proportion of apparently iron-resistant anaemias are caused by inadequate intake of iron.

Other parameters of folic acid deficiency such as increased Figlu excretion, low serum folate levels and hypersegmentation were noted commonly both in anaemic and non-anaemic pregnant women, and were, in part, corrected by folic acid therapy. Thus it was confirmed that these signs are related to folic acid metabolism but since their disappearance as a result of folic acid therapy was not associated with an increase in the haemoglobin they could not be considered significant in relation to anaemia in pregnancy. Increased Figlu excretion, low serum folate levels and hypersegmentation are sometimes found in patients with normoblastic erythropoiesis but they do not provide as good an index of folate deficiency in pregnancy as in the non-pregnant state. A possible difference in the handling of folic acid in pregnancy and the additional factor of foetal requirements and utilisation makes the interpretation of these findings difficult.

Had it been shown that folic acid deficiency in the pregnant women produced other complications of pregnancy, then the prevention of even a mild degree of folic acid deficiency would be of considerable importance. A significant association between megaloblastic anaemia and a history of a previous miscarriage was obtained in the present study, but there is no convincing evidence that folic acid deficiency precedes the onset of and results in abortion. Two maternal complications of pregnancy, viz. haemorrhage and infection, were related to anaemia in pregnancy but the frequency in women with megaloblastic and normoblastic anaemias was the same. These were considered to play a part in the cause of the anaemia and did not appear to be complications of it. No evidence was obtained that folic acid deficiency produces foetal complications; here too the only significant association, i.e. with twins, appeared to be a causal one. Thus it would seem that the significance of folic acid deficiency in pregnancy is related to the accompanying anaemia.

In areas where high parity, poor nutrition and poor socio-economic circumstances are frequently present megaloblastic anaemia will be diagnosed in approximately 4% of pregnant women; in areas such as Oxford, the incidence will be less than that. Prophylactic folic acid will reduce but not abolish this condition and the advisability of using prophylaxis routinely will depend on local conditions and standards of ante-natal care. As Witts (1962) has pointed out, there is a danger that we may be treating laboratory data rather than patients. A rational approach to the prevention of anaemia in pregnancy in an area such as Oxford where megaloblastic anaemia in pregnancy is not common lies in the improvement of dietary standards and the use of prophylactic iron, the use of prophylactic folic acid being reserved for women at special risk.

APPENDIX I

Copy of questionnaire and diet sheet used in pilot study

HOSPITAL No. 30176 66

DEPARTMENT OF HAEMATOLOGY

ANAEMIA OF PREGNANCY SURVEY

Name : Mc Gowan, Audrey

Address : 24, Ash Lane Ambrosedon, Bicester

Age, Years 20 (1) 20-29 (2) 30-40 (3) (40) (4)

Marital status : (M)/S Occupation : H/wife

Husbands occupation : W.D. Policeman Income : Adequate — says no financial difficulty.
Inadequate (5)

General Practitioner's Name : Dr Forbes

Address : Victoria Rd, Bicester.

L.M.P. 27.9.61 Dates of attendance 1 16/4/62.

E.D.D. 8.7.62 2

First seen within 1st trimester (6) 3

2nd trimester (7) 4

3rd trimester (8) ✓

Blood group : O (9) ; (A) (10) ; B (11) ; AB (12) ; Rhesus +ve negative (13)

HISTORY

General : Previous Illnesses ; if significant (14) - usually anaemic

Menorrhagia (15) Cycle 5-6d/30d. Flow heavy

Previous Pregnancies : Number None (16) 1—5 (17-21) (5) (22) 1st 1942 last 1957 +2 miscarriages 1953 1956.

Complications (23) - none

✓ Anaemia (24) - yes. even when not pregnant.

Diet ✓ Good - Appears to be good. Deficient (25), specify :
meat or fish daily, lots salads.

Drugs : (26) specify :

note salicylate intake (27) - none. Occas Beecham powder for cold

Symptoms : Heartburn (28)

Vomiting } (29) Feels nauseous.
✓ Diarrhoea } B.A. 4/d since starting iron 4d ago.

Other (30)

History of Present Pregnancy :

Twins (31) — nō

Complications :

Hypertension (32) None apart from anaemia

Toxaemia (A)

Oedema (B)

Haemorrhage (C)

Virus infection (D)

Pyelitis (E)

Other (F)

EXAMINATIONS:

Code (G) if abnormality present

Nutritional state — 10½ St. Good.

Nails — N

Tongue — N.

TREATMENT :

1. Previous therapy (H), specify : Started iron tbs 4 days ago

2. Drug and dose	Date begun	Date ended	Result
Folic acid 5mg im tds	16⁴/62		
Jectofer 2ml bi-weekly.	30⁴/62.		
9ml in all given. Refused further injections.			

Intramuscular iron given	(I) ✓	
No response to oral iron	(J)	
No response to parenteral iron	(K)	
Blood transfusion	(L) ✓	12⁶/62 20
Folic acid given	(M)	22⁷/62 20.

INVESTIGATIONS :

20 BTF PP Hb=8·8 (120)

Date	16/4/62	30/4/62	17/5/62	28/5/62	16/7/62	4/9/62
Weeks pregnant	25/40	30/40	34/40	36/40	F.T.	6/52 PP
Hb. (A0, A1, A2)	8·4 g	7·8 g	7·5 g	7·5 g	9·6 g	11·7 g
W.B.C. (A3)	9,400	7,200	7,200	8,000	6,200	5,600
R.B.C. (A4)	4·01 m	3·69 m	—	3·31 m	3·99 m	—
Retics (A5)	2%	5·1%	1·1%	2·0%	0·7%	0·3%
P.C.V. (B6, B7)	29%	28%	30%	27%	33%	40%
Platelets (B8)	235,000	195,000	176,000	280,000	125,000	114,000
M.C.H.C. (B9)	29%	27·5	25%	27·5	29%	29%
M.C.V. (C10)	75	76	—	77	83	
C.I. (C11)	0·71	0·72	—	0·78	0·82	
Film (C12, C13, D14-16)	aniso; macro; micro; hypo	as before	aniso; macro wbc-N	aniso, macro Hypot.	aniso.	NAD.

Date	16/4/62	28/5/62		
M.C.D. (D17)	6·8	7·1		
Red Cell Fragility (D18)	Normal			
Iron Binding Capacity (E0)	—			
Buffy Coat (E1) occas giant-	aniso macro platelets hypersegmt.			
Marrow (E2) Abnormal (E3)	Megaloblastic N			
Stainable Iron (E4)	2			
Serum Fe (E5, F6, F7)	35	30		
Serum B12 (F8, F9, G10)	239			
Serum Folic Acid (G11, G12, G13)	—			
Figlu (H14) 1 100mg/ml HCl = 190ml	+ve			
Blood Volume (H15)	—			
Occult Blood (H16)	—			
Xylose Absorption (H17)	—			
Diagnex Test (H18)	—			

CONCLUSIONS :

Anaemia present in 1st trimester	(MC)
2nd trimester	(N)
3rd trimester	(O) ✓
puerperium	(P)
Iron deficiency	(Q) ✓
Vitamin B12 deficiency	(R)
Folic acid deficiency	(S) ✓
Spherocytosis / Haemolysis	(T)
Hydraemia	(U)
Blood loss	(V)
Secondary	(W)
Other	(X)

Post Partum Follow-up :

See table overleaf for results of tests

COMMENTS : Uncooperative;
poor iron taker; ? did not take folic acid.
Refused further injections of Jectofer.

19/7/62 Normal F.T.D. 07 9lb 0oz
normal loss ± 2oz. PP Hb = 8.8g → 20 @ 22/7/62.
Sterilised in puerperium.

DIET : Details Mrs McBowan
RI no 30176.

	Breakfast	Lunch	Tea	Supper
Monday	Tea Toast.	Chicken Rice. Peas	Bread & Butter Cake.	Soup.
Tuesday	Tea Toast.	Rissoles Potatoes Sprouts	Boiled Egg.	Ham Sandwich.
Wednesday	Bacon.	Roast lamb Potatoes Peas.	Sausages & Tomatoes.	Fruit.
Thursday	Tea Toast	Steak Potatoes Tomatoes.	Bread & Butter. Cake.	Fruit
Friday	Tea Toast.	Fish.	Pancakes.	Fruit.
Saturday	Tea Toast.	Curry and Rice Steamed Pudding	Scrambled Eggs.	Soup
Sunday	Toast Egg.	Roast Chicken Potatoes Veg. Rice Pudding	Ham Salad	Soup.

APPENDIX II

Laboratory methods

APPENDIX II

LABORATORY METHODS

The techniques used to estimate the haemoglobin concentration and the packed cell volume, to stain films of the peripheral blood and bone marrow aspirate, are those described in **Practical Haematology** (Dacie and Lewis, 1965) and only a brief outline of the method is necessary.

ESTIMATION OF HAEMOGLOBIN

The cyanmethaemoglobin method was used. A cyanmethaemoglobin standard was obtained from Diagnostic Reagents, Thame, and the photo-electric colorimeter was standardized at 3 g and 18 g/100ml.

DETERMINATION OF PACKED CELL VOLUME

A micro-haematocrit technique was used, the haematocrit tubes being spun for five minutes with a centrifugal force of about 12,000 g.

STAINING METHODS

Both peripheral blood films and bone marrow films were stained using May-Grünwald-Giemsa's stain. A solution of 1% potassium ferrocyanide in 0.1 N-HCl was used to stain for iron in bone marrow films and staining was carried out in the waterbath at 56° C. Counterstaining was carried out with 0.1% aqueous eosin. A control film was stained at the same time since occasional contamination of the films occurred.

Marrow smears were graded according to the amount of iron present in the particles:

0 - None
1 - Very slight
2 - Slight
3 - Moderate
4 - Moderately heavy
5 - Heavy
6 - Very heavy

In a normal bone marrow, marrow iron is graded as 1 or 2 and occasionally 3. Patients with iron deficiency show a marrow iron of 0 or 1 and those

with pernicious anaemia usually have a marrow iron of 3 or more (Rath and Finch, 1948).

BONE MARROW BIOPSY

The manubrium sterni was the site selected for bone marrow biopsy and a Salah marrow puncture needle was used.

SERUM IRON ESTIMATION

The method used is based on that described by Bothwell and Mallett (1955).

Reagents:

1) **Hydrochloric acid:** A 2N solution is prepared by adding 400 ml iron-free water to 100 ml concentrated HCl.
2) **Trichloracetic acid:** 200 g crystalline trichloracetic acid is made up to 1 litre with iron-free water.
3) **Dipyridyl reagent:** A 0.4% solution is prepared by dissolving 0.4 g dipyridyl in 5 ml glacial acetic acid and making the solution up to 100 ml with iron-free water.
4) **Saturated sodium acetate.**
5) **Thioglycollic acid.**
6) **Iron standards:** 0.5 g of iron wire (obtained from Johnson, Matthey & Co., Ltd.) is soaked in 2-3 ml concentrated hydrochloric acid for a few seconds and made up to 500 ml with iron-free water. One ml of this strong iron solution is again made up to 500 ml with iron-free water to make the stock iron solution.
7) **Iron-free water** is obtained using an Elgastat B102 ion exchange system.

Care of glassware: All glassware is cleaned by soaking for at least 24 hours in a solution of chromic acid. It is then washed three times in iron-free water and slowly dried in lined wire baskets in an oven.

Method: 2 ml of 2N-HCl is added to 2 ml of serum in a round-bottomed centrifuge tube. After approximately 10 minutes 2 ml trichloracetic acid and 2 ml iron-free water are added and the solution stirred well with a glass rod. After centrifuging for 15 minutes at 2,5000 rpm; 5 ml of the

supernatant solution is carefully pipetted into another tube. To this, 2.5 ml saturated sodium acetate 0.5 ml 0.4% αα-dipyridyl and one drop of thioglycollic acid is added; these three together are referred to as colour developer.

The standard solutions are prepared as follows: To each of six tubes is added

1.25 ml 2N-HCl 1.25 ml trichloracetic acid 3 ml colour developer	Total volume = 5.5 ml

The six different standards are prepared by adding different amounts of stock iron solution and iron-free water to total 2.5 ml as follows:-

	Standard iron soln. ml		Iron-free water ml	Iron content (μg/ml)
1.	Nil	+	2.5	0 (blank)
2.	0.5	+	2.0	1.05
3	1.0	+	1.5	2.1
4.	1.5	+	1.0	3.15
5.	2.0	+	0.5	4.20
6.	2.5	+	Nil	5.25

The optical density of the unknown and standard solutions is obtained using a spectrometer and a graph of the iron content and optical density of the standard solutions is plotted. From this it is possible to read off the iron content of the unknown solutions and to calculate the iron content of 100 ml serum (5 ml supernatant fluid contained $\frac{5}{8}$ of 2 ml serum = 1.25 ml serum. To calculate the iron content of 100 ml serum it is only necessary therefore to multiply by 80.)

The normal serum iron using this method, for women, is 60-160 μg/100 ml, and for men is 80-175 μg/100 ml.

MICROBIOLOGICAL ASSAY OF SERUM FOLIC ACID ACTIVITY

The method used in this study was that of Waters and Mollin (1961) with certain modifications reported by Spray (1964). A full description of the method follows.

Reagents and glassware: Chemicals of Analytical Reagent

TABLE 52

Stock Solutions for Preparing the Basal Medium for Lactobacillus Casei

Salt solution C

$MgSO_4.7H_2O$	40 g
Ferrous ammonium sulphate, crystalline	2.8 g
$MnSO_4.4H_2O$	8.0 g
Concentrated HCl	4.0 ml
Water to	1,000 ml

Solution A

Vitamin-free casein hydrolysate (enzymatic)*	1,000 ml
Tryptophane	1.0 g
Cysteine hydrochloride	1.0 g
Asparagine	3.0 g
Sodium acetate $3H_2O$	100 g
KH_2PO_4	25 g
K_2HPO_4	25 g
Salt solution C	200 ml
Adenine, guanine, uracil, xanthine	100 mg each
Water to	5,000 ml

Adjust pH to 5.4-5.6 with 40% NaOH solution, store in an ordinary refrigerator in plugged containers.

Solution B

Riboflavin	10 mg
p-Aminobenzoic acid	20 mg
Pyridoxine	40 mg
Thiamine	4 mg
Calcium pantothenate	8 mg
Nicotinic acid	8 mg
Biotin	200 µg
Water to	100 ml

Store under toluene in an ordinary refrigerator.

*Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A.

grade were used when possible, otherwise the purest commercial grade was used. Solutions were made up with water prepared by re-distilling de-ionized water through an all-glass still. Glassware was cleaned by soaking overnight in chromic acid-sulphuric acid cleaning mixture and washing six to eight times in hot tap water and twice in de-ionized water. Aluminium caps were rinsed in hot tap water and de-ionized water. The dispensing syringe was well washed with hot tap water and then with glass-distilled water just before use. With these precautions the only evidence of contamination by exogenous 'folic acid' was occasional tubes showing excessive growth. This was confined to so few tubes that the aberrant readings were clearly distinguishable and could be eliminated.

Basal medium: Three stock solutions were maintained (Table 52). To prepare solution A the casein hydrolysate, sodium acetate, KH_2PO_4, K_2HPO_4, and salt solution C were mixed and the pH adjusted to about 5.4 with 40% NaOH solution. The mixture was boiled for 30 minutes or more until it gave a clear filtrate on filtering through Whatman no. 1 paper. Solid asparagine was dissolved in the hot filtrate. The cysteine HCl was dissolved in water; tryptophane was dissolved in dilute HCl; adenine, guanine, and uracil were dissolved together in hot dilute HCl; xanthine was dissolved in dilute NaOH solution. The solutions were added to the filtrate. The complete medium was prepared for each assay by making up a solution in the following proportions:-

Solution A	100 ml
Solution B	1 ml

(The riboflavin does not dissolve completely and the solution was shaken to produce an even suspension before each removal.)

Dextrose	4 g
Ascorbic acid	200 mg
Water to 160 ml.	

Preparation of serum extracts: Blood was taken by venepuncture using disposable plastic syringes and was allowed to clot for one to three hours in glass containers. The clot was stirred with a glass rod, taking care to avoid haemolysis and the samples were centrifuged. Ascorbic acid was not added to the serum which was stored at -15° C. until

assayed one to 14 days later.

Serum (0.5 ml) was mixed with 0.1 ml freshly prepared ascorbic acid solution in water (22.5 ml per ml) and 2.2 ml 0.2 M sodium phosphate buffer pH 6.1 (750 ml 0.2M NaH_2PO_4 solution + 250 ml 0.2M Na_2HPO_4 solution). The volume was made up to 5 ml with water and the solution was autoclaved at 15 lb pressure for two and a half minutes, cooled, stirred with a glass rod, centrifuged and a clear extract was decanted.

Stock standard folic acid solution: Pteroylglutamic acid was dried over anhydrous $CaCl_2$ and 10 mg was dissolved in 100 ml 0.001 N NaOH solution and stored in a stoppered bottle at 0° C. Comparisons of the growth of the test organism with known amounts of folic acid from fresh and stored solutions showed that there was no appreciable deterioration in the solutions for up to 17 weeks. Nevertheless a fresh solution was prepared every four to six weeks.

Method of assay: Assays were carried out in 5 in. × $\frac{5}{8}$ in. Pyrex test tubes, using four tubes containing identical samples for each standard and each unknown. The standards, prepared from dilutions in water of the stock standard solution, contained 0, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mμg folic acid per tube respectively. Serum extracts (0.5 ml per tube) were pipetted into other tubes and the volume of aqueous solution in every tube was made up to 1 ml with water. Medium (4 ml) was added to each tube with a dispensing syringe (Arnold R. Horwell Ltd.), the tubes were covered with aluminium caps, autoclaved at 10 lb pressure for 6 minutes and allowed to cool.

Maintenance of cultures and inoculating and reading the assays: The test organism was Lactobacillus casei var. rhamnosus (National Collection of Industrial Bacteria, Torry Research Station, Aberdeen, catalogue no. NCIB 6375). The inoculum for assays was prepared using a liquid medium prepared by a modification of the recipe for the complete medium. Ascorbic acid was omitted, 1 μg pteroylglutamic acid was added, and the volume was made up to 200 ml with water. The solution (10 ml) was dispensed into 6 in. × $\frac{3}{4}$ in. tubes which were

plugged with cotton wool and autoclaved at 10 lb pressure for six minutes.

After recovering organisms from the freeze-dried culture using this medium, stab cultures were maintained in agar (Nymon and Gortner, 1946), transferred fortnightly and stored at 0° C. On the evening before setting up an assay, a tube of liquid medium was inoculated from a stab and incubated overnight at 37° C. Next morning 1 ml of the suspension was added to a tube of sterile medium at 37° C. This culture was incubated for four to six hours, centrifuged, the medium was decanted and the pad of organisms was washed four times by decantation with sterile water and re-suspended in sterile water. The suspension was diluted with sterile water until opacity was only just visible, one drop of the diluted suspension was added to each assay tube, and all tubes were incubated for 40 to 44 hours at 37° C.

After incubation the contents of the tubes were shaken and the optical densities were read on a Hilger Spekker photoelectric absorptiometer with neutral grey filters, against a blank of uninoculated medium. The mean density from each set of four tubes was used to calculate the results. The folic acid activity of each unknown was computed from a standard curve relating the amounts of folic acid to the densities for the standards.

Control subjects: The results in 94 non-pregnant, healthy adults, both male and female, varied from 2.1 to 28 mμg per ml (mean 7.8 mμg); except for one value of 28 the highest figure was 17 mμg per ml.

ESTIMATION OF SERUM VITAMIN B_{12}

The method used in the department of haematology is similar to that described in **Practical Haematology**. Difco Bacto micro-assay culture agar, Bacto micro-innoculum broth and Bacto B_{12} assay medium U.S.P. are all used and the organism Lactobacillus leichmannii is obtained from the National Collection of Industrial Bacteria no. 7854.

A 1 in 10 dilution of serum is used and a serum extract is prepared by autoclaving the serum in a sodium acetate buffer at 10 lb pressure for 20

minutes. After centrifugation the supernatant fluid is decanted and assay carried out at two dilutions in three tubes for each sample.

Cytamen 100 Glaxo is used to prepare a standard solution in triplicate of known quantities of vitamin B_{12} from 0 to 250 $\mu\mu$g (eight dilutions in all). 5 ml of freshly prepared B_{12} assay medium is added to the serum extracts and standard solutions and after autoclaving and cooling, one drop of innoculum is added to each tube. All tubes are then incubated at 37° for 36-48 hours.

The turbidity in the standard and unknown tubes is compared with that of the blank (obtained by removing 2 ml of the supernatant fluid from each of the standard tubes and centrifuging until clear) in a spectrophotometer at ± 700 mμ.

A graph of the mean optical density plotted against the vitamin B_{12} concentration of the standard solution is drawn and from this the vitamin B_{12} content of the unknown sera can be calculated.

In the laboratory of the Department of Haematology the results in healthy non-pregnant control subjects range from 150-800 $\mu\mu$g/ml, with a mean of 391 $\mu\mu$g/ml. Values below 100 $\mu\mu$g/ml are definitely abnormal; those between 100 and 150 $\mu\mu$g are equivocal.

URINARY EXCRETION OF FIGLU

FIGLU was estimated in the urine collected between three and eight hours after histidine loading by the technique described by Kohn, Mollin and Rosenbach (1961).

Materials:

1) **Cellulose acetate strips** (Oxoid Ltd.).
2) **Buffer solution:** Pyridine-acetate acid buffer pH 5.3 was made up of pyridine 12.5 ml, glacial acetic acid 5 ml, and distilled water 1 litre.
3) **Location reagent:** Ninhydrin 0.2 g was dissolved in 6 ml of ethanol and made up to 100 ml with diethyl ether.
4) **Ammonia:** A fresh solution of 0.88 ammonia was used for each series of tests.

5) **FIGLU marker:** This was prepared by adding a known amount of pure FIGLU (kindly supplied by Professor A. L. Lubby, New York Medical College) to acidified normal urine. Later, positive urines (acidified and preserved with thymol crystals and kept at 4° C.) which contained known amounts of FIGLU were used instead.

6) **Apparatus:** Glass capillary automatic pipettes (Kirk type, E-mil) which delivered 5 µl urine were used. All separations were performed on a horizontal tank, designed in the hospital workshop for this purpose. The current was supplied by a constant voltage power pack capable of delivering 200 volts.

Method: L-histidine monohydrochloride 15 g partially dissolved in water was given by mouth to the patient after an overnight fast. Food was withheld until one hour after administration of the dose. Fluid intake was restricted during the eight hours of the test to reduce the volume of urine passed.

Three hours after taking the histidine the patient passed urine which was discarded. All urine passed in the next five hours was collected in a bottle to which 1 ml of concentrated hydrochloric acid and a few thymol crystals had been added. The urine was measured and an aliquot kept for analysis.

Technique: The cellulose acetate strip was marked with a soft pencil, the site of application of the unknown and the control urines containing FIGLU being indicated. Care was taken to ensure that the strip was never touched by hand, forceps being used to hold the paper when necessary. After being soaked in buffer (excess moisture being removed by blotting with filter paper) the strip was laid across the shoulder pieces in the tank. Using separate micropipettes, 5 µl of both the test and control urines were applied to the marked spots on each side of the midline. The wicks previously soaked in buffer were put in position, the tank closed and a current of 200 volts applied for 30 minutes.

The strip was then oven-dried for approximately 10 minutes and then cut in half lengthwise. One half only was suspended in a beaker

containing ammonia vapour for 30 minutes, and then dried again in the oven for a few minutes. Both halves were passed through the ninhydrin solution and then laid flat between two pieces of cardboard which were clipped together. After five minutes in the oven and a further half hour to allow for colour development, the strips were examined in front of a bright light and the electrophoretic pattern of the patient's urine compared with that of the control urine. If the FIGLU spot present in the patient's urine was stronger than the control it was possible by serial dilution and further comparison with the control to estimate the approximate amount of FIGLU excreted. With careful technique and experience as little as 20 μg of FIGLU per ml of urine could be detected.

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APPENDIX III

Haematological details of 29 women anaemic at term despite oral iron

Abbreviations used in Appendices III and IV

Film: N = normal
Erythropoiesis: N = normoblastic; T = transitional; M = megaloblastic
gm = giant metamyelocytes
PP = post-partum
BTF = blood transfusion
Pl.pr. = placenta praevia
A.N. = ante-natal

Survey No.	R.I. reg. No.	At diagnosis† Gestation period, weeks	At diagnosis† Hb conc., g/100 ml	MCHC	Film	Serum iron, µg/100ml	Serum folate, mµg/ml	Serum vit. B_{12} µµg/ml	FIGLU	Erythropoiesis	Bone marrow iron	Weeks of iron before diagnosis	Other treatment	Hb at term	Post-partum Hb	Special features
5	34923	30	10.4	31%	Hypochr. Macrocyt.	171		306	Neg	Transit. megalobl.		18	Given blood	10.4	12.9	Bleeding from placenta praevia. LSCS and premature delivery
8	38043	28	10.5	31%	N	77	(5.0)*	235		N	0	3		10.7	10.5	
13	34930	34	9.8	30.5%	Fragmentn.	206	2.3	54				22		10.7	10.4	Took iron poorly. Platelets 91,000. Red cell frag. normal
15	33396	30	10.5	29%	Hypochr.	46		543		N	0	0		10.2	8.9	Ante-partum haemorrhage
47	38287	31	9.9	30.5%	Hypochr.	165		195	Neg	N	0	18		10.7	12.4	Took iron poorly
66	30176	28	8.4	29%	Hypochr. Macrocyt.	30		239	Pos	Megalobl.	2	< 1	Given blood + folic acid	7.5	8.8	Unco-operative. Refused treatment
84	36260	32	10.3	31.5%	N	14	(3.9)	148	Neg	N	0	19	Given blood	9.9	6.4	PPH. Pyelitis
91	38546	34	10.5	31%	N							22		10.7	12.0	B. coli urinary infection
94	36798	16	10.5	29%	N	68	3.6					0		10.1	11.5	Iron begun at 16 wks. No ante-partum response
98	32959	24	10.4	26%	Hypochr.	230		366	Pos	N (twice)	0	Not known	Given blood	8.3	10.2	
117	38844	31	9.8	30.5%	Hypochr.	50			Neg	N		Not known		10.5	11.0	
126	38811	20	9.3	26%	Hypochr. Fragmentn.	250	(3.9)	398	Pos	N		0	Folic acid	10.5	11.1	Thalassaemia. B. proteus urinary infection
132	37496	32	10.8	32.5%	N							12		10.7	10.7	
140	38879	18	10.8	28.5%	N	56	2.1	236				0	Parenteral iron	10.8	10.8	Took iron poorly. LSCS. Hb rose after IM iron in puerperium
150	39034	13	10.8	28%	N	140	3.4	370	Pos	N		0		10.5	11.1	
174	39244	38	10.5	32.5%	N							26	Parenteral iron	9.8	8.7	B. coli urinary infection. Hb rose 3.3g in 2wks following i.m. iron
188	39371	28	7.3	30%	N + rouleaux	80	(1.0)	490	Neg	N (twice)	0	2	Folic acid	10.1	9.6	8% reticulocytosis after folic acid
201	39536	16	10.7	30.5%	Hypochr.		7.2‡ → 4.2					0		10.5	11.8	Sickle cell anaemia
207	39621	30	9.6	27.4%	Hypocnr.	22	3.4	174				6		10.1	10.7	LSCS. Took iron poorly
269	39952	29	10.2	28%	Hypochr.	64	3.1 → 1.3	150	Neg	N	0	20		9.9	11.8	
273	27331	32	9.8	30%	Normocyt. Macrocyt.	66	1.1	260	Pos	Megalobl.	2	4	Folic acid	10.8	None	Twins
284	34781	37	9.5	31%	Hypochr.							11	Parenteral iron	9.8	None	Took iron poorly
301	40042	32	10.8	30.8%	N		2.6					28		10.8	12.1	
326	39491	32	10.5	29.8%	N		7.2					12		10.5	11.7	
349	40291	38	10.1	29.7%	Hypochr. Macrocyt.	30	0.6 → 0.9	235	Pos	Transit. Megalobl.	0	11		10.1	10.2	
362	40482	38	10.8	32.5%	Anisocyt. Normochr.		3.7					8	Parenteral iron	10.8	12.6	Hb rose 1.8 g/100ml in 2 wks following intravenous iron
371	33590	24	10.4	29.5%	N		1.9 → 3.6					0		10.8	13.3	B. coli urinary infection
374	25635	30	10.8	28%	N		1.7					18		10.8	11.4	LSCS
393	40800	40	9.8	32.5%	Macrocyt.		0.8 → 0.6					< 1		9.8	12.6	Delivered before investigation. Hb normal after delivery

*Serum folate results in brackets were estimated using the method of Waters and Mollin (1961)
‡Arrow denotes later result.
†Gestation period and Hb concentration when anaemia presented; other investigations usually carried out soon after.

APPENDIX IV

Details of 120 anaemic pregnant women

33 Women with Megaloblastic Anaemia

R.I. Reg. No.	Age, yr.	Social class	Parity	Previous miscarriages	Blood group	At diagnosis: Gestation period, wk.	At diagnosis: Hb conc g/100 ml.	MCHC	Film	Serum iron, µg/100ml	Serum folate mµg/ml	Serum vit. B_{12} µµg/ml	FIGLU	Erythropoiesis	Bone marrow iron	Weeks of iron before diagnosis	Treatment: PGA	Treatment: Vit. B_{12}	Treatment: i.m. iron	Treatment: BTF	Hb conc., g/100ml: At term	Hb conc., g/100ml: Post-partum	Complications: Maternal: Infection	Complications: Maternal: APH	Complications: Maternal: PPH	Complications: Maternal: PET	Complications: Maternal: None	Complications: Foetal	Complications: Foetal: None	Birth-weight lb. oz.
16713	32	3	5	1	O Pos	P.P.	9.8			1.1	195		M	3	12				+	None	9.8					+			7 6
17281	41	3	2	1	O Pos	36	9.2		Macrocyt.	216	4.0	100	Neg	M	3	Not known	+		+ (at 38wk)	+	12.1	10.1		+ Pl. pr.		+		Dysmaturity		3 13
27331	32	4	2	0	A Neg	32	9.8	30%	Macrocyt. Hyperseg.	66	1.1	260 ‡ → 100	Pos	M	2	4	+				10.8					+		Twins		4 3 6 8
30176	40	3	5	2	A Pos	28	8.4	29%	Macrocyt. Hypochr.	30		239	Pos	M+N	2	< 1	+		+ (at 29wk)	+	7.5	8.8				+			+	9 0
33698	36	3	7	2	A Pos	32	8.3	33%	Macrocyt. Hyperseg.	258		235	Pos	M	3	20	+			+	14.8	12.6	(Epileptic)			+			+	8 2
34837	32	4	1	1	A Pos	38	8.8	27%	Macrocyt. Hypochr.	16	1.0			M+N	3	18	+		+ (at 36 wk)	+	9.6	11.2				+			+	8 0
35216	29	3	1	0	A Pos	33	6.2	30%	Megalobl. Hyperseg.		0.3	181	Pos			0	+				11.4	10.7		+					+	6 15
35492	34	3	6	1	O Pos	39	9.6		Macrocyt. Hyperseg.	193	0.7	115	Neg	M+N	4	Not known					9.6	10.1				+			+	9 2
36165	27	3	1	0	A Neg	39	10.6	29.5%	Macrocyt. Hypochr.	300	1.4	329	Neg	M+N	2	27			+ (at 37 wk)		10.6	11.0	LSCS +						+	7 1
36259	37	5	4	3	O Pos	32	9.5	26%	Anisopoik.	185		164	Pos	M+N	0	4	+				12.2	11.5	+			+		Twins		5.12 & 6.7
36828	26	0	2	0	O Pos	38	4.4	32%	Macrocyt. Hyperseg.	140		100	Pos	M	3	0	+		+ (before 38 wk)	+	12.9	12.7					+		+	7 3
37020	27	0	5	0	A Neg	35	10.2		Macrocyt.				Neg	M	3	0	+				10.4						+	Rh incompatability		8 7
37497	25	3	4	1	O Neg	P.P.	7.7	29.6%	Macrocyt. Hyperseg. Hypochr.	296		174	Pos	M+N	3	16	+				11.0	7.7					+	Stillborn		5 9
37717	36	3	2	3	O Pos	32	10.8	30.5%	Macrocyt. Hypochr.	300	2.3	67	Neg	M+N		1	+	+ (34wk)		+	10.2	9.3			+			Twins		4.13 & 5.11
38035	21	4	1	0	A Pos	P.P.	8.9	29%	Macrocyt. Hypochr.		1.0	178	Neg	M+N	0	16	+			+	11.1	8.9				+			+	8 9
38269	28	3	1	2	A Pos	38	8.5		Macrocyt. Hyperseg. Hypochr.	122	3.4	‡ 94 → 46		M+N	0	1		+ (38wk)		+	6.7	11.0	+				+			6 11
38426	22	5	3	0	AB Pos	P.P.	5.6	27.5%	Macrocyt. Hyperseg.	446		276	Pos	M	0	3	+			+	None	5.6	+				+			7 11
38429	41	4	7	0	A Pos	34	8.0		Macrocyt. Hypochr.	99		182	Neg	M	0	2	+				11.1	11.0				+			+	8 11
38569	35	3	10	0	B Neg	38	9.5		Hypochr.	222			Neg	M	3	Not known	+			+	9.5	11.1	+			+			+	9 11
38594	23	3	0	0	A Pos	32	6.0	23.5%	Macrocyt. Hypochr.	35		388	Pos	M+N	0	2	+				11.5	10.7				+			+	9 8
38637	22	3	0	0	A Pos	36	8.4		Hypochr.			134	Neg	M+N	2	30	+			+	10.8	8.1	+			+			+	9 0
38899	28	3	1	1	A Pos	P.P.	7.4		Macrocyt.		1.4			M	4	0				+	8.0	7.4			+				+	7 0
40679	24	4	0	0	A Pos	37	10.1	32%	Macrocyt.	93	1.5	90	Neg	M+N	0	Not known	+		+ (37wk)		10.2	11.0				+			+	8 1
40716	37	3	5	2	A Pos	36	9.9		Hypochr.		2.8	290	Neg	M	3	6	+			+	10.5	10.1					+		+	7 13
41092	29	0	2	0	A Pos	39	11.1 (10.1)*		Anisopoik. Hyperseg.	117	4.5	141		M		27	+				11.1	12.0					+		+	6 8
42003	22	5	1	0	A Pos	39	11.8 (10.8)*	30.5%	Macrocyt.		2.7	131		M+N		24					12.3	11.8	+				+		+	9 8
42478	31	4	3	1	O Neg	P.P.	9.8		Macrocyt.		1.4	95		M		22	+				9.8	9.8				+		Twins		5.11 & 5.11
43077	27	4	3	0	O Pos	P.P.	8.7		Megalobl. Hyperseg.		2	128				Not known	+		+ (38 wk)	+	9.5	8.7			+				+	7 3
43149	17	3	0	0	O Neg	36	10.1	31.5%	Macrocyt. Hyperseg.		0.7	192		M	2	0	+				12.8	10.0				+			+	8 4
43617	23	3	0	1		P.P.	10.3	30%	Hypochr.	88	2.5	70		M+N	2	12	+				12.0	10.3	+			+			+	8 5
43706	34	5	2	0		30	8.2	29%	Megalobl. Hyperseg.		1.2	305				< 2	+				11.3	12.2	+						+	7 4
43975	19	0	0	0	O Pos	26	5.8	31%	Macrocyt. Hyperseg.		0.2	90		M	3	1	+	+ (26wk)		+	12.5	10.2	+						+	7 10
44147	33	5	4	0	A Neg	P.P.	8.5	27%	Megalobl. Hypochr.		2.2	80				0			+ (P.P.)	+	None	8.5		+	+	+		Twins		6.12 & 7.8

§PGA given after bone marrow biopsy.

*Hb concentration shortly before investigation.

‡Arrow denotes later result.

23 Women with Transitional Megaloblastic Anaemia

R.I. Reg. No.	Age, yr.	Social class	Parity	Previous miscarriages	Blood group	At diagnosis: Gestation period, wk.	At diagnosis: Hb conc. g/100 ml.	MCHC	Film	Serum iron µg/100ml	Serum folate, mµg/ml	Serum vit. B_{12} µµg/ml	FIGLU	Erythropoiesis	Bone marrow iron	Weeks of iron before diagnosis	Treatment: PGA[§]	Treatment: Vit. B_{12}	Treatment: i.m. iron	Treatment: BTF	Hb conc., g/100ml: At term	Hb conc., g/100ml: Post-partum	Complications: Maternal: Infection	Complications: Maternal: APH	Complications: Maternal: PPH	Complications: Maternal: PET	Complications: Maternal: None	Complications: Foetal	Complications: Foetal: None	Birthweight lb. oz.
13769	40	3	9	3	A Pos	31	10.5	27.5%	N	220	6.6	80	Neg	T	1	Not known	+				11.2	12.7							+	9 4
25260	30		5	1	O Pos	32	9.3	32%	Macrocyt. Hyperseg.	103		231	Neg	T	0	2	+				10.8	10.4				+	+		+	6 13
31304	26	3	1	0	A Pos	36	9.9	31%	Hypochr. Macrocyt. Hyperseg.				Neg	T	2	14*	+			+ (37 wk)	12.7	12.8				+	+		+	8 10
32783	27	3	3	0	AB Neg	39	8.3	29%	Macrocyt.	110		200	Neg	T	0	10				+ (39 wk)	9.9	10.5					+	Rh incompat. Bilat. talipes		6 12
33014	27	3	1	0	B Pos	38	8.9		Macrocyt.	29		190		T	0	17	+			+ (38 wk)	8.9	11.9					+		+	8 2
33553	25	3	4	0	B Pos	40	8.1		Hypochr. Macrocyt.	35		135	Neg	T	0	Not known				+ (40 wk)	8.1		+ LSCS			+			+	8 11
34006	42	5	7	0	O Pos	33	8.4		Hypochr.			265	Pos	T		3			+ (P.P.)		10.8	9.8	+				+		+	8 6
34923	27	1	1	0	A Pos	30	10.4	31%	Hypochr. Macro.	171		306	Neg	T		18				+ (33 wk)	10.4	12.9	LSCS	+ (pl.pr.)				Prematurity		3 13
37091	20	0	0	0	A Pos	40	8.9	31%	Hypochr. Macrocyt.	186			Pos	T	1	Not known	+			+ (P.P.)	11.3	11.8		+ (?pl.pr.)			+		+	7 8
37207	27		1	0	O Pos	38	8.0		Hypochr. Macrocyt.				Neg	T	0	Not known				+ (40 wk)	8.0					+	+		+	10 0
37495	29	3	2	1	O Pos	P.P.	8.9	35%	Hypochr. Macrocyt. Hyperseg.	145		172		T (? M)	3	Not known	+				11.8	8.9					+		+	7 11
37716	21	3	0	0	A Neg	36	7.8	28%	Hypochr. Macrocyt.			162	Neg	T	1	<1	+			+ (36 wk)	11.1		+ LSCS				+		+	6 4
38236	17	4	0	0	O Neg	36	11.3 (9.9)*	30.5%	Macrocyt. Hyperseg.	113		110	Neg	T	2	18					11.4					+	+		+	8 4
38512	24	4	1	1	O Pos	34	9.5	31.5%	Hypochr. Macrocyt. Hyperseg.	43	2.0	103		T	0	12	+				11.2	9.8				+	+		+	9 3
38909	29	5	3	0	O Pos	37	8.6			380		141	Neg	T		Not known				+ (38 wk)	11.1	12.2				+		Twins		4 4 4 8
40233	28	3	0	0	A Pos	35	12.0 (10.5)*	33.5%	N		1.8	125		T	2	27	+				11.1	10.5				+		Hypospadius		6 15
40291	30	4	3	0	B Pos	38	10.1	29.7%	Hypochr. Macrocyt. Hyperseg.	30	0.6	235	Pos	T	0	11					10.1	10.2				+	+		+	6 8
40354	23	0	2	0	A Pos	37	8.1	28%	Hypochr. Macrocyt. Hyperseg.		1.0		Neg	T	0	Not known					8.1					+		Prematurity		4 8
40489	24	4	1	1	O Neg	29	9.5	30%	N	135	3.2	76	Neg	T (? M)	3	12	+	+ (33 wks)	+ (<29 wks)		11.1	13.3 (2 mos P.P.)				+	+		+	7 14
41362	30	4	3	1	O Pos	39	6.6	26%	Hypochr.		1.3	405	Pos	T	3	Not known	+		+	+	9.8	12.1				+		Twins		5 6 6 7
42166	21		0	0	O Pos	P.P.	5.4	21.5%	Hypochr. Macrocyt.		1.7	181		T	2	0	+	(all at 39 weeks)		+ P.P.	No A.N. care	5.4	+				+			5 9
43198	17	3	0	0		P.P.	9.6	29%	Hypochr.	60	1.8	216		T	0	0	+				11.5	9.6				+	+		+	7 12
43871	26	3	2	0	O Pos	33	8.5	28%	Hypochr.	28	1.4	115		T	0	13	+		+ (33 wks)		12.6					+	+		+	7 4

[§]PGA given after bone marrow biopsy. *Hb concentration shortly before investigation.

64 Women with Normoblastic Anaemia

R.I. Reg. No.	Age, yr.	Social class	Parity	Previous miscarriages	Blood group	At diagnosis: Gestation period, wk.	At diagnosis: Hb conc. g/100 ml.	MCHC	Film	Serum iron, µg/100ml	Serum folate mµg/ml	Serum vit. B_{12} µµg/ml	FIGLU	Erythropoiesis	Bone marrow iron	Weeks of iron before diagnosis	Treatment: PGA§	Treatment: Vit. B_{12}	Treatment: i.m. iron	Treatment: BTF	Hb conc., g/100ml: At term	Hb conc., g/100ml: Post-partum	Complications: Maternal: Infection	Complications: Maternal: APH	Complications: Maternal: PPH	Complications: Maternal: PET	Complications: Maternal: None	Complications: Foetal	Complications: Foetal: None	Birth-weight, lb.oz.
2217	42	3	2	1	A Pos	32	9.2	33%	Hypochr. Hyperseg.	65		211	Neg	N	0	Little					10.4	10.5					+		+	9 2
15677	39	3	2	0	B Pos	P.P.	10.3	30%	Hypochr.	34	4.3	185		N	0	12†					11.3	10.3					+		+	6 15
24078	30	4	4	0	O Neg	25	6.3	27.5%	Hypochr.	32	7.4			N	0	0					11.1	9.1	LSCS			+		Prematurity		3 -
25914	35	2	2	0	A Pos	40	9.2	30%	Hypochr.				Neg	N	1	Not known					9.2	10.2					+		+	7 4
28861	36	3	2	0	O Pos	34	9.2		Macrocyt. Hyperseg.				Neg	N (few gm)	0	2			+ (36 wk)		11.5	10.4					+		+	6 7
29062	37		6	0	O Neg	32	8.9	29.6%	Hypochr.	16	3.2	245	Neg	N	2	20†			+ (32 wk)		12.4	12.1	+			+		Stillborn		6 9
30042	30	3	3	1	A Neg	36	8.7	28.5%	Hypochr.	84	4.7	300	Neg	N	0	10†					9.1	10.5					+		+	8 10
30239	28	3	3	0	O Pos	35	8.5	28%	Hypochr.	46	1.8	123		N	0	18			+ (35 wk)		9.9	10.5					+		+	8 3
30679	24	3	2	0	A Pos	38	9.3		Hypochr. Hyperseg.				Neg	N	0	14†	+			+	10.7	8.9	+	+					+	7 6
31701	24		2	1	A Neg	32	9.5	30%	Anisopoik.	124	3.5	61		N	0	8	+	+	+ (32 wk)		10.1	11.2					+		+	8 6
32723	19	4	3	0	A Pos	37	10.5	30.5%	Macrocyt.	74		342	Neg	N	0	20					11.1	9.9		+					+	7 2
32959	23	3	2	0	A Neg	25	10.5	29.5%	Hypochr.	230		366	Pos	N(twice)	0	3†				+	8.3	10.2					+	Rh incompatibility Accessory digits		5 8
33396	30	4	3	0	O Pos	32	9.5	29%	Hypochr.	46		543		N	0	2					10.2	8.9		+ (pl.pr.)					+	6 9
34259	24		0	2	O Pos	36	10.1	31%	Hypochr. Macrocyt.				Neg	N	0	Not known	+				10.1					+			+	> 5 8
35001	26	3	2	0	A Pos	31	8.9	28%	Hypochr.	72		165	Neg	N	0	19					11.0	11.9					+		+	9 7
35168	24	3	2	0	A Neg	35	8.8	30.5%	Hypochr.	24	2.8	64 →195‡		N	0	10†			+ (35 wk)		11.8	11.0					+		+	7 5
35444	22	4	1	0	A Neg	17	10.2	32%	Hypochr.		4.9		Neg	N	0	13					12.3	13.0					+		+	7 14
36260	20	3	1	0	A Pos	32	10.3	31.5%	N ITP	14	(3.9)	148	Neg	N		19				+ (P. P.)	9.9	6.4	+		+				+	> 5 8
37201	39	3	4	0	A Pos	39	8.6	28%	Hypochr. Macrocyt.	73		266		N	0	Not known†				+	11.3						+		+	6 10
37211	28		5	1	A Pos	40	9.1	30%	Hypochr. Macrocyt.	105	2.8	184	Neg	N		Not known				+	9.1	11.1	LSCS				+		+	4 11
37215	18	3	0	0	B Pos	P.P.	8.0		Macrocyt. Hyperseg.				Neg	N	1	Not known				+ (P. P.)	12.1	8.0					+		+	6 14
37361	21	3	1	0	O Pos	40	10.8	30%	Hypochr. Macrocyt.	130		290	Neg	N (few gm)	0	25					10.8	9.9	+						+	8 0
37512	19	5	0	0	O Pos	40	8.9	30.5%	Hypochr.	173		270	Neg	N	0	9				+	8.9	10.5					+		+	8 7
37582	20	4	2	0	B Pos	20	9.3	25.5%	Hypochr.	78	3.3		Neg	N	0	0			+ (35 wk)		12.4	11.8					+		+	7 1
37782	23	0	1	0	B Pos	35	11.1 (9.6)	31.5%	N	486		379	Neg	N	0	21					11.5	11.0	+						+	8 6
37883	26	3	1	2	B Pos	33	9.8	30%	Hypochr.	565		320		N		12			+ (31 wk)		11.1	9.0	+		+	+		Twins		5 2 / 6 0
38043	22	3	1	0	O Pos	30	10.4	32%	N	77		235		N	0	5					10.7	10.4			+				+	9 10
38111	26	1	1	1	A Pos	36	7.5		Hypochr.	49			Neg	N	0	0				+	10.5	9.6				+			+	7 3
38114	36	5	6	2	A Pos	40	9.6	30%	Hypochr. Hyperseg.	190			Neg	Macro,N		25					9.6	10.5					+		+	8 0
38120	21	4	2	0	O Pos	37	9.8	28%	Hypochr.	39		160	Neg	N (twice)	0	9†					11.1	11.5					+		+	7 10

§PGA given after bone marrow biopsy. †Iron not taken well. ‡Arrow denotes later result.

Continuation

(Continuation)

R.I. Reg. No.	Age, yr.	Social class	Parity	Previous miscarriages	Blood group	At diagnosis: Gestation period, wk.	At diagnosis: Hb conc. g/100 ml.	MCHC	Film	Serum iron, µg/100ml	Serum folate mµg/ml	Serum vit. B_{12} µµg/ml	FIGLU	Erythropoiesis	Bone marrow iron	Weeks of iron before diagnosis	Treatment: PGA	Treatment: Vit. B_{12}	Treatment: i.m. iron	Treatment: BTF	Hb conc., g/100ml: At term	Hb conc., g/100ml: Post-partum	Complications: Maternal: Infection	Maternal: APH	Maternal: PPH	Maternal: PET	Maternal: None	Complications: Foetal	Foetal: None	Birthweight, lb. oz.
38259	24	3	0	0	A Pos	16	12.4 (10.8)	30%	Hypochr.					N		3					12.0	10.2	+		+	+			+	8 15
38287	21	1	0	0	O Pos	31	9.9	30.5%	Hypochr.	165		195	Neg	N	0	18†					10.7	12.4					+		+	7 10
38329	29	4	0	0	O Pos	40	11.4 (9.9)	29.5%	Hypochr.	133			Neg	MacroN		14					11.4	14.3					+		+	6 5
38369	21	3	1	0	A Pos	30	10.8	32.5%	N		4.6	160	Neg	MacroN	1	23					11.4	12.1					+		+	7 4
38436	24	1	0	0	O Pos	20	7.8	26.5%	Hypochr.	38		300	Neg	N	0	Not known	+				12.4	10.4				+		Prematurity		3 13
38437	27		0	0		38	9.3	32%	Hypochr.				Neg	N (few gm)	0	Not known†					10.4						+		+	9 4
38537	38	5	2	0	A Pos	33	9.5	27.5%	Macrocyt.	41	6.3	160		N	0	3†			+ (38 wk)		11.1	8.8	LSCS				+		+	8 7
38844	32	3	4	0	A Pos	39	10.5	31.5%	Hypochr.	50			Neg	N		Not known					10.5	11.0					+		+	9 7
38845	25	3	0	0	B Pos	P.P.	9.8	32.5%	N	55	4.1	800	Neg	N		18					13.2	9.8	+						+	6 13
39034	36		6	1	B Pos	31	10.1	28%	N	140	3.4	370	Pos	N		18					10.4	11.1					+	Accessory digits		7 8
39200	24		1	0	O Pos	34	10.4	31.5%	N			135	Neg	N		Not known			+ (33 wk)		10.8						+		+	6 0
39255	32	3	1	0	O Pos	18	9.9	28%	Hypochr.	19	3.7	385		N		2					13.8	11.6			+				+	8 3
39300	21	3	0	0	A Pos	35	8.6		Hypochr.	30			Neg	N	0	Not known	+		+ (35 wk)	+	11.8	9.8	+ LSCS						+	7 4
39371	20	5	0	0	A Pos	29	6.7	30%	N	80	(1.0)	490	Neg	N (twice)	0	3	+				10.1	9.6	+				+		+	7 4
39437	16		0	0	O Pos	34	9.5	29%	Hypochr.	47	4.0	165		N		Not known	+		+ (P.P.)		9.9	8.8					+		+	8 12
39773	19	3	0	0	A Pos	36	9.3		Hypochr.	147	3.1	138	Pos	N	0	20				+	8.5	11.5	+			+			+	5 12
39952	29	0	4	0	O Pos	33	9.9	28%	Hypochr.	64	3.1	150	Neg	N	0	20†					9.9	11.8					+		+	9 2
40020	20	4	0	0	O Pos	37	10.4	30.5%	Hypochr. Mecrocyt. Hyperseg.		8.0	140	Neg	N		Not known	+		+ (37 wk)		10.4	12.1	+						+	6 12
40191	36	4	5	1	A Pos	28	10.1	31.5%	N	130	4.4		Neg	N	4	Not known					10.5		+						+	8 5
40280	29	3	2	0	B Neg	34	10.8	30.5%	Hypochr.	24	5.0	195		N	0	8					11.9	13.2					+	Rh incompatibility		8 5
40332	19	3	1	1	A Pos	24	5.6		Hypochr.	37	3.8		Neg	MacroN (few gm)	0	Not known			+ (24 wk)		13.5	12.2					+		+	5 15
40404	18	4	0	0	A Pos	32	11.7 (9.6)	30%	Hypochr.	24	1.0	212	Pos	N	1	6					12.3						+	Dysmaturity		5 9
40799	21	5	0	0	O Pos	P.P.	9.2	31.5%	N		1.8	515		N	0	22			+ (P.P.)		11.9	9.2					+		+	8 5
41538	38	1	2	0	O Pos	31	9.9	29.5%	Hypochr. Macrocyt.		1.4	216		N (few gm)		9	+		+ (38 wk)		12.0	10.2					+	Talipes		7 12
41760	28	2	0	0	A Pos	P.P.	8.9	30.5%	N	36	5.6	180		N		40				+	13.0	8.9		+	+				+	6 12
42426	38	3	0	0	B Pos	P.P.	7.0	30.5%	Hypochr.	30	3.8	165		N		12				+	12.4	7.0	+	+				Exomphalos		6 4
42463	30	3	0	0	A Pos	37	8.6	27.5%	Hypochr.	14	3.8	130		N	0	3			+ (37wk)		8.6	9.6					+		+	7 0
43078	18	0	0	0	A Pos	P.P.	9.6	31%	Hypochr.	38	3.4	250		N	0	12†					11.6	9.6					+		+	6 9
43129	28	2	0	0	A Pos	17	6.9	25%	Hypochr.			164		N	0	0	+		+ (17 wk)		14.3						+		+	7 0
43226	24	3	1	0	A Pos	24	11.9 (8.8)	32%	Hypochr.	48	3.2	240		N		12					11.8	8.8				+			+	6 14
43263	20	4	0	0	O Pos	36	10.1	31%	N	36	8.7	170		N	0	12			+ (37 wk)	+	10.5	9.1	+ LSCS				+		+	8 8
43333	33		4	1	A Neg	26	8.8	27.5%	Hypochr. Hyperseg.	114	5.0	125		N	0	11			+ (26 wk)		11.9	12.0					+	Stillborn		1 10
43507	16	4	0	0	A Neg	37	10.2	29.5%	Hypochr.	34	3.8	110		N	0	9			+ (37 wk)		11.1	12.1					+		+	8 0
43521	27	3	0	1	B Neg	35	9.4	28%	Hypochr.	14	5.3	143		N		14			+ (35 wk)		11.8	11.8					+		+	6 10

§PGA given after bone marrow biopsy. †Iron not taken well.

APPENDIX V

Details of additional 20 women
with normoblastic erythropoiesis

APPENDIX V

Details of 20 Women with Normoblastic Erythropoiesis Not Included in Control Series of 64 Women with Normoblastic Anaemia

R.I. reg. No.	Hb conc., g/100 ml	Serum folate, mμg/ml	Serum vit.B_{12}, μμg/ml	Figlu	Reason for exclusion
31119	12.9	5.9	350	Neg	Non-anaemic control*
32116	11.5	2.5	221	Neg	- ditto -
33740	11.6 (10.4 previously)	-	-	Neg	No follow-up
35135	12.1	-	-	Neg	Non-anaemic control
35750	13.0	2.5	205	Neg	- ditto -
37113	8.4	-	-	Neg	No follow-up
37211	9.2	-	-	Neg	Anaemic in subsequent pregnancy
37994	11.1	-	295	Neg	No follow-up
38871	10.4	-	110	Neg	- ditto -
39100	6.7	-	102	Neg	- ditto -
39730	11.2	2.6	120	Neg	Non-anaemic control
39987	12.7	4.6	288	Neg	- ditto -
40062	8.7	4.4	-	Neg	No follow-up
40150	11.5	2.2	174	Neg	Non-anaemic control
40192	12.4	-	-	Neg	- ditto -
40209	11.8	5.1	522	Pos	- ditto -
40234	13.2	4.3	257	Neg	- ditto -
40532	13.6	10.4	148	Neg	- ditto -
40727	12.3	2.2	220	Pos	- ditto -
40756	13.5	1.2	310	Neg	- ditto -

*Hb $\geqslant$ 11 g/100 ml during pregnancy.

APPENDIX VI

Serum folate results

APPENDIX VI

Relates the Serum Folate To Social Class

Serum folate, mμg/ml	Gestation period: Before 19th week — Social class I & II	III	IV & V	Total	At 28th week — Social class I & II	III	IV & V	Total	After 32nd week — Social class I & II	III	IV & V	Total
0-1.9	4	9	4	17	3	7	6	16	1	7	4	12
2-2.9	15	18	11	44	7	21	21	49	3	11	9	23
3-3.9	6	25	20	51	13	35	25	73	12	34	27	73
4-5.9	18	39	28	85	20	44	29	93	8	30	15	53
⩾6	20	44	37	101	10	27	15	52	4	13	9	26
TOTAL	63	135	100	298	53	134	96	283	28	95	64	187

Relates Parity to the Serum Folate

Serum folate, mμg/ml	Gestation period: Before 19th week				At 28th week				From 32nd week			
	Parity				Parity				Parity			
	0	1-3	> 4	Total	0	1-3	> 4	Total	0	1-3	> 4	Total
0-1.9	8	12	2	22	9	10	3	22	14	22	8	44
2-2.9	21	23	5	49	21	32	2	55	16	28	9	53
3-3.9	35	24	9	68	51	34	5	90	20	16	5	41
4-5.9	60	44	4	108	69	36	7	112	30	26	4	60
⩾6	60	57	4	121	32	32	3	67	13	14	2	29
TOTAL	184	160	24	368	182	144	20	346	93	106	28	227

Relates a History of Previous Abortion or Stillbirth to the Serum Folate

Serum folate, mμg/ml	Gestation period: Before 19th week — Previous misc. or SB			At 28th week — Previous misc. or SB			From 32nd week — Previous misc. or SB		
	No	Yes	Total	No	Yes	Total	No	Yes	Total
0-1.9	15	5	20	19	3	22	29	13	42
2-2.9	38	8	46	42	10	52	44	8	52
3-3.9	47	17	64	80	10	90	31	10	41
4-5.9	90	16	106	98	16	114	54	7	61
⩾6	100	18	118	59	7	66	24	5	29
TOTAL	290	64	354	298	46	344	182	43	225

To Show the Relationship of Birthweight to the Serum Folate

Serum folate, mμg/ml	Before 19th week				At 28th week				From 32nd week			
	Birthweight, lb				Birthweight, lb				Birthweight, lb			
	<5½	5½-8	> 8	Total	<5½	5½-8	> 8	Total	<5½	5½-8	> 8	Total
0-1.9	1	15	6	22	1	12	9	22	2	28	12	42
2-2.9	5	37	7	49	3	38	14	55	3	33	14	50
3-3.9	3	46	18	67	4	65	19	88	1	29	11	41
4-5.9	5	75	28	108	9	81	25	115	3	39	23	65
⩾6	11	75	33	119	6	43	18	67	2	18	10	30
TOTAL	25	248	92	365	23	239	85	115	11	147	70	228

Serum Folate Results During Pregnancy in 23 Mothers whose Child had a Congenital Abnormality

R.I. hosp.No.	Serum folate, mμg/ml	Gestation period, weeks	Abnormality
22285	2.0	21	Genito-urinary malformations; no details
30333	3.6	18	Hydrocephalus. Spina bifida
	3.2	28	
30966	3.5	14	Oesophageal atresia
	4.8	28	
	1.7	32	
	0.8	37	
33659	4.6	11	Spina bifida. Meningocoele
	4.6	28	
37185	4.7	28	Cleft palate and hare lip
38531	3.8	28	Small right accessory auricle
	3.3	36	
39034	3.8	31	Extra digits
	3.4	33	
39671	3.0	28	Mild hypospadius. ? single palmar crease
39859	5.2	12	Imperforate anus
39900	6.0	18	Bat-ears
40532	4.6	25	Fibroelastosis cordis
	10.4	37	
41538	1.4	31	Clubfoot
41923	2.0	24	Pilonidal sinus
41924	2.5	9	Webbed fingers both hands
	3.6	28	
	4.0	32	
	2.4	38	
42032	3.0	16	Abnormality of the penis
	3.2	28	
42129	13.2	21	Omphalocoele
	8.2	28	
42190	5.9	16	Right calcaneovalgus
	4.9	26	
42327	6.8	21	Undescended testicle
	5.9	28	
42358	4.7	20	Anencephaly. Spinal abnormality
	3.5	28	
42426	11.0	22	Exomphalos
	17.2	24	
	12.7	28	
	7.6	38	
42526	6.8	14	Bilateral calcaneovalgus
	6.3	24	
42643	3.2	28	Pyloric stenosis
	2.0	33	
43114	3.5	28	Fibroelastosis cordis
	4.9	32	
	2.6	36	

APPENDIX VII

Controlled therapeutic trial of iron and a small dose of folic acid

APPENDIX VII

CONTROLLED TRIAL OF A SMALL DOSE OF FOLIC ACID IN THE TREATMENT OF ANAEMIA IN PREGNANCY

Materials and methods

During the present study, 52 women attending the ante-natal clinic, at which the author held a special haematology clinic, were admitted to a controlled trial to examine the effect of a small dose of folic acid in the treatment of anaemia in pregnancy. In the majority the Hb concentration at the start of the trial lay below 10.0 g/100 ml and all had a serum iron of less than 60 μg/100 ml.

The trial was carried out blind and compared the effect of treatment with (a) tablets containing 350 mg iron aminoates (35 mg elemental iron) and 50 μg folic acid, the dose being one three times a day, and (b) tablets containing 350 mg iron aminoates only in the same dose. Fifty bottles of each were numbered from 1 to 100 by a system of randomization and only at the end of the trial was the code known. Patients were allocated a number in the order in which they presented at the clinic and were given the corresponding bottle.

A careful check was made at each ante-natal visit to ensure that the tablets were being regularly taken and response to treatment was checked by frequent haemoglobin estimations. The results of this trial are summarized in Table 50.

Bone marrow biopsy was carried out in 10 patients and erythropoiesis was megaloblastic in 2. One patient was only taking iron; the second developed a megaloblastic anaemia in the puerperium after taking iron and 150 μg folic acid daily for 10 weeks.

Riker Laboratories kindly supplied the tablets and arranged for statistical analysis to be carried out by Dr Waterhouse. This showed that

TABLE 50

Results in a Controlled Trial Comparing Treatment with Iron and 150 μg folic acid daily with Iron Alone in 52 Anaemic Pregnant Women

	Number of patients	Mean duration of therapy, wks	Mean Hb conc., g/100 ml		Mean serum folate, mμg/ml	
			At start	At end	At start	At end
Iron and 150 μg folic acid daily	25	9.4	9.65	10.98	4.3	6.8
Iron	27	9.4	9.64	10.83	4.1	3.2

although 150 μg folic acid daily was large enough to produce a significant rise in the serum folate ($P < 0.001$), it was not accompanied by a significant rise in the Hb concentration and was insufficient to prevent the development of megaloblastic anaemia.

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ABSTRACT

The extent to which a deficiency of folic acid is responsible for anaemia in pregnancy and the extent to which such a deficiency might have a harmful effect on the mother or foetus has been examined in the present study.

The subject is introduced with a brief historical review and the way in which laboratory tests of folate deficiency have been developed is described. The scope of the present study, which was begun in January 1962 and continued for a period of three years, is presented.

In Chapter I the relative importance of the different causes of anaemia in pregnancy in Oxford is examined by means of a special survey in the course of which 397 women who attended one ante-natal clinic were interviewed at their booking visit and personal, medical and obstetrical details entered in a questionnaire. The Hb concentration, PCV and MCHC were estimated at this first visit and a peripheral blood film examined; it was ensured that all patients were taking iron by mouth. The Hb concentration was estimated at subsequent visits and if it fell below 11 g/100 ml, a level arbitrarily taken to indicate anaemia, further investigation was undertaken to determine the cause of the anaemia. Anaemia as defined was detected at some stage before delivery in 93 (23.4%) of the 397 women. In the majority of these the anaemia was shown to be due to iron deficiency and the haemoglobin rose above 11 g/100 ml before delivery in response to iron therapy, but in 29 the anaemia was still present near term. Only 6 patients were shown to have unequivocal evidence of folic acid deficiency as indicated by megaloblastic or transitional erythropoiesis in the bone marrow.

The women who had failed to respond to iron therapy were investigated as fully as possible to determine whether folic acid deficiency was responsible. For practical reasons investigation of these 29 women could not be complete in that bone marrow biopsy, serum folate and Figlu examination

was not carried out in all but nevertheless it is clear from the survey that folic acid deficiency was unlikely to be a major factor in the aetiology of anaemia in the majority of patients who failed to respond to oral iron. Other factors such as failure to take iron tablets, hydraemia and infection, were all considered responsible for a persistently low haemoglobin.

In Chapter II the criteria used for the diagnosis of folic acid deficiency based on the bone marrow appearance is described. For the purpose of the present study only those patients who had megaloblastic or transitional megaloblastic changes in the bone marrow were accepted as having unequivocal evidence of folate deficiency. A total of 56 women with bone marrow features which satisfied these criteria were selected for study and a number of clinical and laboratory features found in these patients are compared with two control groups consisting of patients with anaemia and normoblastic erythropoiesis, and non-anaemic controls in whom bone marrow biopsy was not carried out. Clinical features including symptoms and signs, gestation period at diagnosis, socio-economic circumstances, age, parity, diet, blood group and certain maternal and foetal complications of pregnancy are compared in the different groups. It was shown that megaloblastic anaemia is rarely diagnosed before the third trimester and that women with this condition have an altered social class distribution, greater parity and are more likely to have had a previous miscarriage. A significant association between infection and haemorrhage was observed but women with iron deficiency and those with folic acid deficiency were equally likely to have this complication.

Contrary to some other reports in the literature there was no increased incidence of ante-partum haemorrhage, prematurity or congenital malformation. A critical assessment of the available literature indicates that there is insufficient evidence that folate deficiency produces these complications, and that the probable explanation is that in some series these are associated conditions.

A number of laboratory investigations including the Hb concentration, MCHC, macrocytosis and hypersegmentation in the peripheral blood film, serum iron and bone marrow iron, serum folate, vitamin B_{12} and Figlu

excretion were examined and their value in the diagnosis of megaloblastic anaemia in pregnancy is discussed. Iron deficiency was shown to occur in two-thirds of the patients with megaloblastic and transitional megaloblastic anaemia and the relation of iron deficiency and folate deficiency is considered.

The serum folate was shown to be a useful test in the diagnosis of megaloblastic anaemia in pregnancy but was not diagnostic since just over one-third of women with this condition had normal levels and a proportion of women with normoblastic anaemia had low levels. Similarly although a significant association between megaloblastic erythropoiesis and increased Figlu excretion was obtained, there was considerable overlap between the different groups of anaemic women. A serum vitamin B_{12} below 150 $\mu\mu$g/ml occurred significantly more often in women with megaloblastic anaemia and this was shown to be due to the increased number who had values below 100 $\mu\mu$g/ml.

It was shown that all these signs of folic acid deficiency, i.e. macrocytosis, hypersegmentation, low serum folate and serum vitamin B_{12} levels, increased Figlu excretion could be absent in the presence of a megaloblastic anaemia and therefore none could be considered substitutes for bone marrow biopsy. On the other hand these signs were noted in women with normoblastic anaemia and in the case of the last three mentioned, even in those who were not anaemic. This suggested that in some of these pregnant women folic acid deficiency with or without anaemia was present.

In Chapter III the results of serum folate estimations which were carried out on a total of 830 pregnant women are related to a number of other factors which include certain features of megaloblastic anaemia. For the purpose of examining the relationship between the serum folate and the Hb concentration and gestation period, those known to have megaloblastic anaemia of pregnancy were excluded. Nevertheless it was shown that a significant association between a reduced Hb concentration and serum folate levels of less than 4 mμg/ml was present. This was only noted in the last trimester of pregnancy however, which raised the

possibility that folic acid deficiency might be present. Even in women with a Hb concentration of 12 g/100 ml and over the serum folate decreased with advancing pregnancy, although the greatest fall from non-pregnant levels was already present in the first trimester.

For the purposes of all other correlations women with megaloblastic anaemia were not excluded. A direct correlation was noted between reduced serum folate and serum iron levels, suggesting that the fall in both these serum factors was due to a common cause. A significant association between a serum folate below 4 mμg/ml and parity was discovered; women with folate levels of this order early in pregnancy were more likely to have a high parity. All other factors examined, which included social class, history of a previous abortion, ante-partum haemorrhage, congenital abnormalities and a seasonal variation, showed no significant association although a number of trends was apparent. The serum folate was reduced in women expecting twins but this was a small, partially selected, group which included a number of women known to have megaloblastic anaemia.

The possibility that unsuspected folate deficiency was contributing to anaemia late in pregnancy was tested by means of a clinical trial, the results of which are presented in Chapter IV. A total of 360 women were admitted to a controlled therapeutic trial of six different combinations of iron and folic acid at the 28th week of pregnancy. This trial confirmed that treatment with iron produces a significant rise in the Hb concentration of pregnant women but no such benefit was obtained from the use of prophylactic folic acid. There was no difference in the mean Hb concentration of women given folic acid compared with those treated with a placebo, nor were women given folic acid less likely to develop anaemia after the start of the trial; this was clearly dependent on the use of iron. Furthermore mild anaemia, which was already present at the beginning of the trial in 88 women, was not more effectively treated by the addition of a folic acid supplement to iron therapy.

However confirmation was obtained that megaloblastic anaemia, a fall in the serum folate and hypersegmentation were for the most part

prevented by the administration of folic acid.

In the final discussion the specificity of tests for folic acid deficiency in the pregnant woman and the significance of the cellular changes in the peripheral blood and bone marrow associated with this deficiency is considered and the difficulty in making a confident diagnosis of folic acid deficiency in pregnancy discussed. The reasons for choosing megaloblastic anaemia as the sole criterion for the diagnosis are given and the particular features of folic acid deficiency in pregnancy based on this concept which are discussed include the laboratory diagnosis of megaloblastic anaemia, the relationship of folic acid deficiency to iron and vitamin B_{12} deficiency, its incidence and clinical significance to the mother and foetus, the indications for the administration of prophylactic folic acid, and the aetiology of this condition.

It is concluded that this study has shown that severe folic acid deficiency associated with a megaloblastic anaemia during pregnancy is uncommon. For various reasons it was thought that folic acid deficiency might contribute to anaemia in the absence of megaloblastic change. This was not confirmed by the clinical trial which showed clearly that the Hb concentration was not affected by the administration of folic acid. Nevertheless the relationship of two other signs, namely a low serum folate and hypersegmentation, to folic acid deficiency was confirmed and it was shown that folic acid for the most part prevented their occurrence. Since these signs are sometimes found in women with normoblastic erythropoiesis they do not provide as good an index of folate deficiency as in the non-pregnant state. Possibly this is due to the additional factor of foetal requirements and utilisation and an alteration in the maternal handling of folic acid in pregnancy.

The effect of prophylactic folic acid will be to prevent the development of a megaloblastic anaemia and the benefit which will be obtained from its use will depend on the incidence of this condition. Certain pregnant women are at greater risk and the use of prophylactic folic acid to such women is strongly indicated. The advisability of using prophylactic folic acid routinely will depend on local socio-economic circumstances and

standards of ante-natal care. If this is considered worthwhile an effective safe schedule which could be adopted would be the administration of iron and a separate tablet of about 300 μg folic acid daily for the last half of pregnancy.

In an area such as Oxford where prophylactic folic acid has been shown to confer little benefit and the incidence of megaloblastic anaemia is low, it is considered that the rational approach should be dietary instruction, careful ante-natal supervision with emphasis on the importance of taking iron. All cases of anaemia which fail to respond to this regime should be carefully investigated and treated on their merits.

Reprinted from the BRITISH MEDICAL JOURNAL
28 *November* 1964, 2, 1366–1369

Formimino-glutamic Acid Excretion in Anaemia of Pregnancy

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When anaemia develops during pregnancy it is usually due to iron deficiency (Fisher and Biggs, 1955), though physiological anaemia due to hydraemia may play a minor part (Lawrence, 1962). Megaloblastic anaemia responding to folic acid can occur in some patients, but the recognition of this condition in pregnancy may be difficult. Unless megaloblasts can be demonstrated in the peripheral blood or buffy coat, a bone-marrow biopsy is necessary before a definite diagnosis can be made. Even with the aid of bone-marrow biopsy it may be difficult to be certain that haematopoiesis is abnormal.

This investigation was undertaken to see if the measurement of formimino-glutamic acid (Figlu) in the urine could be used instead of bone-marrow biopsy and could provide a method of determining whether folic-acid deficiency was present in any given patient. This test has been found to be useful in detecting folic-acid deficiency in non-pregnant individuals (Kohn *et al.*, 1961 ; Luhby *et al.*, 1959 ; Knowles *et al.*, 1960), but reports of its value in anaemia of pregnancy have been conflicting (Hibbard, 1962 ; Chanarin *et al.*, 1963).

Material and Methods

The urinary Figlu excretion has been estimated in 102 pregnant women and correlated with the haematological state, including the bone-marrow appearance, in every case, and the serum folic-acid activity (F.A.A.) and serum vitamin-B_{12} levels where available. The patients were seen in the Nuffield Department of Obstetrics between July 1961 and November 1963 and were not a random sample of pregnant women. A considerable number had been treated with oral iron for some time and were referred because of poor response ; some had had no previous iron therapy. Of the 102 women, 20 had a haemoglobin concentration of 11 g./100 ml. or more, which we regard as being within the normal range in the later months of pregnancy. The remainder showed a varying degree of anaemia ; in 22 it

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was mild (10 to 10.9 g./100 ml.), in 23 moderate (9 to 9.9 g./100 ml.), and in 37 severe (less than 9 g./100 ml.).

Patients were investigated at different stages of pregnancy but the majority were in the last trimester.

Figlu Excretion.—Figlu was measured in urine collected between three and eight hours after ingestion of 15 g. of L-histidine monohydrochloride, using the low-voltage electrophoresis method of Kohn *et al.* (1961). A solution of pure Figlu of known concentration was run in parallel to provide a quantitative estimation of excretion. Increased excretion was thought to be present if the amount of Figlu excreted in the urine exceeded 2 mg./hour.

Serum Folic-acid Activity.—This was measured by microbiological assay using *Lactobacillus casei* as test organism by Spray's (1964) modification of the method described by Waters and Mollin (1961). The normal range in healthy non-pregnant adults is from 2.1 to 28 mμg./ml., with an average of 7.8 mμg./ml.; the lowest level is lower than that found by other techniques.

Serum Vitamin B_{12}.—The serum vitamin-B_{12} levels were estimated according to the method of Boczarow (1961) using *Lactobacillus leichmanii.* In our laboratory the normal range is 150–800 μμg./ml.

Bone-marrow Biopsy.—Smears from each patient were examined by two independent observers before the Figlu results were known, and erythropoiesis was classified as normoblastic, megaloblastic, or "transitional." In the absence of frank megaloblastosis transitional erythropoiesis was regarded as being present if erythroblasts with some but not all of the features of the classical megaloblast were seen. Usually multiple or frequent Howell-Jolly bodies and/or giant band cells and metamyelocytes were noted in addition. As these changes are not found in simple iron deficiency and in non-anaemic pregnant women they are thought to represent abnormal erythropoiesis.

Results

Relation of Figlu Excretion to Haemoglobin Concentration.—Table I shows that the incidence of increased Figlu excretion was significantly greater in patients with a haemoglobin concentration of less than 9 g./100 ml. ($P<0.05$). Women who

TABLE I.—*Figlu Excretion Related to Haemoglobin Concentration*

Haemoglobin Concentration (g./100 ml.)	Normal Figlu	Increased Figlu
<9 g. (<61%)	25	12 (32%)
9–9·9 g. (61–67%)	20	3 (13%)
10–10·9 g. (68–73%)	19	3 (14%)
11–11·9 g. (74–81%)	8	2 (20%)
12 g. & over (>81%)	9	1 (10%)

were not anaemic were as likely to have increased excretion as those who were moderately so.

Relation of Figlu Excretion to Type of Erythropoiesis.—Figlu excretion is related to the type of erythropoiesis in Table II. Patients who excreted increased amounts of Figlu were more likely

TABLE II.—*Relation of Figlu Excretion and the Bone-marrow Appearance*

Figlu	Megaloblastic Erythropoiesis	Transitional Erythropoiesis	Normoblastic Erythropoiesis	Total
Normal	10	12	59	81
Increased	8	4	9	21
Total	18	16	68	102

to have megaloblastic or transitional erythropoiesis ($P<0.05$). The correlation is not a good one, however, since 43% of patients with increased excretion had normoblastic marrows and more than half of the patients with megaloblastic or transitional marrows had normal Figlu excretion.

Relation of Figlu Excretion to Serum Folic-acid Activity.—The Figlu excretion is correlated with the serum F.A.A. in 41 patients (Table III). Patients with increased Figlu excretion

TABLE III.—*Figlu Excretion Related to Serum Folic-acid Activity*

Serum Folic-acid Activity	Normal Figlu	Increased Figlu
Normal (2·1–28 mμg./ml.) ..	26	5
<2·1 mμg./ml.	5	5

were more likely to have a low serum F.A.A. than those with normal excretion. Nevertheless, half of the patients with low serum F.A.A. had normal Figlu excretion. When Figlu excretion and serum F.A.A. are related to the type of erythropoiesis there is a considerable overlap (Fig. 1). However, four of the five patients with increased Figlu excretion and a low serum F.A.A. had megaloblastic or transitional erythropoiesis.

Relation of Figlu Excretion to Serum Vitamin-B_{12} Levels.—Serum vitamin-B_{12} levels were estimated in 69 women and the results correlated with Figlu excretion (Table IV). Patients with normal Figlu excretion had a greater chance of having a low serum vitamin-B_{12} level than those with increased excretion irrespective of the type of erythropoiesis. The association of normal Figlu excretion with low vitamin-B_{12} levels

TABLE IV.—*Relation of Figlu Excretion to the Serum Vitamin B_{12}*

Serum Vitamin B_{12}	Normal Figlu	Increased Figlu	Total	Significance of Difference
Normal (150–800 μμg./ml.) ..	31	17	48	$\chi^2 = 4{\cdot}9085$.* n = 1.
Low (<150 μμg./ml.)	19	2	21	P <0·05. Significant

* With Yates's correction.

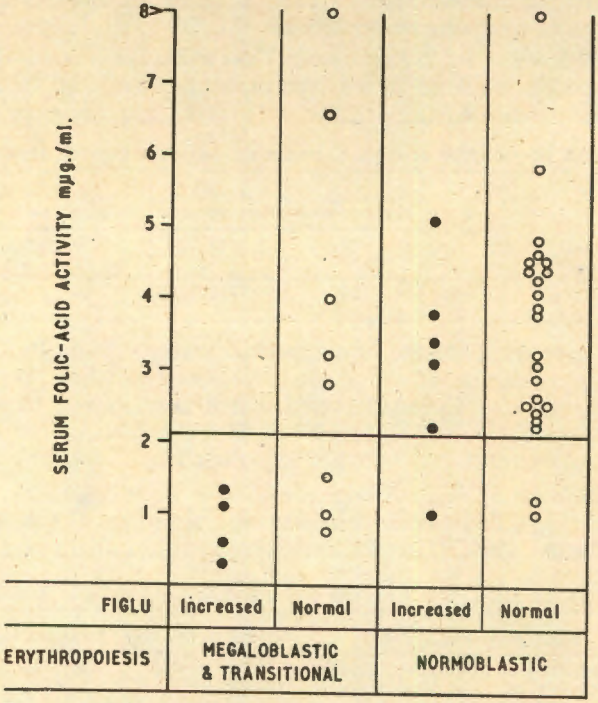

FIG. 1.—Figlu excretion related to the serum folic-acid activity and erythropoiesis.

is statistically significant only when erythropoiesis is megaloblastic or transitional (Table V ; Fig. 2). Furthermore, it shows that megaloblastic or transitional erythropoiesis was found as often in association with an abnormally low serum vitamin-B_{12} level as with an increase in Figlu excretion.

Relation of Figlu Excretion to Both Serum F.A.A. and Serum Vitamin-B_{12} Levels.—Fig. 3 shows that there was no clear-cut relation between serum F.A.A. and vitamin-B_{12} levels, but does again demonstrate the absence of increased Figlu excretion when the serum vitamin B_{12} was low. The small number of patients

TABLE V.—*Relation of Figlu Excretion, Serum Vitamin-B_{12} Levels, and Erythropoiesis*

Erythropoiesis	Serum Vitamin B_{12}	Normal Figlu	Increased Figlu	Total	Significance of Difference
Megaloblastic and transitional ..	Normal	6	9	15	$\chi^2 = 4{\cdot}965$.* n = 1.
	Low	10	1	11	P < 0·05. Significant
Normoblastic	Normal	25	8	33	$\chi^2 = 0{\cdot}277$. n = 1.
	Low	9	1	10	P > 0·05. Not significant

* With Yates's correction.

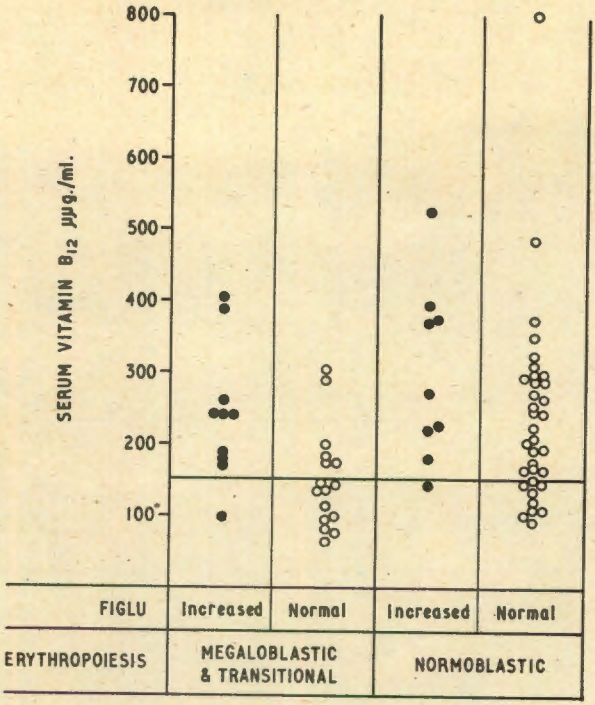

FIG. 2.—Figlu excretion related to serum vitamin B_{12} and erythropoiesis.

with megaloblastic or transitional erythropoiesis and increased Figlu excretion all had a low serum F.A.A. and a normal serum vitamin B_{12}. Only one out of the eight who had similar erythropoiesis and a normal Figlu excretion had a low serum F.A.A. and normal serum vitamin B_{12}.

Relation of Figlu Excretion to Iron Deficiency.—There was no correlation between Figlu excretion and iron deficiency. In the 68 patients with normoblastic erythropoiesis evidence of iron deficiency was found in 40 ; six of these had increased excretion, compared with 3 of the 28 patients in whom there was no evidence of iron deficiency.

Discussion

The results demonstrate that the measurement of Figlu excretion after histidine-loading is no substitute for bone-marrow biopsy in detecting megaloblastic or transitional erythropoiesis during pregnancy. Although patients with increased excretion are more likely to have megaloblastic or transitional erythropoiesis than those in whom the excretion is normal, a substantial number of patients with megaloblastic or transitional erythropoiesis excrete normal quantities of Figlu

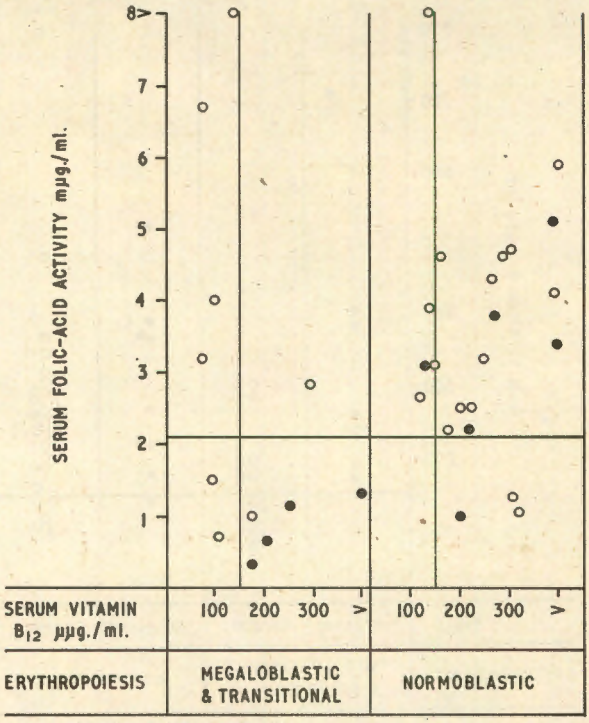

FIG. 3.—Figlu excretion related to serum folic-acid activity, serum vitamin B_{12}, and erythropoiesis.

in the urine. This confirms the finding of Chanarin *et al.* (1963), who have shown that the absorption and excretion of histidine are altered in pregnancy, but is in marked contrast to that of Hibbard and Hibbard (1963), who considered that normal Figlu excretion excludes the possibility of megaloblastic erythropoiesis in pregnancy.

Although the measurement of the serum F.A.A. was not diagnostic it was found to be a better guide to the type of erythropoiesis than the Figlu excretion. In some patients with anaemia and transitional or megaloblastic erythropoiesis the Figlu excretion was increased and the serum F.A.A. was low, as one would expect if the anaemia were due to folic-acid deficiency. An unexpected finding was that a significant number of patients with normal Figlu excretion had low serum vitamin-B_{12} levels when compared with those in whom the Figlu excretion was increased (Table IV). This was most striking in patients who had a transitional or megaloblastic bone-marrow, but a similar tendency was found in those with normoblastic erythropoiesis. Unfortunately, both serum F.A.A.

Furthermore, 3 of the 20 patients with a normal haemoglobin concentration had increased Figlu excretion, a proportion similar to that found by Berry *et al.* (1963), who did serial estimations of urinary Figlu in a group of normal pregnant women. It is clearly important to determine whether a normoblastic anaemia which responds to treatment with folic acid does occur in pregnancy. We believe that this point can be settled only by means of a controlled therapeutic trial, and such a trial is in progress.

Summary

In the present study the urinary excretion of Figlu has been estimated in 102 pregnant women and correlated with the haematological state, including the bone-marrow appearance in every case and the serum folic-acid activity and serum vitamin-B_{12} levels where available. Estimation of Figlu excretion was of little value in the diagnosis of the type of anaemia and was no substitute for bone-marrow biopsy; the serum folic-acid activity was found to be a better guide to the type of erythropoiesis. A number of patients were found to have low serum vitamin-B_{12} levels in association with normal Figlu excretion and a significant proportion of these had megaloblastic or transitional erythropoiesis.

We are grateful to Professor L. J. Witts for helpful criticism and advice and for allowing us to use the facilities in his department for the folic-acid assays, which were carried out under the direction of Dr. G. H. Spray; to Professor Chassar Moir and Mr. Mosteyn Embrey, who kindly allowed us to study patients under their care; and to Dr. R. Wright for all his help. This study has been financed by a grant from the Nuffield Committee for Advancement in Medicine.

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Printed in Great Britain by Fisher, Knight and Co., Ltd., Gainsborough Press, St. Albans.

and serum vitamin-B_{12} levels were available on only a limited number of patients, but Fig. 3 shows that patients with a megaloblastic or transitional marrow who had normal Figlu excretion usually had either a low serum vitamin B_{12} alone, or a low serum vitamin B_{12} and a low serum F.A.A.

The association of low serum vitamin-B_{12} levels and anaemia in pregnancy is being recognized more frequently as more pregnant women have their serum vitamin-B_{12} levels estimated (Ball and Giles, 1964). All five cases described by Ball and Giles with megaloblastic erythropoiesis and subnormal vitamin-B_{12} levels responded to folic acid. This suggests that a true vitamin-B_{12} deficiency was not present and that the folic-acid deficiency had caused the serum vitamin-B_{12} level to become subnormal. In our series there were four patients with transitional or megaloblastic erythropoiesis and normal Figlu excretion in whom the serum vitamin B_{12} was low and serum F.A.A. was normal. One of these was treated with folic acid 5 mg. t.d.s., but there was no haematological improvement after four weeks of therapy. Injections of vitamin B_{12} were then given with good results. Malabsorption of vitamin B_{12} can probably be excluded as the cause of the deficiency in this patient, as absorption of radioactive vitamin B_{12} was normal several months after delivery.

The cause of the low serum vitamin-B_{12} levels found in pregnancy is obscure. A progressive decrease in the serum vitamin-B_{12} levels of normal women as pregnancy progressed was found by Lowenstein *et al.* (1962), but the total circulating serum vitamin B_{12} did not change significantly A low serum vitamin B_{12} was associated with megaloblastic erythropoiesis in a high proportion of their cases, however, and they felt that this probably indicated true vitamin deficiency. Vitamin-B_{12} absorption has been shown to be normal in megaloblastic anaemia in pregnancy by Badenoch *et al.* (1955). It is therefore unlikely that these low levels are due to poor absorption, but rather are the result of excessive utilization by the foetus or are secondary to a deficiency of folic acid. This may not be true in every case, however, as one patient with low serum vitamin B_{12} investigated before this study started has since been shown to have a true absence of intrinsic factor in spite of her haemoglobin returning to normal after delivery.

There is evidence to suggest that folic-acid deficiency may exist during pregnancy without megaloblastic erythropoiesis developing (Chanarin *et al.*, 1959), in which case it is likely that anaemia due to folic-acid deficiency may be commoner than is at present thought. If this is so it would be of interest to know whether increased Figlu excretion gives an indication of such deficiency. Our own data suggest that this is unlikely, as there is no correlation between Figlu excretion and serum F.A.A. in patients with a normoblastic bone-marrow (Fig. 1).

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Reprinted from

The Journal of Obstetrics and Gynaecology of the British Commonwealth

Vol. 73, No. 2, April 1966

J. Obstet. Gynaec. Brit. Cwlth.
April 1966. Vol. 73. pp. 191–196.

A CONTROLLED CLINICAL TRIAL OF PROPHYLACTIC FOLIC ACID AND IRON IN PREGNANCY

BY

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A SURVEY carried out in this hospital to determine the magnitude of the problem of anaemia during pregnancy in Oxford revealed that in spite of prophylactic iron therapy, 12 per cent of women near term had a haemoglobin concentration of less than 11 g. per 100 ml. Overt megaloblastic anaemia due to folic acid deficiency was not common, however, and was found in approximately one woman in every hundred. The possibility that folic acid deficiency might also be a factor in producing non-megaloblastic anaemia was raised by finding increased excretion of formimino-glutamic acid in the urine and low levels of folic acid activity (F.A.A.) in the serum of pregnant women with normoblastic erythropoiesis (Chisholm and Sharp, 1964). These observations together with those of Chanarin *et al.* (1959), who showed that unsuspected folic acid deficiency can exist in normal, and especially multiple, pregnancies suggested that treatment with folic acid might prevent or correct anaemia which occurred in women already taking iron.

Several studies have been reported of the use of prophylactic folic acid in different doses during pregnancy and a resulting reduction in the incidence of megaloblastic anaemia observed, (Lowenstein *et al.*, 1955; Francis and Scott, 1959; Giles and Burton, 1960; Dawson *et al.*, 1962; Chanarin *et al.*, 1965). The use of small amounts of folic acid is considered by some to lessen the risk of precipitating subacute combined degeneration of the cord in a pregnant woman with unsuspected Addisonian pernicious anaemia but it has been only partially effective in the prevention of megaloblastic anaemia (Dawson *et al.*, 1962; Chanarin *et al.*, 1965). Since the minimal effective dose for the prevention of megaloblastic anaemia is not known, a trial was designed (*a*) to test the effectiveness of a large and a small dose of folic acid in maintaining a normal level of folic acid activity in the blood, and (*b*) to see if folic acid was effective in raising the haemoglobin in the absence of frank megaloblastic erythropoiesis, or if its sole value was to prevent the occasional case of megaloblastic anaemia in pregnancy.

The high incidence of mild anaemia at term noted in women who were apparently taking iron had led to doubts about the value of routine prophylactic administration of iron. While physiological haemodilution might be partly responsible for this, the work of others (Fisher and Biggs, 1955; Lawrence, 1962) had indicated that hydraemia in pregnancy was not an important cause of anaemia, and it was felt that the value of routine iron therapy in pregnancy needed reassessing. A group of women not given iron therapy was therefore included in the trial.

DESIGN OF THE TRIAL

Method of Selection

Women attending the antenatal clinic at their first visit before the 28th week of pregnancy were asked to participate in a clinical trial to investigate the best method of preventing anaemia during pregnancy.

If already receiving iron they were asked to stop taking their tablets. Patients who had a haemoglobin of less than 11 g. per 100 ml. (74 per cent) and serum iron of less than 60 μg per 100 ml. were not included in the trial and were treated immediately.

All the women were asked to reattend when 28 weeks pregnant and were then allocated at random to one of six treatments, provided (*a*) they had not taken any haematinics in the preceding 8 weeks and (*b*) their haemoglobin was 10·2 g. per 100 ml. (70 per cent) or more.

Several women had a haemoglobin below this level but because they had a normal serum iron were nevertheless included in the trial. It was thought that if any benefit was to be obtained from the empirical use of folic acid then this group would be most likely to show it. In all there were 50 patients who were not included in the trial because iron deficiency anaemia was already present by the 28th week of pregnancy. These were treated immediately with iron and subsequently followed up; only one developed a megaloblastic anaemia.

Treatment Groups

Various combinations of ferrous gluconate and folic acid with appropriate placebo tablets were used as shown in Table I.

Half the patients were treated with ferrous gluconate 5 gr. (300 mg.) three times daily and half with a placebo tablet indistinguishable from the ferrous gluconate tablet. In addition to the iron or placebo tablets, patients were given one tablet daily containing a low dose of folic acid (500 μg.) or a high dose of folic acid (5 mg.) or a folic acid placebo. One in six patients therefore received neither iron nor folic acid. Bottles containing the tablets had been numbered by random selection at source and the code was not known while the patients were still on trial.

Investigations during the Course of the Trial

The capillary haemoglobin was checked before delivery usually at fortnightly intervals and finally early in the puerperium. At 28 weeks and again at least once before delivery a venous sample of blood was taken and the haemoglobin, haematocrit, mean corpuscular haemoglobin concentration, serum iron and serum folic acid activity (F.A.A.) estimated. A film was examined if the haemoglobin fell below 11·0 g. per 100 ml. Treatment was considered to have failed if the haemoglobin fell to 9·5 g. per 100 ml. or less, and the patient was withdrawn from the trial. Unless the anaemia was characteristic of iron deficiency, further investigations including a serum vitamin B12 estimation and bone marrow biopsy were carried out and then the necessary corrective treatment was instituted.

Methods

It was intended that every patient should have at least one venous sample of blood examined after treatment had been started and before delivery. Several days before this visit was due, a letter was sent asking the patient to stop taking her iron and vitamin tablets 24 hours before coming to hospital.

The serum folic acid activity was estimated using a modification of the method of Waters and Mollin (1961) described by Spray (1964). The normal range in healthy non pregnant adults is from 2·1—28 mμg. per ml. with an average of 7·8 mμg. per ml.

The serum iron was estimated by the method described by Bothwell and Mallett (1955) the normal range for women being 60–160 μg. per 100 ml.

The serum vitamin B12 levels were estimated according to the method described by Meynell *et al.* (1957) using *Lactobacillus leichmannii.* In this laboratory the normal range in non pregnant adults is 150–800 $\mu\mu$g. per ml. but results between 100 and 150 $\mu\mu$g. per ml. are considered to be equivocal.

Results

Three hundred and sixty women were admitted to the trial and Table I gives the number of patients in each treatment group.

Haematological findings at the start of treatment

Table I records the mean haemoglobin, serum iron and serum F.A.A. as well as the incidence of low serum levels of iron and F.A.A. at 28 weeks. As judged by these parameters randomization appears to have resulted in roughly comparable groups.

Haemoglobin levels at the end of the trial

The results in the 360 women after therapy are shown in Table II. Where it was necessary to give additional therapy, the last haemoglobin before this was started is recorded. The mean haemoglobin in the three groups of women treated with iron rose by 0·95 g. per 100 ml. and differs significantly from that of the three groups treated with placebo iron, where the mean haemoglobin fell by 0·41 g. per 100 ml. (difference of the mean increase in haemoglobin =

1·36 g.; overall standard error (s.e.) = 0·075; $p < 0·001$).

The use of folic acid did not have the same beneficial effect. There was no difference between the mean haemoglobin of the groups treated with folic acid (either in the high or low dosage) and that of the groups treated with placebo folic acid. Iron and 5 mg. folic acid together produced a rise in haemoglobin of 1·04 g. per 100 ml. compared with a rise in haemoglobin of 0·96 g. per 100 ml. in the group treated with iron alone. The difference was not significant (difference of the mean increase in haemoglobin = 0·08 g.; overall s.e. = 0·14; $p > 0·05$).

Amongst these 360 women were 88 who had a mild anaemia when 28 weeks pregnant but were

TABLE I

Results of Investigations in the 6 Groups Before the Start of Treatment

	Iron Therapy					
	Placebo			Ferrous Gluconate		
Dosage of folic acid	Placebo	500 μg.	5 mg.	Placebo	500 μg.	5 mg.
Number of patients	60	58	59	60	61	62
Mean Hb. g./100 ml.	11·62	11·58	11·55	11·42	11·35	11·49
Overall mean Hb.		11·59			11·42	
Mean serum Fe. μg./100 ml.	72	80	76	72	77	78
Number with serum Fe. < 60 μg./100 ml. ..	22	14	13	21	20	16
Mean serum F.A.A. mμg./ml.	4·3	4·5	5·1	4·4	4·8	4·6
Number with serum F.A.A. < 2·1 mμg./ml.	5	4	5	5	3	2

TABLE II

Results of Investigations in the 6 Groups after Treatment

	Iron Therapy					
	Placebo			Ferrous Gluconate		
Dosage of folic acid	Placebo	500 μg.	5 mg.	Placebo	500 μg.	5 mg.
Mean Hb. g./100 ml.	11·23	11·03	11·27	12·38*5	12·21*3	12·53
Overall mean Hb.		11·18			12·37	
Number with Hb. < 11 g. per 100 ml. ..	20	26	19	3	5*2	2
Mean serum Fe. μg./100 ml.	60	57	67	86	89	102
Number with serum Fe. < 60 μg./100 ml. ..	37	34	26	6	11	10
Mean serum F.A.A. mμg./ml.	4·0	14·6	30·1	4·3	13·6	28·2
Number with serum F.A.A. < 2·1 mμg./ml.	9	—	—	6	—	—

* Iron tablets not taken.

The number of patients who did not take iron is indicated next to the asterisk.

not excluded then either because they had a normal serum iron level or because the haemoglobin lay between 10·2 g. per 100 ml. and 11·0 g. per 100 ml. If the response to therapy of these 88 is considered separately (Table III) it can be seen that even in mildly anaemic women the empirical use of folic acid does not bring about any improvement in the haemoglobin which could not be produced by iron alone. Judging by the response to iron therapy it is apparent that most were indeed iron deficient although this was not always obvious at the time.

Development of anaemia during the trial

The number of patients who developed an antepartum haemoglobin of less than 11 g. per 100 ml. after treatment had begun is shown in Table IV. Thirty-five patients not treated with iron became anaemic before delivery and the dose of folic acid made no difference to the number of anaemic patients in each group. Only one patient taking iron became anaemic but her haemoglobin only fell from 11·1 g. per 100 ml. to 10·9 g. per 100 ml.

The serum F.A.A. and serum iron level related to therapy

The effectiveness of the different dosage of folic acid in raising the serum F.A.A. of the 345 patients in whom it was measured can be seen in Table II. The mean serum F.A.A. of patients treated with the 500 μg. dose was more than

TABLE III

Haemoglobin Concentration at Term Related to Treatment in 88 Women Whose Haemoglobin was Less than 11·0 g. per 100 ml. Before Therapy

	Iron Therapy					
	Placebo			Ferrous Gluconate		
Dosage of folic acid	Placebo	500 μg.	5 mg.	Placebo	500 μg.	5 mg.
13 g./100 ml. and over	—	—	—	3	1	3
11–12·9 g./100 ml.	5	1	3	11	10*[1]	13
10–10·9 g./100 ml.	6	5	4	3	2*[1]	2
<10·0 g./100 ml.	4	7	4	—	1	—
Total number of patients	15	13	11	17	14	18

* Iron tablets not taken.

TABLE IV

Haemoglobin Levels in 37 Women who Developed Antepartum Anaemia After 28 Weeks

	Iron Therapy					
	Placebo			Ferrous Gluconate		
Dosage of folic acid	Placebo	500 μg.	5 mg.	Placebo	500 μg.	5 mg.
10–10·9 g./100 ml.	9	10	8	—	2*[1]	—
<10·0 g./100 ml.	1	4	3	—	—	—
Total number of patients	10	14	11	—	2	—

* Iron tablets not taken.

three times that of those given placebo folic acid and the increase was even greater in those treated with 5 mg. No patients given folic acid had serum levels below 2·1 mμg./ml.

As one would expect the mean serum iron was higher in the iron supplemented group (Table II). (The serum iron level of patients who could not take their iron tablets was not included here). Folic acid therapy does not appear to have a marked effect on the serum iron of the treated patients; and the reverse also appears to be true. It is interesting to note, however, that the greatest rise in the serum iron level occurred in those patients treated with iron and 5 mg. folic acid.

Erythropoiesis and serum vitamin B12 levels related to therapy

Bone marrow biopsy was carried out on 29 patients. Only two were found to have megaloblastic erythropoiesis; both were taking placebo iron and placebo folic acid and both had a serum F.A.A. of less than 2·1 mμg./ml.

Nine of the 29 women on whom a bone marrow biopsy was performed had a low serum vitamin B12 level and of these one had megaloblastic erythropoiesis. In the groups being treated with folic acid there were fewer patients with low levels of vitamin B12 and the mean serum vitamin B12 level was higher. The numbers in each group, however, are too small for accurate analysis.

Discussion

The results of this trial confirm earlier work which showed that pregnant women who take iron will have a higher haemoglobin concentration at term than those who do not. Fifty women could not be included in this trial because iron deficiency anaemia was already established by the 28th week of pregnancy. A further 88 were mildly anaemic at this stage but were included for reasons already given; in addition there were 35 women given placebo iron, whose haemoglobin fell below 11·0 g. per 100 ml. in the last trimester. Almost all of these 173 women had received no iron therapy and this high incidence of anaemia re-emphasizes the prevalence of iron deficiency and confirms the need for prophylactic iron during pregnancy.

The results do not show a comparable benefit from the prophylactic use of folic acid. The lack of effect of folic acid supplements on the haemoglobin in spite of good absorption and a marked rise in the serum F.A.A. would seem to indicate that a subclinical folic acid deficiency does not materially contribute to anaemia in pregnancy in the absence of frank megaloblastosis.

The incidence of megaloblastic anaemia in the present study was low but it should be pointed out that Oxford city and the surrounding area has an affluent industrial and agricultural population and as it has been shown that inadequate diet plays a part in the ætiology of megaloblastic anaemia in pregnancy (Lowenstein *et al.*, 1955; Giles and Burton, 1960) it is likely that these economic and social factors may have contributed to this result.

Due to this low incidence the dose of folic acid required to prevent frank megaloblastic anaemia of pregnancy could not be determined with certainty. No women taking folic acid were found to have megaloblastic erythropoiesis. The rise in the mean serum F.A.A. in those women receiving folic acid seems to indicate that either dose used should be adequate for prophylactic therapy. However, megaloblastic anaemia responding to folic acid therapy can occur in patients with normal serum F.A.A. (Waters and Mollin, 1962; Chisholm and Sharp, 1964; Chanarin *et al.*, 1965) and therefore this question of dosage can only be decided by further trial in an area where the incidence of megaloblastic anaemia is high.

Only eight women taking iron had a haemoglobin of less than 11 g. per 100 ml. at term and of an additional eight who were unable to tolerate the iron, one quarter developed mild anaemia; the overall incidence of anaemia at term in women given iron was therefore approximately 5 per cent. This compares favourably with the 12 per cent incidence noted in the preliminary survey in which prescriptions for iron were given out regularly but the intake of tablets not checked. Since the improvement is independant of treatment with folic acid, it would appear to be due to close supervision of iron therapy and suggests that mild anaemia in pregnancy apparently not responding to iron is commonly due to inadequate iron intake.

Hydraemia of pregnancy alone was not a significant factor in the production of anaemia

in the women studied. Mild anaemia at term without any signs of iron deficiency was found in only 5 out of the 360 women and only one of these was taking iron.

Since the empirical use of folic acid produces a rise in the serum F.A.A. but no corresponding rise in haemoglobin the benefit resulting from its use in pregnancy will depend on the incidence of megaloblastic anaemia in a particular area. It is doubtful if the routine use of folic acid to prevent the occasional case of megaloblastic anaemia is indicated where the incidence is low. During pregnancy, women with iron deficiency anaemia are more likely to become folic acid deficient and it has been shown that, by maintaining the haemoglobin at a higher level, adequate iron therapy alone will reduce the risk of megaloblastic anaemia developing (Chanarin, *et al.*, 1965). It would seem therefore that a more practical approach to the prevention of megaloblastic anaemia in pregnancy would be to reserve the use of prophylactic folic acid for those women who are found to be iron deficient in the last trimester of pregnancy and for those who fail to respond to adequate oral or parenteral iron therapy.

Summary

The results of a controlled therapeutic trial in 360 pregnant women of iron and folic acid in different combinations are presented. Patients treated with iron in the last trimester had a significant rise in haemoglobin whereas the mean haemoglobin of those treated with placebo iron fell. The mean haemoglobin of patients treated with folic acid did not differ from the mean haemoglobin of patients receiving placebo folic acid and treatment with folic acid made no difference to the number of women who were anaemic at term.

Only two cases of megaloblastic anaemia were seen in the present trial and neither received folic acid or iron. Both the high and the low dose of folic acid were effective in raising the serum folic acid activity substantially, but a trial along similar lines in an area where megaloblastic anaemia is common will be necessary to determine the minimal effective dose of folic acid required to prevent this condition.

In view of the lack of any demonstrable effect of folic acid on the haemoglobin in the absence of frank megaloblastic erythropoiesis there would appear to be no indication for the routine use of folic acid during pregnancy in areas where megaloblastic anaemia is uncommon.

Acknowledgments

I am grateful to Dr. A. A. Sharp for his support during the trial; to Professor L. J. Witts for advice and for allowing me facilities in his department; to Professor Chassar Moir for allowing me to study patients under his care, and to Dr. Wright for help in the preparation of this manuscript.

This study was financed by a grant from the Nuffield Committee for Advancement in Medicine. The folic acid was supplied without charge by Lederle Laboratories and the placebo iron tablets were made up by the Bayer Products Company and Sandoz Ltd.

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