

ANAEMIA IN PREGNANCY*

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The haemoglobin (Hb.) concentration falls during normal pregnancy as a result of the greater expansion of plasma volume than red cell volume, but the extent of this dilution or 'physiological' anaemia has been greatly exaggerated in the past (Hyttén and Leitch, 1964). It was not realised that about half the population of even the wealthiest communities is iron deficient (Banerji et al., 1968) and there was the belief that the greater increase of red cell mass in pregnant patients receiving iron supplements was the result of a pharmacological action, a view which has been shown to be mistaken (de Leeuw and Lowenstein, 1966). Pregnant women of all races should maintain a haemoglobin concentration above 11.0g./100ml. whatever their race or environment (World Health Organisation, 1969), and greater concentrations should be maintained at higher altitudes.

The problem is immense in India. A study in one village near Delhi revealed that 52% of pregnant women had Hb. below 10g./100 ml. and 80% had Hb. below 11g./100 ml. (Sood and Ramalingaswami, 1969); these figures may be held to be representative of all rural India (Devi, 1966).

Pregnancy anaemia is universal, but communities may be classified into four groups according to the incidence and severity of anaemia.

1. Communities where anaemia is a major cause of maternal mortality.

The first report of anaemia as a common cause of maternal death came from Madras, where it was the cause of 35% of all deaths in the Sir Ramaswami Mudaliar lying-in-hospital during the 1914-1915 (Mudaliar, 1915). Today 20% of maternal deaths in Madras—and other parts of India—are attributable to anaemia, a figure which has remained unchanged for the last 30 years (Menon, 1968; Masani, 1969). These patients die of congestive cardiac failure or a state of irreversible shock (Harrison, 1967, Harrison, 1969). But this is only part

* Based on a talk given in the Central Health Laboratory, Kathmandu on 19th March 1969. Submitted for publication 24th June 1969

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of the picture, as anaemic women are less able to survive haemorrhage, infection or other stresses. The importance of anaemia as a contributory cause of death is difficult to assess, but probably represents another 20% of deaths in India (Menon, 1968), and it is worth noting that 90% of all deaths in Maharashtra occur when the Hb. is less than 7.0g./100 ml. (Masani, 1969).

Anaemia does not have any direct association with pre-eclampsia or eclampsia, as some authors have suggested, but there is a condition of 'pseudo-eclampsia' in severely anaemic patients, who have raised systolic blood pressure (the diastolic pressure remaining normal), albuminuria and oedema (Mudaliar and Menon, 1942; Scott and Govan, 1949; Hendrickse and Watson-Williams, 1966).

2. Communities where anaemia causes foetal loss, but maternal loss is uncommon.

There was a 55% mortality amongst pregnant Nigerian women with packed cell volume (PCV) 13% or less and in congestive cardiac failure. The establishment of blood transfusion facilities and the introduction of exchange blood transfusion has reduced maternal mortality to less than 40%, and these are the women admitted *in extremis* or who have other complications (Fullerton and Turner, 1962). Foetal loss remains high (30 to 40%) (Ojo, 1965), and Ibadan may be said to be in transition from group 1 to group 2. Kuala Lumpur is in a similar situation, where anaemia is no longer a common cause of death (12 per 1000 anaemias) at least in those who attend hospital, but is still accompanied by a high rate of foetal loss (15.5%) (Lourdenadin, 1964).

3. Communities where mild to moderate anaemia is common.

Anaemia is a frequent complication of pregnancy in the western world, but is a negligible factor in maternal mortality and is rarely listed as the immediate cause of foetal loss. For example, the incidence of anaemia (Hb. < 10.0g./100ml.) in one antenatal clinic in Western Australia is 10% (Fleming 1969). It is the commonest - if not the most serious - complication of pregnancy; iron deficiency persists in spite of oral medication and remains a constant, recurring and tedious problem. The main areas of debate and research are into the possible relationships between anaemia *per se* and folate deficiency on the one hand and disturbed placental function, placental growth, abortion, *abruptio placentae*, premature labour and low birthweight on the other (Klein, 1962; Hibbard and Hibbard, 1963; Martin et al., 1964; Martin et al., 1967; Beischer et al., 1968a; Beischer et al., 1968b).

4. Communities where pregnancy anaemia is unknown.

No honestly conducted survey has ever revealed such a community. Even in the wealthiest countries, sideropenia is ubiquitous, except for south and west Africa, where anaemia is common from other causes; forty-three per cent of livers of traffic accident victims in the United States of America contained no intracellular haemosiderin (Banerji et al., 1968), and two-thirds of nulliparous women in Texas (Scott and Pritchard, 1967) and Finland (Vartiainen et al., 1967) were shown to have iron stores insufficient to meet the needs of pregnancy.

AETIOLOGY

Iron deficiency

Sixty-six per cent of livers from Indian medicolegal autopsies contained no haemosiderin (Banerji et al., 1968). Surveys have shown that usual Indian diet provides between 15 to 30 mg. of iron daily (Devi, 1966), which should be adequate to meet requirements outside pregnancy, but the total dietary iron does not represent the amount available for absorption (Jacobs and Greenman, 1969). It is probable that much of the iron is not available in the bulky Indian diet rich in phosphates and phytates (Lourdenadin, 1964; Devi, 1966; Sidhu et al., 1967), and that this is the major cause of the nation-wide sideropenia. Iron deficiency anaemias are precipitated in the sideropenic community by various factors, the relative importance of which may be assessed from the series of Chatterjea (1964) in Calcutta; nutritional iron deficiency accounted for 21%, hookworm infection 42%, uterine haemorrhage 18%, haemorrhage from other sites 15% and hookworm plus other causes of haemorrhage 3% of 28 males and 42 females with iron deficiency anaemia. This series included only patients with pure iron deficiency, and so excluded those with malabsorption syndrome who have multiple deficiencies; up to 60% of patients with tropical sprue have iron deficiency anaemia (Baker and Mathan, 1968; Jeejeebhoy et al., 1968).

To all these factors is added the burden of pregnancy, and especially the strain of pregnancy before the completion of growth. Anaemia in pregnancy has multiple aetiology, but in India about one half of all anaemias are purely or predominantly due to iron deficiency (Devi, 1966) and only 6 out of 47 consecutive anaemic women had stainable interacellular iron in their bone marrows (Sidhu et al., 1967).

Folate deficiency.

There are widely differing estimates of the incidence of megaloblastic anaemia in pregnancy in India; Devi (1966) and Vyas et al (1959) place the incidence between 3 to 5% of anaemias, but laboratories in southern, eastern and northern India have reported that megaloblastic erythropoiesis occurs in between 40 to 60% in all pregnancy anaemias (Chatterjea, 1966a; Karthigaini et al., 1964; Sidhu et al., 1967). The discrepancy is likely to be the result of differences of technique, and well-known masking of megaloblastosis by iron deficiency. The higher estimates are probably correct, because of the exhaustive investigations which these workers performed, and because such figures are in accord with experiences in Europe, North America and Africa (Chanarin et al., 1965; Lowenstein et al 1966; Fleming et al., 1969).

Megaloblastic anaemia in pregnancy is almost the result of folate deficiency, but the significance of vitamin B₁₂ deficiency in pregnancy in Indians will be discussed below

Three quarters of folate compounds in a European mixed diet are polyglutamates with a chain of up to 7 glutamic acid residues; these are absorbed and utilised to about one-third of the extent of monogluamates, which make up the remaining folate (Per ryand Chanarin 1968). The folates of the vegetarian diet of Hindus will be predominantly in the polyglutamate forms, but there should be adequate utilizable folate except that folates are heat

labile and destroyed by long cooking. The frequency of folate deficiency in different races reflects their cooking habits; Indians overcook green vegetables (Karthigaini et al., 1964) and have a higher incidence of megaloblastic anaemia in pregnancy in Malaysia and Singapore, whereas the Malays have lower incidence and the condition is almost unknown amongst the Chinese, who eat vegetables lightly cooked (Tasker et al., 1956; Told and Kam, 1965 Kwa and Gaw, 1968) The Thais eat their vegetables more lightly cooked than do the Chinese, and the serum folate activity in normal Thais is even higher than that encountered amongst well-nourished Europeans; folate deficiency is unknown in Bangkok except for occasional Chinese patients (Dr. Supa Na-Nakorn, personal communication).

The fields and markets of Nepal are green with masses of leafy vegetables, but Nepalese cooking is similar to Indian; although the food is delicious, nothing survives which is delicious, nothing survives which is recognizable to a European eye as a green vegetable. Macrocytic anaemias are reported to be common (Dr. Gurubacharya, personal communication) and it is probable that there is widespread folate deficiency.

Tropical sprue is of particular interest in the Indian subcontinent as the folate polyglutamates need the secretion of conjugases by active normal jejunal epithelial cells (Booth et al. 1968)

The main cause of folate deficiency in pregnancy is simply intake not meeting high requirement, but other factors may play a part. The serum folate of a normal male adult is maintained by PGA 50 μ g. per day, but a supplement of 100 to 500 μ g. per day is needed to meet the increased requirement of pregnancy (Willoughby, 1967). Erythroid hyperplasia secondary to haemolysis or haemorrhage leads to further folate demands and haemoglobinopathies or post partum haemorrhage are often the precipitating factors of megaloblastic anemia (Hendickse and Waston-Williams, 1965). High folate requirements do not end with delivery, but are continued by the process of lactation (Shapiro et al; 1963).

The theory of a metabolic block in folate metabolism in pregnancy is an old one to explain the many megaloblastic anaemias which do not respond to therapy. Most of these unresponsive anaemias are the result of infections or haemolysis; folic acid treatment causes a reversion to normoblastic erythropoiesis, but the haematocrit does not rise. However, there are certain conditions in which folate metabolism may be disturbed. Iron deficiency leads to reduced formimino-transferase activity and apparent folate deficiency in pregnancy (Chanarin et al., 1965; Vitale et al., 1966). Infection (particularly urinary infections) can precipitate "megaloblastic arrest" of erythropoiesis (Chanarin and Davey, 1964), possibly because dihydrofolic acid reductase is inactive with pyrexia (Panders and Rupert, 1965).

The anaemia of protein deficiency as in kwashiorkor is a normoblastic hypoplastic anaemia (Sood et al., 1965; Adams et al, 1967) and there is no good evidence that protein deficiency is a cause of megaloblastosis—a view which is still held by some (Upadhyay and Verma, 1969) — although the deficiencies of protein and folate often occur in the same patient (Ghitis et al., 1963).

Vitamin B₁₂ deficiency.

The requirements for vitamin B₁₂ of pregnancy and lactation are not sufficient to deplete normal vitamin B₁₂ stores, but maternal serum vitamin B₁₂ falls throughout pregnancy and may be below 100 pg./ml in about 2% of normal pregnant women (Temperley et al., 1968).

Vitamin B₁₂ deficiency reduces fertility and the chances of pregnancy continuing if fertilization does take place (Jackson et al., 1967), but there are at least two situations in temperate climates where the serum vitamin B₁₂ may fall to below 50pg./ml. The commoner situation is where folate deficiency results in a partial immobilization of body stores of vitamin B₁₂; the patients have a megaloblastic anaemia, low serum folate and serum vitamin B₁₂, but treatment with small doses of folic acid only is followed by a reversion of marrow morphology to normoblastic and a rise of the serum Vitamin B₁₂ to normal levels (Johnson et al., 1962). Secondly and extremely rarely, true Addisonian pernicious anaemia in relapse may be encountered during pregnancy (Armstrong et al., 1968).

The question of vitamin B₁₂ deficiency in pregnant Indians is complicated by two factors—the low intake by Hindu vegetarians (Chatterjea 1966a) and tropical sprue (Jeejeebhoy et al., 1968). The daily requirement of vitamin B₁₂ is small (about 1 pg.) and is met usually by a vegetarian diet which contains milk products, such as curds and legumes (Baker, 1967). Well water in rural India is another source of vitamin B₁₂ presumably from faecal contamination (Professor S.J. Baker, personal communication). Pure dietary deficiency is rare in southern India, but stores are low and tropical sprue may be followed by frank deficiency within a few months (Baker, 1967). Some women with mild malabsorption and low stores become pregnant; the demands of pregnancy lead to depletion and the women give birth to infants with deficiency (Baker et al., 1962; Jadhav et al. 1962). Chatterjea (1966a) has classified megaloblastic anaemias in pregnancy in Calcutta as due to folate deficiency in 70%, folate and vitamin B₁₂ deficiency in 20% and pure vitamin B₁₂ deficiency in 10% of patients, but this may over estimate the incidence of vitamin B₁₂ deficiency as the classification was from serum assay results, which can be misleading (Johnson et al., 1962). True vitamin B₁₂ in pregnancy appears to be confined to eastern and southern India; Sidhu et al. (1967) found no relationship between serum vitamin B₁₂ and bone marrow morphology in pregnancy.

Thalassaemias and haemoglobinopathies

The most frequent causes of anaemia in pregnancy after the nutritional anaemias are the thalassaemias and haemoglobinopathies. The genetics, chemistry and manifestations of these conditions have been reviewed extensively by Lehmann and Carrell (1969) and Weatherall (1969); it is sufficient to describe the pathology of β thalassaemia, α thalassaemia, Hb E and Hb S and their interactions in as much as they affect the course of pregnancy.

β -thalassaemia. This term covers a group of conditions where synthesis of β chains of adult haemoglobin is reduced. The homozygous state results in severe anaemia, hypochromic

red cells, numerous target cells, high levels of haemoglobin F and iron-overload, the condition is almost invariably fatal in childhood and no instance has been described of a patient with thalassaemia major becoming pregnant.

The anaemia of the heterozygous form is primarily one of ineffective erythropoiesis, and adults of both sexes have an average Hb. concentration 2-3g/100ml. lower than normal (Valentine and Neel, 1948). The minimum haemoglobin concentrations during otherwise uncomplicated pregnancy are in the range of Hb. 7.5-11.0g./100ml. (Fleming and Lynch, 1969). The peripheral blood picture is variable, but shows hypochromia and a degree of abnormality greater than would be expected from the haemoglobin concentration. The level of Hb. A₂ is usually raised, but is sometimes normal, especially when there is coincidental iron deficiency (Wasi et al., 1968). Patients may be iron deficient, iron sufficient or iron loaded, but haemosiderosis is rare except when there has been over-enthusiastic treatment with parenteral iron or blood transfusions (Williams and Siemsen, 1968). Patients may be folate deficient, especially during pregnancy. The balance between placental and foetal growth is disturbed, urinary oestrogen excretion is low, foetal distress is twice as common and there is an increase in the perinatal deaths in pregnancies complicated by thalassaemia (Hocking and Ibbotson, 1966; Beischer et al., 1968a; Beischer, 1968b; Fleming and Lynch, 1969).

α -thalassaemia. The inheritance of depressed α -chain synthesis is a complex subject, but there are three genotypes of importance. An individual with the heterozygous inheritance of α -thalassaemia₁ will have about 6% Hb. Barts (the tetramer γ_4) in the cord blood; Hb. Barts disappears as γ_4 chain synthesis declines and β chain synthesis increases, but very little or no Hb. H (β_4) is formed. Heterozygous α thalassaemia₁ is characterised in adult life by red cells showing slight hypochromia, microcytosis, anisocytosis, poikilocytosis, target cells and reduced osmotic fragility; the subjects are not anaemic unless there is some other cause, usually iron deficiency. The haemoglobin generally remains above 11g./100ml. in pregnancy, unless there is coincidental iron deficiency or haemorrhage, but the occasional patient has anaemia for which no other cause is found (Pootrakul et al., 1967a; Pootrakul et al 1967b).

The second genotype has been designated α -thalassaemia₂, α thalassaemia₂, or Hb.H disease. The α -thalassaemia₂ gene is silent or associated with only slight reduction of α chain synthesis (Kan et al., 1967). but in conjunction with α -thalassaemia₁ gene there is considerable reduction of α chain synthesis and inefficient erythropoiesis with haemolysis. About 26% Hb. Barts is present at birth and about 5-30% Hb. H during adult life (Pootrakul, 1967b; Weatherall, 1968). The severity of anaemia is variable (Hb. 4.0-14.0g/100ml) (Booth, 1966), but is generally intermediate between that of β -thalassaemia major and β thalassaemia minor. It is characterised by hypochromia, microcytosis, poikilocytosis, target cells, a high reticulocyte count and numerous Hb. H inclusion bodies. Reports of Hb. H disease during pregnancy show that the Hb. concentration is between 4.3 and 10.0g./100 ml. (Ryan et al., 1961; Booth, 1966; Mingot and Julliens, 1967b; Kwa and Grw, 1968; Alessio et al., 1968) with a high incidence of megaloblastosis (Kwa and Gaw, 1961).

The third important genotype is α thalassaemia, α thalassaemia. This homozygous condition is invariably fatal in utero or within a few minutes of birth, and accounts for about half of all cases of hydrops foetalis seen in Thailand (Pootrakul et al., 1967a). Eighty to one hundred per cent of the total cord blood haemoglobin is Hb. Barts (Pootrakul et al., 1967b).

The effect of maternal α -thalassaemia on the survival of the normal foetus is not known, but the complications of Hb. H disease in pregnancy are likely to be greater than those of β thalassaemia minor.

Haemoglobin E The heterozygous inheritance of Hb. E does not cause anaemia in the non-pregnant or pregnant subjects. The homozygous state (Hb. EE) is one of partially compensated haemolytic anaemia in the non-pregnant (Hb. 10.0–12.0 g./100 ml) (Huchns and Shooter, 1965) and like all haemolytic states is liable to be complicated by folate deficiency, especially in pregnancy. Haemoglobin E differs from Hb. A. by substitution on the β chain, so there is interaction with β thalassaemia, but not α thalassaemia. Hb. E β thalassaemia is the commonest haemoglobinopathy in hospital practice in Thailand (Bhamarapravati et al., 1967) and in pregnancy is a cause of severe anaemia, folate deficiency and premature labour (Lie-Injo et al., 1959).

Haemoglobin. S. Sickle cell anaemia differs from other haemoglobinopathies not only in the severity of anaemia, but also in the complications of tissue infarction. Sickle cell trait does not cause anaemia and is rarely a cause of infarction, but Hb. SS, Hb. SC and Hb. S/ β thalassaemia are serious conditions, their effects on pregnancy are similar and may be considered together (Fullerton et al., 1965; Hendrickse and Watson-Williams, 1966).

Most patients give a history of episodes of bone pains and of abdominal pain especially over the area of the spleen, and some will have noticed that they had dark urine and yellow eyes at various times. The patients are seen during pregnancy with the symptoms of severe anaemia, bone pain, jaundice, pyrexia or other complications. The commonest period of gestation on presentation is towards the end of the second trimester, as patients with anaemia are usually seen early in pregnancy, and patients with infarction are usually near term. The spleen is palpable in about 60% of patients, and the liver in nearly 50%.

Over 30% of patients have Hb. 9.0 g/100 ml. or less on first attendance in pregnancy; almost all will have some evidence of folate deficiency and megaloblastic erythropoiesis becomes increasingly common as pregnancy proceeds. Anaemia may develop extremely rapidly if folic acid supplements are not given; the haemoglobin can drop from about 7 g./100 ml. to less than 1 g./100 ml. in less than a week. Severe and sudden anaemia without folate deficiency can occur following acute sequestration of red cells in the spleen and liver near the time of delivery; the liver and spleen increase in size rapidly, and the haemoglobin drops 4 to 5 g./100 ml. in 24 hours.

Increased sickling of cells in the venous blood leads to packing of capillaries with aggregates of sickled cells and bone pain in crisis. This occurs more frequently during pregnancy, and particularly near the time of delivery. The pain is characteristically in the

shaft of the long bones or in the back, but moves from bone to bone. The onset is usually sudden and lasts for several days. Bone pain crisis is probably not fatal in itself, but bone marrow infarction can be followed by fat and marrow embolism. There is a sudden development of dyspnoea, systolic hypertension and albuminuria without dependent oedema. The appearance of these symptoms and signs is a matter of emergency, and should not be confused with the signs of eclampsia of pregnancy. Infarction may be complicated by osteomyelitis.

Incidence of haemoglobinopathies in Asia.

The frequency of these conditions in the Indian sub-continent has been reviewed by Chatterjea (1966b) and there have been extensive studies in Thailand. β -thalassaemia trait occurs in about 3-4% of the population across the whole of the northern part of India and throughout south-east Asia (Huehns and Shooter 1965; Chatterjea, 1966b). The incidence of α -thalassaemia is difficult to assess because of the normality of adults with the trait, and has been variously estimated in Thailand as between 3% and 21% (Tuchinda et al., 1959; Pootrakul et al., 1967a). Haemoglobin E occurs in Thailand overall in 13.6%, but the frequency reaches 50% towards the Cambodian border. Haemoglobin S is common among various tribal and aboriginal communities in India and may be seen rarely in other Asians.

Regrettably, no work has been performed yet within Nepal; the incidence of thalassaemia amongst Nepalese in Calcutta has been estimated to be as high as 13.6%; Hb. H and Hb. E have been reported in Gurkhas (Chatterjea, 1966b).

LABORATORY INVESTIGATION

The essential laboratory investigation of anaemia in pregnancy does not involve elaborate apparatus or expense. Routine haematological methods are very informative, but should be supported by the bioassay of folate and vitamin B₁₂ and haemoglobin electrophoresis. Haematological studies of rural and urban communities and of hospital admissions must precede systematic treatment and prevention of pregnancy anaemia in Nepal.

Peripheral blood examination.

The basis of investigation is the examination of the peripheral blood smears, with particular emphasis being placed on hypochromia, target cells, macrocytosis and hypersegmentation of the neutrophil polymorphs.

Hypersegmentation of the polymorphs was the earliest haematological sign in experimental nutritional folate deficiency (Herbert, 1962), and its usefulness in diagnosis has been confirmed in both pregnant and non-pregnant patients (Chanarin et al., 1965; Hoffbrand et al., 1966). Hypersegmentation was found to be a sensitive sign of folate deficiency in non-anaemic pregnant Nigerians (Fleming et al., 1968), and from the second trimester onwards, showed a close correlation with megaloblastosis in marrow specimens collected at term. However, when there was severe anaemia, a shift to the left predominated over and completely masked the expected shift to the right. This was probably action by the bone marrow to hypoxia.

Bone marrow biopsy

Regrettably, the idea still lingers in some places that marrow biopsy is something extraordinary and slightly heroic; one reason for this is the anachronistic survival of the sternum as the site of puncture. Biopsy of the sternal marrow is alarming to patients, it can cause anginal pain, and is potentially dangerous, especially in unskilled hands, because of the vital structures immediately behind the bone. The site of choice in the adult is the anterior iliac crest; this causes little apprehension, is painful only momentarily and there are no vital structures nearby.

There is agreement amongst competent haematologists on the characteristics of florid megaloblastic change, but there is often an incomplete transition from normoblastic erythropoiesis in pregnancy, and there is disagreement as to what are the earliest morphological changes which indicate significant deficiency. Giant metamyelocytes, dissociation of nuclear and cytoplasmic maturity and excess Howell-Jolly bodies have been described as the earliest signs, but Chanarin and his co-workers dismiss many of these minor changes as being both without significance and impossible to describe consistently (Chanarin et al., 1965). The present writer believes that the minor changes do represent folate (or vitamin₁₂) deficiency and as long as they can be described consistently on two slides viewed blind, they should be reported; it should be understood, however, that these small degrees of megaloblastic change cannot be the cause of severe anaemia.

The examination of bone marrow smears is the sheet anchor of investigation and there is no valid reason for omitting its performance on every anaemia in pregnancy. It is also valuable in the diagnosis of the unexpected - leukaemia or aplastic anaemia - and the iron status of patients may be assessed from the marrow more accurately than by serum iron estimations.

Bioassay of blood folate and vitamin B₁₂

A certain amount of false mystique has surrounded bioassay techniques. The methods need to be established by a well-qualified person, but once established, the repetitive work of routine assay can be performed by an intelligent person with only primary education, as long as supervision is adequate. The most common cause for failure is lack of cleanliness.

The method of choice for serum folate activity (SFA) assay is the aseptic addition technique, which avoids any destruction of folate activity by heat (Harper, 1965). An aseptic addition technique of red cell folate assay has been devised; whole blood is diluted 0.1 ml. into 2 ml. ascorbic acid solution (1g./100 ml.). The resulting haemolysate is assayed in exactly the same manner as serum (L. Comley and A. F. Fleming, unpublished). Serum vitamin B₁₂ assay using *Euglena gracilis* or radio-isotope dilution are fashionable, but assay by *Lactobacillus leichmannii* is simpler and adequate for clinical and medical research purposes (Fleming, 1968).

Serum folate results show a close correlation with megaloblastosis in pregnancy, but there are two problems of interpretation, (i) low SFA with megaloblastic erythropoiesis and (ii) normal SFA with megaloblastic erythropoiesis which responds to folic acid therapy. The first situation is probably explained by the decline of SFA throughout apparently normal pregnancy, reflecting placental transfer and high requirements; it may be argued that SFA is the most sensitive test, showing abnormal results long before there is megaloblastosis. The second situation where about 10% of folic-acid responsive megaloblastic anaemias have normal SFA is probably explained by an inadequate or irregular intake of folate; there is tissue depletion, but a normal SFA from being topped up.

The red cell folate also falls during normal pregnancy and about 20% have low levels in some western countries. A low red cell folate always indicates significant deficiency, provided that vitamin B₁₂ deficiency has been excluded, and is the best biochemical test available (Hoffbrand et al., 1966).

The serum vitamin B₁₂ concentration declines during apparently normal pregnancy and is below 100pg./ml in about 2% of normal women; very low vitamin B₁₂ concentrations may result from folate deficiency or true vitamin B₁₂ deficiency (see above). A therapeutic trial of physiological dose of folic acids (50-200pg. per day) (Thirkettle et al., 1964) will be followed by a full haematological response and a rise of serum vitamin B₁₂ to normal if the abnormalities are all result of folate deficiency.

Diagnosis of haemoglobinopathies.

A study of the red cell appearance and the bone marrow with staining for iron will often be sufficient for the recognition of a congenital abnormality of haemoglobin synthesis (Watson-Williams, 1968). This will be confirmed and categorised by haemoglobin electrophoresis. Once again, the apparatus and reagents are not expensive or the skills difficult to acquire (Graham and Grunbaum, 1963).

SUMMARY

Anaemia is still the direct cause of about 20% of maternal deaths in India and probably contributes to a further 20%. Deficiencies of iron, folate and vitamin B₁₂ and the haemoglobinopathies are the most important of anaemia. The aetiology and frequency of these factors in India and south east Asia are reviewed.

The basis of laboratory investigation of anaemia in pregnancy is the microscopic examination of blood films and bone marrow smears. Further studies include bioassay of blood folate and vitamin B₁₂ and haemoglobin electrophoresis. None of these studies are expensive or need skills difficult to acquire.

Haematological studies of rural and urban communities and of hospital admissions must precede the systematic treatment and prevention of anaemia in Nepal.

I am grateful to the Federation of Obstetric and Gynaecology Societies of India for their hospitality in Bombay, to the Ford Foundation and the Arnold Yeldham and Mary Raine Medical Research Foundation, University of Western Australia, for generously meeting the expenses of travel, and to many other individuals and institutions who made a four week tour of India and south-east Asia both profitable and enjoyable.

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