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The prevention of anaemia in pregnancy in primigravidae in the guinea savanna of Nigeria

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Two hundred Hausa primigravidae at Zaria were divided into five groups in a randomized double-blind trial of antenatal oral antimalarial prophylaxis, and haematinic supplements. Group 1 received no active treatment. Groups 2 to 5 were given chloroquine 600 mg base once, followed by proguanil 100 mg per day. In addition, group 3 received iron 60 mg daily, group 4 folic acid 1 mg daily, and group 5 iron plus folic acid. Forty-five per cent were anaemic (haemoglobin (Hb) $< 11{\cdot}0\ g\ dl^{-1}$) at first attendance before 24 weeks of gestation, and malaria parasitaemia (predominantly *Plasmodium falciparum*) was seen in 27%, of whom 60% were anaemic. The mean Hb fell during pregnancy in group 1, and seven patients in this group had to be removed from the trial and treated for severe anaemia (packed cell volume (PCV) $< 0{\cdot}26$). Only five patients in the other groups developed severe anaemia ($P = 0{\cdot}006$), two of whom had malaria following failure to take treatment. Patients in group 1 had the lowest mean Hb at 28 and 36 weeks of gestation, and patients receiving antimalarials and iron (groups 3 and 5) had the highest Hb at 28 weeks, but differences were not significant, possibly due to removal from the trial of patients with severe anaemia. Anaemia ($Hb < 12{\cdot}0\ g\ dl^{-1}$) at six weeks after delivery was observed in 61% of those not receiving active treatment (group 1), in 39% of those protected against malaria but not receiving iron supplements (groups 2 and 4) and in only 18% of patients receiving both antimalarials and iron (groups 3 and 5). Folic acid had no significant effect on mean Hb. Proguanil was confirmed to be a highly effective causal prophylaxis. Prevention of malaria, without folic acid supplements, reduced the frequency of megaloblastic erythropoiesis from 56% to 25%. Folic acid supplements abolished megaloblastosis, except in three patients who were apparently not taking the treatment prescribed. Red cell folate (RCF) concentrations were higher in subjects with malaria, probably due to intracellular synthesis by plasmodia. Infants of mothers not receiving antimalarials appeared to have an erythroid hyperplasia. Maternal folate supplements raised infants' serum folate and RCF. Fourteen per cent had low birth weight (< 2500 g), and the perinatal death rate was 11%; the greatest number were in group 1, but not significantly. A regime is proposed for the prevention of malaria, iron deficiency, folate deficiency and anaemia in pregnancy in the guinea savanna of Nigeria.

Anaemia in pregnancy is a major health problem in tropical countries, because it is extremely common and is strongly associated with maternal and foetal morbidity and mortality. The World Health Organization has supported several large trials of antenatal oral supplements of iron and folic acid for the prevention of anaemia in pregnancy in, for

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example, Mauritius, western Asia, the Indian subcontinent and south east Asia (Sood *et al.*, 1975; Baker and DeMaeyer, 1979; Fleming, 1982). Conclusions may be summarized briefly: prophylactic supplements equivalent to elemental iron 30–60 mg per day are sufficient to prevent anaemia in populations whose iron stores are generally adequate; in populations whose women have low or no iron stores, supplements should be 120–240 mg per day, but in some countries, such as India, up to half the women may still be anaemic (haemoglobin (Hb) $< 11{\cdot}0$ g dl^{-1}), as there is not sufficient time for repletion of iron stores before delivery (World Health Organization, 1972; Sood *et al.*, 1975). Supplements of folic acid 500 μg per day are recommended in almost all parts of the world: in seemingly well-nourished populations this will prevent the development of the preanaemic state of megaloblastic erythropoiesis (Fleming *et al.*, 1974); supplementation can result in an increase of the average Hb in populations where folate deficiency is common (Baker and DeMaeyer, 1979).

The aetiology of anaemia in pregnancy in tropical Africa is dominated by haemolysis due to malaria, especially in primigravidae (Gilles *et al.*, 1969; Fleming *et al.*, 1984). Trials of antimalarial prophylactics and supplements of iron and folic acid have been conducted so far only on a small scale. Morley *et al.* (1964) administered pyrimethamine 50 mg monthly to pregnant women at Imesi, in western Nigeria, and observed an average increase of birthweight of 157 g compared to unprotected pregnancies; they did not record, however, maternal Hb levels. Serum folate activity (SFA) was higher on average in protected primigravidae in Ibadan than in those unprotected against malaria, and this was ascribed to a reduction in the demand for folate following the prevention of haemolysis (Fleming *et al.*, 1968). In the primigravidae who were receiving supplements of iron and protection against malaria, the 85% frequency of folate deficiency at delivery was halved by the administration of folic acid 5 mg per fortnight in the second trimester and 5 mg per week in the third trimester (Fleming *et al.*, 1968). It was recommended that pregnant women in western Nigeria should receive a single dose of chloroquine 600 mg base at first antenatal attendance, pyrimethamine 25 mg per week, ferrous sulphate 200 mg per day and folic acid 5 mg per day (in the absence of cheap preparations containing 500 μg). Gilles *et al.* (1969) studied the effects of antimalarials, also on primigravidae in Ibadan: 24 out of 38 not receiving antimalarial protection developed haemolytic anaemia (packed cell volume (PCV) $< 0{\cdot}28$), compared to one subject in 19 protected women, anaemia in this patient being due to folate deficiency. In contrast, in Kampala, Uganda, administration of chloroquine did not affect the maternal Hb, but was associated with a higher mean birthweight, in a community with low levels of parasitaemia (Hamilton *et al.*, 1972): the folate content of food and SFA levels were high, so that supplements were not necessary, and supplementary iron was probably the most important antenatal therapy.

The importance of antimalarial protection has been accepted by both patients and obstetricians in West Africa, and later work has concentrated more on the prevention of deficiencies. Osifo (1970) noted a rise of Hb following folic acid but not iron supplements in a town near to Ibadan. In contrast, iron deficiency appears to have become increasingly common in Ibadan, and iron supplements during pregnancy were followed by increases of Hb or PCV; ferrous sulphate 200 mg per day was recommended for the non-anaemic, and 200 mg three times per day for the anaemic (Ogunbode *et al.*, 1980). The largest survey to date, on 621 subjects, was conducted in Monrovia, Liberia, where malaria control has been exercised since 1969 (Jackson and Latham, 1982): it was found that supplements equivalent to 60–180 mg of elemental iron per day were followed by similar rises of average Hb, which were not enhanced by folic acid or antimalarials.

Observations have been published earlier on 228 young primigravidae seen before the twenty-fourth week of gestation, at Zaria, in the guinea savanna of Nigeria (Fleming *et al.*, 1984). Forty-three per cent were anaemic (Hb $< 11{\cdot}0$ g dl^{-1}): the commonest association was *Plasmodium falciparum* parasitaemia, in 28% of all and 40% of anaemic women. Iron

deficiency was diagnosed, by haematological measurements only, in 18% of all and 25% of anaemic women. Fourteen per cent were folate deficient, but this did not correlate with anaemia. The roles of malaria, folate deficiency and iron deficiency in the aetiology of anaemia have been defined further in the same patients, by a double-blind trial of antimalarial prophylactics and supplements of iron and folic acid. The results are discussed in this paper, and recommendations are made as to the best antenatal regime to prevent anaemia in this population.

MATERIALS AND METHODS

Subjects Studied

The patients selected were (i) Hausa women living in Zaria and planning to deliver in Zaria, (ii) pregnant for the first time, (iii) at less than 24 weeks of gestation, as estimated by the height of the *fundus uteri*, and (iv) the wives of unskilled or semiskilled men. Women were excluded if it was ascertained that they had already taken any antimalarial treatment or haematinics during the pregnancy. Other patients excluded at the time of initial attendance were one with hydatidiform mole, one with haemoglobin SC disease, two with anaemia (PCV < 0·30), and two with proteinuria.

The purpose and design of the study was approved by the Medical Ethical Committee of Ahmadu Bello University; the trial was explained in the Hausa language to the patients or to their husbands or guardians, and their informed consent was obtained.

Clinical, haematological and parasitological data on first attendance of 228 successive subjects meeting the entry criteria, have been discussed in the earlier communication (Fleming *et al.*, 1984). The youth and small size of the 200 subjects finally included in the trial are shown by their mean age, height and weight (Table 1). The patients gave no history of significant disease and none was found by physical examination, except that one patient was deaf (group 4), one had a clinically unimportant ventricular septal defect (group 1), one had a mild glossitis (group 3) and the spleen of one patient (group 5) was palpable 5 cm below the costal margin. The mean period of gestation for all patients was 18·5 weeks, and there were no significant differences between the five treatment groups with respect to gestational period, weight, height or age (Table 1).

TABLE 1

Clinical observations on 200 Hausa primigravidae at their first attendance at antenatal clinic

	Treatment groups				
	1 *Mean ± s.d.*	*2* Mean ± *s.d.*	*3* Mean ± *s.d.*	*4* Mean ± *s.d.*	*5* Mean ± *s.d.*
Age (years)	16·3 ± 2·0 (36)	15·9 ± 2·0 (39)	16·2 ± 2·0 (38)	16·4 ± 2·4 (37)	15·5 ± 1·4 (39)
Height (cm)	157·5 ± 5·3 (36)	155·9 ± 5·4 (39)	156·0 ± 5·5 (38)	154·7 ± 6·0 (37)	156·2 ± 5·2 (39)
Weight (kg)	49·2 ± 6·1 (36)	50·1 ± 6·0 (36)	50·6 ± 5·9 (38)	47·7 ± 5·3 (37)	49·9 ± 6·0 (39)
Fundal height (weeks)	18·6 ± 4·4 (40)	18·4 ± 3·5 (40)	18·9 ± 4·0 (40)	18·3 ± 3·9 (40)	18·8 ± 4·5 (40)

Numbers of observations are shown in parentheses.

TABLE 2

Treatment schedules, each administered orally to five groups of 40 Hausa primigravidae, from first antenatal attendance to six weeks postpartum, in a double-blind trial

Treatment group	*Chloroquine**	*Proguanil†*	*Iron‡*	*Folic acid§*
1	0	0	0	0
2	+	+	0	0
3	+	+	+	0
4	+	+	0	+
5	+	+	+	+

* Chloroquine 600 mg base once at first attendance.
† Proguanil 100 mg per day.
‡ Elemental iron 60 mg per day as ferrous sulphate.
§ Folic acid 1 mg per day.

Study Design

The first ten successive patients were included in a pilot study: they were treated in exactly the same manner as the patients in the study, except that all received the treatment of group 5 (Table 2).

Two hundred subsequent successive subjects were randomly allocated to one of five treatment groups using a random numbers table (Diem and Lenter, 1970). Neither the researchers nor the patients were aware of the treatment allocated until after the completion of the study. Five further patients were removed from the trial because of anaemia (PCV < 0·30) developing in the first week, 12 patients defaulted after only the first or second visit to the antenatal clinic, and one was found to be mentally subnormal and unable to follow instructions. These 18 patients were replaced in the trial by others; this was arranged by a moderator (Dr. B. M. Greenwood), who was not otherwise involved in the research, but had access to the treatment allocation code for this purpose. The five treatment groups, of 40 subjects each, received oral chloroquine sulphate and proguanil tablets (ICI) and spansules containing ferrous sulphate and folic acid (Smith, Kline and French), following the schedules shown in Table 2: the manufacturers supplied active tablets or spansules and the placebos*, which could not be distinguished by sight. The patients were instructed to bring any unconsumed medication to the clinic at every visit, so allowing for some check on their compliance to instructions.

At first attendance, the patients were questioned as to their ages, their husbands' occupations, their previous medical history and present symptoms: they were examined, and their height, weight, height of *fundus uteri*, blood pressure and presence or absence of palpable hepatosplenomegaly were recorded. Patients were examined by an obstetrician at least once every two weeks up to the 36th week of gestation, and subsequently every week until delivery: weight, fundal height and blood pressure were measured and urinalysis was performed at all visits.

Patients were encouraged to deliver in the hospital; the period of gestation, evidence of foetal distress, mode of delivery, complications and maternal outcome were recorded. (Some of these data were recorded also on those who delivered at home.) The infants were

*Placebo gelatin capsules consisting of starch, talcum, kaolin, sucrose and colouring.

examined, and the birthweight, Apgar score at two minutes, foetal complications, sex, outcome and age and weight at discharge from hospital were recorded. It was intended to record data on the appearance, weight and histology of the placentae, but this was frequently overlooked.

The mothers and infants were examined for complications and the infants were weighed at six weeks after delivery.

A Hausa-speaking female social worker was employed to trace and recall for observation or treatment any patient who failed to attend the clinic on her day of appointment, or any patient in whom the laboratory investigations revealed serious anaemia (PCV $<0{\cdot}30$) or malarial parasitaemia.

Patients were removed from the trial regimens and treated with antimalarials, iron and folic acid if (i) the PCV fell to $<0{\cdot}26$ or, (ii) malarial parasitaemia persisted for more than two weeks, or (iii) if they failed to take one third or more of the medication prescribed.

Laboratory Investigations

Haematological observations were performed at first attendance, 28 weeks and 36 weeks of gestation, at delivery and six weeks postpartum, following the techniques described by Dacie and Lewis (1975). These included the Hb concentration, red cell indices, and total white cell count (WBC) by the Coulter ZF system: for technical reasons (most commonly, fluctuating electrical power supply), manual methods for Hb, PCV and WBC had to be substituted on some occasions. Thin blood films were examined; the red cell morphology was recorded semi-quantitatively (0, +, ++, +++) for each of anisocytosis, macrocytosis, microcytosis, poikilocytosis, hypochromia, polychromasia and other abnormalities, including target cells, spherocytes, elliptocytes and nucleated red cells; the differential white cell count was performed and the percentages of neutrophils which were unsegmented or hypersegmented (five or more lobes) were recorded. The reticulocytes were recorded both as percentage and as absolute counts. A thick blood film was examined over 200 oil-immersion fields, and the percentage of fields positive recorded for each of *P. falciparum* asexual forms, *P. falciparum* gametocytes, *P. malariae* and *P. ovale* (Molineaux and Gramiccia, 1980). Bioassay of serum and red cell folate (SFA and RCF) was performed, using *Lactobacillus casei* as the test organism (Fleming, Comley and Stenhouse, 1971). Serum vitamin B12 was measured at first attendance and at 36 weeks of gestation, using *Lact. leichmannii* as the test organism (Fleming, 1968*a*). Hb electrophoresis on cellulose acetate was performed at first attendance (Lehmann and Huntsman, 1975). Faeces were examined microscopically for parasites at around 28 weeks of gestation. Bone-marrow was aspirated from the anterior iliac crest within 24 to 48 hours of delivery, or when a patient was removed from the trial because of anaemia or persistent malaria: two smears were stained by the May, Grünwald and Giemsa method and one by the Prussian blue reaction for iron, which was recorded on an ordinal scale (Bothwell *et al.*, 1979); the slide stained for iron was used also for the identification of malarial pigment in the macrophages.

Blood of the infants for all haematological investigations was collected by free flow from the placental end of the cut umbilical cord.

For the purposes of this study, anaemia has been defined as Hb $<11{\cdot}0\ \mathrm{g\,dl^{-1}}$ during pregnancy and at delivery, Hb $<12{\cdot}0\ \mathrm{g\,dl^{-1}}$ at six weeks postpartum, and Hb $<14{\cdot}0\ \mathrm{g\,dl^{-1}}$ in the newborn.

Statistical Methods

The effects of the different treatment regimens on haematological and other measures were investigated by comparing the five treatment groups at each stage of the trial; that is, at entry to the trial at around 18 weeks of gestation, at 28 and 36 weeks of gestation, at delivery, and at six weeks postpartum.

Table 3 shows the various comparisons that were made between the treatment groups. To examine the effect of iron supplementation, groups 3 and 5 were compared with groups 2 and 4, as the treatments given to those in the former groups were identical to the treatments given to those in the latter groups apart from the addition of iron to groups 3 and 5 (Table 2). Similarly the effect of folic acid was examined by comparing groups 2 and 3 with groups 4 and 5. The third comparison in Table 3 was made to determine whether any interaction between iron and folate effects existed. The effect of antimalarials was examined using two comparisons. First, group 1 was compared with group 2, the treatments given to patients in these groups being identical apart from the addition of antimalarials to group 2. Secondly, patients in group 1 were compared with those in groups 2 to 5 combined: this was done only when there was no marked effect of iron or folic acid on the variable being examined.

To test each comparison for statistical significance, *t*-tests were performed for continuous variables, and chi-squared tests for binary variables (with a continuity correction). For binary variables for which any expected frequency was less than 5, Fisher's exact test (2-sided) was performed.

Logarithmic transformations were made for SFA, RCF, serum B_{12} and reticulocyte counts before analysis, as the distributions of these variables were skewed, but they have been re-transformed to the original scale for presentation.

TABLE 3
Comparison between treatment groups

		Treatment groups				
Contrast	*Effect*	*1*	*2*	*3*	*4*	*5*
1	Iron		−	+	−	+
2	Folate		−	−	+	+
3	Iron/folate interaction		+	−	−	+
4	Antimalarial	−	+			
5	Antimalarial	−	+	+	+	+

RESULTS

Initial Laboratory Studies

There were no significant differences in any haematological measurement between the five groups when the patients were entered into the trial (Table 4). Ninety-one patients (45·5%) were anaemic. Four patients only had totally normal red cells on the blood film; most commonly seen were moderate (++) anisocytosis, with mild (+) macrocytosis, microcytosis and polychromasia, as described previously (Fleming *et al.*, 1984). Six subjects had elliptocytosis not associated with anaemia. Fifty-one (25·5%) had sickle cell trait, with nine to 12 individuals in each of the five groups: two had Hb-AC (groups 1 and 5).

Malaria, predominantly *P. falciparum*, was observed in 53 (26·5%) of subjects. There was only one significant difference in frequency of parasite density between the treatment groups; *P. falciparum* gametocytes were more frequent in groups 3 and 5 (to receive iron supplements) than in groups 2 and 4 (not to receive iron supplements) (Table 5). None had *P. ovale*. Thirty-

TABLE 4

Haematological observations on 200 Hausa primigravidae at their first attendance at antenatal clinic

	Treatment groups					
	1 *Mean ± s.d.*	*2* Mean ± *s.d.*	*3* Mean ± *s.d.*	*4* Mean ± *s.d.*	*5* Mean ± *s.d.*	*Significance*
Hb ($g\ dl^{-1}$)	11·2 ± 1·4 (40)	11·2 ± 1·4 (40)	11·0 ± 1·8 (40)	11·4 ± 1·5 (40)	11·5 ± 1·8 (40)	N.S.
PCV	0·35 ± 0·05 (39)	0·36 ± 0·05 (37)	0·35 ± 0·06 (38)	0·36 ± 0·04 (38)	0·35 ± 0·05 (39)	N.S.
MCHC ($g\ dl^{-1}$)	32·5 ± 2·0 (39)	31·7 ± 2·6 (37)	31·9 ± 3·1 (38)	31·8 ± 2·2 (38)	32·6 ± 1·9 (39)	N.S.
MCV (fl)	83·6 ± 6·3 (39)	84·4 ± 7·7 (37)	84·2 ± 9·1 (38)	84·1 ± 6·4 (38)	84·8 ± 5·8 (39)	N.S.
RBC ($\times 10^{12}\ l^{-1}$)	4·16 ± 0·65 (39)	4·23 ± 0·65 (37)	4·19 ± 0·72 (38)	4·27 ± 0·56 (40)	4·18 ± 0·67 (39)	N.S.
MCH (pg)	27·2 ± 2·8 (37)	26·9 ± 3·1 (36)	26·7 ± 3·5 (38)	26·8 ± 2·6 (40)	27·6 ± 2·2 (39)	N.S.
Five-lobed neutrophils (%)	0·8 ± 1·2 (40)	0·6 ± 0·9 (40)	0·9 ± 1·4 (40)	1·2 ± 1·8 (40)	1·1 ± 1·9 (40)	N.S.
	Mean 95% CI	Mean 95% CI	Mean 95% CI	Mean 95% CI	Mean 95% CI	
Reticulocytes ($\times 10^{9}\ l^{-1}$)	36 6–204 (39)	40 8–200 (37)	47 10–214 (38)	40 9–182 (40)	41 0–170 (39)	N.S.
SFA ($\mu g\ l^{-1}$)	9·3 2·7–32·4 (39)	7·8 2·8–21·4 (37)	8·9 2·7–29·5 (40)	7·8 2·7–22·4 (39)	8·3 3·0–22·9 (40)	
RCF ($\mu g\ l^{-1}$)	331 100–1096 (36)	339 117–977 (34)	372 129–1072 (34)	269 68–1072 (37)	324 79–1318 (39)	N.S.

Numbers of observations are shown in parentheses.
CI = CI for single observations.

two (60·4%) with malaria were anaemic, compared to 59 (40·1%) without parasitaemia ($\chi^2 = 5{\cdot}64$; $P = 0{\cdot}002$).

Three subjects had microfilariae of *Mansonella perstans* detected in the blood at initial attendance or later. One subject had haematuria associated with *Schistosoma haematobium*. Stool examination (performed on 65 subjects at around 28 weeks of gestation) revealed ova of hookworm in three, *Entamoeba coli* in seven, *E. histolytica* in two, *Ascaris lubricoides* in four and a flagellate in one subject. All three with hookworm were in group 1; infestations were judged to be light in two and heavy in one; two were anaemic during the trial, but iron was present in the bone marrow of one of these (not recorded in one) and also in the non-anaemic subject with a heavy hookworm load. The other protozoa and helminths were found evenly distributed amongst all five treatment groups and did not seem to be influencing the frequency or severity of anaemia.

TABLE 5

Malarial parasitaemia observed in 200 Hausa primigravidae at their first attendance at antenatal clinic

	% High-power fields +ve	Treatment groups 1 No.	2 No.	3 No.	4 No.	5 No.	Total No.	Total %
P. falciparum asexual forms	0	33	30	31	31	30	155	77·5
	<16	2	5	0	1	0	8	4·0
	16–100	5	5	9	8	10	37	18·5
P. falciparum gametocytes	0	37	35	32	38	32	174	87·0
	<16	3	5	8	2	8	26	13·0
P. malariae all forms	0	40	40	38	39	40	197	98·5
	<16	0	0	2	1	0	3	1·5

P. falciparum gametocytes: groups 3+5 *v.* 2+4, $\chi^2 = 3{\cdot}91$; $P = 0{\cdot}05$.

Compliance by Subjects

Patients who attended the clinic only once or twice were excluded from analyses and replaced in the trial (see above). A further 42 subjects left the trial before delivery and another 30 failed to attend the postnatal clinic: the pattern of defaulting did not seem to be related to the treatments received (Table 6). Only 89 subjects delivered in hospital. Some patients did not report on the day of an appointment, but continued in the trial on their return: missed attendances, and hence gaps in their therapy, showed no pattern related to the treatment received (Table 6).

Anaemia during the Trial

SEVERE ANAEMIA

Twelve subjects were removed from the trial and treated because their PCV fell below 0·26: severe anaemia developed in three before 28 weeks of gestation, in four between 28 and 36 weeks and in five from 36 weeks to delivery. Seven of the anaemic subjects were in group 1 and five were in the remaining groups (group 1 *v.* groups 2 to 5 combined: $P = 0{\cdot}006$). Severe anaemia was associated with the absence of antimalarial protection, as seven were so affected in group 1 compared to only one patient in group 2 ($P = 0{\cdot}06$). Furthermore, two of the

TABLE 6
The compliance of patients to the antenatal supplementation trial

	Treatment groups					
	1	*2*	*3*	*4*	*5*	*Total*
DEFAULTERS						
<28 weeks gestation	1	0	2	2	2	7
28–36 weeks gestation	4	3	5	3	5	20
36 weeks gestation to delivery	5	2	4	3	1	15
Postpartum	4	6	7	7	6	30
Total defectors	14	11	18	15	14	72
MISSING ATTENDANCE						
<28 weeks of gestation						
1 week	5	9	5	7	9	37
2 weeks	2	1	5	3	3	14
3–5 weeks	1	4	4	2	3	14
28–36 weeks of gestation						
1 week	7	10	4	5	6	32
2 weeks	4	2	4	0	4	14
3–5 weeks	4	8	1	8	2	23

anaemic patients in other groups, supposedly taking antimalarials, had parasitaemia at the time of their severe anaemia.

Anaemia was regarded as persistent if the Hb was less than 10·0 g dl^{-1} after four weeks treatment with antimalarials, iron and folic acid. Four patients had persistent anaemia, three of whom were in group 1 (not significant). Persistent anaemia was associated with a high reticulocyte count ($>200 \times 10^9\,l^{-1}$) in three of the four subjects.

MILD OR MODERATE ANAEMIA

The frequency of anaemia declined from the first attendance to delivery, being observed in 91 out of 200 (45·5%) at first attendance, 64 of 170 (37·6%) at 28 weeks, 40 of 127 (31·5%) at 36 weeks and 19 out of 80 (23·8%) at delivery: there were no significant differences between treatment groups (but it is to be noted that 12 subjects with severe anaemia were removed from the analysis and treated at different times).

Anaemia was observed in 37 out of 107 subjects (34·6%) postpartum, and was associated significantly with the absence of antimalarial prophylaxis (61%) and of iron supplements (39%) (Table 7). Folic acid supplements did not appear to affect the prevalence of anaemia postpartum.

Red Cell Indices and Reticulocytes

ANTIMALARIAL EFFECT

Among patients not receiving antimalarials, the mean Hb concentration was lower than that of the other groups at 28 and 36 weeks of gestation and at six weeks postpartum (Fig. 1), but no difference achieved significance, possibly due to the removal of seven patients in group 1 with severe anaemia. The mean RBC in group 1 at 36 weeks (3·99 (S.E.M. = 0·15) $\times 10^9\,l^{-1}$; $n=20$) was significantly lower ($P=0·04$), than that of group 2 (4·35 (S.E.M. = 0·09) $\times 10^9\,l^{-1}$; $n=30$), but this difference was not consistent at other times of the trial.

TABLE 7

Frequency of anaemia ($Hb < 12{\cdot}0\ g\ dl^{-1}$) in Nigerian primigravidae, six weeks after delivery; the effect of antimalarial prophylaxis, iron supplements and folic acid supplements

		No antimalarials (Group 1)		*Antimalarials (Groups 2, 3, 4, 5)*			
		No.	*%*	*No.*	*%*	χ^2	*P*
ANTIMALARIAL EFFECT	Anaemic	11	61·1	26	29·2		
	Non-anaemic	7	38·9	63	70·8	5·40	0·02
	Total	18		89			
		No antimalarials (Group 1)		*Antimalarials only (Group 2)*			
		No.	*%*	*No.*	*%*		
	Anaemic	11	61·1	9	33·3		
	Non-anaemic	7	38·9	18	66·7	2·34	0·13
	Total	18		27			
		Iron received (Groups 3, 5)		*No iron (Groups 2, 4)*			
		No.	*%*	*No.*	*%*		
IRON EFFECT	Anaemic	7	17·5	19	38·8		
	Non-anaemic	33	82·5	30	61·2	3·85	0·05
	Total	40		49			
		Folic acid received (Groups 4, 5)		*No folic acid (Groups 2, 3)*			
		No.	*%*	*No.*	*%*		
FOLIC ACID EFFECT	Anaemic	15	35·7	11	23·4		
	Non-anaemic	27	64·5	36	76·6	1·08	N.S.
	Total	42		47			

TABLE 8

Stainable intracellular iron in the bone marrow collected after delivery or when withdrawn from the trial

	Iron supplements received (groups 3, 5)		*No iron supplements (groups 1, 2, 4)*	
Marrow iron	*No.*	*%*	*No.*	*%*
0	11	39·3	28	49·1
+	10	35·7	17	29·8
++	6	21·4	9	15·8
+++	1	3·6	3	5·3
Total	28		57	

IRON EFFECT

Subjects in groups 3 and 5 receiving iron supplements had highest mean Hb at 28 weeks of gestation and six weeks postpartum (Fig. 1), but these differences were not statistically significant. Iron supplements also had no consistent or significant effect on other red cell indices.

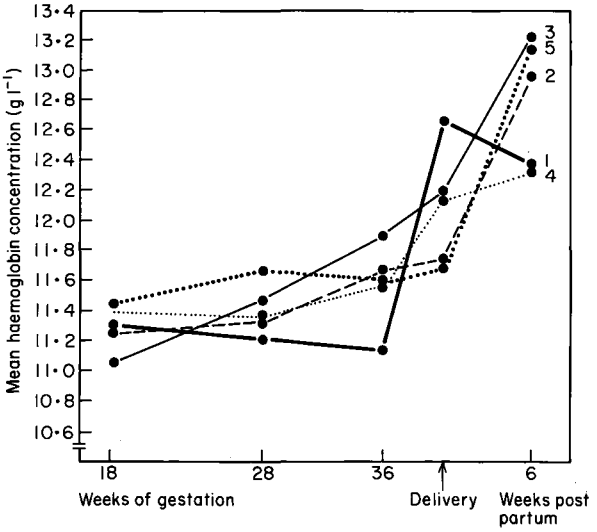

Fig. 1. Mean haemoglobin in Nigerian primigravidae allocated to five treatment groups throughout pregnancy (see text).

NUMBER OF OBSERVATIONS

Group	*18 weeks*	*28 weeks*	*36 weeks*	*Delivery*	*6 weeks postpartum*
1	38	36	22	11	18
2	39	37	31	19	27
3	39	33	22	13	20
4	40	33	25	21	22
5	40	31	27	16	20

FOLIC ACID EFFECT

Supplements of folic acid did not appear to influence the Hb (groups 4 and 5 in Fig. 1) or other red cell indices. No interaction of iron effect and folate effect could be detected.

Malaria

At 28 weeks of gestation, malaria was observed in nine (25·0%) of 36 patients in group 1, and in only three (2·2%) of 137 in the other four groups ($P<0·001$). At 36 weeks, five (22·7%) of 22 blood films from group 1 were positive, compared to two (1·9%) in 106 subjects from the four groups receiving antimalarials ($P=0·003$). One patient in group 1 was removed from the trial because of persistent parasitaemia at 32 to 35 weeks of gestation, and treated. At the time of delivery, 67 films were examined, and only one was positive (from group 4). At the postnatal visit, nine out of 107 films were positive (groups 1, 2 and 5 one each, groups 3 and 4 three each).

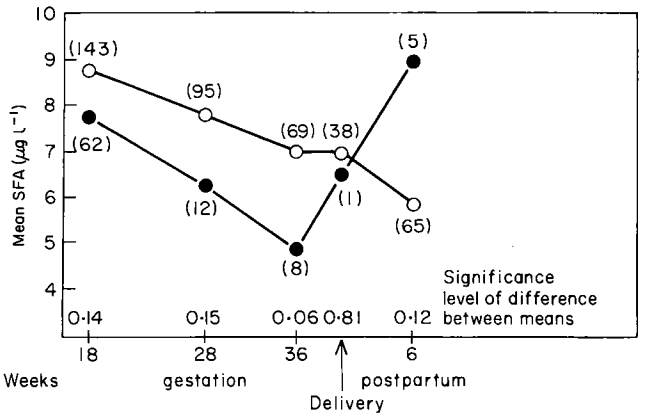

Fig. 2. Mean serum folate activity (SFA) in Nigerian primigravidae with or without malarial parasitaemia. Numbers of observations are shown in parentheses. ●, With malarial parasitaemia; ○, without malarial parasitaemia.

Plasmodium falciparum parasitaemia was recorded in 14 instances in subjects who were supposed to be taking prophylaxis. Eight had parasitaemia only at their postnatal visit, two of whom were known not to be taking treatment. Three had intense parasitaemia at 28 to 36 weeks of gestation, one of whom was severely anaemic. One had persistent gametocytes and severe anaemia at 21 weeks gestation, and two had moderate parasitaemia but no anaemia. Eight of these patients with malaria were supposedly on folic acid supplements, but low SFA in five indicated that they were not taking their medications.

Malarial pigment was recorded in 24 (24·5%) of 98 bone marrows, ten of which were from 20 marrows collected from patients in group 1 ($\chi^2 = 7{\cdot}19$; $P = 0{\cdot}008$).

Intracellular Iron in Bone Marrow

Iron supplements decreased the frequency of sideropenia at the time of delivery, but this was not statistically significant. Eleven (39·3%) of 28 subjects receiving iron supplements had no stainable intracellular iron in the bone marrow; this compared to 28 (49·1%) of 57 subjects not receiving iron ($\chi^2 = 0{\cdot}39$; N.S.) (Table 8). The absence of stainable iron was more common in women who were anaemic at any stage of pregnancy (30 out of 61 or 49·1%) than in those with normal Hb throughout (nine out of 24 or 37·5%), but not significantly so.

Serum Folate, Red Cell Folate and Megaloblastosis

SFA and RCF values were comparable in the five groups at first attendance (Table 4). Subsequently, SFA and RCF were raised above physiological levels in most subjects in groups 4 and 5, and results have not been analysed further. SFA and RCF declined during pregnancy in subjects in groups 1, 2 and 3. The mean SFA did not differ significantly between group 1 and group 2, but the mean RCF was consistently higher in group 1 than in group 2 throughout pregnancy, though none of the differences was statistically significant.

The mean SFA was consistently lower in subjects with malarial parasitaemia at first attendance, 28 and 36 weeks of gestation and delivery (Fig. 2). In contrast, the mean RCF was consistently higher in those with malarial parasitaemia at all stages of pregnancy and the puerperium (Fig. 3). No difference was statistically significant.

Ten out of 18 subjects in group 1 had megaloblastic erythropoiesis (Table 9). Protection against malaria without folic acid supplements (groups 2 and 3) more than halved the frequency of megaloblastosis, and this was reduced further by the addition of folic acid supplements. Seventeen (26·6%) of 64 women who were ever anaemic during pregnancy had

megaloblastic erythropoiesis, as compared with four (16%) out of 24 who were never anaemic (not significant).

Three subjects supposedly receiving folic acid supplements and antimalarials had frankly megaloblastic erythropoiesis (Table 9). In all three, the SFA was low and two had intense malarial parasitaemia, suggesting that none of the three had been taking the prescribed treatment.

TABLE 9

Megaloblastic erythropoiesis at time of delivery or when removed from trial, following different antenatal treatment regimes in Nigerian primigravidae

	No treatment (group 1)		*Antimalarials, no folic acid (groups 2, 3)*		*Antimalarials, plus folic acid (groups 4, 5)*	
	No.	%	*No.*	%	*No.*	%
Normoblastic	8	44·4	24	75·0	36	92·3
Megaloblastic	10	55·6	8	25·0	3	7·7
Total	18		32		39	

Antimalarial effect (group 1 *v.* 2+3), $\chi^2 = 3{\cdot}44$; $P = 0{\cdot}06$
Folic acid effect (groups 2+3 *v.* 4+5), $\chi^2 = 2{\cdot}81$; $P = 0{\cdot}09$

Serum Vitamin B_{12}

Mean serum vitamin B_{12} declined from 462 ng l^{-1} ($n = 128$) at the time of first attendance to 432 ng l^{-1} ($n = 75$) at 36 weeks of gestation (within subject comparisons; $P = 0{\cdot}003$): in no patient did levels reach those suggesting deficiency.

Complications of Pregnancy

ABORTION

Six patients aborted: all were in groups 4 and 5, receiving folic acid supplements (groups 2 and 3 *v.* groups 4 and 5; $P = 0{\cdot}03$).

HYPERTENSION, PRE-ECLAMPSIA, ECLAMPSIA

Eight patients had hypertension without other symptoms or signs. Twenty-one were diagnosed as having pre-eclampsia and six developed eclampsia. No association with treatment was evident.

OTHERS

Two patients were admitted to hospital for observation because of abdominal pain (groups 1 and 4). One patient (group 1) developed hydramnios.

INFECTIONS

Patients developed the following infections or evidence of infection during the course of pregnancy, besides malaria and other parasitic infestations discussed above: respiratory tract infections (8), significant bacteriuria (5), skin abscess (1), pyrexia of unknown origin (2) and positive VDRL tests (2). There were no apparent associations with treatment groups.

Delivery

Eighty-nine women were delivered in hospital, but some information was obtained from a further 75 patients who were visited by the social worker following home delivery. The mean period of gestation was 39·0 (S.D. 2·4) weeks; there were no significant differences between the five treatment groups.

Nine patients had cephalopelvic disproportion and three had delay in the second stage of labour. Other complications included previously undiagnosed twins (1), umbilical cord around the neck (1), intrapartum haemorrhage (1) and primary postpartum haemorrhage (4).

Twenty-two subjects required assisted deliveries, of whom only four were in groups 4 and 5 receiving folic acid (groups 2 and 3 *v.* groups 4 and 5: $\chi^2 = 3{\cdot}79$, $P = 0{\cdot}05$) (Table 10).

TABLE 10
Mode of delivery by Nigerian primigravidae

	Treatment group					
Mode of delivery	*1*	*2*	*3*	*4*	*5*	*Total*
Normal vaginal	27	29	25	29	28	138
Assisted, forceps	1	2	2	1	1	7
Assisted, breech		1	1			2
Caesarian section	3	3	3	2		11
Craniotomy	1	1				2
Total	32	36	31	32	29	160

TABLE 11
Clinical and haematological observations on infants born to Nigerian primigravidae

	n	*Mean*	*S.D.*
Birthweight (g)	87	2849	476
Placental weight (g)	13	538	144
Weight at about 4 days (g)	36	2746	556
Weight at 6 weeks	89	4265	760
Hb ($g\,dl^{-1}$)	64	14·4	2·8
PCV	62	0·44	0·09
MCHC ($g\,dl^{-1}$)	62	32·6	2·5
MCV (fl)	50	102	8·6
RBC ($\times 10^9\,l^{-1}$)	54	4·39	0·88
MCH (pg)	54	33·1	3·7
Five-lobed neutrophils (%)	66	0·45	0·98
		Mean	*95% confidence limits*
Reticulocytes ($\times 10^9\,l^{-1}$)	52	100	38–263
SFA ($\mu g\,l^{-1}$)	43	14·5	5·0–41·7
RCF ($\mu g\,l^{-1}$)	31	457	174–1216
Vitamin B_{12} ($ng\,l^{-1}$)	13	823	214–3090

Postnatal Complications

By six weeks postpartum, the following complications had been recorded in one patient each: secondary postpartum haemorrhage, lower respiratory tract infection, skin abscess, caesarian section wound sepsis, meningitis and pyrexia of unknown origin.

The Infants

BIRTHWEIGHT

There was no significant difference in mean birthweight between the treatment groups. The mean birthweight in group 1 (2723 g) was 132 g lower than the mean of groups 2, 3 4 and 5 combined (2855 g) (N.S.). The mean birthweight of infants born to 28 women who had malarial parasitaemia at any time during pregnancy (2814 g) was 51 g lower than in 59 infants of women who never had malaria detected (2865 g) (N.S.).

FOETAL DISTRESS AND APGAR SCORES

Foetal distress was recorded during labour in four instances; one infant died later. The Apgar score was recorded at two minutes in 50 infants: seven scored 3 or less, but there was no association with treatment groups.

COMPLICATIONS AT BIRTH

Three infants were judged to be premature, of whom two died; four suffered intrapartum asphyxia, of whom one died; one had haemolytic disease of the newborn due to foeto-maternal group ABO incompatability; one had intracranial damage, and died; one had talipes. The number are too small to relate these complications to maternal antenatal treatment.

HAEMOGLOBIN AND RED CELL INDICES

Results are summarized in Table 11; only the Hb, SFA and RCF seemed to have been affected by the treatment administered to the mothers. The mean Hb ($15{\cdot}8\,g\,dl^{-1}$) was higher in group 1 compared with the remainder ($14{\cdot}0\,g\,dl^{-1}$; $P=0{\cdot}04$). Only two of 14 group 1 infants were anaemic, compared to 21 of 50 of all other infants ($\chi^2=2{\cdot}54$; $P=0{\cdot}11$). Maternal folic acid supplements were associated with higher mean SFA in groups 4 and 5 ($18{\cdot}6\,\mu g\,l^{-1}$) compared with groups 2 and 3 ($11{\cdot}7\,\mu g\,l^{-1}$; $P=0{\cdot}01$). The mean RCF was also raised ($562\,\mu g\,l^{-1}$ compared to $417\,\mu g\,l^{-1}$), but this was not significant. None of 58 cord blood specimens showed malarial parasitaemia.

COMPLICATIONS BY SIX WEEKS OF LIFE

Three infants developed severe diarrhoea, of whom two died. Other infections recorded included ophthalmia (2) and skin sepsis (1). Three infants had failed to thrive. Two had umbilical hernias. No association with maternal treatment groups was evident.

PERINATAL MORTALITY

There were 16 (10·5%) perinatal deaths amongst the 152 infants in whom the outcome is known. The maternal complications most frequently associated were pre-eclampsia and eclampsia (8) and prolonged labour due to cephalopelvic disproportion (5). Five infants were premature and two suffered postnatal gastro-intestinal infections. A greater number of patients (5) were in group 1 than in any other group: this was not statistically significant, but it should be noted that three women unprotected against malaria showed no other maternal complications contributing to the loss of their infants.

The Placentas

Only 13 placentas were examined (Table 11). Malarial pigment was observed in two (groups 3 and 5), one of which was associated with low birthweight (2250 g).

DISCUSSION

Periodic attendance at antenatal clinic and taking therapy regularly are still not accepted practices among Hausa women around Zaria. They are subservient, and can leave their homes only with their husbands' consent: if a husband travels away, even for weeks, a woman may not be allowed to go to the clinic. Those who do receive antenatal care often deliver at home. These social customs imposed constraints upon this trial and have limited the usefulness of the results. Despite the employment of a social worker, whose main duty was tracing patients and trying to persuade them to attend and to take therapies as prescribed, 12 patients did not attend again after the first or second visits: a further 72 did not continue until the postnatal visit. Treatment was often not taken (Table 6). Only 89 women out of 200 delivered in the hospital.

In circumstances in which some patients leave a trial prematurely and some do not attend for clinical visits and measurements regularly, the possibility must be considered that this may introduce some bias into the results of the trial. Of special concern in this respect are those patients who were withdrawn because of severe anaemia or persistent malaria. Most such patients were in group 1 and, following withdrawal, these patients were given the treatment regimen of patients in group 5, that is antimalarial protection and full supplementation. Haematological measurements made on these patients after withdrawal have not been included in the analysis. There are cogent arguments for both including and excluding such measurements in analyses. Patients were more closely monitored in this trial than is likely in normal clinical practice, and thus it could be argued that persistent malaria and severe anaemia were detected more quickly than might normally be the case. Thus the haematological results on such patients following withdrawal are unlikely to be representative of patients who might normally receive no antimalarials or other supplements in pregnancy. On the other hand, it might be argued that as far as the patients in the trial are concerned, we should be interested in their haematological fate according to the treatments they actually received, which included treatment changes following adverse measurements. Schwartz and Lellouch (1967) have discussed such problems in the context of clinical trials, but it does not seem to us that there is a straightforward solution with respect to the present trial. We have chosen to exclude measurements after the treatment was changed, and this affects mostly group 1. It should be borne in mind, therefore, that the haematological measurements presented for this group, especially in the latter stages of pregnancy, are biased by the prior exclusion of patients with severe anaemia.

Patients who defaulted from the trial were investigated with respect to their final Hb measurement before leaving the trial, and appeared to have, on average, higher Hb concentrations than those who did not default. However, as the pattern of defaulting was comparable over the five treatment groups, although the defaulting phenomenon may induce bias in estimating absolute levels in any one treatment group, any comparison between groups should not be invalidated. This holds true for all variables examined.

Forty-five per cent of the young primigravidae were anaemic at the time of first attendance and 27% had malarial parasitaemia. *Plasmodium falciparum* malaria was the commonest cause of anaemia diagnosed, being observed in 35% of the anaemic women. Other infections (viral, bacterial, protozoal, or helminthic) could not be shown to be making significant contribution. The mean Hb fell for women in group 1 until 36 weeks of gestation (Fig. 1). This group received no protection against malaria, while the Hb tended to rise following first attendance in all groups who were allocated antimalarials. Of 12 patients who

were removed from the trial because of severe anaemia (PCV < 0·26), seven were from Group 1 and a further two had malarial parasitaemia following failure to take treatment: any of the three remaining patients with severe anaemia may also not have complied, but not shown parasitaemia on the day that anaemia was diagnosed. In four of these 12 patients, anaemia persisted after four weeks' treatment with antimalarials, iron and folic acid; it is likely that these patients had a persistent haemolytic anaemia, triggered by malaria and associated with hypersplenism or immune haemolysis (Fleming and Allan, 1969; Facer, 1980; Fleming, 1981). Slow recovery from anaemia of malaria is shown also by the high prevalence (61%) of anaemia in group 1 at six weeks postpartum (Table 7), by which time resistance to malaria should have returned to its non-pregnant high level.

Chloroquine resistant strains of *P. falciparum* have not been reported in the area of this study, and 600 mg base in a single dose was effective in clearing parasitaemia. The frequency of parasitaemia continued during pregnancy at over 20% in the unprotected women, but was seen in only five out of 243 blood films in women prescribed proguanil, and these were almost certainly from women not complying with instructions. This work confirms the efficacy of proguanil as a causal prophylactic, recently emphasized by Olsen (1983). The choice of prophylactic antimalarial for administration during pregnancy remains controversial (Bruce-Chwatt, 1983). In our opinion, the use of chloroquine as a prophylactic is to be condemned, as low concentrations in the blood, which will result from irregular dosage, favour the emergence of resistant strains (Nguyen-Dinh and Trager, 1978), and so hastens the day when this highly effective treatment will be rendered useless. Although the two drugs act in the same way, pyrimethamine appears to be less effective than proguanil (Olsen, 1983), probably because a tablet taken once a week is more easily forgotten or lost if the patient has vomiting or diarrhoea. Pyrimethamine is also less safe than proguanil, as overdosage can follow misinterpretation of instructions. Sulphonamide-containing mixtures (Fansidar, Maloprim) are not recommended because of possible effects on the blood forming tissues of the foetus (World Health Organization, 1982). We recommend daily proguanil 100 mg per day, or 200 mg for patients with height above 170 cm, as it is effective, accepted by the patients, and non-toxic.

Earlier work has shown that protection against malaria by pyrimethamine prevented haemolytic anaemia and its consequent erythroid hyperplasia and high demands for folate, so that the SFA fell less sharply in protected than in unprotected pregnant women (Fleming *et al.*, 1968). The same tendency was seen in the present work (Fig. 2), but numbers were small and differences did not achieve significance. However, antimalarial prophylaxis clearly reduced the frequency of megaloblastic erythropoiesis from 56% to 25% at delivery (Table 9). Paradoxically, malarial parasitaemia was associated with high RCF (Fig. 3). This phenomenon was discussed in an earlier publication (Fleming *et al.*, 1984), and is most probably due to synthesis of folates by the malaria parasites, sufficient to raise the RCF and confuse diagnosis, but not to contribute significantly to the host's nutrition.

Abdalla *et al.* (1984) have studied Gambian children with severe anaemia due to *P. falciparum* malaria: their bone marrows showed dyserythropoiesis, ineffective erythropoiesis, some ringed sideroblasts and occasional giant metamyelocytes. However, the deoxyuridine suppression tests indicated that these changes were not the consequence of folate or vitamin B_{12} deficiency. While it may be true that some megaloblastic-like changes seen, especially in children, may be the results of a metabolic disturbance, as yet not understood, there remains sound evidence that malaria makes a major contribution to the folate-deficiency and frank megaloblastosis which commonly complicate pregnancy in West Africa (Fleming, 1968*b*; Fleming *et al.*, 1968; Fleming, 1970; Fleming, 1981).

Groups receiving iron supplementation had higher Hb levels at 28 weeks of gestation and six weeks postpartum, but were not distinct from other groups at other times (Fig. 1). At six weeks postpartum, 39% of groups 2 and 4 (no iron) were anaemic, compared to 18% of

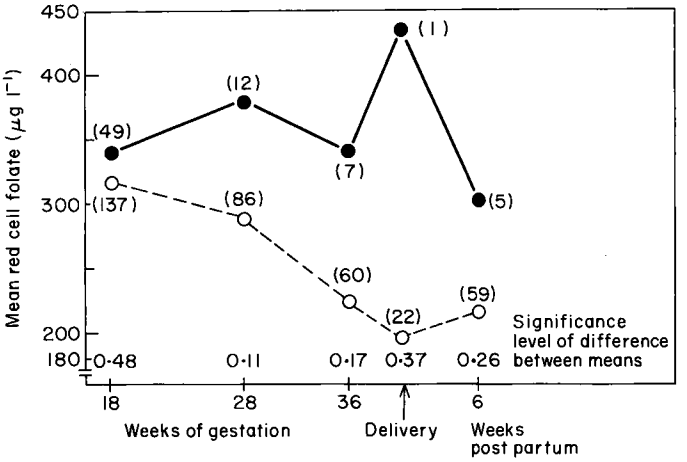

Fig. 3. Mean red cell folate (RCF) in Nigerian primigravidae with or without malarial parasitaemia. Numbers of observations are shown in parentheses. ●, With malarial parasitaemia; ○, without malarial parasitaemia.

groups 3 and 5 (iron supplemented) (Table 7): thus it is possible that the benefit of iron supplements may not be confined to the present pregnancy, but could be carried over to any subsequent pregnancy. However, 39% of those supplemented had no stores of stainable intracellular iron at delivery (Table 8), and it is concluded that oral iron 60 mg per day is inadequate as an antenatal supplement. Iron status has been assessed in pregnant women in Zaria recently by measurements of red cell-protoporphyrin, serum-transferrin saturation and serum-ferritin (Isah *et al.*, 1985): they found that the frequency of anaemia declined with parity, being 52% in primigravidae, 48% in women para 1 to 4, and 40% in grande multigravidae, but that iron deficiency increased with parity from 18% to 29% and 35% respectively in the three groups. It is recommended that antenatal supplements should be equivalent to elemental iron 120 mg per day, but that this should be increased to 180 mg or 240 mg in grande multigravidae or in any women who show evidence of iron deficiency.

Supplements of folic acid 1 mg per day had no significant effect on the mean Hb (Fig. 1) or on the frequency of anaemia (Table 7). Its administration prevented megaloblastic erythropoiesis, except in patients who failed to comply (Table 9). The subjects were young primigravidae (Table 1) many of whom were still growing. Folic acid supplements were associated with a significant reduction in the need for assisted labour (Table 10). Evidence has been presented in another publication that folic acid supplements during pregnancy enhance growth of young patients and reduce the frequency of cephalopelvic disproportion (Harrison *et al.*, 1985).

An association between the administration of folic acid and abortion has not been reported in any other study, and probably represents a chance finding in the present study.

Supplements of folic acid 500 μg per day are probably sufficient in subjects who are protected against malaria, as recommended by the World Health Organization (1972).

Vitamin B_{12} deficiency did not occur, and the relatively high serum levels are usual for Nigerians (Fleming, 1968*a*).

Fourteen per cent of infants on whom there were records were of low birthweight (<2500 g) and the perinatal death rate was 11%. The data were insufficient to show whether any of the antenatal interventions were reducing these high figures, although five out of 16 perinatal deaths occurred in group 1. Infants born to mothers who were not protected against malaria had higher mean Hb and less anaemia than other infants. It is possible

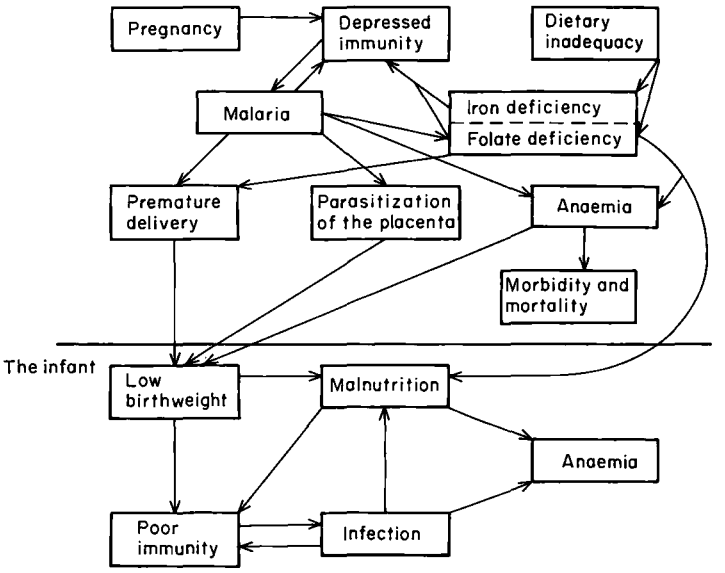

Fig. 4. The pathophysiology of malaria, iron-deficiency, folate-deficiency and anaemia during pregnancy.

that maternal parasitaemia and haemolysis stimulated an erythryoid hyperplasia in the foetus. Folic acid supplements improved the folate status of the infants.

The impact of malaria, dietary deficiency and anaemia on the mother and the infant are shown schematically (Fig. 4). There is a physiological depression of cell mediated immunity during normal pregnancies, probably necessary to allow tolerance of the foetus (Petrucco *et al.*, 1976). One consequence of this is the increase of both frequency and density of *P. falciparum* parasitaemia from the second trimester onwards, especially in first pregnancies (Gilles *et al.*, 1969; Bruce-Chwatt, 1983). Malaria is itself immunosuppressive, and may be complicated by viral or bacterial infections (Greenwood and Whittle, 1981*a*). Folate deficiency results from the high demands following malarial haemolysis and erythroid hyperplasia, and from dietary inadequacy (Fleming *et al.*, 1968; Fleming, 1970; Fleming, 1981). Iron deficiency is caused by a low bioavailability of iron from the food, in millet and guinea corn consumed by the population under study (Oomen, 1975). Both iron deficiency and folate deficiency further depress immunity, iron deficiency through effects on cell-mediated immunity and neutrophil myeloperoxidase, and folate deficiency by causing neutropenia and atypical nuclear division by lymphocytes (Brabin, 1982; Fleming and Werblińska, 1982). The mother has then entered a vicious cycle of depressed immunity, malaria and nutritional deficiency, and develops anaemia. The profoundest anaemias show a combination of haemolysis and megaloblastosis, and once the PCV is less than 0·13 there is a high maternal mortality (Fullerton and Turner, 1962).

The pyrexia of malaria can precipitate premature labour (Gilles *et al.*, 1969; Bruce-Chwatt, 1983). Malaria infects the placenta, and causes low birthweight (Archibald, 1956; Morley *et al.*, 1964; Jelliffe, 1968; Gilles *et al.*, 1969; McGregor *et al.*, 1983; Watkinson and Rushton, 1983; Bruce-Chwatt, 1983). Anaemia from any cause persisting throughout pregnancy, results in a degree of foetal hypoxia; there is an hypertrophy of the placenta (Beischer *et al.*, 1970), but this compensation is inadequate and there is low urinary oestrogen excretion and intrauterine growth retardation (Beischer *et al.*, 1968). The prevalence of low

birthweight is related directly to the severity of anaemia (Harrison and Ibeziako, 1973; Yusufji *et al.*, 1973; Garn *et al.*, 1981). Foetal distress is common; Apgar scores of 3 or less are observed in up to 20% of infants when the maternal Hb has been constantly in the range 7·0–10·0 g dl^{-1} (Fleming, 1973). When the maternal Hb is less than 7·0 g dl^{-1} and untreated, perinatal mortality rises sharply to more than 30% (Tasker, 1958; Llewellyn-Jones, 1965; Fleming, 1968*b*; Fleming, 1974). Folate deficiency *per se* leads to premature delivery and supplements are followed by an increase in the average duration of pregnancy and birthweight (Baumslag *et al.*, 1970; Rolschau *et al.*, 1979; Blot *et al.*, 1982). Low birthweight is associated with the development of nutritional deficiencies: total iron stores of infants are directly related to birthweight, and infants of low birthweight have a rapid rate of growth and quickly expend their iron stores (Stockman and Oski, 1978; Dallman *et al.*, 1980; Hågå, 1980); similarly, infants with low birthweight have small stores and high demands for folate, and are liable to develop deficiency (Stockman and Oski, 1978). The folate status of infants is also more directly related to the status of the mother, as shown in the present work and by Blot *et al.* (1982); folate in breast milk is diminished by folate deficiency of the mother and by maternal malaria (Osifo and Onifade, 1980). Infants born to iron deficient mothers have low iron stores, as demonstrated by serum ferritin levels (Kelly *et al.*, 1978), and iron reserves of children are higher at two months of age when mothers have received iron supplements during pregnancy (Blot *et al.*, 1980). Low birthweight is strongly associated with poor immunity and high susceptibility to infections (Boxer, 1978). Neonatal infections lead in turn to further depression of immunity, and to malnutrition, through mechanisms of anorexia, malabsorption, high demands for nutrients, loss of nutrients into the gastrointestinal tract, and disturbances of metabolism (Fleming and Werblińska, 1982). Protein energy malnutrition leads to loss of non-specific immune defences and cell mediated immunity in particular (Greenwood and Whittle, 1981*b*): depression of immunity by folate and iron deficiency are discussed above, and folate deprivation during intrauterine life and breast feeding may be of particular significance (Brabin, 1982). The infant has then completed the same cycle of poor immunity, infection and malnutrition as the mother, but in the infant, who has an immature immune system and critical demands for nutrition, the cycle is much more vicious.

CONCLUSION

It is recommended that in the guinea savanna of Nigeria, anaemia in pregnancy be prevented by the following oral regime:

1. Chloroquine 600 mg base, once at first attendance at the antenatal clinic;
2. Proguanil 100 mg per day, or 200 mg if height above 170 cm, until six weeks after delivery;
3. Ferrous sulphate equivalent to iron 120 mg per day, to be increased to 180 mg or 240 mg in grande multigravidae or others proven to be iron deficient;
4. Folic acid 500 μg per day.

The widespread and successful application of this regime, accompanied by education of women to follow prescribed treatment, could result in improvements in the health of pregnant women and their infants who would derive the advantages of greater maturity and better nutrition at birth. These benefits to the well-being of the community would be relatively inexpensive.

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