EFFECTS OF IRON AND FOLATE SUPPLEMENTATION ON
HAEMATOLOGICAL PARAMETERS, BIRTHWEIGHT
AND NEO-NATAL GROWTH IN A
HIGHLAND POPULATION
OF KENYA

Submitted in Partial Fulfilment of Requirements
of Master of Science in Applied Human Nutrition at
University of Nairobi, Kenya.

by

Helena Perry

Date: 11th June, 1993
DECLARATION

I, Helena Perry, hereby declare that this thesis is my original work and has not been presented for a degree in any other University.

Helena Perry

Date: 11th June, 1993

The thesis has been submitted for examination with our approval as University Supervisors:

Dr. J. W. Muita  Dr. A. M. Omwega
(Lecturer)      (Lecturer)

Date: 12/6/93  Date: 12/6/93

Department of Food Technology and Nutrition,
Unit of Applied Nutrition,
University of Nairobi.
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ABSTRACT

Anaemia during pregnancy as a result of iron and folate deficiencies is a major threat to women, particularly in developing countries. Because of the extra demands that pregnancy imposes on iron and folate status of the mother, and because of the traumatic effects of such deficiencies, WHO recommends supplementation of these micronutrients. This study evaluated the effects of iron and folate supplementation in 100 healthy, pregnant outpatients of the Kikuyu Hospital outside Nairobi. The subjects were similar with respect to ethnicity and socioeconomic factors.

Subjects were divided into 4 groups in a randomized, double-blind trial. Group A received a placebo, group B a combination of 150 mg. iron and 0.5 mg. folate, group C folate only and group D iron only. Supplements were given in slow-release capsules. Participants were non-anaemic (Hb > 10 g/dl) and 25-30 weeks pregnant on enrolment. Treatments were given for a minimum of 4 weeks before delivery and patients were also encouraged to continue taking the capsules until 4 weeks post-partum.

Haematological indices of haemoglobin (Hb), haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), erythrocyte morphology, as well as birthweights and neonatal weight gain were used to evaluate the effects of the supplementation. The higher than expected attrition rate may be due to reasons of fees payable at the hospital, distances for some patients to travel and perhaps
choosing to deliver elsewhere at the last minute. At the time of enrolment, 27% of the women showed macrocytosis. Diet had no association with initial Hb or the change in Hb during the study. However, 52% of women who showed initial macrocytosis were consuming a diet poor in iron and folate.

The average birthweight was 3178 grams. None of the treatments had any association with birthweights. The two factors that affected birthweights were mothers' initial weight at the time of enrolment and mothers' weight gain during the last trimester.

Folate supplementation had no significant effect on parameters tested. When age, parity, height, diet and socioeconomic factors were adjusted for, only the group receiving iron alone showed a significant rise in Hct and Hb (p < 0.005).

Iron is an essential supplement during pregnancy in most communities. The highland population of Kenya generally have a diet rich in iron and folate compared with many other African peoples. As a consequence, pregnancy anaemia may not be a major public health problem in this population. Although the results of this study support the routine supplementation with iron during pregnancy in this highland population, the benefit of additional folate was not clear and further studies evaluating iron and folate status during pregnancy and possible influencing factors in this region are recommended.
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Erythropoiesis - the production of red blood cells.

Haemoglobinopathy - a state of illness referring to a imperfection in the production of haemoglobin.

Haematocrit - packed cell volume.

Macrocytosis (megaloblastosis) - A state of ill health, when the average size of circulating erythrocytes is greater than normal, often caused by folate deficiency, preventing the normal development of red blood cells.

Normocytosis - a state of normal size circulating erythrocytes.

Microcytosis - a state of ill health where red blood cells are unusually small, often due to eg. iron deficiency anaemia.

Mean corpuscular volume - The average percentage or volume of red blood cells in the blood.
Mean corpuscular haemoglobin - Haemoglobin concentration in grams per litre to the red blood cell count in millions per cubic millimetre.

Mean corpuscular haemoglobin concentration - The ratio of haemoglobin to the packed cell volume.

Primigravida - first time pregnant

Primipara - first time parent

Multigravida - many times pregnant

Multipara - parent of many children
CHAPTER 1
INTRODUCTION

In most African countries, anaemia during pregnancy is a major health problem associated with maternal morbidity and mortality. Anaemias caused by iron and/or folate deficiencies are the most prevalent nutritional problems today, and yet they are easily prevented (INACG., 1984; Gebie et al., 1970; Fleming, 1986; Royston and Armstrong, 1989; Dallman, 1989; Scrimshaw, 1991). Iron and folate supplementation is cost-effective, and improvement in pharmaceutical technology has resulted in both well tolerated, inexpensive and palatable medication which improves compliance.

1.1 Background

Two thirds of women of childbearing age in developing countries suffer from iron deficiency. Iron deficiency during pregnancy is an obstetrical complication in which maternal morbidity, prenatal and perinatal infant mortality and prematurity are significantly increased. The rapidly growing foetus requires a large supply of iron, which is obtained from the iron stores of the mother (Scrimshaw, 1991). However, the mother and her foetus, have two independent systems controlling iron metabolism. If the mother has iron deficiency anaemia, it may not necessarily affect the iron status of her newborn baby. But even though maternal iron and folate deficiencies have relatively little effect on the haemoglobin
concentrations in the newborn, they are known to increase the incidence of low birth weight and perinatal mortality, and therefore supplementation programmes are generally well justified in most communities. Royston and Armstrong (1989), reported that many researchers agree with the statement that iron supplements are commonly required irrespective of socioeconomic status or maternal parity.

Knowledge regarding folate requirements and techniques for measuring levels are not as precise as is the case of iron. The WHO Technical Report No. 503 (1972), recommended an intake of 0.8 mg per day for pregnant women, because of the extra need for folate during pregnancy. This level is almost impossible to achieve in any habitual diet, however perfect and balanced it may seem. Final status of folate metabolism is best measured as serum and red cell folate concentration, by microbiological or radioisotopic dilution assay. These assays are complicated and often difficult if not impossible to accomplish in any field study, since the access to a well equipped laboratory may be limited (Shojania, 1984). However, other haematological responses, such as haematocrit and mean corpuscular volume, in combination with erythrocyte morphology findings, may suffice in detecting macrocytosis. It is important, however, to bear in mind other reasons for macrocytosis than pregnancy and folate deficiency. These may be inherited diseases, e.g. sideroblastic anaemia or thalassaemia, or acquired diseases, e.g. cancers, AIDS, drug or parasite activated haemoglobinopathies. Macrocytosis during pregnancy is most commonly caused by a deficiency of folate and/or vitamin B12 (cobalamin) deficiency. Women with frank B12 deficiency, however, are usually infertile (see section 2.9)(Eastham, 1985; Omondi, personal
1.2 Overall aim

The purpose of this study was to provide a deeper insight into the responses of iron and folate supplementation during pregnancy in women in a highland area of Kenya, and to provide health planners with evidence on whether this kind of supplementation is beneficial. This information could assist in the improvement of antenatal care, not only in the community under study, but for pregnant women in many parts of eastern Africa, where altitude and diet are similar, and haemoglobinopathies are noted commonly (Young et al., 1974).

1.3 Statement of the problem

Anaemia is a condition of diminished oxygen-carrying capacity of the blood due to a reduction in the number of red cells or in their content of haemoglobin or both. There are two major types of anaemia, namely those caused by blood loss eg. bodily trauma, peptic ulcer or hookworm infestation, and those caused by impaired formation of and/or destruction of red blood cells for various reasons, eg. disturbances of bone marrow function or deficiency of micro-nutrients essential for erythropoiesis. The added burden of pregnancy is known to increase the risk and incidence of both iron and folate deficiency anaemias. This is particularly important in developing countries or in poor communities, where the diet may be inappropriate or inadequate. Folate deficiency is a geographical burden in Kenya. Malaria is not endemic, nor is sickle cell anaemia a problem in this particular area of Kenya.
The anaemia that occurs during pregnancy may develop due to increased requirements for micronutrients, impaired absorption, insufficient dietary intake and increased fetal demands or a combination of any or all of these factors (Bentley, 1985; Holly, 1965). The progress and outcome of the pregnancy, with respect to both mother and baby, are affected by the state of anaemia, which can easily be corrected in the majority of cases. Corrective measures can take place in a number of ways. One is by nutrition education, which may prove successful, particularly in a situation where finances are virtually unlimited, and where the lack of sufficient iron and folate in the diet is mainly due to ignorance. Supplementation can also be done through an intervention scheme, where the staple foods of a community at risk are fortified with the micronutrients. Dietary modification and fortification are the most effective long-term strategies for alleviating nutritional anaemias. Another way to supplement the diet of individuals at risk, is to administer iron and/or folate via the oral route, or in severe cases of deficiency, via the parenteral route.

Iron and folate pills are given routinely to pregnant women in many parts of the world, and have proven to be simple and cost-effective. In Kenya, UNICEF have had a programme of oral iron and folate supplementation, but the coverage has been limited. Maternal and Child Health (MCH) clinics distribute oral iron supplements only, but no data are yet available on these programmes’ efficacy or patients’ compliance.
1.4 Objectives

The objectives of this study were:

1) to determine the effects of iron and folate supplementation on some haematological parameters during pregnancy in a cohort of pregnant women attending the antenatal clinic at Kikuyu Hospital, near Nairobi, Kenya.

2) to determine the effects of iron and/or folate supplementation on the birthweights of the infants born to participants in the study.

3) to determine the effects of iron and/or folate supplementation on the neo-natal growth rate of the babies of the participants in study.

1.5 Hypothesis

The working hypothesis that was employed for this research was, that iron and/or folate supplementation during the last trimester of pregnancy would have a positive effect on mothers’ haematological parameters, birthweights of babies and/or the neo-natal weight gain, in a cohort of pregnant outpatients of the Kikuyu Hospital, near Nairobi, Kenya.

1.6 Benefits of the study

It is anticipated that the findings of this research will benefit the Kikuyu Hospital Management and Administration, as well as other hospitals and health care institutions, both in the public and private sector, in relation to planning intervention policies for antenatal and maternity health care delivery systems in this area. The findings of this study will also be publicised in local and
international professional publications.

6
CHAPTER 2

LITERATURE REVIEW

2.1 Causes and consequences of anaemia

Major causes of anaemia in tropical Africa are iron deficiency, folate deficiency, bacterial and parasitic infections and haemoglobinopathies. Anaemia is often multifactorial, with the different causes interacting in a vicious cycle of depressed immunity, infection and malnutrition in a synergistic mode. Iron is essential for life, yet iron deficiency is often subclinical and/or non-anaemic. Two thirds of women of child-bearing age in developing countries are said to suffer from iron deficiency, although it is found to be relatively rare in geographical areas where dietary iron intake is high (Bentley, 1985; Fleming, 1986; Dallman, 1989; Scrimshaw, 1991; Royston and Armstrong, 1989).

Iron deficiency anaemia is characterized by a reduction of the mass of circulating haemoglobin (Hb), decreased concentrations of haemoglobin and serum iron and the complete absence of iron reserves. When the haemoglobin concentration falls, oxygen supply to vital organs declines, and the person begins to feel general weakness, tiredness, dizziness and headaches. Pallor of the skin and of the mucous membranes, as well as the nail beds and tongue, becomes noticeable when Hb drops to 7 g/dl or below (Royston and Armstrong, 1989).
2.2 Iron Metabolism

Iron is a constituent of haem (which is the prosthetic group of the organic compound porphyrin) and is present in haemoglobin, myoglobin and a variety of enzymes. Iron containing compounds can be grouped into: 1) functional compounds which are haemoglobin, myoglobin, enzymes and transferrin and 2) storage compounds which are ferritin and haemosiderin. Of these, haemoglobin is by far the largest constituent (INACG, 1984).

Human iron metabolism functions to supply sufficient iron to the bone marrow for haemoglobin synthesis. In women, iron balance is influenced by the effects of menstruation and pregnancy. In the normal state, the total amount of body iron ranges from 3.5 to 4.0 gm, and the haemoglobin concentration from 12 to 15 gm %. The liver, spleen and the bone marrow store 1 gm of iron as ferritin or haemosiderin. Apparently, haemoglobin is the last of the iron complexes to be affected by iron deficiency. Only 10% dietary iron is absorbed, and the place of absorption is the duodenum of the small intestine. The rate of absorption falls as the haemoglobin concentration approaches normal and this must also be taken into consideration in planning oral iron therapy (Holly, 1965; INACG, 1984).

An important relationship exists between storage iron (ferritin or haemosiderin) and haemoglobin concentration, for as long as there is iron in storage the bone marrow is capable of maintaining a normal haemoglobin level. The quantity of iron required for haemoglobin synthesis ranges from 25 to 40 mg/day. The same quantity is normally returned each day to the iron pool as the older erythrocytes
are haemolyzed and the haemoglobin undergoes degradation (Lind, 1983).

The absorption of iron is a complex process that is influenced by the intestinal mucosa, the amount and chemical nature of iron in the ingested food, and a variety of factors that increase or decrease the bioavailability. Absorption of iron is increased by the presence of ascorbic acid, and reduced in the presence of phosphates, phytates and tannin. Vitamin C and fructose both increase the absorption, by enhancing the conversion of oxidized ferric iron (Fe$^{+++}$) to the reduced form, ferrous iron (Fe$^{++}$). The status of the iron stores themselves has an important regulatory effect, i.e. absorption being decreased when stores are adequate (NAS, 1980; Kreutler, 1980; Lind 1983). Also, if a meal contains both haem iron (from animal sources) and non-haem iron (from vegetable sources), the former will improve the absorption of the latter (Scrimshaw, 1991).

2.3 Dietary sources of iron

Dietary iron is available in two forms, the haem and the non-haem compounds. Forty percent of the total iron in all animal tissues, including meat, liver, poultry and fish is classified as haem iron. The remaining 60% of iron in animal tissues, and all the iron of vegetable products are classified as non-haem iron. Food sources rich in iron are meat products in general but organ meats in particular, as well as eggs, lentils, nuts, green and leafy vegetables (NAS, 1980).
2.4 Anaemia during Pregnancy

The classification of anaemia according to haemoglobin values may vary and are arbitrary. The WHO (1989) however, recommended using 11 g/dl as the cut-off point, for pregnant women living at sea level. Severe anaemia is diagnosed when the MCHC value falls below 60%.

Women are especially prone to iron deficiency anaemia during pregnancy, and therefore supplementation programmes must be given careful consideration. In pregnancy, iron deficiency anaemia is associated with an increased risk of low birth weight, prematurity, and perinatal mortality. Severe anaemia resulting in heart failure, shock, or some opportunistic infection are common causes of maternal death in developing countries. Anaemic women are also more likely to suffer complications during anaesthesia and surgery, as well as antepartum and post-partum haemorrhage (Royston and Armstrong, 1989; Lind, 1983; Scrimshaw, 1991).

Iron absorption increases progressively as gestation advances. Approx. 300 mg of the absorbed iron is diverted directly to the fetus, with most of this transfer occurring in the last trimester of pregnancy. Literature suggests that this transfer crosses the placenta against a concentration gradient, bound to transferrin, where there is inadequate intake. Depletion of iron stores occurs as pregnancy advances. Women with repeated, frequent pregnancies are therefore predisposed to low iron stores. The circulating level of Hb does not always reflect the true iron status of the mother, since normal Hb levels are found in mothers with low
iron stores. This is so, because circulating levels are replenished from the stores until these are depleted (Holly, 1965; Lind, 1983; Desai, 1992).

Anaemia does not usually feature in early pregnancy. The Hb concentration remains stable until about the 16th week, following which there is a progressive fall until a low point is reached during the second trimester. A further reduction is uncommon in the third trimester except in those who develop iron deficiency (Lind, 1988; INACG, 1984).

A woman in late pregnancy absorbs 6 mg. iron per day, and the increased demand for iron is most difficult to satisfy however varied and balanced the daily intake is. Negative iron balance is therefore inevitable in later pregnancy unless a woman starts pregnancy with substantial iron reserves of more than 200 mg. It therefore follows that iron depletion would occur if iron supplements are not given (Bentley, 1985).

Haemodilution, a process by which the plasma volume increase is proportionally greater than the cell mass, is the cause of *physiological anaemia* in pregnancy. During pregnancy, there is an increase in red cell size independent of folate status, accompanied by bone marrow erythroid hyperplasia. Any expansion of the circulating red cell mass is likely to drain maternal iron stores, which may lead to iron deficiency (Holly, 1965; Bentley, 1985). In its maintenance of erythropoiesis, the foetus acts as a true parasite. It assures its own production of haemoglobin by drawing iron from the mother, such that iron deficiency does not necessarily
result to an anaemic infant at birth. Many authors claim that no relationship exits between the iron status of mothers and their newborn babies, but there is a general agreement that the most common effect of iron deficiency anaemia in the infant is prematurity (Pritchard et al., 1969; Worthington-Roberts et al., 1985; Morton et al., 1988).

In a study at a high altitude in Quito, Ecuador by Yepez et al. (1987), it was found that 46% of the women studied had iron deficiency anaemia at the time of delivery. The authors found, in contrast to many others including those quoted above, a correlation between maternal iron stores, as assessed by serum ferritin concentration, and cord blood haemoglobin.

Ho et al. (1987) carried out studies in Taiwan, and reported that pregnancy produced a considerable degree of iron depletion in more than half of the previously non-anaemic women (in a sample of 221 normal, pregnant women at term). Subclinical iron deficiency as determined by serum ferritin levels, was found in 15.4% of pregnant, normal, non-supplemented women. The authors also found that multiparity, but not age, increased the occurrence of iron deficiency. Many authors claim that Hb levels decrease during pregnancy and that pregnant women require iron supplements irrespective of socioeconomic status or parity (Isah et al., 1985; Gofin et al., 1989; Guerra et al., 1990).

Dimperio (1990) stressed that generally, preconceptional nutrition assessment and intervention is essential for optimal pregnancy outcome. It is also emphasized
that the attainment of an appropriate pre-pregnancy weight is crucial to the success of a subsequent pregnancy. This, however, seems to be an unrealistic goal in most parts of the world. The importance of nutrition counselling though, is obvious.

All the above mentioned authors seem to agree that pregnancy produces a considerable degree of iron depletion, and that negative iron balance and anaemia can cause severe complications, particularly during the last part of pregnancy.

2.5 Diagnosis of iron deficiency in pregnancy

Various authors have recommended different methods of determining anaemia during pregnancy. Serum ferritin concentrations is currently regarded as being one of the most reliable ways of estimating iron stores (Lind, 1983; Assami, 1988; Horn, 1988). Liljestrand (1986), claimed that the Mean Corpuscular Haemoglobin Concentration (MCHC) was a better method to detect iron deficiency anaemia, while Hercberg et al. (1985) proposed that it appears that no single iron parameter monitors the entire spectrum of iron deficiency, and that only a combination of indicators, depending on the objectives of the study, the characteristics of the study population and the working conditions, is required to measure the body iron content of individuals and to evaluate the iron status of the population.

The International Nutritional Anemia Consultative Group, however, stated that
the most important single measurement is the haemoglobin concentration (INACG, 1984). Royston and Armstrong (1989) pointed out, that although the WHO Expert Committee recommend an arbitrary cutoff point for diagnosing anaemia at Hb <11 g/dl, some obstetricians in developing countries feel that 10 g/dl would be more appropriate and practical. The reasons were not given, but presumably, the lower cutoff point seems justified since more notable physiological changes occur below this lower level.

Ring et al. (1988) did a study in a German location and found that following iron prophylaxis, haemoglobin concentration, haematocrit and erythrocyte count were significantly higher at the time of birth. However, obstetrically significant parameters (eg. prematurity and haemorrhages) were unaffected. The authors do not consider routine iron prophylaxis to be necessary in the German community studied.

Bentley (1985) stated that Hb values falling below 11 g/dl should be regarded as abnormal, even if microcytosis is undetected. Serum ferritin of less than 12 micrograms/l is an indication for iron prophylaxis. Bentley further said, that serum iron concentration is not the best indicator of iron status, but that the most reliable indicator is maternal haemoglobin concentration. The iron status of the newborn infant is determined by the degree of maturity, the birth weight and cord haemoglobin concentration. Infants born to mothers with evidence of exhausted iron stores have a significantly lower cord serum ferritin concentration than those born to mothers with detectable iron stores. The author supported iron
supplementation with some restriction. Iron overload is hardly a hazard for women in reproductive years, but large doses of parenteral iron should rarely be required and blood transfusions should nowadays be necessary only in exceptional cases.

Kaufer et al. (1990) considered a claim that serum ferritin concentration below 20 micrograms/l is the cutoff point for considering oral iron administration. They also describe that during early pregnancy, women with high serum ferritin usually had decreased Hb. In women with a low serum ferritin however, the Hb value increased during the first few weeks of pregnancy. These authors stipulated that this finding confirms the haemodilution process of early pregnancy and that iron deficiency tends to develop during this period. It also suggests that there is an increased utilization of iron stores, but if serum ferritin is low, iron stores are not available for this physiological response. They recommend pre-conceptional iron supplementation for women with low ferritin levels.

It appears that most authors agree, that there is no single criterion for the best measurement of iron status, but many studies have used haemoglobin effectively. Since Hb is the last of the haematological variables to suffer, it seemed a reliable tool for this study, particularly considering the lack of other sophisticated, analytical equipment.

2.6 Treatment of iron deficiency anaemia during pregnancy

During pregnancy, growth of the foetus and the uterus, leads to an increase in the
demand for many nutrients, including iron and folate. Many studies have demonstrated the beneficial effects of iron supplementation on haemoglobin levels in pregnant women.

Seyal and Rehana (1982) undertook a study in India using exactly the same commercial iron/folate slow-release preparation as in the current study. However, they did not use a control group. They sampled patients on enrolment and found that the Hb was an average of 12.6 g/dl. Treatment was initiated at 18 weeks into gestation, and patients were sampled again after 10 and 20 weeks. They found that the Hb in patients taking the capsules rose by 0.7 g/dl after 10 weeks and 1.4 g/dl after 20 weeks. Compliance was reported to be good, and none of the participating patients complained of any side-effects. These researchers found that the type of supplement given in this study was superior to other conventional iron pills or simple time-release preparations.

Fisher et al. (1989) in a study in Leipzig, Germany, looked at older women and multiparous women as well as women with infections of urinary tract and found that they had an elevated risk of anaemia. In their study, weight increase was lower in anaemic pregnant women than in those who were not. The authors recommended early treatment of anaemia in pregnancy using a combination of iron and folic acid.

In a study by Reddiah (1989), the optimum dose of supplemental iron for prophylaxis against anaemia in pregnancy was found to be 120 mg. elemental iron,
given as ferrous sulphate daily. The National Nutritional Anaemia Prophylaxis Programme in India had recommended a lower dose of 60 mg. Reddiah also reported that many other authors found that side effects, eg. headaches, nausea and gastro-intestinal problems, increased with increasing doses of iron.

A dramatic response to oral iron therapy can not always be anticipated, but it is known that the initial haemoglobin level is positively related to the response. Even if the woman has a normal haemoglobin level early in pregnancy, however, it does not reflect the status of iron reserves and the subsequent course of the haemoglobin (Holly, 1965).

Hallberg et al. (1979), looked at treatment over the years for iron deficiency in Sweden and said that four factors were identified as responsible for the improved iron status of Swedish women that has occurred since the mid-60s. These were a) the increased use of oral contraceptives, i.e. leading to decreased menstrual iron loss b) the impact of general iron fortification of flour c) the widespread use of ascorbic acid supplements and d) the greater prescribing of iron tablets.

A study by Thane et al. (1982), however, considered the state of body iron stores as a result of supplementation. They found that women given 120 mg or 240 mg of oral ferrous sulphate with or without folate, for 12 weeks during the last trimester, responded with a significant increase in serum ferritin. Patients with folate did not respond better than those without. They did not find any differences in final Hb levels among the supplemented groups, and authors
concluded that serum ferritin may be a better indicator of iron.

Dawson et al. (1987) administered vitamin/mineral/iron supplements during pregnancy and for 12 weeks postpartum, and they found that patients maintained maternal iron stores, the need for additional iron medication was eliminated and that the development of iron deficiency anaemia was prevented. The authors stressed that iron supplementation during late pregnancy is essential.

Fochi et al. (1985) tested the blood of women after 50 days of treatment with four different iron preparations. While three products (including a combination of iron and folate) all produced similar efficacy and were well tolerated, the fourth which was pure ferrous sulphate induced gastro-intestinal side-effects.

Gofin et al. (1989) looked at the comparison of iron treatment versus prophylaxis, and found that in the supplementation group, no significance was found between compliance and age, education, social class or parity. The mean decrease of Hb and haematocrit (Hct) between 2nd and 3rd trimester was smaller in the supplementation group than in the treatment group. The mean Hb and Hct levels during the third trimester were higher for good compliers than for poor compliers.

Most authors favour a combination of iron and folate during supplementation, but the recommended dosages and types of administration vary.
Worthington-Roberts et al. (1985) and Bailey (1990), described folic acid (folate or folacin) as a B-vitamin, which has a vital function in all aspects of DNA and RNA synthesis. If it is lacking, cell division cannot proceed normally. Consequently, this leads to abnormal erythrocyte morphology and a reduction in haemoglobin concentration. The production of red blood cells (erythropoiesis) is impaired, resulting in megaloblasts being released from the bone marrow causing macrocytosis. Many factors compromise folate status, among which pregnancy is most important, but also biological maturity and socioeconomic status, chronic use of alcohol, drugs and smoking. Megaloblastic anaemia is often an obstetrical complication. Worthington-Roberts et al. (1985), also claimed that solid proof relating folate deficiency to adverse pregnancy outcome may never evolve from human studies, so they stress that judgements related to clinical practice must be made on the basis of limited data coupled with common sense.

However, even as early as 1968 many authors reported severe consequences of folate deficiencies during pregnancy. Evidence suggested that a lack of folate may be associated with abruptio placentae, spontaneous abortion, preeclampsia, prematurity, fetal malformations and subnormal infant development (Iyengar, 1970; Anon., 1968; Kreutler, 1980; Pippard, et al. 1988). Adequate folate nutrition is essential for optimal fetal growth and erythropoiesis, and even a marginal deficiency of this nutrient may produce adverse effects in the rapidly developing fetus (Blocker et al. 1989; Shojania, 1984).
In recent years much research has gone into folate supplementation during pregnancy. It has been recommended by WHO (1972) and INACG (1989) and many other authors. The British Medical Research Council’s Vitamin Study Research Group (MRC, 1991) carried out a recent, multi-centre prevention trial in 7 countries. Supplementation was initiated with folate or 7 other vitamins around the time of conception to determine whether neural tube defects (anencephaly, spina bifida, encephalocele) could be prevented. The research group firmly recommended folic acid supplementation preconceptionally for all high risk women, and that public health measures should be taken to ensure that the diet of all women in reproductive years contains an adequate amount of folic acid (Vergel et al., 1990; MRC, Morbidity and Mortality, Weekly Reports, 1991).

In spite of the several reports about the damaging effects of folate deficiency on foetal growth from around the world, there is at present no routine administration of supplemental folate in Kenya.

2.8 Diagnosis of folate deficiency

Folate deficiency causes megaloblastic anaemia, which complicates pregnancy and is common wherever nutrition is inadequate. Symptoms, if present at all, are non-specific, for example tiredness. Rarely, sore mouth, and/or sore tongue and even purpura may be present. An accompanying urinary tract infection is common. One of the hallmarks of megaloblastic anaemia is macrocytosis manifest in a raised MCV. More useful information may sometimes be obtained from the blood film. However, the only way to establish a firm diagnosis of megaloblastic
anaemia is by bone marrow biopsy (Bentley, 1985).

Bailey (1990) described how the first stage of folate depletion can be assessed by measuring serum folate levels, which drop prior to tissue depletion, and which are paralleled by a reduction in red blood cell folate.

Ball et al., (1964) carried out a study in Stoke-on-Trent, England, using an assay with *Lactobacillus casei*, and found that there is a significant fall in the serum folic acid level during pregnancy, reaching its lowest level at term. They found that cases with florid megaloblastosis showed the most marked depression of B12 and folic acid activity.

Iron and folate deficiencies frequently coexist. Iron deficiency is even believed to be a precursor for folate deficiency. However, Chanarin and Rothman (1988), claim that iron does NOT have a direct effect on folate status in pregnancy, and that the association of iron deficiency anaemia and megaloblastic anaemia in pregnancy is the result of poor nutrition, and that there is no cause-effect relationship.

Young (1974) stated, that the differentiation between megaloblastic anaemia during pregnancy due to folic acid or vitamin B12 deficiency may be very difficult (depending on available resources) and therefore, oral folic acid should be administered.
Folate deficiency diagnosis is often complicated and many studies report of different diagnostic methods. In view of the relative lack of resources for a proper diagnosis in Kenya, it was decided that macrocytosis as detected by microscopy, would suffice as a diagnostic tool for this study.

2.9 Treatment of folate deficiency during pregnancy

Bentley (1985), recommended treatment of folate deficiency by the administration of pteroylglutamic acid 0.5 mg. daily. It has been estimated that pregnant women require 3.3 - 3.6 μg. folate per kg of body weight daily with an additional 300 μg. per day. Cobalamin (B12) deficiency could possibly be masked by folate treatment. Bentley (1985) also confirmed that folate can indeed aggravate neuropathy due to cobalamin (B12) deficiency, but he also stated, as the WHO Technical Report (1972), that patients with that degree of cobalamin deficiency are infertile, and that pernicious anaemia is generally a disease of old age. It was pointed out, that the hypothetical danger with giving routine folate supplements during pregnancy is vastly outweighed by the benefits of such prophylaxis. In developing countries, there is little doubt that supplementation with folate and iron is beneficial (Bentley, 1985), (see also p. 45).

High prevalence of both iron and folate deficiency anaemia during pregnancy also exist in Latin America, due to the insufficient diet and the extra burden of pregnancy (Layrisse et al. 1988).

Patients with severe iron deficiency anaemia were treated with intravenous iron-
dextran or intravenous iron and folic acid in a study by Dommisse et al. (1982). There was no difference in the rate of response or the eventual total response in the two groups, suggesting that iron therapy does not unmask or produce a relative folic acid deficiency.

Pritchard et al. wrote in 1969, that the role of maternal folate and iron deficiencies in pregnancy was underestimated, and looked into their role in pregnancy wastage, abruptio placentae, fetal malformation and abortion. They concluded, however, that the fetus and the placenta quite effectively parasitize folate and iron from the mother even when she is grossly deficient in these nutrients.

2.10 Dietary sources of folate

Most foodstuffs contain some folate and most green vegetables, lentils, eggs and nuts are good sources though liver and yeast are particularly rich in folate (see Appendix 1). It is not unusual that up to 80% of folate activity is lost during cooking and storage (NAS, 1980).

2.11 Controversies

There have been many controversies regarding iron and folate supplementation, particularly in developed countries. Many obstetricians believe that it is not necessary to supplement in areas where pregnant women consume a nutritionally adequate diet. However, as has been reported above, most authors are in strong support of iron and folate supplementation during pregnancy in developing
countries, where diets are often inadequate.

Two contradictory opinions were expressed by Hibbard (1988) and Horn (1988) in the same issue of the British Medical Journal. The editorial comment supported the argument of Hibbard, that only those in whom there is a clear indication for iron or folate supplementation should receive treatment. Horn, on the other hand, stresses that the cost entailed in identifying those women most at risk is making routine supplementation for everyone cost-effective. Horn also mentions problems with laboratory examinations. The single most reliable predictor of iron deficiency anaemia is serum ferritin concentration. No similar simple predictor exists for megaloblastic anaemia due to folate deficiency. Although Horn supports routine prophylactic treatment, the author rightly claims that this does not replace the need for dietary advise and haematological monitoring, wherever possible.

Another controversy is that of iron and folate supplementation interfering with the body's ability to absorb zinc. Zinc is another vital nutrient needed in pregnancy. Meadows et al. (1983) found a decrease in oral bioavailability of zinc after a two-week supplementation period, which suggests that zinc absorption in the intestine is decreased because of competition with the iron. They therefore claim, that zinc depletion may be induced by oral iron supplementation. The participants in this study were not pregnant.

Simmer et al. (1987), also argued, that iron-folate supplements in pregnancy may
be harmful, since they inhibit the absorption of zinc, and maternal depletion of zinc is strongly associated with intrauterine growth retardation. Swanson and King (1987), pointed out that pregnancy adapts the body to many changes. These include increased zinc absorption, reduced endogenous zinc loss, redistribution of tissue zinc and an efficient maternal-fetal zinc transfer. These authors felt that the relationship between zinc status and pregnancy outcome remains an open question, and many studies contradict each other (Mukherjee et al. 1984, Simmer et al. 1987, Ronbidge et al. 1983, Swanson and King, 1987).

2.12 Studies in the African Region.

Fleming et al. (1986), examined 200 Hausa primigravidae in Nigeria after giving haematinic supplementation. Malaria prophylaxis was also administered, and that alone reduced the frequency of megaloblastic erythropoiesis from 56% to 25%. However, folic acid supplements totally abolished megaloblastosis, but without significantly effecting the mean Hb.

Assami et al. (1988), in a study in Algeria found the prevalence of iron deficiency anaemia to be 45%. These authors also report that the mean prevalence of anaemia for pregnant women in North Africa is estimated at 45%, and that iron deficiency is the single most important cause of anaemia. The Algerian diet is poor in haem iron and non-haem absorption promoters, e.g. vitamin C. Folate status was assessed using red cell folate concentrations, and the frequency of folate deficiency was 51.9%. It must be noted, however, that plasma folate can be low during pregnancy, even if tissue levels are normal. The Algerian study
found most women to have low iron and folate stores at the beginning of their pregnancies. In a study in South Africa on pregnant coloured women, the authors found a high frequency of iron deficiency anaemia during the first trimester, but folate deficiency and B12 deficiencies did not appear to be significant problems (Lamparelli et al., 1988).

Jackson and Latham (1982) carried out a study in Liberia. They divided 621 patients in their last trimester into 4 treatment groups and a control group. The group given iron and folate did not respond any better than the iron group alone. Parasitic infestation and haemoglobinopathies did not appear to be significant contributors to the iron deficiency or anaemia in this population. There was a low prevalence of malaria due to effective control measures. The authors reported, that a household food consumption study in Liberia found that diets were below those recommended by WHO/FAO for both iron and folate and that non-haem iron was predominant.

Isah et al. (1985), carried out a study in Nigeria, and they concluded that anaemia and iron deficiency were seen most frequently in the third trimester. Baumschlag et al. (1970), were among the first to propagate folate supplementation, as a result of research done among the Bantu tribe in South Africa. They found that folate supplementation during pregnancy was associated with a significant reduction in the incidence of prematurity. The authors strongly recommended routine supplementation with folic acid to prevent megaloblastic anaemia, particularly in communities which subsist on a suboptimal diet.
Hercberg et al. (1987), looked into the haematological consequences in infants of anaemic mothers in Benin. In this study, 55% of pregnant women were found anaemic according to WHO standards. Iron deficiency anaemia was found in 83% and folate deficiency in 48% among the anaemic. Iron was screened for using serum ferritin, transferrin saturation or erythrocyte protoporphyrin levels, while folate status was determined by red cell folate estimation. Hb concentration and mean corpuscular volume were significantly lower in babies born of iron deficient mothers. In Cameroon, Coulibaly et al. (1987) studied effects of migration on nutritional status of pregnant women, and found that the women from the north of the country did not have much anaemia, nor iron and folate deficiencies, which was believed to depend on the favourable nutritional conditions in that area.

Liljestrand et al. (1986), reported from a multi centre study in Mozambique, where they found, contrary to many other authors, that nulliparous women were more prone to anaemia. The cause for this, was believed to be the lower socioeconomic status for this group of women, i.e. they did not eat as well as others, due to traditional beliefs and customs, where age and parity are hierarchical.

Also in Kenya, some research into pregnancy anaemia has taken place over the years. Gebie et al. (1970) presented results from a study carried out at the Kenyatta National Hospital, where they found that anaemia in pregnancy is associated with high maternal mortality and morbidity and leads to premature
labour with consequent high foetal mortality. The authors also reported, that in general, the pathogenesis of pregnancy anaemia differs from place to place and is environmentally determined. The same authors conducted a study on 240 pregnant outpatients. Moderate anaemia in Nairobi was noted in 25% and severe anaemia in 10%. They found that 11% had serum folate levels below 3.0 ng/ml, which according to WHO (1968) is the absolute lower level of normal. Twenty percent of the women showed either a folate or a B12 deficiency, or a combination of the two at first attendance. Authors pointed out that more research is necessary on pregnancy anaemia in East Africa, where iron deficiencies play a major role, but B12 deficiency is rare.

Mati et al. (1971), studied pregnancy anaemia in Nairobi, Kenya, and they concluded that it is indeed an important obstetric problem in this area. The most common type found was megaloblastic anaemia, followed by iron deficiency anaemia. They also found that nearly half the cases of megaloblastic anaemia were associated with malaria. These cases were primarily haemolytic anaemia with a secondary megaloblastic change due to depletion of folic acid. They have also found that studies among hospital patients in the Kenyan highlands report that parasites and haemolytic anaemia due to malaria are not common. Brabin et al. (1986), studied folacin concentrations in malaria during pregnancy in a rural, antenatal clinic in Kenya. They found low serum folacin having poor specificity for low RBC folacin concentrations. They also found that multigravidae had lower values than primigravidae.
Jansen et al. (1987), reviewed most work done in the field of nutritional anaemia in Kenya. They stated that high altitude affects haemoglobin values by activating haemopoiesis in the individual. The resulting increase in haemoglobin values is proportionally greater than the corresponding rise in the number of red blood cells. Few guidelines are given as to adjustment for altitude regarding Hb levels. Young et al. (1974) suggested adding 0.4 g/dl for every 300 meters above sea level. Other authors present similar ideas. Jansen et al. also reported of a study in a Nairobi slum, where haemoglobin values proved to be rather satisfactory, (11-17.3 g/dl, with a mean of 12.5 g/dl). Iron intakes were 72% of the RDA for pregnant women, being 14 mg of iron per day, but mainly from vegetables.

It was further reported that in a study from Machakos District, mild to moderate anaemia occurred in about 1/3 of pregnancies. In rural areas of Kenya, the mean pregnancy Hb was only 9.2 g/dl, and the large majority of pregnant women were below the age of 20. Teenage pregnancies are a heavier strain on female iron resources than pregnancies later in life, when growth and expansion of blood volume have ceased. Jansen and colleagues believed that macrocytosis is seasonal in character (increased during November - April both in Nairobi and at the coast). Since this kind of anaemia also is secondary to malaria, seasonal variations may be connected with the occurrence of this disease Mati et al., (1971).

Research on anaemia during pregnancy has been reported above from Nigeria, Liberia, Algeria, Benin, South Africa and Mozambique. Also from Kenya, studies
have been quoted above, concluding that pregnancy anaemia is highly prevalent and should justify further research into the problem. Both Young et al. (1974) and Gebie et al. (1970), recommended further studies of prevalence, causes and consequences of anaemia during pregnancy in Kenya. Although many studies have been carried out, and reviewed by Jansen et al. (1987), they are not yet conclusive, and further research is well justified.
CHAPTER 3
MATERIALS AND METHODS

3.1 Study Site

The study was carried out at the Kikuyu Hospital, situated about 25 kms northwest of Nairobi city centre, near the village of Thogoto, at an altitude of approximately 2,100 meters above sea level. The hospital has twenty maternity beds, and delivers an average of 1700 babies per year. According to hospital records, the months of August and September constitute the peak season for nativity in this hospital. The catchment area has a radius of about 25 kms, which includes the villages of Kikuyu, Dagoretti Market, Muguga, Kabete, Gikambura and Thogoto. Some patients use public transport to reach the hospital, but many walk from their homes to the health facility. The Antenatal Clinic (ANC), which runs daily, Monday through Friday 8.30 am. - 4 pm., is staffed by midwives. A resident obstetrician handles special cases, referred as needed by the clinicians on duty. The largest attendance for the ANC is between 8 am and 12 noon, Monday to Friday.

The hospital is a private institution operated by the Presbyterian Church of East Africa (PCEA) and a minimal charge for hospital services is levied. For registration at the Antenatal Clinic, the charge is 35 Kenya Shillings at the time of the study. On admission, a deposit of 300 Kenya Shillings was required, and a daily charge of 100 Kenya Shillings was the usual fee (1 $ US = 32 Kenya...
Shillings, June 1992). Special treatment, surgery and drugs attracted additional charges. After a normal delivery, the mother spent about 24 hours in the maternity ward before discharge.

3.2 Study Design

A prospective study was carried out, in which a cohort of pregnant women attending an outpatients clinic was followed up. This study was a clinical trial assessing the effects of iron, folate and placebo supplementation on certain maternal and foetal outcomes (see Fig. 1 for Study Flow Chart). The study was double-blind, such that the patients and those administering the drugs, as well as the laboratory technicians were unaware of the type of drug given. Repeated blood samples were obtained during the course of the project, and certain parameters to measure haematological values were examined; white blood cells (WBC), red blood cells (RBC), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and haematocrit (Hct). Microscopy for erythrocyte morphology and malaria parasites was also carried out.

Permission had been sought and received from the Ethical and Research Committee of Kenyatta National Hospital/College of Health Sciences. This permission, however, stated that only patients with Hb levels equal to or above 10 g/dl could be included in the study, for ethical reasons. The patients were enrolled on the basis of a simple haemoglobin test performed at the small, simple laboratory of the hospital. Because of poor sensitivity in that test, a small number
of patients with haemoglobin below 10 g/dl were unintentionally included in the study.

It was decided to use haemoglobin as the main diagnostic tool in this study, because it is an indication of the iron status, and because the test could be carried out within the realm of this study's resources. Other authors have also recommended it as an appropriate diagnostic tool. Ronbidge et al., (1983).
Fig. 1 FLOW CHART FOR STUDY DESIGN

Study population: Pregnant out-patients of Kikuyu Hospital, Antenatal Dept.

Patients participating: all pregnant women reporting to clinic with gestation 25-30 weeks and Hb > 10g/dl.

Informed consent sought

Assignment to drug treatment by randomization.

Antenatal check-up, morbidity notes taken, blood collection and analyses.

Participants return to clinic 4 weeks later. New supplies of drugs given. Antenatal check-up, morbidity notes taken, blood collection and analyses.

Data analysis.

Participants return to give birth. Morbidity notes taken. Blood collected and analysed from mother and baby.

Data analysis.

Participants return for post-natal check-up, immunization and weighing of baby.

Data analysis.
3.2.1 Study Population

The study population consisted of clients attending the ANC at the Kikuyu Hospital. Most pregnant women appear at the ANC for their first check-up at the end of the second or beginning of the third trimester of pregnancy. The hospital serves a certain spectrum of social classes and ethnic groups. Social class was classified into three groups depending on occupational income, i.e. high, middle and low income earners (Civil Service Review Committee, 1992; Kenya Subsidiary Legislation, 1992). Due to its location, the major ethnic group attended here is Kikuyu. A few Luo, Luhya, Kamba and Masai patients are also seen.

3.2.2 Enrolment of subjects

During the patient’s first visit to the antenatal clinic, the criteria used for inclusion into the study were:

1. that she had a haemoglobin level equal to or above 10 g/dl
2. that her gestation stage was 25 - 30 weeks
3. that she had no diagnosed complication associated with the pregnancy
4. that informed consent was given (see Appendix 1).

The laboratory facility at the hospital was used to estimate the haemoglobin level of the participants, so as to make certain that no severely anaemic patient was included. Because of low sensitivity of the test at the hospital laboratory, a
small number of patients were erroneously included in the study with haemoglobin levels below 10 g/dl.

It was decided that the consent form would be in English, since the majority of patients were able to speak English (as determined during the pilot study).

### 3.2.3 Sampling method

Patients were randomized into treatment groups using the Randomly Permuted Blocks strategy (Fleiss, 1986). Treatments were of four kinds: 1) placebo, 2) iron and folate combination, 3) folate only and 4) iron only. These were randomly assigned to patients independently within consecutive series of enrollees. A random permutation table was used for this purpose (see Appendix 2). This method allows for the control of the effects of prognostic variables in response to treatments.

### 3.2.4 Determination of sample size

The estimation of the sample size was done taking into account the difference in haemoglobin between the means of the treatment groups and the placebo that needed to be known to be statistically significant. An equal importance was also given to the difference in birthweights between babies born to mothers taking placebo and those taking iron and/or folate treatment. A minimum sample size for each group was estimated using a formula described below, as provided by INACG, (1984):
\[ n = \frac{2\sigma^2 (Z_\beta + Z_\alpha)^2}{\Delta^2} \]

where \( \Delta \) = the difference in haemoglobin (Hb) and the difference in birthweights between the means of the treatment group and the placebo that is considered to be of biological importance, \( \sigma \) = standard deviation. The standard deviation for both haemoglobin and birthweights were calculated using data collected during the preliminary study. The required confidence coefficient was determined to be 95%. \( Z_\alpha \) is the normal deviate corresponding to the confidence coefficient of 95%, 1.96. \( Z_\beta \) is the normal deviate corresponding to a power of 90%.

It was decided that a difference of 1.5 g/dl in the mean haemoglobin values between recipients of the placebo and the treatment groups and 300 g. for birthweights needed to be shown to be statistically significant (Neumann & Bwibo, 1987; P. Stanfield and A. Wilson, personal communication, 1991).

Consequently, the applied formulas are:

- for Hb: \[ 2(1.63)^2 (1.96 + 1.28) = 25 \quad \frac{1.5^2}{1.5^2} \]
- for Birthweight: \[ 2(304)^2 (1.96 + 1.28) = 22 \quad \frac{300^2}{300^2} \]

On the basis of these calculations, it was decided that a sample size of 25 in each treatment group would be sufficient. The attrition rate was difficult to predetermine, but it was estimated, on the basis of the pilot study, that a dropout
rate of no more than 10% would occur. Possible reasons for dropping out of the study were predicted to be cost of maternity care at the hospital, choosing to deliver elsewhere, illness, migration or lack of cooperation.

3.2.5 Treatment groups

Treatment groups were identified as follows:

A. PLACEBO

B. COMBINATION

(150 mg. ferrous sulphate +

0.5 mg. folate)

C. FOLATE ONLY (0.5 mg)

D. IRON ONLY

(150 mg. ferrous sulphate)

ONE CAPSULE TO BE TAKEN DAILY STARTING AT THE TIME OF ENROLMENT, UP TO THE TIME OF GIVING BIRTH, AND FOR SIX WEEKS AFTER DELIVERY.
Treatment groups (see above) were divided as follows: A for placebo, B for a combination of folate (500 micrograms) and iron (150 mg. ferrous sulphate, all slow release), C for folate only (500 micrograms) and D for iron only (150 mg. ferrous sulphate). Tiny pellets of each preparation were packed into dark and light green spansules of identical appearance. Treatments were dispensed to patients in a plastic container with one hundred spansules. The containers were also marked with A, B, C or D, with a space for name and project number. The patients and the hospital staff were not told of the treatment type before, during or after the study. The time of treatment was initially 4 weeks, started on the day of enrolment, and continued until the time of birth. All patients were encouraged to continue supplementation for 6 weeks after delivery, and supplies were given to cover that period. The capsules supplied by SmithKline Beecham were of a slow-release kind, and have proven to give minimal side effects and therefore improve compliance in comparison with traditional iron/and or folate supplements. Compliance was assessed by the investigator by checking the capsule containers on the second visit, and again at birth. Compliance was also confirmed with the patients verbally, and it is believed that with a background of a friendly and honest rapport, the investigator could assess the compliance for most patients in the study, by talking to the subjects on the first, second and consecutive visits. The participants were generally complying very well to the treatment regimen allocated, and their attitude can be described as keen and rigorous, and the taking the capsules did not present a problem. Similar findings were presented in another study using the same preparation (Seyal and Rehana, 1982). The "spansules" pass through the gastric cavity in their entirety, and they
3.3 Preliminary Study and Pilot Phase

For the purpose of establishing the characteristics of the population for this study, a preliminary study was carried out using hospital records during the month of October, 1990. The following information was obtained, which was then used to calculate the sample size: 1) age 2) parity of mothers, 3) birthweights, 4) gestation time and haemoglobin levels at first visit and 5) types of delivery. Forty-eight recent medical records from the maternity ward were randomly selected and checked for these parameters. An attempt was also made to establish drop-out rates between the first antenatal visit and birth, according to available medical records.

A pilot study was then undertaken in January 1991, to complement the preliminary study. The main purpose was to prepare the investigator and the staff of the Kikuyu Hospital for the tasks involved in the research project. Other purposes were to test the format of the dietary interviews, the appropriateness of the predesigned questionnaire and the logistics and methodology of the study design. The selection of participants in the pilot study followed the same pattern as that for the main study (see above). The selected patients were interviewed using a previously designed questionnaire that included questions on dietary frequency. On the basis of the pilot study interviews, certain alterations were made to the questionnaire. Venous blood was collected by the midwives and this
procedure was slightly altered and subsequently improved for the main study. Cord blood was not collected in the pilot study, but the midwives were instructed on the correct procedure.

3.3.1 Training of midwives

The chief hospital administrator, the resident obstetrician and the matrons in charge of midwifery staff had previously been approached for and given their approval for the study to take place. During the pilot phase, four midwives were briefed on the interview technique, which was also discussed and practised. It was emphasized that the pregnant women should be encouraged to be honest about their dietary intake, their compliance or otherwise with the treatment programme, and any other information given to the investigator or the medical staff. The establishment of a friendly rapport was also stressed. The investigator performed all interviews with English speaking patients, while the midwife on duty was present to assist with translation into Kikuyu or Kiswahili if needed.

The midwives were also instructed about correct blood sampling technique, sterile precautions and the handling of the collection bottles once filled.

3.4 Data Collection

3.4.1 Questionnaire

Using a structured questionnaire, information was obtained from the patients (see Appendix 3). It was decided, on the basis of observations during the pilot
phase, that the questionnaire and consent form would be in English, since the large majority of patients attending the hospital could speak English. The following information was sought: name, hospital information number, area of residence, marital status, source of income and occupation, parity, age of the last born child, date of last menstrual period, morbidity, height and weight. On each patient's information sheet was also recorded the coded type of treatment given (A, B, C or D), her project number and the date of her next appointment. Her height was recorded at the first visit to the clinic, and her weight was recorded at both the first and second visit. After registration, pregnant outpatients were asked to provide a urine specimen and a peripheral blood sample, both of which were examined immediately at the hospital laboratory. The results were recorded and used for enrolment of study cases. Patients were enrolled in the study on the basis of a Hb value of 10g/dl or above. If the urine contained bacteria, protein or glucose, this was noted by the investigator in the Patient Information Sheet under morbidity and the patient also subsequently received attention from the clinician on duty.

### 3.4.1.1 Socioeconomic Status

Patients were not asked directly to state their income, but rather, who supplied the family income. If she was single, she was to state if she herself or anybody else supplied financial resources. She was asked to describe the occupation of the income earner. On the basis of this information, the occupations were grouped according to income levels based on the classifications: unskilled, skilled or professional. Incomes were hence estimated using guidelines from the Civil
3.4.1.2 Dietary Frequency Interview

The Patient Information Sheet also contained a brief dietary frequency interview. Commonly eaten food items were grouped into dairy products, starch and cereals, vegetables, fruits, nuts and legumes, fats and meats. This classification was pre-tested and found to be useful and appropriate relating to the women's nutritional status with respect to weight and haematological parameters.

The investigator performed the dietary frequency interview, during which the patient was asked about the frequency of consuming different food stuffs. The structure of the interview was adjusted after the pilot study. Patients were asked questions about the frequency of consuming certain foods, and the answers were categorized as 1) every day; 2) more than once a week; 3) seldom; or 4) never.

The results from the dietary interviews were analyzed using a statistical package and interpretations were done. An evaluation of the patients' diet was made to quantify iron, folate and vitamin C content, on the basis of the type of foodstuff consumed. Quantities of foodstuffs were estimated by the patient.

The patients' diets were classified into three groups for the purpose of data analysis for this study, with particular regard to the iron, folate and vitamin C.
contents of the food: 1) poor; 2) fair; 3) good (see Appendix 4). This classification was made using the WHO recommendations and the US NRC's Recommended Dietary Allowances for iron, folate and ascorbic acid during pregnancy (NAS, 1980; WHO, 1972).

Dietary intakes that comprised at least one normal portion size of dairy products, starch, green vegetables, citrus fruits, nuts or legumes and meat, fish or eggs per day were classified as "good". This diet was estimated to contain a minimum of 0.5 mg. folate/day, which is the recommended dietary allowance for adults. During pregnancy, however, the RDA is 0.8 mg. folate/day. The RDA for iron for women of childbearing age is 18 mg./day. There is no RDA for iron during pregnancy, except the recommendation that supplementation ranging from 30 - 60 mg./day should be given to any habitual American diet. With regard to vitamin C (ascorbic acid), the recommended daily allowance is 80 mg/day. Consequently, in this study, a "fair" diet contained an estimated 0.25 mg. of folate, 10 mg. iron and around 50 mg. ascorbic acid. A "poor" diet contained anything between 0 and 0.25 mg. folate daily, iron between zero and 10 mg/day and vitamin C zero to 30 mg/day.

3.4.2 Clinical examination

3.4.2.1 Anthropometry

Patients' heights were measured and recorded in centimetres (cms) using a fixed tape measure and a headboard, providing an accuracy of 1 centimetre (Lohman
Weight was measured and recorded in kilograms (kgs) with an accuracy of 0.5 kg using a bathroom scale, which was calibrated every morning before the clinic opened. Weighing was done on the first antenatal visit and again four weeks later. The weights of the new born infants were measured and recorded, using a Salter Baby Weighing Scale with an accuracy of 0.1 kg.

3.4.2.2 Blood pressure measurement and medical history interview

The patient’s blood pressure was measured and a midwife conducted a medical history interview which included questions about the date of the first day of her last menstrual period (if known) and brief obstetrical history. All the data were recorded on individual medical records. Relevant data were transferred to the individual Patient Information Sheet by the investigator.

3.4.2.3 Gestation assessment

The midwife checked the record for the date of the last menstrual period. She proceeded to palpate and measure the patient’s abdomen using a tape measure between the symphysis pubis and the fundus to determine the fundal height and expected date of delivery. A foetoscope was used to examine the status and foetal heart beat. From this examination, the midwife was able to estimate the date of delivery with an accuracy of plus or minus two weeks (Pritchard, 1980).

Gestation at the time of first attendance at the MCH clinic and at the time of
enrolment was generally high, and commonly women did not turn up until even into the third trimester. However, during the last trimester of pregnancy, the most stressful time occurs for foetal growth and demands on the mother's nutritional status to supply this rapid development. Since anaemia due to iron and folate deficiency is commonly instigated at this time, it was decided that the study period would cover the participants' gestation from approximately the 26th week onwards.

3.4.2.4 Blood sampling

The methodology for haemoglobin estimation used at the Kikuyu hospital's laboratory was the "Sahle's" method, which applied the technique of photometry. This method depends on the conversion of haemoglobin to cyanmethaemoglobin (HiCN). A volume of 0.20 ml blood was added to a 4 ml solution of 200 mg potassium ferricyanide and 50 mg potassium cyanide in 1 litre of water. The mixture was then allowed to react for ten minutes. The HiCN has a maximum absorbency at a wavelength of 540 nm. The absorbency of the sample was then measured against the diluent as a blank in a matching cuvette. The haemoglobin concentration of the sample was then determined by reference to a standard calibration curve or chart, observing the point at which the photometer reading (R) intercepts the calibration line and extrapolating this to the horizontal axis C (INACG, 1984).

For the collection of blood for the haematological examination, a medical tourniquet, cotton swabs dipped in methanol, disposable 2 ml syringes (Medical
Manufacturers Ltd., Kenya) and disposable 21G-1 1/2" needles (Misiwa Medical Industry Co, Ltd., Japan) were used. Collected blood samples were transported and stored in 5 ml glass bottles, sterilized and coated with the anticoagulant ethylenediaminetetraacetic acid (EDTA). Each bottle was labelled with the patient's name and the project number. Rubber gloves were used by the midwife who collected the blood, and all needles were discarded carefully as instructed by the hospital administration. Two ml of blood were collected by venipuncture from the patients' arm by the assisting midwife on three occasions during the course of the study, as follows:

1) during the first antenatal visit; 2) 4 weeks later; and 3) immediately before the time of delivery. A blood sample was also collected from the cord of the newborn baby. The blood samples were directly transferred to pre-prepared collecting tubes. These were then inverted, and the patients' name, number and the date were carefully recorded on the labels. The tubes were kept at room temperature until they were taken for analysis to the Medical Research Centre (MRC) later the same day. Any samples that could not be examined the same day were stored in a refrigerator at 2-8° C.

3.5 Haematological analysis

A complete haematological analysis was undertaken for each blood sample by the Medical Research Centre (MRC), Nairobi. Each blood sample underwent a quantitative, semi-automated haematological analysis using an electronic unit, the Coulter Counter model M530 (Coulter Electronics Ltd., Luton, Beds., LU3 3RH,
The reagents used in operating the M530 included an azide-free, isotonic electrolytic diluent (Isoton II), used for diluting whole blood. Isoton II was also used to rinse the instrument. The lysing agent used was an azide-free product, Zap-Oglobin, that rapidly lysed erythrocytes, freeing native haemoglobin and reducing cellular debris to particle size level that did not interfere with leucocyte counts. Isoterge, a cleaning agent, was used to clean all glassware and tubing of the M530 model. As calibrator and controls, the Coulter Electronics Co. Ltd., also supplied M-CAL™ Calibrator, 4C Abnormal Low Cell Control and 4C Abnormal High Cell Control. During the operating mode WBC/HGB, the sample dilution ratio was 1:500 and included the lysing agent. The sample was placed on the platform beneath the WBC aspirator tip. When the WBC bath filled with diluent for the rinse, the HGB blank was read. When the sample was delivered to the bath, the HGB concentration was measured. For the RBC/HCT and RBC/MCV mode of operation, the sample dilution was 1:50,000. A RBC aspirator tip was used, and the sample was delivered to the bath. For 15 seconds the piston pump pulled the sample through the aperture. The data gathered was sent to the Analog card where RBC, HCT, and MCV were shown the display card (see Appendix 5). For the measurement of haemoglobin concentration, a beam of white light from an incandescent lamp passed through an optical filter, which was a centre transmission wavelength of 525 nm. The photocurrent thus generated was proportional to the transmittance of the contents of the cuvette at the chosen wavelength. The current was converted to a voltage that was sent to the Analog card, and there the voltage was converted to absorbency. From the Analog card's HGB computer circuit, the data was sent
via a digital card, to the display card (see Appendix 5) (Instruction manual for the Coulter Counter, Model M530, 1983).

Microscopy was carried out on thick blood films for malarial parasites and on thin blood films for erythrocytic status.

3.6 Data Analysis

The data analysis was carried out on an IBM PS2 Model 30 computer, using Statistix 3.1., and interactive statistical analysis program for microcomputers, produced by Analytical Software Ltd., USA. Harvard Graphics software program was used for the graphical presentation, and Word Perfect for the word processing.

The statistical tests performed for the analysis of the collected data were: One-way analysis of variance, analysis of covariance, regression analysis, chi-square tests, correlation analyses, Student’s T distributions. Cross tabulations, frequency distributions, histograms, scatterplots, percentiles, Whisker and box plots have also been computed for the descriptive statistics.

3.7 Limitations

The more anaemic an individual, the greater is the response which may be produced by supplementation. However, this project was limited in this respect and for ethical reasons, and only non-anaemic patients could participate.

There were certain problems with attendance and adherence to appointments.
On days with very heavy rainfall, many patients opted out of coming to the ANC. Compliance is a common problem in supplementation studies, particularly among out-patients. In this study, however, the majority of participants gave an impression of good compliance.

A high degree of accuracy and precision was difficult to obtain in the food frequency interview. The reasons were that the diet information obtained was only approximate. Patients' ability to recall eaten items and quantities thereof was sometimes limited. Our knowledge of nutrient requirements is also approximate. The dietary frequency interview was performed only on enrolment, and not again later in the study. There is a chance that participants may have altered their diets conscientiously or subconscientiously during the course of the study, perhaps caused by an extra awareness of eating habits or food intake in general. In the study population, however, this is believed to have made a minimal impact on outcome variables, if at all.

Tables of food contents with regard to iron and folate are limited in accuracy. The foods analyzed can differ in variety, geographic source, season of the year and subsequent processing, preparation methods and storage, all of which affect their nutrient content.

The increased requirement of iron during pregnancy cannot be met by the iron content of habitual diets in the United States or other industrialized countries, and the US NRC therefore recommends supplementation ranging from 30 to 60
mg daily, on top of the dietary intake (NAS, 1980). It is important to remember that the usage of American Recommended Dietary Intake may not be very appropriate outside the USA and particularly not in an African setting. The reasons for this are many, but include the differences in food production and handling. At the time of this study, no appropriate food tables were yet available in Kenya for local food stuffs and their micronutrient contents.

No consideration was given in this study to the possible effects of intestinal parasites among participants, due to the lack of time and resources. It is possible and likely that an infestation with gastro-intestinal parasites would negatively affect the haematological status of participants. However, the statistical impact of these effects would be minimized due to the randomization procedure of the study.

It may be difficult to properly differentiate between macrocytosis caused by vitamin B12 deficiency and folate deficiency, particularly when access to sophisticated laboratory services is limited. If the resources are able to detect a B12 deficiency, it is important to supplement with this vitamin so as to diminish the risk of potentially serious neurological complications, that can be masked by supplementation with folate. However, many authors, including WHO (1972), Young (1974) and Bentley (1985), stated that the macrocytosis seen during pregnancy most commonly was caused by folate deficiency, and for this reason they supported routine administration with folate.
Nairobi is non-endemic for malaria, but blood samples were checked for malaria parasites and none was found positive. It must be noted, however, that Jeliffe (1968), stated that thick blood films will only reveal malaria as is present in peripheral blood at the time of sampling, and that in pregnant women placental infection may be present even in spite of a negative reading.
CHAPTER 4

RESULTS

4.1 Summary of demographic characteristics

One hundred healthy, pregnant outpatients at the Kikuyu Hospital’s MCH clinic were included in the study and its analysis. There were two still births and one birth of twins occurring during the course of the study among those originally enrolled, but these were excluded from the analysis for logistical reasons. The majority of the participants were ethnically homogenous. Eighty-three percent of the study population were married and 17% were single. The population studied was young, with 18% falling in the age group below 20 years of age, and only 10% were above 30 years old. The median age was 23 years (Fig. 2). The average height was 160.6 cms. The majority of participants were of good height with only 4% being of a height less than 150 cms, which is regarded as a potential obstetrical hazard (Pritchard, 1980) (Fig.3). Weight was measured on enrolment and again 4 weeks later to determine whether average weight and/or weight gain of mothers had any association with birthweights (see below, 4.1.11). The average weight was 64.3 kgs.

Participants were divided into three groups depending on socio-economic status (SES). The parameter used in this classification was family income based on occupation of head of household, making use of guidelines from the Civil Service Review Committee, Office of the President, Nairobi, 1992, and The Kenya
Subsidiary Legislation, Regulation of wages and conditions of employment act, 1992. The study population consisted of 13% unskilled heads of family, earning K. Sh 0 - 1080/- per month, 54% skilled heads of family earning 1080 - 2439/- per month, and 33% professional heads of family earning a minimum of 2440/- per month.

The characteristics of the study population at the time of enrolment are summarized below (Table 1).

**TABLE 1: DESCRIPTION OF THE STUDY POPULATION ON ENROLMENT**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>mean</th>
<th>s.d.</th>
<th>range</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE (years)</td>
<td>100</td>
<td>23.7</td>
<td>4.2</td>
<td>14-33</td>
</tr>
<tr>
<td>HEIGHT (cms)</td>
<td>100</td>
<td>160.6</td>
<td>6.2</td>
<td>142-178</td>
</tr>
<tr>
<td>WEIGHT (kgs)</td>
<td>100</td>
<td>64.3</td>
<td>9.5</td>
<td>46.6-95</td>
</tr>
<tr>
<td>GESTATION (weeks)</td>
<td>100</td>
<td>28.8</td>
<td>2.4</td>
<td>23-35</td>
</tr>
<tr>
<td>PARITY</td>
<td>54</td>
<td>2.04</td>
<td>1.06</td>
<td>1-5</td>
</tr>
</tbody>
</table>

In Fig. 2 and 3 below, the age and height distributions are shown. It can be seen, that the study population were young, with a median age of 23 years. It can also be seen that the heights of participants were mainly distributed between 150 and 170 cms.

**4.2 Categorisation of study groups**

As a result of the randomization procedure, each of the four different treatments
AGES OF PARTICIPANTS

No. of patients

Age range (years)

FIG. 2

HEIGHTS OF PARTICIPANTS

No. of Patients

Height (cm)
were administered to an equal number of patients, with an equal distribution in the age categories (Table 2).

TABLE 2: DISTRIBUTION OF STUDY POPULATION BY AGE AND TREATMENT CATEGORY

<table>
<thead>
<tr>
<th>AGE</th>
<th>PLACEBO</th>
<th>COMBINATION</th>
<th>IRON</th>
<th>FOLATE</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>3</td>
<td>2</td>
<td>6</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>20-24</td>
<td>12</td>
<td>9</td>
<td>10</td>
<td>10</td>
<td>41</td>
</tr>
<tr>
<td>24-29</td>
<td>8</td>
<td>9</td>
<td>8</td>
<td>6</td>
<td>31</td>
</tr>
<tr>
<td>&gt;30</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>24</td>
<td>25</td>
<td>26</td>
<td>100</td>
</tr>
</tbody>
</table>

4.3 Effects of treatment on haematological parameters

The haemoglobin estimation carried out at the hospital laboratory was not very accurate, and although a criterion for enrolment was a haemoglobin level equal to or above 10 g/dl, it happened that some patients with lower values got included. The haemoglobin levels of the pregnant women on enrolment ranged between 8.9 g/dl and 16.1 g/dl, but a majority of the women, (69%), had an Hb level between 11 g/dl and 13 g/dl. Only a small percentage, (9%), had Hb levels above 13 g/dl. Treatments were randomly distributed among the women, and there were no significant differences in the Hb levels on enrolment between the different treatment groups, as tested in a one-way analysis of variance (p=0.62).

In table 3 below, the study cases are distributed according to their haemoglobin levels on enrolment and treatment group. Table 4 depicts the distribution of
patients and haemoglobin levels at the time of giving birth.

TABLE 3: DISTRIBUTION OF PATIENTS BY HAEMOGLOBIN LEVEL (on enrolment) AND TREATMENT GROUP

<table>
<thead>
<tr>
<th>Haemoglobin:</th>
<th>PLACEBO</th>
<th>COMBINATION</th>
<th>IRON</th>
<th>FOLATE</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;11 g/dl</td>
<td>2</td>
<td>5</td>
<td>8</td>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td>11-13 g/dl</td>
<td>20</td>
<td>19</td>
<td>15</td>
<td>15</td>
<td>69</td>
</tr>
<tr>
<td>14-16 g/dl</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>24</td>
<td>25</td>
<td>26</td>
<td>100</td>
</tr>
</tbody>
</table>

TABLE 4: DISTRIBUTION OF PATIENTS BY HAEMOGLOBIN LEVEL (at the time of birth) AND TREATMENT GROUP

<table>
<thead>
<tr>
<th>Haemoglobin:</th>
<th>PLACEBO</th>
<th>COMBINATION</th>
<th>IRON</th>
<th>FOLATE</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;11 g/dl</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>11-13 g/dl</td>
<td>11</td>
<td>12</td>
<td>7</td>
<td>7</td>
<td>37</td>
</tr>
<tr>
<td>&gt;14 g/dl</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>18</td>
<td>15</td>
<td>12</td>
<td>57</td>
</tr>
</tbody>
</table>

The patients that took iron only (150 mg. ferrous sulphate) supplements showed a significant increase in mean haemoglobin levels compared with controls (p = 0.05), as found using one-way analysis of variance. However, the patients that took iron (150 mg. ferrous sulphate) in combination with folate, did not show the same mean increase. This is depicted in the line graph of Fig. 4 below, and also in the histogram of Fig. 5.
Fig. 4  Observed Haemoglobin Level of Participants by Treatment over the Study Period
Fig. 5  Mean Change in Hb levels in Response to Treatments
The only haematological parameters investigated that significantly responded to treatment, as tested in one-way analysis of variance, were haemoglobin and haematocrit. A statistical difference was found with a value of $p = 0.05$, when looking at the association between treatment and mean change in haemoglobin, and similarly a value of $p < 0.05$ for the mean change in haematocrit (see Tables 5 and 6 below).

**TABLE 5: MEAN CHANGES IN HAEMOGLOBIN BETWEEN THE TIME OF ENROLMENT AND BIRTH (ANOVA)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean</th>
<th>n</th>
<th>group variance</th>
<th>s.e. of mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>placebo</td>
<td>0.21</td>
<td>12</td>
<td>1.034</td>
<td>0.29</td>
</tr>
<tr>
<td>combination</td>
<td>0.58</td>
<td>18</td>
<td>0.735</td>
<td>0.2</td>
</tr>
<tr>
<td>folate</td>
<td>0.44</td>
<td>15</td>
<td>1.415</td>
<td>0.31</td>
</tr>
<tr>
<td>iron</td>
<td>1.36</td>
<td>12</td>
<td>1.443</td>
<td>0.35</td>
</tr>
</tbody>
</table>

$p = 0.05$

**TABLE 6: MEAN CHANGES IN HAEMATOCRIT BETWEEN TIME OF ENROLMENT AND BIRTH (ANOVA)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>mean</th>
<th>n</th>
<th>group variance</th>
<th>s.e. of mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>4.07</td>
<td>12</td>
<td>22.22</td>
<td>1.36</td>
</tr>
<tr>
<td>Combination</td>
<td>3.63</td>
<td>18</td>
<td>11.93</td>
<td>0.81</td>
</tr>
<tr>
<td>Folate</td>
<td>1.01</td>
<td>14</td>
<td>30.25</td>
<td>1.47</td>
</tr>
<tr>
<td>Iron</td>
<td>6.9</td>
<td>11</td>
<td>34.56</td>
<td>1.77</td>
</tr>
</tbody>
</table>

$p < 0.05$
The scattergram in Fig. 6 below, shows the close correlation between haemoglobin and haematocrit on enrolment, which was also to be expected.

Fig. 6  Haemoglobin and Haematocrit Correlation on Enrolment

% HAEMATOCRIT

<table>
<thead>
<tr>
<th>HAEMOGLOBIN (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.0</td>
</tr>
<tr>
<td>9.0</td>
</tr>
<tr>
<td>10.0</td>
</tr>
<tr>
<td>11.0</td>
</tr>
<tr>
<td>12.0</td>
</tr>
<tr>
<td>13.0</td>
</tr>
<tr>
<td>14.0</td>
</tr>
</tbody>
</table>

It is noted from these tables, that women with an initial low haemoglobin
mean that supplementation, showed a mean increase of over 2 g/dl as
opposed to those with an initial high haemoglobin with a negligible
increase.
It is well documented that patients with low haemoglobin levels respond better to iron supplementation than those with optimal haemoglobin levels. Although this study was limited in its ability to include patients with Hb. values less than 10 g/dl for ethical reasons, a few patients with low initial Hb values were included as a result of inaccuracy of Hb testing at the hospital laboratory, where equipment was limited. The response to treatment showing the mean change in haemoglobin of these "out-lying" groups can be seen in Tables 7 and 8 below.

It is noted from these tables, that women with an initial low haemoglobin receiving iron supplementation, showed a mean increase of over 2 g/dl as opposed to those with an initial high haemoglobin; with a negligible increase.

TABLE 7: MEAN CHANGE IN HAEMOGLOBIN MEASURED IN WOMEN WITH AN INITIAL LOW HB (< 11 g/dl)

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>mean</th>
<th>s.d.</th>
<th>s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Combination</td>
<td>5</td>
<td>1</td>
<td>0.68</td>
<td>0.3</td>
</tr>
<tr>
<td>Folate</td>
<td>5</td>
<td>-0.1</td>
<td>0.95</td>
<td>0.42</td>
</tr>
<tr>
<td>Iron</td>
<td>3</td>
<td>2.03</td>
<td>1.15</td>
<td>0.66</td>
</tr>
</tbody>
</table>
TABLE 8: MEAN CHANGE IN HAEMOGLOBIN MEASURED IN WOMEN WITH AN INITIAL HIGH HB (> 13 g/dl)

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>mean</th>
<th>s.d.</th>
<th>s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>placebo</td>
<td>4</td>
<td>-0.75</td>
<td>1.18</td>
<td>0.59</td>
</tr>
<tr>
<td>combination</td>
<td>4</td>
<td>0.28</td>
<td>1.08</td>
<td>0.54</td>
</tr>
<tr>
<td>folate</td>
<td>2</td>
<td>0.8</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>iron</td>
<td>4</td>
<td>0.3</td>
<td>0.8</td>
<td>0.66</td>
</tr>
</tbody>
</table>

4.4 Other haematological indices

The response to treatment was measured in other haematological indices: Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Volume (MCV). One way analysis of variance was used to test for significant change in these variables. None of the changes observed had any association with treatments given. For normal values of haematological parameters, please refer to Appendix 5.

4.5 Effects on birthweights

Birthweight was measured in 86 babies born at the Kikuyu Hospital. The mean birthweight was 3178 grams (s.d. = 398 grams), ranging from 2268 grams to 4081 grams (see Fig. 7).
Fig. 7 Distribution of New Born Infants by Birthweights

No. of babies

<table>
<thead>
<tr>
<th>Birthweights (kg)</th>
<th>&lt; 25</th>
<th>25 - 30</th>
<th>30 - 35</th>
<th>35 - 40</th>
<th>&gt; 40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count</td>
<td>4</td>
<td>26</td>
<td>38</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>No. of babies</td>
<td>51</td>
<td>40</td>
<td>30</td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 9: Relatioship between Predictor Variables and Birthweights
Five babies (5.8%) had birthweights equal to or less than 2500 grams. There was no statistical difference found in birthweights when looking at the effects of the 4 treatments given, as found using regression analysis (p = 0.22).

Only two variables were found to be significantly associated with the birthweights when considered on their own in a regression analysis, and these were: mothers' weight on enrolment (p = 0.01) and mothers' weight gain during one month of the last trimester (p = 0.02).

The data for age, height, diet, parity and the final haemoglobin values were also analyzed. None of the variables were significantly associated with birthweight either on their own or when considered together in a multiple regression as seen in table 9 below:

### TABLE 9: RELATIONSHIP BETWEEN PREDICTOR VARIABLES AND BIRTHWEIGHTS.

<table>
<thead>
<tr>
<th>Predictor Variables</th>
<th>Coefficient</th>
<th>Std Error</th>
<th>Student's t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>3166.5</td>
<td>2301.6</td>
<td>1.38</td>
<td>0.18</td>
</tr>
<tr>
<td>Diet</td>
<td>12.6</td>
<td>86.4</td>
<td>0.15</td>
<td>0.88</td>
</tr>
<tr>
<td>Age</td>
<td>-25.9</td>
<td>20.4</td>
<td>-1.27</td>
<td>0.22</td>
</tr>
<tr>
<td>Height</td>
<td>3.9</td>
<td>13.4</td>
<td>0.29</td>
<td>0.77</td>
</tr>
<tr>
<td>Parity</td>
<td>-110.7</td>
<td>69.4</td>
<td>-1.59</td>
<td>0.12</td>
</tr>
<tr>
<td>Hb at time of birth</td>
<td>17.7</td>
<td>40.0</td>
<td>0.44</td>
<td>0.66</td>
</tr>
</tbody>
</table>

degrees of freedom = 27  overall F = 2.06  p = 0.1  R squared = 0.28
4.6 Effects of treatments on neonatal weight gain

Neonatal weight was measured and recorded from 24 infants when revisiting the MCH clinic of the Kikuyu Hospital 6 weeks after birth. The average neonatal weight measured was 4662 grams, (s.d. 396 grams), ranging from 4000 grams to 5700 grams. The average neonatal weight gain was measured to evaluate the effect of treatments. The average was 1387 grams (s.d. 347 grams), ranging from 686 grams to 2117 grams. Using a one way analysis of variance test, no association was found between treatment and neonatal weight gain (p = 0.24), having adjusted for birthweight.

4.7 Diet

Although not truly a "Kikuyu staple", ugali is now commonly eaten in this community, and is a dish with boiled maize meal. This is often eaten together with sukuma wiki which is a green, leafy vegetable, and sometimes with meat. Githeri is a dish with mixed beans and white sweet corn, sometimes also containing onions and oil. Irio (sometimes called mukimu) contains boiled green vegetables, commonly mixed with beans and sweet corn, and mashed with potatoes. Meat is eaten less commonly, and the consumption of meat is related to income earned, as is also stated by den Hartog and van Staveren (1985).

The results of the analysis of dietary data obtained in the food frequency interview were used as proxy indicators of iron, folate and vitamin C intake. It was found that the study population consumed an average of 25 - 30 mg. of iron daily. For the purpose of this analysis, the diets were classified as "poor", "fair"
or "good", with respect to the contents of iron, folate and vitamin C (NAS, 1980, Statens Livsmedelsverk, 1986).

Thirty-two percent of the women were consuming a "fair" diet, and 42% were on a diet classified as "poor" with regard to iron, folate and vitamin C content, as shown in Table 10 and Fig. 8 below. Quality of dietary intake was found to be similarly distributed amongst patients in the different treatment groups on enrolment.

### TABLE 10: DISTRIBUTION OF PARTICIPANTS' SOCIOECONOMIC STATUS (INCOME EARNED BY HEADS OF HOUSEHOLDS) AND QUALITY OF DIET

<table>
<thead>
<tr>
<th>QUALITY OF DIET</th>
<th>SOCIOECONOMIC STATUS</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LOW</td>
<td>MEDIUM</td>
</tr>
<tr>
<td>POOR</td>
<td>6</td>
<td>22</td>
</tr>
<tr>
<td>FAIR</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>GOOD</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>TOTAL</td>
<td>13</td>
<td>55</td>
</tr>
</tbody>
</table>
Fig. 8 Diet and Socioeconomic Status of Participants

No of patients

Contents of Iron, Folate & Vit C
A chi-square test was done to see if there was any association between diet and initial erythrocyte morphology findings. The results are shown in Table 11 below.

**TABLE 11: DIET AND ERYTHROCYTIC MORPHOLOGY ON ENROLMENT**

<table>
<thead>
<tr>
<th>MACROCYTIC STATUS</th>
<th>DIET QUALITY</th>
<th>POOR DIET</th>
<th>FAIR DIET</th>
<th>GOOD DIET</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORMAL</td>
<td></td>
<td>21</td>
<td>18</td>
<td>19</td>
<td>58</td>
</tr>
<tr>
<td>SLIGHT</td>
<td></td>
<td>4</td>
<td>7</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>SEVERE</td>
<td></td>
<td>16</td>
<td>7</td>
<td>4</td>
<td>27</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>41</td>
<td>32</td>
<td>24</td>
<td>97</td>
</tr>
</tbody>
</table>

The overall chi-square was 9.28, degrees of freedom 4, and p < 0.05, indicating that there was indeed an association between patients’ diet and their status of macrocytosis on enrolment. The same test was also done for the final morphology examination results. No association was found between macrocytic status and diet at the time of giving birth (p ≥ 0.52).
4.8 The Age of the Lastborn

Fifty-four mothers reported the age of the lastborn, and the average was 49.6 months, ranging between 12 and 99 months. Forty-six participants were primagravidae.

4.9 Cord Blood

Blood was collected from the umbilical cord of 31 newborn babies. The average haemoglobin was 15.39 g/dl., (s.d. = 1.75), ranging between 12.3 - 20.4 g/dl. No effect of treatment was found on cord blood haemoglobin in a one way ANOVA test (p = 0.87). There was no association between cord blood haemoglobin and the mothers' haemoglobin at the time of birth (p = 0.44).

4.10 Erythrocyte Morphology

On enrolment, the haematologist examined erythrocyte morphology by microscopy, as a diagnostic method for folate deficiency during pregnancy, and divided the samples into normal, slight, moderate or severe macrocytic status according to haematological guidelines (Eastham, 1985). The findings are summarised in Fig. 9 below.
Fig. 9 Erythrocyte Morphology-
Macrocytic Status on enrolment

- Normal: 61%
- Slight: 12%
- Moderate/Severe: 27%
A chi-square test was not used for testing treatment and morphology response, because of the small numbers in each cell. However, trends can be ascertained, as shown in Table 12 below, suggesting that treatment had little effect. Only 7 women showed any improvement in macrocytic status after treatment with folate with or without iron, and only 3 showed improvement as a result of iron supplementation. One patient taking placebo showed an improved macrocytic status.

**TABLE 12: MORPHOLOGIC RESPONSE TO TREATMENT**

<table>
<thead>
<tr>
<th>Morphologic Response:</th>
<th>control</th>
<th>combination</th>
<th>folate</th>
<th>iron</th>
<th>total</th>
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</thead>
<tbody>
<tr>
<td>deterioration</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>the same</td>
<td>8</td>
<td>14</td>
<td>10</td>
<td>10</td>
<td>42</td>
</tr>
<tr>
<td>improvement</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>total</td>
<td>12</td>
<td>18</td>
<td>15</td>
<td>13</td>
<td>58</td>
</tr>
</tbody>
</table>

4.11 Mothers' weight on enrolment and after one month

Participants were weighed on enrolment and again 4 weeks later. The average weight on enrolment was 64.3 kgs, ranging between 46 - 95 kgs. The average weight gain was 2.02 kg (s.d. = 2.2). One person lost 4.5 kg during that time, and the maximum weight gained was 9.5 kgs. Mothers' initial weight as well as their weight gain during one month of the study both affected birthweights significantly (p < 0.01 and 0.02 respectively) when considered on their own in a regression analysis (see above section 4.5).
4.12 Malarial parasites

Each blood sample was also checked for malarial parasites, in view of the fact that malaria causes haemolysis and could affect the participants' haemoglobin and macrocytic status. No blood smear was found to have signs of malarial parasites, and it would therefore appear, that malaria is not a problem in this population. This corresponds to other reports from highland Kenya (Jansen et al., 1987; Mati et al., 1971).

4.13 Other parameters investigated

Gestation at the time of first attendance at the MCH clinic and at the time of enrolment was generally late, but this is the common practice in this and other MCH clinics of Kenya (A. Wilson, P. Stanfield, personal communication, 1991, M. Murioki, personal communication 1992).

The estimated average time of gestation was 28.8 weeks at the time of enrolment. Time of gestation at the time of enrolment had no association with the birthweights or change in Hb levels, as tested in linear regression analysis (p = 0.9).

The average parity in this sample was low, 2.04 children per woman. Forty-six participants were primigravidae. Parity had no significant association with initial or final haemoglobin values, nor did it have any association with birthweights or neo-natal weight gain.

The period in which the participants were taking the supplements was measured
in weeks in the 86 patients who delivered babies at Kikuyu Hospital out of the study population (the remaining 14 participants did not turn up to give birth at the hospital). The mean time for receiving supplementation was 8.1 weeks (s.d. = 2.4).

4.14 Confounding variables

Analysis of covariance was used to test whether certain variables acting as covariates, would affect the change in haemoglobin or the birthweights. These variables were: age, height, parity, age of last born, treatment time, weight gain and initial haemoglobin. It was found that only the initial haemoglobin was associated with a change in final haemoglobin level, and that height and women's weight gain significantly affected birthweight as covariates.

4.15 Attrition

The attrition over the course of the project became larger than anticipated. Reasons for this could be that patients sometimes changed to another ANC unit, miscarried, gave birth at home, or chose to deliver at another hospital without notification.

Seventy-one patients turned up for the second antenatal visit and blood sample. The attrition rate for births was 14%. Although 86 patients delivered their babies at the hospital, only 57 last blood samples were obtained. A statistical analysis was done, using chi-square tests to find out whether patients who dropped out of the study had certain characteristics, or if the reason for dropping out could be
explained by certain variables. Parity and initial haemoglobin values had no association with dropping out. Socioeconomic status also had no association with dropping out of the study \((p = 0.11)\), but it was found that the largest frequency of drop-outs came from the socioeconomic group 2, i.e. the middle income earners. The highest proportion of women who turned up for delivery and third blood sample at the hospital were from socioeconomic group 3. Considering marital status and its relation to attrition, a chi-square test gave a value of \(p = 0.2\), indicating that this variable also had no association with the dropping out of the study.

### 4.2 Further analysis

Further analysis was carried out and the results are presented in the final statistical model (see page 75 below). One way analysis of variance was first used to look for significance of treatment on haemoglobin levels. At least one statistically significant difference was found between the means. Having established that this was the case, further examination was done to compare the mean changes in haemoglobin between the treatment groups. Iron was found to be significantly different from the means of the other three treatment groups. Although there was NO significant difference in the haemoglobin levels on enrolment, it was decided to use the initial Hb level as a covariate in further analysis. Using a regression analysis, it was shown that even after accounting for the variation due to initial haemoglobin levels, the treatment with iron still had a significant effect.
In summary, the **Final Statistical Model** (see next page), shows that three parameters were tested for significance. These were 1) Initial haemoglobin level (Hb1), 2) treatment (DAWA) and 3) interaction between treatment and initial haemoglobin level. In the first part of the model, it was found that all three parameters were significant. *The Error* contains all variation in change in haemoglobin that are not explained by those three parameters. In conclusion, this model tested and found that initial haemoglobin levels, treatment with iron only, and an interaction between those two, explained the significant increase in haemoglobin.
**Fig. 10  FINAL STATISTICAL MODEL**

**Change in Hb = HB, DAWA**

\[ HB_1 = \text{initial haemoglobin} \]
\[ \text{DAWA} = \text{treatment} \]
\[ \text{DAWA}^* H_b = \text{interaction between initial haemoglobin and treatment} \]

57 observations 43 missing

Change in Hb = HB\(^3\) - HB\(_1\)

Change in Hb = missing when HB\(_3\) = missing

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>Pr &gt;F</th>
</tr>
</thead>
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<tr>
<td>model</td>
<td>7</td>
<td>24.303</td>
<td>3.4719</td>
<td>3.84</td>
<td>0.0021</td>
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<tr>
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<td>44.3314</td>
<td>0.9047</td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>68.635</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ R^2 = 0.3541 \]

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>TYPE I</th>
<th>MS</th>
<th>F</th>
<th>Pr &gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB(_1)</td>
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<td>5.1379</td>
<td>5.137</td>
<td>5.68</td>
<td>0.024</td>
</tr>
<tr>
<td>DAWA</td>
<td>3</td>
<td>9.8207</td>
<td>3.2735</td>
<td>3.62</td>
<td>0.0194</td>
</tr>
<tr>
<td>DAWA * HB(_1)</td>
<td>3</td>
<td>9.34495</td>
<td>3.1149</td>
<td>3.44</td>
<td>0.0237</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>TYPE III</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
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</thead>
<tbody>
<tr>
<td>HB(_1)</td>
<td>1</td>
<td>8.316057</td>
<td>8.316</td>
<td>9.19</td>
<td>0.0039</td>
</tr>
<tr>
<td>DAWA</td>
<td>3</td>
<td>10.5042</td>
<td>3.501</td>
<td>3.87</td>
<td>0.0146</td>
</tr>
<tr>
<td>DAWA * HB(_1)</td>
<td>3</td>
<td>9.344</td>
<td>3.1149</td>
<td>3.44</td>
<td>0.0237</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Means</th>
<th>iron only</th>
<th>fefol</th>
<th>folate only</th>
<th>placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.358</td>
<td>0.578</td>
<td>0.440</td>
<td>0.208</td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>18</td>
<td>15</td>
<td>12</td>
</tr>
</tbody>
</table>

DF = Degrees of freedom  
SS = Sum of squares  
MS = Mean square
CHAPTER 5

DISCUSSION

The objectives of this investigation were to measure the effects of iron and/or folate supplementation on haematological parameters, birthweights and neo-natal weight gain in a highland population of Kenya. This was to provide data on iron and folate status during pregnancy, and whether supplementation of these nutrients would be beneficial to the expectant women living in this highland area of the country. The anticipation was also, that the results could be useful to antenatal clinics, policy makers and health workers in the area.

5.1 Study population characteristics

The average heights and weights of the study population correspond to those of previous studies of Kikuyus as reported by Jansen et al. (1987). The heights ranged between 142 - 178 cms, with an average of 161 cms. Only 4% of the study population were below 150 cms, which is regarded as an obstetrical risk. The weights on enrolment averaged 64.3 kgs, ranging between 46 - 95 kgs.

The participants in this study were notably young, with a median age of 23, ranging between 14 - 33 years of age (s.d. 4.2). Fifty-nine percent of participants were below 24 years old and only 10% were above 30 years old. This probably had an influence on variables such as socioeconomic and marital status and parity, and because of the young age, many women had not yet had time to get married.
(17% were single), develop a family or attain a secure or elevated socio-economic status.

It was also important to determine the effects of socioeconomic status on certain variables, particularly dietary intake, since it is well known, according to Jansen et al. (1987) and den Harthog and van Staveren (1985), that food consumption is related to household characteristics, notably social class. The study population was divided into low, middle and high income earners as assessed depending on the occupation of the head of the household (section 3.2.2). The study results did not clearly indicate that the socio-economic status had any effect on the quality of diet consumed, with equal proportions from the low and the high income earners consuming a diet classified as "poor". However, the trend shows that the middle and higher income earners (27% and 28% respectively) were consuming a diet of higher quality than the lower income earners (15%) (see Table 10). The only nutrients considered in the dietary analysis of this study were iron, folate and vitamin C, because of their importance in erythropoiesis. It is therefore possible that the findings do not totally correspond with those of other investigators, as reviewed by Jansen et al. (1987). These authors reviewed a multitude of different food studies, historical and contemporary, but quality of diet in those studies commonly referred to caloric and/or protein intake. The results of the present study would probably have shown a statistical significance regarding the relationship between socio-economic status and dietary intake, if the sample size had been larger.
However, it is noteworthy, that the socio-economic classification of participants showed 13% as low income earners, 55% as middle income earners and 32% as high income earners. Perhaps as a consequence of this, these women had chosen voluntarily to attend a private hospital (the Kikuyu Hospital), rather than government services which are more affordable.

Parity in the study population was low, (2.04), but this is likely to be a reflection of the young age. The total fertility rate per woman in Kenya is very high, and was reported from the Kenya Contraceptive Prevalence Study in 1984 to be 7.7 (UNICEF, 1989). The total fertility rate was also estimated in the Kenya Demographic and Health Survey (KDHS, 1989) to be 6.7. Other factors that may influence family size, eg. educational status of women, were not investigated in this study.

5.2 Haematological parameters

It was decided, that haemoglobin levels as determined by a Coulter counter analysis, would be the main criterion used for evaluation of iron status. This type of analysis was readily available at the time of the study. The state of iron metabolism, looking at serum iron concentration, total iron-binding capacity and storage of iron in the bone marrow was not necessary in this study, since it is well known, as stated by Holly, (1965), that pregnancy in itself precipitates iron deficiency anaemia, and haemoglobin is the last of the iron complexes to suffer. Haemoglobin tests are comparatively easy and inexpensive. Folate status was more difficult and access to biological assays or bone marrow biopsy was neither
practical nor necessary. Microscopy of erythrocyte morphology was considered sufficient for the purpose of this study.

At the time of giving birth, the last blood sample was to be collected from the mother, and also from the cord of the newborn baby. Even though there were 86 births taking place at the hospital, blood samples were only collected from 54 mothers at the time of giving birth. Possible reasons were that the attending midwives could not always manage to collect blood, perhaps due to time and/or staff shortages, if the birth occurred during the night shift or over week-ends. Occasionally the woman in labour also refused to have blood taken from her vein, because of pain or unwillingness for other reasons. The attrition related to cord blood collection was very high, resulting in only 31 of the newborn babies being sampled. The mean haemoglobin value for the cord blood was 15.39 g/dl, ranging between 12.3 and 20.4 g/dl, which corresponds to normal ranges reported by Eastham (1985).

Iron supplementation had a significant effect on haemoglobin and haematocrit levels. This finding corresponds with those of most other studies eg. Fleming, 1986, Reddiah et al., 1989, de Benaze et al., 1989, Holly, 1965, Lind 1983, Royston and Armstrong, 1989. Frank anaemia (Hb < 7 g/dl) did not appear to be a common problem among the outpatients of the ANC of the Kikuyu Hospital, and no non-experimental analysis of anaemic patients could be carried out in the present study, due to lack of resources. A large, collaborative study (between the Universities of California/Los Angeles campus/and Nairobi) carried out in Embu
found anaemia due to hookworm infestation during pregnancy to be very common in that area (Neumann and Bwibo, 1987). No intestinal parasites were examined in the present study, but only the presence of malarial parasites in blood was studied, due to resource restrictions.

However, even though the participants in the present study were not anaemic on enrolment, the results suggest that haemoglobin levels were not optimal and hence it was possible to improve them with oral iron supplementation.

It was unexpected to find the group receiving iron and folate in combination, not to respond significantly to treatment. They had received the same dosage of iron as the group receiving iron alone. The explanation of this is not obvious, because no literature has been found reporting that folate in any way inhibits the absorption of iron, in fact, most authors state the opposite (Young, 1974, Bentley, 1985, Swanson and King, 1987, Assami et al., 1988). It is possible, however, that the non-significant effect in the group receiving the combination treatment was due to the small sample size. It could also be possible, that compliance may have been poor among certain women in this group, although this was not evident during the course of the study.

With regard to the effects of folate, Jackson and Latham (1982) did a study on anaemic pregnant women in Liberia, and found that supplemental iron on its own produced as strong an effect on haemoglobin and haematocrit as when given together with folate. Similar results were also found by Thane and Tehein
Charoenlarp et al. (1988), Domisse et al. (1982) and Chanarin et al. (1988). All this evidence points to the fact that folate has no effect on haemoglobin levels, even though it may correct macrocytosis. The largest decrease (although not statistically significant) in MCV occurred in the group receiving folate, which was also expected since this group responded with decreasing macrocytes as a result of the folate supplementation.

The dynamic processes of iron and folate metabolism and their interaction of these during pregnancy, however, is not conclusively documented and warrant further research.

Seventeen percent of the study population had macrocytosis on enrolment, while it only affected 6-8% of the participants in the Embu study (Neumann and Bwibo, 1987). The diagnostic methods were not identical, and the results can therefore not be compared. The morphologic response to treatment in the present study did not show statistically important improvements, but it is possible that results would have been different if a more specific test for folate deficiency had been used.

### 5.3 Birthweights and neonatal weight gain

The attrition rate became larger than anticipated in this study, and only 86% of participants turned up to give birth at Kikuyu Hospital. The reasons for pregnant participant women not turning up to give birth there, could have been that they miscarried, gave birth at home, or chose to deliver at another hospital, without notification. These events were unfortunately not under the control of the investigator.
However, of the 86 births that occurred at the Kikuyu Hospital, and the average birthweight was 3178 grams, which corresponds well with findings of other authors, eg. Pertet (1981) and many studies reviewed by Jansen et al. (1987). Only 5.8% were equal to or below 2500 grams, which is the WHO cut-off point for perinatal high risk infants. Neumann and Bwibo (1987) stated that birthweight is an excellent indicator of the pregnancy outcome and a powerful determinant of perinatal, neonatal and infant morbidity and mortality. The same study also found that a large number of factors affect birthweights, eg. nutritional status during pregnancy, and the mothers' lifelong nutritional experience. These investigators found that women who have higher weights and greater fat stores give birth to heavier babies. Similar findings were obtained in the present study, where mothers' weight on enrolment and their weight gain during one month of the study period were both significantly associated with birthweights.

Resources were not available in the present study to incorporate detailed examination on whether the newborn infants were delivered at full term or not, but judging from the incidence of low birthweights, it appears likely that only a very small percentage may have been born premature.

The neonatal weight gain on average corresponds to that recommended by UNICEF (1989). Breastfeeding of infants is common practice in Kenya, and the majority of the mothers in the study population were also breastfeeding. The average weight gain of the infants was 1387 grams, (S.d. = 347 grams) ranging between 686 grams and 2117 grams. None of the supplementations had any significant effect on neo-natal growth.
5.4 Diet and weight gain during the last trimester

It was decided that a dietary survey should be included in this project. Nutritional status is to a large extent determined by food habits. However, during pregnancy, the growing foetus, placenta and increased blood volume put extreme pressures on the expectant mother, in particular with regard to iron and folate intakes. The diets of the pregnant outpatients were therefore examined, so as to obtain a proxy indicator of intakes of iron, folate and vitamin C (the main promoter of iron absorption). All these micronutrients are known to affect erythropoiesis, and this had to be considered when looking at iron and folate status during pregnancy. Intakes were generally lower than the Recommended Dietary Allowances of USA (during pregnancy). In Kenya, there are no local recommendations of dietary intakes during pregnancy, and the RDAs can therefore only serve as guidelines. Dietary patterns differ by ecological zones and ethnic habitation (see section 4.7). Many food consumption studies have been reviewed by Jansen et al. (1987), most using the 24 hour dietary recall method. For this study, it was decided that a food frequency questionnaire covering the last week’s dietary intake would be more appropriate. Food frequency questionnaires have been widely applied because of ease and speed of data collection and handling. Different combinations of responses to questions are used to calculate indices of certain nutrients consumption. Results of studies have indicated that the use of frequency interviews alone is appropriate when the objective of data collection is to establish subjects’ relative intake of specific nutrients (Samet et al. 1984). The results from the present study showed clearly, however, that the intake of the crucial nutrients was below the recommended
levels during pregnancy, which also corresponds with the findings of many other studies carried out in Kenya by eg. Pertet, Woldeghiorghis and Kogi, as reviewed by Jansen et al. (1987).

Seasonality in food crop production obviously affects dietary intake, and the same authors also reported that seasonality affects not only caloric intake, but primarily the dietary intake of retinol equivalents and ascorbic acid. Seasonality regarding the availability of folate and vitamin C would also have affected dietary intake in the present study, and it is possible that different results may have been achieved if the study had been carried out at another time of the year. Citrus fruits and green, leafy vegetables for example, seemed to be available throughout the study period, however. Dietary interviews were only conducted at the time of enrolment. It is also possible, although unlikely, that the food consumption pattern would have changed in the study population over the course of the project. This could have happened as a result of eg. increased awareness, or changed availability of food items.

Reports from many studies in Kenya (as reviewed by Jansen et al. 1987) show very modest weight gain during the third trimester, in comparison with the present study, where the average weight gain was 2.02 kgs. The range in this study, however, as compared with those reviewed by Jansen et al., was very large; between a weight loss of 4.5 kgs and a weight gain of 9.5 kgs. Reasons for this may be manifold, and it is well documented that eg. nutritional experience during childhood and adolescence, nutritional status at the onset of and throughout
pregnancy as well as past and present morbidity, all affect maternal weight gain and morbidity. The reports from the Embu study (Neumann and Bwibo, 1987) stated that only 11% of pregnancy weight gain during the third trimester was accounted for by food intake, indicating that in spite of nutritional shortcomings, the pregnant women may physiologically adapt to difficult circumstances and still produce infants with an acceptable birthweight.
CHAPTER 6
CONCLUSIONS AND RECOMMENDATIONS

The objective of this study was to determine whether supplementation with iron and folate during the last trimester of pregnancy had any effects on participants' haematological parameters, birthweights and neonatal weight gain of babies born to those participants. To achieve this, a cohort of healthy, pregnant outpatients of the Kikuyu Hospital’s Antenatal Clinic was selected. The participants were given one of four randomly-administered supplements including a placebo for control.

6.1 Conclusions

The working hypothesis that was employed for this research was: "Iron and/or folate supplementation during the last trimester of pregnancy will have a positive effect on one or all of the following parameters: mothers' haematological values, fetal growth, birthweights of babies or the neo-natal weight gain." The working hypothesis can be retained as far as the haematological values of haemoglobin and haematocrit are concerned, since the results show that participants supplemented with iron had significantly increased levels. Supplementation had no significant effect, however, on fetal growth, birthweights or neonatal weight gain of the babies born to participants, and therefore the second part of the hypothesis must be rejected.
The conclusions that can be drawn from this study, are that pregnant women of Highland Kenya do benefit from iron supplementation, since the study showed statistically important improvements in haemoglobin and haematocrit values as a response to treatment with oral iron. It is likely, that if truly anaemic patients could have been examined in large numbers, the significance of iron supplementation on haemoglobin levels would have been even greater.

6.2 Recommendations

1) The evidence that folate supplementation is beneficial to haematological parameters, birthweights and neo-natal growth cannot be considered conclusive in this study, and further research into this area, using much larger sample sizes in multiple centres is recommended. However, since macrocytosis is a common complication of pregnancy, and the recent literature now unanimously supports folate supplementation during pregnancy to protect the foetus and the mother, it seems of benefit to combine folate with iron in routine supplementation during pregnancy. The additional cost of adding folate to iron is also minimal (estimated at approximately 12% extra production cost), and it is believed a majority of pregnant women in the area would benefit from such supplementation. The mode of administration of preference is "slow release" capsules, and the recommended dosage is 150 mg. ferrous sulphate and 0.5 mg. folate daily.

2) Further research into the subject of anaemia during pregnancy in various
parts of Kenya is recommended, as well as studies looking into the micronutrient contents of locally produced and prepared food items, to facilitate improvement in levels of micronutrient retention.

3) It is also recommended that MCH and antenatal clinics around the country aim to increase the general awareness of the importance of good nutrition during pregnancy. Optimum nutritional status during pregnancy is one of the key elements of safe motherhood and the administration of supplemental iron and folate during this period has been shown to be of irrefutable benefit to pregnant women worldwide.
REFERENCES


Appendix 1: Consent Form

CONSENT form: Kikuyu Hospital Antenatal Outpatients

Name: ............................................................................................

Hospital ID #: ...........................................................................

...................................................... hereby consent to participate in the experimental study of The effects of iron and folic acid supplementation on pregnancy anaemias, birthweights and postnatal performance. The study has been explained to me by the searchers and/or a midwife of the Kikuyu Hospital.

........................................ Date and signature of patient

................................................ Signature of investigator/informant
## Appendix 2:

### RANDOM PERMUTATIONS OF THE FIRST 100 INTEGERS

<table>
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<td>D</td>
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<td>B</td>
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<td>8</td>
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<td>C</td>
<td>80</td>
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</table>

Ref. Fless, J. L. *The design and analysis of clinical experiments*. Wiley Series USA, 1996
Appendix 3

PATIENT INFORMATION SHEET AND FOOD FREQUENCY QUESTIONNAIRE

Today's date: Treatment:
Name: Hospital ID #:
Height: cms Weight: kgs Age:
Husband's or own occupation:
Kikuyu Hospital Hb estimation: g/dl MRC: g/dl
Parity: Age of last child:
LMP: EDD: Gestation: Next visit:

Below is a list of foods: Please tell me how often you eat them:
(1) every day (2) more than once a week (3) seldom (4) never

- Milk ...........................................
- Masiwa lala ................................
- Cereals ......................................
- Ugali, uji ...................................
- Bread ........................................
- Rice .........................................
- Pasta ........................................
- Green, leafy vegetables
  (eg sukuma wiki, spinach, cabbage) ........
- Carrots .....................................
- Other vegetables (eg potatoes, tubers) ... 
- Githeri (maize, beans + onions) ...........
- Citrus fruits (eg oranges, limes) ...........
- Other fruits ................................
- Nuts and seeds, eg. groundnuts .......... 
- Legumes, eg. dried peas, mung beans, lentils 
- Cooking fat, margarine or butter .........
- Eggs ........................................
- Fish ........................................
- Meat (chicken, beef, pork, goat) .........
- Organ meats (eg. liver, kidney) ...........

2nd visit date:
morbidity:
compliance:
## TABLE OF FOOD CONTENTS

Source: Statens Livsmedelsverk, Stockholm, 1986

Contents of Folate, Iron and Ascorbic Acid per 100 grams:

<table>
<thead>
<tr>
<th>Food Item</th>
<th>Folate (ug)</th>
<th>Iron (mg)</th>
<th>Vit. C (mg)</th>
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<tbody>
<tr>
<td>Milk</td>
<td>5</td>
<td>0.07</td>
<td>-</td>
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<tr>
<td>Beans (for githeri)</td>
<td>133</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Potatoes</td>
<td>10</td>
<td>0.4</td>
<td>12</td>
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<tr>
<td>Dark, green veg. (ave)</td>
<td>194</td>
<td>3</td>
<td>60</td>
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<tr>
<td>Carrots</td>
<td>14</td>
<td>0.24</td>
<td>6</td>
</tr>
<tr>
<td>Other veg.</td>
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<td>12</td>
</tr>
<tr>
<td>Citrus fruits</td>
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<td>0.17</td>
<td>50</td>
</tr>
<tr>
<td>Other fruits (ave.)</td>
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<td>-</td>
<td>30</td>
</tr>
<tr>
<td>Meats (ave.)</td>
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<td>12</td>
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<tr>
<td>Liver</td>
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<td>25</td>
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<tr>
<td>Kidney</td>
<td>80</td>
<td>7.2</td>
<td>9</td>
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<td>Groundnuts</td>
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<td>Eggs</td>
<td>21</td>
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<tr>
<td>Bread</td>
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## Appendix 5: Coulter Counter Record Card

**HAEMATOLOGY**

**COULTER COUNTER**

**COULTRONICS FRANCE S.A. - Morancy FRANCE**

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<tr>
<th>TEST</th>
<th>NORMALS</th>
<th>Coulter Counter&lt;sub&gt;♂&lt;/sub&gt;</th>
<th>Coulter Counter&lt;sub&gt;♀&lt;/sub&gt;</th>
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<td>WBC x 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>4.8-10.8</td>
<td>4.8-10.8</td>
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</tr>
<tr>
<td>RBC x 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>4.7-5.1</td>
<td>4.2-5.4</td>
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<tr>
<td>Hgb&lt;sub&gt;g&lt;/sub&gt;</td>
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<tr>
<td>Hct %</td>
<td>42-52</td>
<td>37-47</td>
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</tr>
<tr>
<td>MCV µ&lt;sup&gt;3&lt;/sup&gt;</td>
<td>80-94</td>
<td>81-99</td>
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<tr>
<td>MCH µg</td>
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<tr>
<td>MCHC %</td>
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**Differential**

- POLY.
- STAB.
- LYMPH.
- MONO.
- EOS.
- BASO.
- BLAST CELLS
- MYELOCYTES
- METAMYELOCYTES
- NRBC/100 WBC
- ANISOCYTOSIS
- POIKILOCYTOSIS

**REMARKS:**

- CBC
- Hgb
- Hct
- OR in AM
- WBC
- EMERGENCY

**NOTES**

- PLASMA VISC
- PLATELET
- RETIC
CERTIFICATE OF ANALYSIS

Sample : Ferrous Sulphate and Folic Acid Placebo Capsules

Batch No : 1
BPBR No : 1759
Date of Manufacture : 10 December 1990
Expiry Date : 10 December 1993

Appearance

Uniformity of Weight

Average Weight : 464.03 mg

Disintegration

Absence of Ferrous Sulphate : None detected

Absence of Folic Acid : None detected

Analytical Development Ref No : 90RD 4462

Complies with Analytical Development Specification No. DP 230/B/1

Size No. 1 hard gelatin capsule with a dark green body and a light green cap. The capsule contains white spherical pellets.

Complies with EP

Signed

D J Evans, BSc, C Chem, FRSC
Manager - Analytical Development

Date : 8 Feb 1991
CERTIFICATE OF ANALYSIS

Sample: Ferrous Sulphate and Folic Acid Capsules

Batch No: 1
BPBR No: 1762
Date of Manufacture: 10 December 1990
Expiry Date: 10 December 1993

Appearance:
Size No. 1 hard gelatin capsule with a dark green body and a light green cap. The capsule contains a mixture of red, yellow and white spherical pellets.

Identity:
Positive for Iron and Folic acid

Uniformity of Weight:
Complies with EP

Average Weight:
472.00 mg

Disintegration:
5.5 mins

Ferrous Sulphate Content:
150.4 mg/cap

Ferrous Sulphate Release Rate:

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<th>Time</th>
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<td>8%</td>
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<td>3 hours</td>
<td>90%</td>
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<tr>
<td>4.5 hours</td>
<td>95%</td>
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</table>

Folic Acid Content:
0.485 mg/cap

Analytical Development Ref No:
9ORD 4455

Complies with Analytical Development Specification No. DP 153/B/1

Signed: D J Evans, MSc, C Chem, FRSC
Manager - Analytical Development

Date: 11 Feb 1991

0165q/17
CERTIFICATE OF ANALYSIS

Sample: Folic Acid Capsules 0.5 mg
Batch No: 1
BPBR No: 1760
Date of Manufacture: 10 December 1990
Expiry Date: 10 December 1993

Appearance
- Size No. 1 hard gelatin opaque capsule with a dark green body and a light green cap. The capsule contains a mixture of yellow and white spherical pellets.

Identity
- Positive for Folic acid

Uniformity of Weight
- Complies with EP
- Average Weight: 427.69 mg
- Disintegration: 4.0 mins

Absence of Ferrous Sulphate
- None detected

Folic Acid Content
- 0.506 mg/cap

Analytical Development Ref No
- 90RD 4460

Complies with Analytical Development Specification No. DP 569/A/1

Signed

D J Evans MSC, C Chem, FRSC
Manager - Analytical Development

Date: 11 Feb 1991

0165q/16
CERTIFICATE OF ANALYSIS

<table>
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<tr>
<th>Sample</th>
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**Appearance**

Size 1 hard gelatine capsule with dark opaque green body and light opaque green cap containing a mixture of red and white pellets.

**Identity**

Passes specified chemical tests

**Uniformity of Weight**

Complies with BP

**Average Weight**

464.12 mg

**Ferrous Sulphate Content**

149.9 mg/cap

**Disintegration**

5.0 mins

**Ferrous Sulphate Release Rate**

Complies with specification

**Analytical Development Ref No**

90D 4458

Complies with Analytical Development Specification No. DP 568/A/1

Signed ........................................

D J Evans MSc, C Chem, FRSC
Manager - Analytical Development

Date 8th Feb 1991

0165q/14