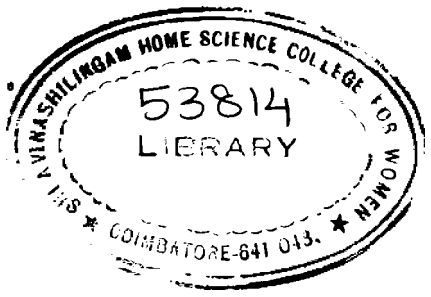
INTAKE AND AVAILABILITY OF FOLIC ACID FROM THE DIETS OF A GROUP OF EXPECTANT WOMEN AND THE IMPACT OF SUPPLEMENTATION

By

HEMALATHA, K.P.A.



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I INTRODUCTION

Child bearing imposes strain on maternal tissue and it is important that the would be mothers lead a healthy life throughout pregnancy. One of the major factor that promotes health and well-being both of the mother and the baby in the womb is whole some nourishing food (ICMR 1971). Accumulated evidence indicates clearly that faulty nutrition during pregnancy effects both the pregnant woman and the developing foetus.

The growing foetus is not a parasite to the mother from the nutritional point of view surviving and growing normally at any maternal expense. Rather it appears that both organisms are symbiotic and are affected by the quality of the maternal diet. On the other hand, if the mother becomes sufficiently depleted nutritionally, foetus may suffer to spare the mother (Helen et al., 1972).

Many studies have given conclusive evidence that the ability of a woman to nurture a healthy foetus depends to a great extent on her own nutritional status not only during pregnancy but during her whole preconceptional age. The prenatal care, the pre pregnant weight, as well as weight gain during pregnancy all exert strong influence on the outcome of pregnancy (Habitch et al., 1973).

It is evident therefore that during pregnancy not only the quantity but also the quality of food consumed is important. In addition to the calories, mother needs a greater quantity of better quality protein to facilitate or ensure adequate foetal growth and maintenance, (Munro (1974).

Studies in Guatemala among village Indian women of uniformaly low socio-economic status have indicated that supplemented protein and energy intake during pregnancy are associated with increased birth weights and decreased mortality and morbidity among new born (Habich, 1974). Heggin (1974) also found a positive association between infant birth weight, maternal weight gain, and protein energy intake. Poor prenatal nutrition is also reflected in the low vitamin and mineral stores of the new born.

Anaemias of pregnancy is one of the major health problems in developing countries with anaemia being directly responsible for 20 per cent of maternal deaths (Menon, 1967). Besides it is also responsible for the high incidence of premature and low birth weights, thus increasing the prenatal mortality and morbidity (ICMR, 1975).

Infants of anaemic mothers are frequently born with a subnormal iron endowment while infants born to mothers treated with oral iron during pregnancy exhibit higher serum iron value than the control group (Benjamin, 1978).

Several studies have shown that improved nutrition of mothers during pregnancy has profound effect on the infants birth weight, chances of survival, and learning ability (Monacha, 1972). Women poorly nourished prior to pregnancy is much more subject to complications of pregnancy such as toxaemia, hypertension, anaemia and premature births. (Robert et al., 1974).

Information regarding intestinal absorption of folic acid during pregnancy is conflicting. In addition in these studies increase in plasma folate level after the ingestion of an oral doses of crystalline folate has been used as a measure of folate absorption (NIN 1975).

Signs attributed to deficiency of B complex vitamins are frequently seen in pregnant women belonging to the low income group, particularly during the last trimester (NIN, 1975). Folic acid deficiency is far more likely to occur and the consequences may be far more serious than any of the other potential vitamin deficiencies guarded against pregnancy. The reports of the Hibbard (1964) suggested that megaloblastic anaemia in pregnancy as indicated by serum

folic acid level below 3 ng/ml occur in 65 per cent of the pregnant women from the low income group in the last trimester of pregnancy (Iyengar, 1971). The serum and cell folate concentration of all supplemented pregnant women tend to fall as pregnancy advances. (Beaton et al., (1976).

Colsey and Kline (1972) reported that maternal nutritional status has greater influence on the birth weight of the infant than even the genetic factors. Under their circumstances the observation that supplementation even in the last few weeks of pregnancy have a favourable influence on the pregnancy outcome assures greater significance.

An initial study on iron requirement of pregnant women in the last 16 weeks by Iyengar (1975) indicated that infants born to mother receiving 300 µg of folic acid in addition to iron were heavier than the infants born to unsupplemented groups. In a subsequent study on folic acid requirement of the same income group it was observed that increasing the intake of folic acid from 100 to 300 µg promoted both fetal growth and maintenance of the tissue stores. Hence supplementation of folic acid in pregnancy is essential to maintain folacin status particularly among the low income group.

Studies on the effect of food supplementation during pregnancy by Jacobson (1974) appear to indicate a favourable relationship. The study conducted by Iyengar (1972) indicated that nutritional supplements given to undernourished expectant mothers even late in pregnancy can significantly influence the birth weight of their infants. Hence the objective of nutrient supplementation during pregnancy is to ensure optimum health of the mother and child.

The present study aims at finding out the availability of folic acid from the diets of a group of expectant women belonging to the low income category. It is also hoped that the study will throw some light on the impact of supplementation of folic acid and iron together during the last trimester on the folacin nutritional status of these mothers.

II REVIEW OF LITERATURE

Review of literature pertaining to this study on the intake and availability of folic acid from the diets of a group of expectant women and the impact of supplementation is discussed under the following headings:

A. Effect of nutrition on pregnancy outcome

B. Prevalence of Anaemia among pregnant women

C. Role of folic acid in pregnancy

D. Studies on supplementation of folic acid in pregnancy

E. Assessing the extent of folate availability from food items.

A. Effect of nutrition on pregnancy outcome:

One of the major factor that promotes health and well being of the mother and the baby is wholesome nourishing food. It is essential from the point of view of the mother as well as of the infant that the special nutritional demands of pregnancy are adequately met (Gopalan et al., 1972).

A study conducted in NIN (1972) showed that most of the babies born in India, particularly in rural areas and among those belonging to low-socio-economic group weigh only, about 2.6 kg. Surveys conducted in various parts of

our country indicate that the birth weight of infants born to mothers belonging to the poor socio-economic group are generally less than those of infants born to mothers of high socio-economic group. Thus the poor state of nutrition of the low-socio-economic group is reflected in low birth weight of the offspring (ICMR, 1971). Gopalan (1973) showed that induction of various nutritional deficiencies singly or in combination have shown to produce congenital malformation, increase fetal wastage, resorption and growth retardation of the offspring.

Jelliffe (1976) emphasises that maternal nutrition affect both the new-born and estrogestate foetus directly on the birth weight level, fetal stores of nutrients and the laying down of adequate lactation reserves.

In a study of the assessment of nutritional status of Indian mothers Srikantia and Iyengar (1975) found that when the nutrient intake is compared with recommended allowances the intake of all nutrient was low. Toxemia of pregnancy has been related to dietary intake but proof of cause and effect was lacking. Women on poor diet with deficient calorie and protein content have shown to have a markedly high incidence of toxemia (Duke Enstes 1960).

Maternal malnutrition has shown to result in higher frequency of pregnancy wastage through abortion miscarriages and still births. In addition, faulty diet and poor nutrition of mothers can lead to premature termination of pregnancy. These babies face a number of risk soon after birth. They are liable to develop bleeding injuries in the head during and after delivery because the head is soft and not well moulded and fine blood vessels are not well formed and are fragile. Heat regulation is usually defective because they have little subcutaneous fat and also temperature regulating centre in the brain are not well developed. As the suckling and swallowing reflexes are not well developed feeding problem arises. Sometimes because of low blood sugar level they also develop frank convulsions. This may lead to permanent brain damage (NIN, 1974).

In a survey carried out in India, the poor women gained around 6 kg during pregnancy starting with an initial weight of 42 kg. This is extremely low in terms of desirable weight (Venkatachalam, 1962). Undernutrition and malnutrition among mothers especially in the developing countries contribute towards impaired fetal and infant health and vitality (WHO, 1965).

Gopalan (1972) showed an increased frequency of pregnancy termination by abortion or still birth in malnourished women. WHO (1976) reported that out of 22 million lowbirth weight infants born in a year in the world, 21 million are born in developing countries and 10 million of them are small for-date babies.

Thomsan et al., (1963) indicate that good health and nutrition are correlated with good reproductive performance. Improving the nutrition of the mother directly improve the birth weight of the baby. Increase in birth weight among babies born to poor Indian women who were given during the third trimester, of pregnancy a qualitatively and quantitatively superior diet (Iyengar, 1968).

Zamenhoff (1968) reported that the pregnancy of rats fed a diet that contained 8 per cent casein for one month prior to mating and gestation weighed 30% less at birth than the offspring of controls fed an inadequate amount of protein and brain weight was reduced to 23% in the experimental group. Hurely (1968) reported when female rats were kept on zinc deficient diet during the entire 21 days of gestation, the litters were small both in number and size, and the body weight being approximately the half of the normal.

A longitudinal study by Mahrun (1969) indicates the possibility of inter-relationship between maternal malnutrition, still birth, gestational complications and possibility of blindness among children.

Serum levels of the heat stable alkaline phosphatase showed a great increase at terms in the women of lower income group resulting in an inverse correlation between the enzyme activity and birth weight of the infant. These observation can indicate that placental function is altered considerably in maternal nutrition.

A study conducted in NIN (1974) showed that inadequacy of protein, calories and calcium in pregnancy have been shown to reduce the bone density. Besides affecting the health of the new born, an inadequate diet affect the mothers own health. When her diet does not supply the nutrients essential for her child, the child in the the womb withdraws them from the tissue of her own body. This tissue depletion weakens her, so that, she is unable to cope with the strain of child bearing. Poor maternal diet create not only the risk of serious complications during gestation but often results in a difficult and sometimes a prolonged labour (NIN 1978).

An inadequate food intake during the late pregnancy had a marked effect on rat fetal lung growth and development by inhibiting cellular proliferation, differentiation and development of epithelial and macrophagic cells. (Adamson et al., 1978).

Since pregnancy is a period of stress for women, for particularly those from poor community, a properly balanced diet is essential. In view of their severe consequences, nutritional disorders in expectant women should be corrected and in fact adequate preventive care must be taken at the earliest possible opportunity.

B. Prevalence of anaemia in pregnancy

Anaemia is one of the most common complications of pregnancy. It results from the inability of the erythropoietic tissue to maintain a normal haemoglobin concentration on account of an inadequacy or lack of iron, folic acid, vitamin B_{12}, protein, and ascorbic acid (Beaton et al., 1986) and WHO, 1978). About one in five of our expectant mothers suffer from severe degree of anaemia as judged by their haemoglobin level. About 25 per cent of maternal deaths are attributed to anaemia. Fifty to sixty per cent of all pregnant women in the poor income group have haemoglobin level below 10 g/ 100 ml during the last three months of pregnancy (Rajalakshmi, 1975).

In India, 47 per cent of the pregnant women in general are anaemic and 40 per cent of these suffer from folate deficiency (Gopalan, 1977). Every alternate women in the third trimester of pregnancy and 50 per cent of children below the age of five years are anaemic (Gopalan, 1977); and ICMR, 1977).

The amount of iron provided by an Indian diet is around 20 mg day (Vasishtha, 1976), a level that may be apparently adequate. However phytate and the low calcium and ascorbic acid content of poor Indian diets have now shown to restrict the availability of iron.

Maternal anaemia appears also to be related to the birth weight of the infant. The mean birth weight of full term infant, in a series of 1000 mothers with level of haemoglobin above 11 g/100ml. was found to be 2800g. While, the mean birth weight of full term infant born to anaemic mothers with level of haemoglobin content below 6.5 gm/100ml was found to be 2000g. Nearly 45 per cent of total infant death in the poor income groups is accounted by neonatal deaths, and greater majority of them occur in infant with birth weight of 2000g or less (Gopalan, 1975).

In temperate zone 10 to 15 per cent of the menstruating women and from 20 to 30 per cent of pregnant women

may exhibit iron deficiency anaemia (WHO, 1971). A survey conducted on 1000 women in Tamil Nadu, haemoglobin levels of less than 12g /100ml were found in 56 per cent of the pregnant women. In 15 per cent of the pregnant women the levels were less than 8 gm/100ml. Iron deficiency as indicated by low serum iron level and decrease transferritin concentration was found in 95 per cent of the pregnant women (WHO, 1968).

Numerous survey of the prevalence of anaemia in different parts of the our country such as Calcutta, Madras, Delhi and Vellore showed that 30-70 per cent of women in later part of pregnancy have level of haemoglobin below 10 per cent (Nutrition society of India report of the study group on Nutritional anaemia, 1968).

Megaloblastic anaemia in pregnancy is less frequent than that due to iron deficiency. Both Beaven et al., (1966) and Lowenstein et al. (1966) found Megaloblastic features in the marrow in 25 per cent of over 500 pregnant women. Temperely et al., (1968) found megaloblastic in 30 per cent of 50 women. Little (1962) found Megaloblastic anaemia is some eight times more frequent in twin pregnancy.

There is a fall in the white blood folate level in normal pregnancy, and low whole blood folate value were found in anaemic pregnant women (Hansen, 1964). Varndi et al., (1966) found reduced red cell folate level in 24 per cent of pregnant women. Lowenstein et al., (1966) as found in 30 per cent out of 148 pregnant women.

C. Role of folic acid in pregnancy

Various investigations have indicated the possible relationship between folacin deficiency and complications of pregnancy. It may be dependent on the severity of the deficiency and or the period of pregnancy (Pritchard, 1970). Folacin deficiency reportedly causes chromosomal abnormalities like chromosomalcontraction and ostromare spreading (Hibbard, 1965).

Wordsworth (1973) had pointed out that folacin deficiency, early in pregnancy may interfere with the normal development of placenta. He had suggested that folacin deficiency of pregnant women is related to the incidence of a number of complications in pregnancy such as abortion, abruptive placenta and malformation. The improvement in birth weight of babies born to folacin supplemented mothers appear to be due to an increase in size, cell number and the protein content of the placenta (Rajalakshmi, 1975).

Maclain (1975) found the importance of folacin intake for brain maturation. Folacin deficiency during pregnancy showed abnormal or delayed development in one or more of the general areas tested. Most had developmental defects in the gross motar area.

An increased risk of accidental haemorrhage was noted in megloblastic anaemia of pregnancy (Nourichan, 1960). An increased incidence of premature labour before the thirty eight week in women with megaloblastic anaemia of pregnancy was noted by Gateby (1966).

Martin (1965) found a direct relationship between serum folic acid level and threatened abortion. Herta (1965) showed a direct quantitative relationship in chicks between the oviduct response and the dietary level of folic acid. Hibbard and Hibbard (1964) had suggested that megaloblastic anaemia of pregnancy is associated with a increased risk of mothers and the infants.

Since the placentation and normal formation of foetus occur in early pregnancy, there is evidence of increased requirement of folic acid in seventeenth and eighteenth weeks of pregnancy. Proper development of foetus and efficient implatation in endometrium fundamentally depend on normal metabolism of cells, in which folic acid play

vital part. Folic acid necessary for synthesis of essential nucleic acid, deficiency of folic acid manifested growing tissues (Nutr. Review) 1968).

Folic acid supplementation after onset of pregnancy does not reduce pregnancy wastage. Therefore that supplementation of folic acid be started no later than the onset of pregnancy or preferably before (Hibbad, 1964).

D. Supplementation of folic acid in pregnancy

The possibility that maternal folate deficiency may have an adverse effect on the products of conception is of great interest and importance. The frequent findings especially in deprived populations of biochemical and cytological changes suggesting maternal folate deficiency have resulted in the trend towards routine folic acid supplementation.

From the studies of Lowenstein et al., (1966) it would appear that the ingestion of a diet containing approximately 80µg of folate activity daily would prevent maternal folate deficiency but would not prevent a high incidence of folate deficiency. Gatenby (1960) reported that to prevent all evidence of folate deficiency in pregnancy, requires either a diet containing approximately 160µg folate activity

supplemented with atleast 100ug of folic acid/day or a diet containing approximately 80ug of folate activity supplemented with 200µg folic acid/ day.

Chanarin et al., (1968) have reported that red cell stores in pregnant subject, receiving supplement of 100µg of folic acid daily were higher than in control subjects. Hansen et al.,(1967) and Willoughby (1967) have found that supplements of 300 to 500 µg of folic acid daily was necessary to raise red cell folate level in pregnant ladies and that 100µg had no such effect. On the other hand, it has been suggested by Herbert (1968) that folic acid requirements during pregnancy is around 300µg/day.

It had been reported that pregnant women receiving 300 to 500ug of folic acid in addition to elemental iron during the later half of pregnancy delivered babies who were heavier than those who were born to mothers who had received only elemental iron. The total amount of DNA was higher in the folic acid supplemented group as a result the placenta being heavier. The total protein content was significantly higher in folic acid supplemental group (NIN, 1974).

Hansen and Ryhe (1967) determined the amount of folate required to maintain whole blood folate levels throughout pregnancy. A daily supplement of 50µg of folic acid in the second half of pregnancy did not have any obvious effect

on the whole blood or serum folate levels. Hundred µg folate daily maintained these values, and higher doses resulted in marked elevation of both. Chanarin et al., (1968) found that 100ug of folate each day resulted in a rise in red cell folate which was maintained in the last trimester. Dawson (1966) found a rise in serum folate levels when 150 µg folate were given daily.

Fleming et al., (1968) reported that at delivery the mean red cell and serum folacin value of Australian women who had received 500ug supplemental folic acid during the last half of pregnancy were three and four times greater than the values of non supplemented women. Six weeks after delivery both values remain elevated.

In a study on iron requirement of pregnant women in the last 16 weeks indicated that infants born to the mother receiving 500ug of folic acid in addition to iron were heavier than the infants born to nonsupplemented mothers or to those receiving iron supplement alone (Iyengar, 1975).

Coursey and Kimal (1972) reported that maternal nutrition status has greater influence on the birth weight of the infant. WHO (1971) recommended that pregnant women should be given 500ug daily supplements of folic acid during second and third trimester of pregnancy.

The benefits derived from widespread folic acid supplementation during pregnancy have not been determined. Folic acid will eradicate megaloblastic anaemia due to folic acid deficiency, but whether it would reduce pregnancy wastage such as abruptive placenta and other late pregnancy, fetal malformations and abortions remains to be established.

2. Availability of folic acid

There are number of ways of assessing folate absorption in man. None of these methods measure the absorption of natural food folate in the amounts available from normal diet. There were considerable difference among individuals in their ability to absorb folate from different foods especially lime, beans and cabbage. Availability of folacin in some foods varied between 0 and 100 per cent. The investigators were unable to explain an availability of more than 100 per cent for different foods in some individuals (Retief, 1969).

Studies of Baugh et al., (1971) using labelled polyglutamate have suggested that the absorption of polyglutamate is inversely proportional to the length of the glutamyl chain-Godwin et al., (1970) using a similar labelled compound reported that synthetic poly-glutamate and mono-glutamate of folic acid are absorbed with nearly same efficiency.

Availability of folate from Bengal gram and green gram was studied by Lakshmi et al., (1969). Availability of folate from Bengal gram and green gram was around 70 per cent while that from spinach was about 63 per cent. Availability from the tomato and banana was relatively lower, approximately 50 per cent being absorbed. Folate from the brewers yeast was the least available of the foods tested. There were considerable variations between individuals regarding the absorption of folate from different foods and also in the same individual (NIN, 1975).

Urinary folate excretion may be quite variable from day to day even in the same person. Intestinal pH different cooking procedure, and even because of inhibitory substances in the diet which may have selective efficiency for various folate conjugate. Excretion pattern suggested that total folic acid was absorbed from the dietary item such as calf liver, spinach, green peas, but the total folate was less available from cauliflower, pumpkin and tomatoes (Rentif, 1964).

Hardcastle (1968) showed that there are considerable variation between different foodstuffs and also that in the same individuals in their availability.

It appears that during pregnancy the renal tubules do not absorb folacin as they do normally. Hytten (1971) found that healthy pregnant women excreted four times as much folacin as they did during 6th week post partum.

Studies of folic acid by jejunal perfusion indicate that glucose increases folic acid absorption in human (Jenson et al., (1971). Chung et al., (1961) found that a high-cost diet contained 193µg of folate daily, a low-cost diet 157µg and a poor diet 47µg. A rural Puerto Rican diet was thought to supply 300µg folate and an urban one 650µg folate.

Folate content of complete food collection was assayed by Butterworth et al., (1968). They showed that the diet of a healthy pregnant woman contained about 160µg of free folate and 67µg of total folate per day.

Nelson et al., (1975) concluded that the availability of folacin in orange juice was equivalent to that of synthetic folic acid, 60 and 58 per cent respectively. Colman et al., (1975) also found that increment in serum and red cell folacin values of pregnant women who consumed

a mains meal containing 500µg folic acid/day was similar to that of women who took 300µg of folic acid in tablet form.

Tamura et al., (1976) found the poor availability of folacin from orange juice. Tamura et al., (1973) found that egg yolk and romaine lettuce did not affect the availability of added pteroyl heptaglutamate but orange juice definitely inhibited the added pteroyl-triheptaglutamate.

III EXPERIMENTAL PROCEDURE

The experimental procedure pertaining to this study on the intake and availability of folic acid from the diets of a group of expectant women and the impact of supplementation is presented under the following headings:

A. Selection of the samples

B. Assessment of the food and nutrient intake of the expectant women

1. Food weighment survey
2. Nutrient analysis

C. Administration of folic acid supplements

D. Assessment of selected nutritional profiles of the expectant women

1. Folic acid availability
2. Serum folic acid levels
3. Haemoglobin levels
4. Serum iron levels

A. Selection of the samples

A maternity centre where every Tuesday and Friday there was antinatal clinic and the expectant women came regularly for health checkup was selected as it was easy to contact the women regularly.

A total of 20 expectant women were chosen for the study. Care was taken to see that the selected mothers were in their 6 to 7th month of pregnancy and their age ranging from 20 to 28 years. All the selected mothers belonged to the low socio-economic groups. It was considered enough to follow the mothers from their third trimester, as maximum growth occur in the third trimester of pregnancy (Ramalingaswamy, 1975). The expectant women selected also had to be from Coimbatore city to facilitate the weighment survey in order to assess their dietary intake.

B. Assessment of the food and nutrient intake of expectant women

1. Food weighment survey

In order to assess the actual food and nutrient intake of the expectant women in their homes, it was considered from the point of view of accuracy to conduct actual weighment of the foods eaten, and collect an aliquot portion of the food for nutrient analysis using biochemical methods.

Accordingly, the investigator visited the homes of the selected expectant women and created rapport. All the foods eaten on a particular day, by each expectant woman was weighed at each meal time and one tenth of

the food eaten was collected. The food thus collected for each expectant woman was pooled for the day, homogenised, and analysed for different nutrients on the following day. Ascorbic acid content of the diet alone was estimated immediately after pooling.

2. Nutrient analysis

From aliquots of homogenised food, nutrients like folic acid, ascorbic acid and iron were analysed. Ascorbic acid and iron were estimated by standard procedures given by Omar. (1965).

Folic acid was analysed microbiologically using the organism L. casei following the procedure of NIN (1981). Both free folate and total folate was estimated by this method and the details of the procedure adopted in the study is presented in the Appendix A.

C. Administration of supplements

All the 20 selected expectant women were given 200mg of iron and 500µg of folic acid in the form of tablet for a period of one month. Investigator visited the women regularly both at home and in the clinic to ensure regular consumption of the nutrient supplements.

D. Assessment of selected nutritional profiles of the expectant women

The nutritional profiles of the expectant women were assessed on the basis of folic acid intake through dietary analysis, folic acid availability serum folate levels haemoglobin levels, and serum iron levels before and after supplementation.

1. Folic acid availability

By far, the review of literature on the measurement of folate availability in humans (Fleming, 1973, Retif, 1969, Jansen Steenland 1973 and Srikantia and Babu, 1976) have indicated that measurement of excretion in urine against dietary folate give a measurement of availability of folate. Hence in order to assess the folic acid availability, 24 hour urine was collected for each expectant women on the same days when her dietary intake was assessed.

The urine was collected under toluene and from that, one ml the sample was taken for analysedof the folic acid concentration. The procedure followed was as given by Babu (1976) and is appended in Appendix A urine was analysed only for free folate as Tamura (1974) reported that there was no increase in folic acid content on treatment

with conjucare. From the data of folate activity in food as measured by food weighment survey and urinary excretion the availability was calculated using the following formula:

$$\text{Availability per cent} = \frac{\text{Folate content in diet} - \text{urinary excretion}}{\text{Total folate content in diet}} \times 100$$

2. Serum folic acid levels

In order to assess the serum folate levels of the expectant women before and after supplementation of folic acid and to correlate it with dietary folate 5ml of the blood was collected from each women before supplementation i.e. when dietary intake survey was done, and one month after supplementation. Serum was separated from the collected blood and the folate level were estimated microbiologically using the procedure of NIN (1974).

3. Haemoglobin levels

It was thought interesting to estimate the haemoglobin levels and wherever possible to correlate with folic acid availability and iron intake, as such data on expectant women is not readily available. Hence using the cyanmethaemoglobin method of Varley (1972), haemo-

globin was estimated on all expectant women before and after supplementation.

4. Serum iron levels

Total serum iron level was estimated using the procedure of Ramsay (1954, 1958). 0.5 ml of the serum sample was taken for the analysis. This was also done before and after supplementation.

IV RESULTS AND DISCUSSION

The results pertaining to this study on intake and availability of folic acid from the diets of expectant women are discussed under the following headings:-

A. Family profile of the selected women

B. Nutrient content of the diets of the expectant women

   1. Folic acid content of the diets of the expectant women

   2. Protein, iron and ascorbic acid content of the diets

C. Availability of folic acid from the diets of the expectant women

D. Impact of the supplements on the certain indices of expectant women

   1. Serum iron level

   2. Haemoglobin level

   3. Serum folic acid level

A. Family profile of the selected women

The family profile of the selected 20 women all belonging to low socio-economic group were gathered by interviewing the women personally for background information. The data thus gathered is presented in the following paragraphs.

The details regarding family pattern of 20 selected expectant women is given in Table I.

TABLE I

FAMILY PATTERN

Pattern of family	Number	Percentage
Nuclear family	18	90
Joint family	2	10
Total	20	100

Among the 20 families 18 belong to nuclear family and two were living in joint family. All the families belonged to low socioeconomic strata with their income ranging from Rs. 50 to Rs. 250 per month per family. Table II presents the size of the family.

TABLE II

SIZE OF THE FAMILY

S.No.	Number of family members	Number	Percentage
1.	2 - 4	15	75
2.	4 - 6	5	25
	Total	20	100

Table II reveals that 75 per cent of the families had 2 to 4 members where as 25 per cent had 4 to 6 members in the family.

The occupational status of the families of selected women in this study is presented in Table III.

TABLE III

OCCUPATIONAL STATUS

S.No.	Occupation	No.	Percentage
1.	Professional	-	-
2.	Clerical	5	25
3.	Coolies	8	40
4.	Others	7	35
	Total	20	100

Majority (40 per cent) of the families were coolies and they were daily or weekly wage earners. Thirty five per cent of the families had varied occupations like carpentary, petty shop, tea shop and the likes. Only 25 per cent of the families were engaged in clerical jobs.

History of previous pregnancy and delivery of the selected women is given in Table IV.

TABLE IV

HISTORY OF PREVIOUS DELIVERY

	Numbers	Percentage
Full time	29	96.6
Pre-maturity	1	3.4
Abortions	-	-
Total	30	100.00

Of the mothers studied, 96.6 per cent of the deliveries had been normal full time delivery and only 3.4 per cent had been premature cases. This indicates that the selected sample have had apparently normal delivery and thus forms a good sample for studying folic acid availability in their system.

TABLE V

FOLATE CONTENT OF THE DIETS OF THE EXPECTANT WOMEN

Subject No	Folate content in/µg		Subject No	Folate content in µg	
	Free folate	Total folate		Free folate	Total folate
1.	134	470	11	131	438
2	266	950	12	147	307
3	170	567	13	132	308
4	225	600	14	141	353
5	156	992	15	115	380
6	205	620	16	138	350
7	198	462	17	90	362
8	170	460	18	112	378
9	165	460	19	112	428
10	137	546	20	109	402
Mean free folate = 193.2µg			Mean total folate=470µg		

It is evident from Table V that the folate content of the diet ranges from 112 µg to 266µg of free folate and 307 to 950 µg of total folate in this study having the mean value of 193.2µg for free folic acid and 470µg for total folic acid.

In a study conducted by Channarin (1968) with 16 healthy pregnant ladies, the total folic acid intake was 675µg/day and before conjugase treatment the value was 160µg. The range for total folate was 198-1615 µg/day and for free folate

58 - 256 µg/day. Another study conducted by Mundle (1967) gave a value of 223 µg as free folate/day for normal women.

Analysis of food served in London hospital showed a free folate content of 117 µg/day and total folate of 487µg daily (Chararin et al, 1968). A rural Puerto Rocian diet as studied by Santini et al, (1962) supplied 380µg of folate and of urban one 650µg folate. Jukes (1961) suggests a value of 400µg of folate in the diets.

The folic acid content in the diets of the selected expectant women studied in this investigation gave values nearer to those values obtained by other workers cited above. Eventhough the diets of expectant women were lacking in green leafy vegetable other vegetables and fruits, the consumption of liberal quantity of pulses accounted for the folic acid content of the diets. But this amount does not seem to be enough to maintain the normal folacin in pregnant women. Gatey (1960) reported that to prevent all evidence of folate deficiency in pregnancy requires either a diet containing approximately 160µg folate activity supplemented with 100µg of folic acid/day or a diet containing approximately 80µg of folate activity supplemented with 200µg folic acid/day.

In any case, the free folate levels of the expectant women of low income group studied here would not be sufficient in pregnancy and hence need supplementation with folic acid.

2. Protein, iron and ascorbic acid content of the diets

The mean protein, iron, and ascorbic acid contents of the diet of the expectant mothers studied is presented in Table VI. Their individual value is appended in Appendix B Figure 1 also gives a comparison of the intake of these nutrients as against the recommended allowances of ICMR (1976).

TABLE VI

PROTEIN, IRON AND ASCORBIC ACID CONTENT IN THE DIET OF THE EXPECTANT WOMEN

	Protein g	Iron mg	Ascorbic acid mg
Intake	30.6	19.4	22
Recommended daily allowance ICMR (1976)	55.0	40.0	40

The diets of these expectant women particularly for the low income group is low in their protein content when compared with ICMR (1976) recommended values. There exists a deficit of 14.7g of protein. Nutrient intake of expectant mothers of low income group were studied by Srikantia and

PROTEIN IRON AND ASCORBIC ACID CONTENT IN THE DIET OF THE EXPECTANT WOMEN.

Scale
x-axis 1cm = 10 percent

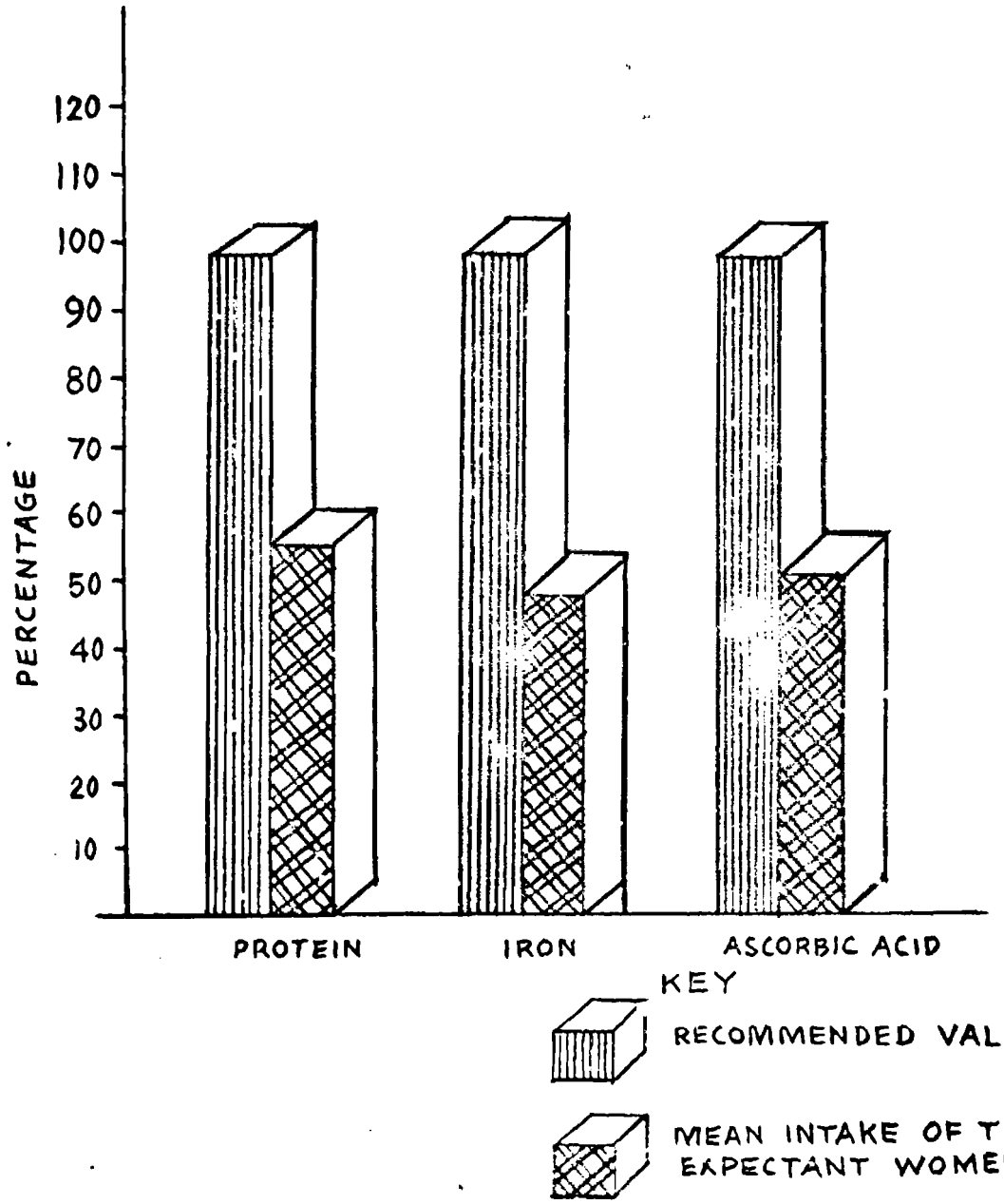

FIG. 1

Iyengar (1970) and it was deficient particularly in protein, the deficit being approximately 15g/day. These values support the present study in which the protein deficit is 14.7g.

Iron content in the diet of the expectant women studied also were lower than the recommended allowances. In a study conducted by Passricha (1958), it was observed that the amount of iron provided by an average Indian diet of poor income group was about 18 - 22 mg/day. This level apart from being inadequate coupled with the low calcium and ascorbic acid content in the diet of low income group decreased the availability of iron (Apte and Venkatachalam, 1962).

The mean ascorbic acid content in the diet was 22mg/ day which was deficient by 18mg when compared with ICMR recommended allowances. Ascorbic acid is essential for iron availability. Thus the availability of nutrients by the selected expectant women in this investigation is low not only in folate, but also in the related nutrients like protein, iron and ascorbic acid.

B. Availability of folic acid from diets of expectant women

Table VII presents the percentage availability of folic acid from the diet of the 20 expectant women.

TABLE VII

AVAILABILITY OF FOLIC ACID

Subject No.	Percentage of availability	Subject No.	Percentage of availability
1	39.3	11	56.6
2	72.4	12	29.2
3	30.8	13	29.5
4.	29.2	14	31.7
5	56.1	15	37.6
6	41.3	16	55.2
7	36.3	17	52.7
8	58.1	18	36.9
9	25.3	19	46.2
10	43.2	20	60.2

Mean availability = 43.1 per cent

Standard deviation = 43.1 ± 13.9

The availability of folic acid from the diets of the selected 20 expectant women ranged from 25.8 to 72.4 per cent with a mean value of 43.1 per cent. Information regarding the intestinal absorption of many nutrients during pregnancy is limited. Available information regarding absorption of folic acid during pregnancy is conflicting (NIN, 1975). There were considerable difference among individuals in their ability to absorb folacin from different foods. Availability of folacin in some foods varied between 0 and 100 per cent (Reid, 1969). It appears that during pregnancy, renal tubules do not absorb folacin as well they do normally. Mythen (1971) found that pregnant women excreted four times as much folacin as they did during 6th week of postpartum. Studies done at National Institute of Nutrition (1977) proved that the requirement of folic acid in pregnancy is increased due to excess of folic acid excretion in urine. Denko et al (1946) found that the daily excretion varied between 220 and 397µg/day with a mean of 310µg.

In this study, the mean availability of folic acid from the diets of the low income expectant women was 43.1 per cent. 20 Availability of folic acid from the diet is lower in these group, since their diet consisted mainly of cereals

and pulses. Most of them did not include green leafy vegetables in their diets. This may be the reasons for the decreased availability. Lakshmi et al (1969) studied the availability of folate from Bengal gram and green gram because these two are frequently consumed as part of the diet by majority of the people belonging to low and middle group. Also these two are rich sources of this vitamin. Availability from the two legumes averaged around 70 per cent.

Tamura and Stockstand (1975) suggested the presence of conjugase in some foods. The presence of conjugase have been reported in foods like Orange juice, in yeast and nucleic acid (Hoesenberg et al., 1971; and Tamura et al, 1973).

Viewing the above factors it may be postulated that non-inclusion of fruits and vegetables and the high cereal based diets of low income group may be responsible for the low folic acid availability in these expectant women belonging to lower socio-economic group.

C. Impact of the supplementation on certain indices of the expectant women

Having studied the intake and availability of folic acid and the intake of the related nutrients like protein, iron and ascorbic acid, it was thought of interest to follow up these selected women in their last trimester of pregnancy. The women were getting regular antinatal care in the maternity health centre and there by where recipients of folic acid and iron supplementation. Initially the serum iron levels, haemoglobin levels and serum folic acid levels of these women were estimated. Thereafter, the impact of supplementation of folic acid and iron on the above indices over a period of one month was studied. For want of time data for one month supplementation could alone be investigated in the present study. The impact of supplementation on these indices is clearly depicted in Fig. 2.

1. Serum iron level:

Based on serum iron level of 20 women studied before and after supplementation were grouped according to INCAP definition as high, medium and low risk groups and is presented Table VIII.

IMPACT OF SUPPLEMENTATION ON HAEMOGLOBIN, SERUM IRON AND SERUM FOLIC ACID LEVELS.

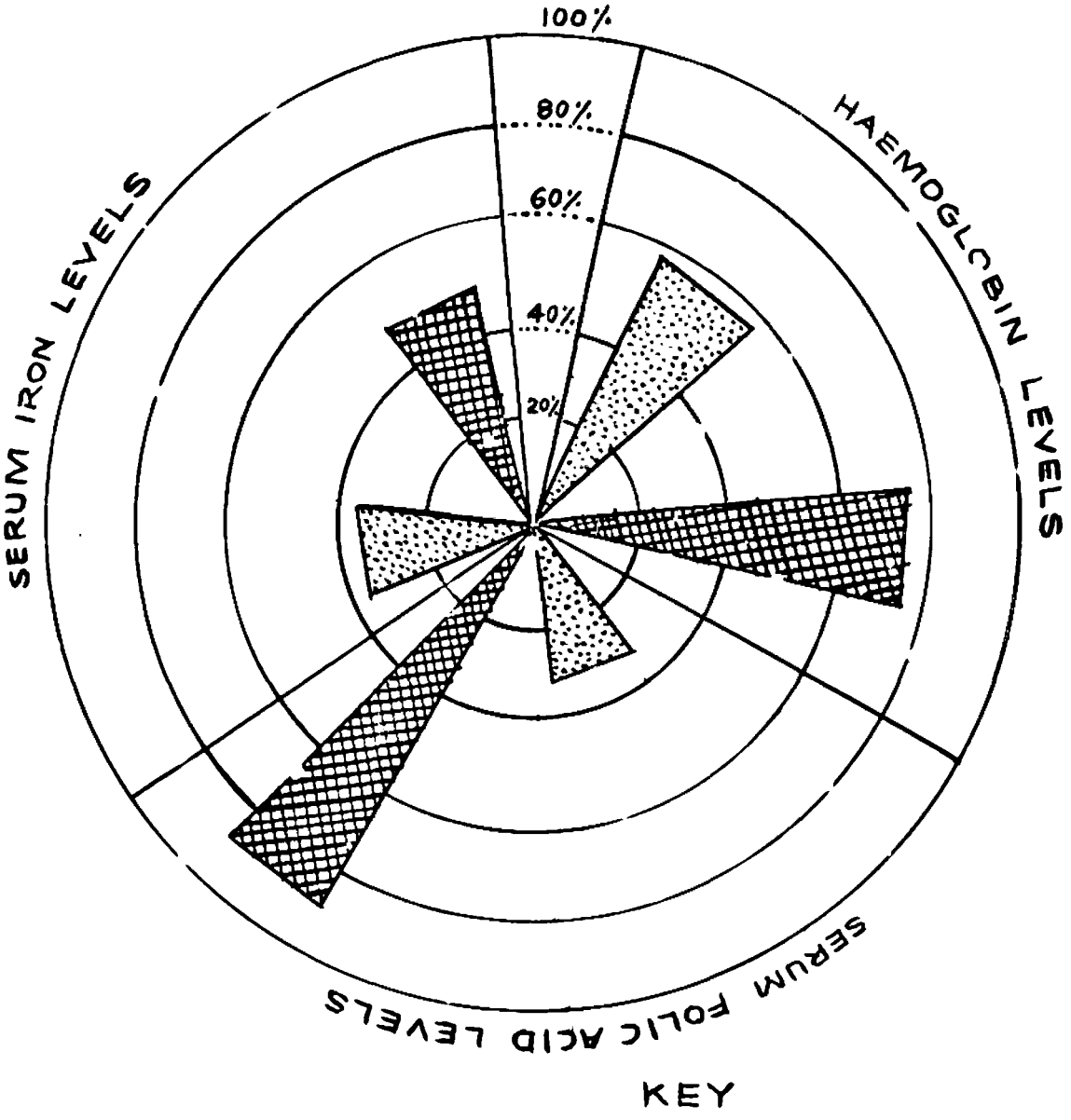

FIG. 2

TABLE VIII

SERUM IRON LEVELS OF THE EXPECTANT MOTHERS

INCAP Definition	Before supplementation		After supplementation		't' value
	No. of women	Percentage	No. of women	Percentage	
High risk (<45µg/100ml)	14	70	—	—	
Medium risk (45–59.9 µg/100ml)	6	30	3	15	27.6**
Low risk (>60µg/100ml)	—	—	17	85	
Total	20	100	20	100	
	Mean = 37.6µg/10ml		Mean = 76.0µg		
	S.D. = 37.6 ± 6.4		S.D. = 76±11.8		

** Highly significant at 1 per cent level.

Before supplementation, 70 per cent of the women were in the high risk group (<45µg/100ml) and 30 per cent were in medium risk group (45-59.9µg/100ml). None of these women were in the low risk group. Even within the short period of one month of supplementation with iron and folic acid, the serum iron level increased. Consequently, 85 per cent of the women were shifted to the low risk category (>60µg/100ml) and the remaining 15 per cent were in the medium risk category. None of them were in the high risk category. This impact of supplementation when analysed statistically were significant at 5 per cent level. The individual serum iron values of each expectant women before and after supplementation is given in Appendix C. The mean value of serum iron of expectant women before supplementation was 37.6µg/100ml and it rose to 76µg/100ml within one month of supplementation. Apte and Iyengar (1970) have reported serum iron level of 68µg/100ml for normal expectant mothers. With longer period of supplementation, there is a definite possibility of serum iron levels of these low income group expectant women raising to the suggested values.

2. Haemoglobin levels:

Percentage of women classified as deficient, low and acceptable as per ICMMD (1965) standards their haemoglobin levels from among the expectant women studied here is

given in Table IX. The individual haemoglobin level before and after supplementation along with mean is given in Appendix II.

TABLE IX

HAEMOGLOBIN LEVELS OF THE EXPECTANT WOMEN

Level 1963	Before supplementation		After supplementation		Groups compared 't' value
	No. of women	Percentage	No. of women	Percentage	
Deficient (< 9g)	18	90	—	—	
Low (9g-10.4g)	2	10	7	35	25.6**
Acceptable (10.5g-12.9g)	—	—	13	65	
Total	20	100	20	100	
Mean	8.98g		10.97g		
S.D.	0.38 ± 0.20		10.9% ± 0.74		

** Highly significant at 1% per cent level.

It is clearly evident from the Table that before supplementation with iron and folic acid, 90 per cent of the women had a positive deficiency of haemoglobin (9g/100ml) and 10 per cent of them had low haemoglobin level (9-10.4g/100ml). After supplementation, 65 per cent of them had acceptable haemoglobin levels of 10.5-12.9g/100ml and remaining 35 per cent were in the category of low haemoglobin level. The mean value before supplementation was 8.36g/100ml and one month after supplementation the mean value rose to 10.97g/100ml. This increment in haemoglobin level is statistically significant at one per cent level.

According to Gopalan (1971) and Kanigar et al., (1975) haemoglobin estimation carried out in various parts of the country on expectant mothers show that about 90 per cent of them are anaemic i.e. they have a haemoglobin level of 10g/100ml. In a survey conducted with 1000 women in Tamil Nadu, haemoglobin levels less than 12g/100ml were found in 56 per cent of the pregnant women. In 15 percent of the pregnant women the levels were less than 8g/100ml (Nutrition Society of India, Report of study group of nutrition Society of India, 1968). Gopalan (1972) reported a value of 9.9g/100ml of haemoglobin in the third trimester

of pregnancy for Indian women. The recommended values of haemoglobin for expectant mothers by WHO (1975) is 11g/100ml. In this study, supplementation over a period of one month with iron and folic acid rose the haemoglobin levels to the range of 10.5 to 12.9g/100ml.

Iron supplementation when combined with a supplementation of folic acid is much more effective in raising the haemoglobin levels than iron given alone. Devadas et al., (1970) reports haemoglobin level 10.7g for expectant women of Coimbatore district. The results of the present study is in line with the values reported by Devadas et al., Supplementation over a longer period, like over the entire period of third trimester might further increase the haemoglobin values and thus place these women in good haemoglobin status.

3. Serum folic acid levels:

The mean serum folic acid levels before and after supplementation and the classification of the women studied as high risk, medium risk, and low risk (WHO 1968) groups is presented in Table X. The individual serum folate levels of the expectant women is given in Appendix X.

TABLE X

SERUM FOLIC ACID LEVELS OF THE EXPECTANT WOMEN

Specification by WHO (1968)	Before supplements		After supplements		't' value
	No. of women	Percentage	No. of women	Percentage	
High risk (< 3mg/ml)	6	30	—	—	
Medium risk (3 - 6mg/ml)	14	70	—	—	15.8**
Low risk (> 6mg/ml)	—	—	20	100	
Total	20	100	20	100	
Mean	3.3mg/ml		10.1mg/ml		
S.D.	3.3 ± 0.68		10.1 ± 1.32		

** Highly significant at 1 per cent level.

It is evident from Table X that 30 per cent of the women were in the high risk group (< 3ng/ml) and 70 per cent in the medium risk group (3-6ng/ml) before supplementation. One month after supplementation with iron and folic acid all of them could be classed belonging to the low risk group (76ng/ml). The mean value for the serum folate level before supplementation was 3.3 ng/ml and it rose to 10.1ng/ml.

one month after supplementation. This increment is statistically significant at one per cent level. Iyengar (1971) has reported that 65 per cent of the pregnant women from low income groups in India have serum folate level below 3ng/ml. The values obtained in this study in slightly higher but quite comparable as reported by Iyengar (1971). Mudaliyar et al (1969) (1969) had obtained serum folate levels of 11 - 14 ng/ml for healthy expectant women and ≤ 6ng/ml for anaemic expectant women. Values obtained in this study before supplementation is very similar to those reported in the literature and it is highly encouraging to note that supplementation with iron and folic acid even for a short period resulted in bringing up the serum folate levels of these expectant women.

When the haemoglobin levels and serum folate levels were correlated for values before and after supplementation (Table XI), it was found that there exists a positive correlation between serum folate and haemoglobin levels (r = + 0.683, and + 0.032).

TABLE XI

CORRELATION BETWEEN HAEMOGLOBIN AND SERUM FOLIC ACID LEVELS

Correlation	r	t
Serum folate level and Hb level		
Before supplementation	0.663	5.427
Serum folate level and Hb level After supplementation	0.032	0.1383

The correlation between the food folate and serum folate (Table XII).

TABLE XII

CORRELATION BETWEEN FOOD AND SERUM FOLATE

Correlation	r
Correlation between food folate and serum folate	+ 0.173

was also positive (r = + 0.173).

From the foregoing discussion, it is evident that there exists a wide variation in the intake of folic acid from the diet as consumed commonly by expectant women. The availability of folic acid thus, consumed was also depended upon various factors like the variety of food eaten, possible presence of inhibitory substances in cereals and the like. These factors coupled with the socio-economic status of these low income category of women reflected in low intake of folic acid rich foods in the predominantly cereal based diet of these women. This apparent low intake of folate and non availability resulted in lower serum folate levels. Apart from being low in folic acid content, the diet of these women also were also low in protein, iron and ascorbic acid, the nutrients which compliment each other in the absorption of these nutrients in human system, consequently the majority of the women studied had risk levels of serum folate, serum iron and deficient levels of haemoglobin. The fact that supplementation even over a shorter period of time helped remarkably in raising the standard of these nutrients in the system of the women studied indicate that supplementation with iron and folic acid definitely improves the nutritional

status of these expectant women. Apart from the supplementation of iron and folic acid in the form of tablet, the possibility of supplementation of these nutrients from food sources in the diets commonly consumed by these women opens out new areas of research and also would enlightend the common man as to where they stand in terms of their nutritional status and how one could improve by modifying their own dietary pattern.

V SUMMARY AND CONCLUSION

The main objectives of this investigation was to find out the availability of folic acid from the diets of a group of expectant women belonging to low income category. Twenty expectant women belonging to low-income group, attending regularly, an antinatal clinic, and all in their third trimester of pregnancy, belonging to Coimbatore city were selected as the participants in this study.

In order to assess the folic acid intake and availability from the diets of these expectant women, a weighment survey was conducted initially in the families of selected expectant women. Simultaneously an aliquots of each food item consumed by the expectant women was collected for the entire day, pooled, homogenised and samples drawn for the analysis of folic acid, protein, iron and ascorbic acid. On the same day, 24 hours urinary collection was made, sample drawn and analysed for free folate. This enabled, the computation of the availability of folic acid from the diets of selected expectant women. In order to study the impact of supplementation of folic acid and Iron given in the antinatal clinic, for the same mothers, serum folate, serum Iron and haemoglobin levels were estimated initially and one month after supplementation. The salient findings are:-

1. The mean folic acid content of the diets of the expectant women studied were 193.2µg of free folate and 470µg of total folate. These values were well with in the range reported in the literature.

2. The mean intake of protein, iron, and ascorbic acid were 30.8g, 19.4mg, 22mg respectively. Intake of all these nutrients were nearly 15 to 50 per cent deficit when compared to recommended allowances of ICMR (1976).

3. The mean availability of folic acid from the diets of the expectant women studied were 43.1 per cent.

4. Initially 70 per cent of the women studied were in the high risk group as far serum iron level and 30 per cent in low risk group. One month after supplementation, only 15 per cent were in medium risk group and 85 per cent in low risk group. This increment in serum iron level was significant at one per cent level.

5. The mean initial Haemoglobin level of the expectant women studied was 8.9g/100ml. One month after supplementation, the mean value increased to 10.97g/100ml and this increment was significant at one per cent level.

6. The mean serum folic acid level was 3.3ng/ml initially and it rose to 10.1ng/ml one month after supplementation, this increment was significant at 1 per cent level.

7. There existed a low positive correlation between serum folate level and haemoglobin level before (r = ±0.683) and after (r=+0.032) supplementation. food folate and serum folate (r = + 0.173)

A few problem and limitations were encounted by the investigator while undertaking this study. Standardisation of the technique itself was a challenge as it was a new effort in the laboratory. Secondly, since it involves expectant women. Collection of food, urine and serum for analysis to be limitted only to a small sample due to want of time, because collection from human subject especially during pregnancy. And above all microbiological assays involved, lengthy and laborious procedure. These the choice of small sample.

It may however be well concluded that, this investigation can be taken as beginning of a series of investigation that could follow. This study clearly brings out the fact that there is a wide variation in the intake and availability of folic acid from the diets commonly consumed by the expectant women. Availability of folic acid into

a great extent dependent upon various factors. And in an predominantly cereal based diet it is very low. Coupled with low intake of folic acid, low availability from such diets, and the low intake of associated nutrients like protein, iron and ascorbic acid, expectant women under this socio-economic category are classed as women belonging to high risk, and non acceptable category when their serum folate, serum iron, and haemoglobin levels are considered. Supplementation with iron and folic acid tablet even over a short period of one month resulted in a remarkable improvement. Hence it leads one to postulate that, supplementation of commonly consumed diet of the expectant women with the foods rich in hematopoietic nutrient within then socio-economic, cultural reach could well improve their nutritional profile of these women.

This leads to new areas of research and few recommended ones are:

1. Study of folate intake and availability from the diets of various population group on a large sample.

2. Study of the effect of possible factors in foods that affect the absorption and availability

3. Influence of processing including cooking on folate availability

4. Supplementation studies with synthetic and natural foods containing haematopoietin nutrient on the availability of folate in different population group.

BIBLIOGRAPHY

Adams on I.Y.R. and CURL, D.L. 1978)

Retarded development of neonatal rat lung by natural Nutrition" Journal of Histo chemistry and cylo-chemistry, Vol. II, pp. 401-408.

Apte, S.V. and Iyengar, L. 1972

"Composition of Human Foetus" Brit. J. Nutr. vol. 27, pp.305.

Baugh, C.M. Krumdieck, C.L. Baka, H.J. and Butter worth, C.E. 1971.

"Studies on absorption and Metabolism of folic acid" J. Clin. Invest. Vol. 50, pp. 200.

Babu, S. and Srikantia, S.G. 1976.

Availability of folate from foods" Am, J. Clin, Nutr. Vol. 29, pp. 376-37

Benjamin, F.F. Bassen, A. and Mayer, L.W. 1966

"Serum levels of folic acid, Vitamin B_{12} and Iron in Anaemia of pregnancy" Amer. J. Obstect Gynecol 96, pp. 310.

Benten, G.H. and Bengoa, J.M. 1976

"Nutrition in preventive medicine The major deficiency Syndromes epidemiology and approaches to control", WHO Geneva Monograph Series No. 62, pp. 63-64.

Bruce, B.S.

"Nutrition in pregnancy" 12th edition by G.J. Geneva will sandev, 2, Philadelphia, pp. 203.

Butterworth, C.E., 1968

"Absorption and mal absorption of dietary folate" Am, J. Clin. Nutr. vol.21, pp. 1121-1127.

Colman, R. Green, R. and Metas, J. 1975

"Prevention of folate deficiency by food furtification", Am. J.Cl. in Nutr. Vol. 28, pp. 459-464.

Coursey, H.G. and Kima, K.S. 1972 — "Relationship of Maternal Nutrition and weight, gain to Newborn Infant" J. Obstect Gynaecol, Vol. 139, pp. 460-465.

Chanarin, I. Rothman, D. Ward, N., and Berry, J., 1960. — "Folate Status and requirement in pregnancy" Brit. Med. J. Vol. 2, pp. 390.

Dawson, D.W. 1966 — "Microdose of folic acid in pregnancy J. Obstect. Gynaecol, Br. Commonw, Vol. 73, pp. 44-88.

Dento, C.N. Grudy, W.E. Porter, J.W. Berryman, G.H. Friedemann, J.F. 1949. — "The urinary excretion of B complex vitamin in the urine and faeces of several normal adults". Archs. Bio-Chem. Bio-Phys. Vol. 10, pp. 38.

Devadas, R.P. Shanbagavalli, R.N. and Vijayalakshmi, R. 1970. — "The impact of applied nutrition Programme on the Nutritional Status of the selected Women," Ind. V. of Nutr. and diets.

Fleming, A.F. Martin, J.D. Habret, R. and Westlake, A.J. 1974 — "Effects of iron and folic acid anti-natal supplements on maternal haemotology and felal, well being, "Med. J. Aust. vol.2, pp. 429-435.

Gateby 1956 — "Diet and anaemia", The anaemia of pregnancy in Dublir' Proc. Nutr. Soc. vol. 15, pp. 115.

Gerson, C.D. Cohen, N. Hepner, G.W., Brow, N. — "Folic acid absorption in Man. Enhancing effect of glucose" Gastroenterology Vol. 61, pp. 224-227.

Herbert, V and Jano wits H.P. 1971

Gopalan, C. 1972 — "Nutrition in Pregnancy' Tropical Doctor, Vol. 2, No. 4 pp. 1-3.

Gopalan, C. (1973) — "Symposion on Maternal and child Nutrition" Proceedings of the nutrition society of India No. 14, pp. 32-33.

Gopalan, C. 1973 — 'Public Health Nutrition Programme" Report of the task force on nutrition', Proceeding of the nutrition society of India, NIN Hyderabad, No. 18, pp. 80.

Gopalan, C. 1975. — 'Anaemia as a public health problem' Nutrition (Published by NIN), vol. 9, No. 2, pp. 2-11.

Gopalan, C. 1977 — 'The Fight against Malnutrition in the world' WHO Chronicle, Vol. 31, No. 7, pp. 276-227.

Hansen, H.A. Nordiquest, P. and Sourandai, P. — 'Magnloblastic anaemia and neurological disturbances combained with folic acid deficiency Acta Med, Scand, 176 pp. 243.251.

Hansen, M. and Ryhe.S. 1967 — "Folic acid dosage in prophylatic treatment during pregnancy". Acta, Obstect, Gynecol Suppl. Vol. 7, No. 46, pp. 107.

Habitch, A. Yesbrough, C. Lachilig, A. Klein, R.E. 1973. — "Nutrition Report of Internatural" vol. 17, pp. 31.

Herbert, V. 1968 — 'Nutritional requirements for vitamin B_{12} and Folic acid" Amer, J. Clin, Nutr., No. 2, pp. 743.

Hibbard, B.M.
1964 'The role of folic acid in pregnancy with particular reference to anaemia abruption and abortion'. J.Obstet Gynaecol B.Commonw. 71, pp. 529-542.

Hibbard, B.M. and Hibbard
1965 'Folic acid and reproduction' Acta, Obstect. Gynecol 46, Supplem 7, 61-69.

Hoorichane, B. Coyles V. and Druky, M.I.
(1960) Megaloblastic anaemia of pregnancy J. In. Med. Assoc. 47.

Hundle, A.D.F.
1967. 'Folate contents of Hospital diet" M.D. thesis University of London.

Hurely
1968 "The consequences of fetal impoverishment" Nutrition Today, Vol. 3, No. 4, pp. 3-10.

ICMR
1971 'Nutrition for mother and child", ICMR special report series No. 41, pp. 5.

ICMR
1975 "Anaemia in pregnancy" ICMR Bulletin, Vol. 5, No. 11, p. 41-4.

ICMR
1975 Maternal Nutrition effect on fetal Nutrition, ICMR Bulletin Vol. 7, No. 12, p. 1-7.

ICNND
1963 Manual of Nutrition Surveys' International Committee on Nutrition for National Defence p. 236.

Iyengar, L.
1968 'Urinary Oestrogen excretion in undernourished pregnant Indian Women, Amar, J. Obstect Gynecol B. Commonw Vol. 102, pp. 834-838.

Iyengar, L.
1975

"Influence of diet on outcome pregnancy" Proceedings of 9th International Congress of Nutrition, Vol. 2, pp. 93.58.

Jellief (
1976)

"World trends in Infant feeding" Am. J. Clin. Nutr. Vol. 29, No.1 pp. 1231, 1234, 1227.

Jacobson, H.L.
1964

Report of a Workship on Nutritional Supplementation and the outcome of Pregnancy" National Research Council, Washington, pp. 63.

Kerrigar, K.A.
and Bernard, R.J.
1975

"Nutritional status of anaemic tea bicters from Ceylon" Proceedings of International Congress of Nutrition. Keyto Japan pp. 597.

Lakshmi, V.
and
Ramasastri, B.V.
1969.

'Folic acid content of Indian foods of plant origin" J. Nut. and Dietetics, No. 6, pp. 200.

Lillis E.N.
1968

"Obsterical aspects of Megaloblastic anaemia of pregnancy" J. Obstect. Gynacol. Br. Commonw. 69, pp. 37 36.

Lowrstein
Cantlie, R.
Ramos, O.
and
Baaten, L.

'The incidence and prevention of folate deficiency in pregnant clinic population' Can. Med. Assoc. Vol. 95, pp. 797-806.

Markkanen, T.
1968

"Absorption tests with normal folate material in controls and gastrectomised patients" Am. J. Clin, Nut. Vol. 21, pp. 473-481.

Macclain, G.
1975

'Distribution of folic acid coensyme and folate dependent enzyme in mouse brain". J. Neno, Chem. Vol. 24, pp. 719-722.

Mehran 1968 — 'To eat to see' Nutrition Today Vol. 3, No. 1, pp. 2-8.

Mormcha, S.L. 1972 — "Malnutrition and Retarded human Development" Springfield Thomas P, p. 123.

Mudaliyar, A.L. Menon, M.K.K. 1969 — "Clinical obstetrics Orient Diagnosis" VI edition pp. 220-227.

Munro, N.N. 1974 — American Journal of Clinical Nutrition Vol. 27, pp. 55.

Nelson, E.W. Sterij, R.R. and Cerda, J.J. 1975. — "Comparative bio-availability of folate and vitamin from synthetic and natural source" Am.J. Clin. Nut. Vol. 28, pp. 1014-1019.

NIN 1971 — Manual of Laboratory technique Folic acid assay, pp. 59-62.

NIN 1974 — Effect of folic acid supplements during pregnancy on birth weight of infants" ICMR, Annual Report, pp.99-104.

NIN 1975 — NIN Annual report, pp. 21

NIN 1977 — "Influence of dietary cereals and Millets on absorption of folate Annual report ICMR pp. 26-27.

Nutrition Review 1967 — "Folic acid and pregnancy" Vol. 25, pp. 325.

Pritchard, J.A. 1962 — 'Megaloblastic anaemia during pregnancy and the puer perium' Am. J. Obstect. Gynecol. No. 100 pp. 341-345.

Ramsay, W.N.M. 1958 — 'Advance clinical chemistry' edited by Sabokka H. and Stewart C.P. Accademic Press New York.L.

Rajalakshmi,K. 1975 — "Folic acid absorption in pregnancy" J. of obstet Gynecol, Vol.22, pp. 332-336.

Rajalakshmi,K. 1975 — "Nutritional disorders in expectant Mothers" 'Nutrition' (Published by NIN) No.2, pp. 3-8

Retif,L. 1969 — "Urinary foltate excreation after ingestion of pteroyl glutamic acid and food folate" Am.K.Clin. Nut.Vol.22, pp. 362.

Shanthimi,R. Berger,P.M. Sheety,T.W. A Wiles,J. and Davila,I. — "Folic acid activity in putero Racian foods". J.Am.diet-Assoc.Vol.141, pp.562.

Srikantia,S.G. and Iyengar,L. 1972 — "Effect of Nutritional supplements in pregnancy on birth weight of the new born" proceeding of Nutrition Society of India pp.27, 31-32.

Swaran,and Parvathia.G. — "A Survey of dietary intake in groups of pregnant and lactating women". Ind.J.Med.Res.Vol.46, pp.605.

Tamura,T. Shin,Y.S. Williams,M.A. and Stockstan,E.L.R. — "The availability of folate in man" Effect of orange Juice supplement on intestinal conference" Br.J.Haemated,Vol.32, pp. 123-133.

Thomson, A.M. and Billewicz, W.Z. 1963 "Maternal Physique and reproductive efficiency proc. Nutr. Spc. Vol. 22, pp. 5.

Temperly, I.J. Mohan, M.J.M. and Gateby, P.B.B. 1964 "Serum folic acid level in pregnancy and them relationship to Magaloblastic marrow change. Br. J. Haemtol.

Venkatachalam, P.S. 1962 "Maternal Nutritional Status and its effect on the new born", WHO Bulletin, XXIV, pp. 193-201.

WHO 1968 "Nutritional Anaemias," WHO Technical Series, No. 4053 pp. 21-23, 35.

WHO 1975 "Control of Nutritional anaemias with Special reference to Iron deficiency" WHO tech. report series, No. 580, pp. 16-17.

WHO 1971 'Nutritional Anaemias' WHO technical report series No. 503, pp. 128.

WHO 1978 WHO Chronicle, 32, No. 6, pp. 231-232.

WHO 1978 "Global events in the field of Nutrition" WHO chronical, Vol. 32, No. 8, pp. 314.

Varley, H. 1969 Practical clinical Bio-Chemistry Arnold Heinemann Publishers (India) 4th edition pp. 586.

Willoughby, M.L.N. 1968 'An Investigation of folic acid requirement in pregnancy' Br. J. Haematol. Vol. 13, pp. 503-509.

Baradi, S. Abbot, D. and Elwis, A. 1966 Correlation in peripheral white cell and bone marrow change with foeal level in pregnancy and their clinical significance" J. Clin Path, 19, pp. 33.

Wordsworth, G.R. 1973 "Some historical aspects of knowledge about folate deficiency" Nutrition, Vol. 27 pp. 17-22.

Zamenhoff, R.J. 1968 'DNA and Protein in neonatal brain' Science, No. 160, pp. 322.

APPENDICES

APPENDIX A

MICROBIOLOGICAL ASSAY OF FOLIC ACID

Aim

Estimation of folic acid by Microbiological assay.

Principle:

Microbiological methods are based on the observation that certain micro-organisms require specific nutrients for growth. Using a basal medium complete in all the aspects except for the nutrient under test, growth responses of the organism, are compared quantitatively with standard and unknown solutions.

Either the mold or the turbidity produced by the organism is measured to determine the extent of growth and these by the amount of nutrient in the test solution.

Organism used:

Lactobacillus casei Atcc 7469.

1. Reagents Required:

1. Solution A:

Dissolve 25g each KH_2PO_4 and K_2HPO_4 in distilled water and make up the volume to 250ml.

2. Salt solution B:

Dissolve the following in distilled water, add 5 drops of con Hcl and made up the volume to 250ml.

$MgSO_4$ 7H20	-10.0gms
Nacl	- 0.5gms
$FeSO_4$ 7H20	-0.5gms
MgSO4 H20	- 0.5gms

3. Vitamin solution:

Thiamine hydrochloride	-20mg
Nicotinic acid	-20mg
P.amino benzoic acid	-10mg
Ca.pantothenate	-20mg
Pyridoxine hychochloride	-50mg
Biotin solution 100µg/ml	-1ml

Dissolve the above in about 100ml distilled water and add to this solution, 20mg riboflavin in distelled water and prepared with the use of few drops of acetic acid and, gentle warming if necessary. Finally make up the volume of vitamin solution to 200ml.

4. L. Tryptophan solution 0.2%:

Dissolve 1g L. Tryptophan in distilled water using a few drops of conc Hcl, and makeup the volume to 500ml.

5. L. Cystine solution 0.8%:

Dissolve 5g of L. Cystine in distilled water using a few drops of non-Hcl and make up the volume to 500ml.

6. D. Lalanine solution - 2%:

Dissolve 2g of DL.alanine in distilled water and make up the volume to 100ml.

7. Xanthin solution - 0.2%

Dissolve 200mg of xanthin in distilled water using a few drops of conc NH_4 and make up the volume to 100ml.

8. Adenine, guanine and uracil solutions

200mg of each adenine Hcl, guanine So_4, and uracil in distilled water using few ml of conc Hcl and make up the volume to 100ml.

9. Peptone solution - 4%:

Dissolve 4g of DEFCO peptone in distilled water and adjust the pH of the solution to 3.0 using con Hcl and make up the volume to 100ml. add to this solution 2g of activated

charcoal stir for 30 minutes and filter. Repeat the treatment with charcoal and filtration two or more time.

10. Protein hydrolysate solution - 10% vitamin free:

Dissolve 100g of Hydro protein, a hydrolysate obtained from Bengal community, co; Ltd., Calcutta, in about 800ml distilled water with aid of heat if necessary. Adjust the PH of the solution to 3.5 using 40 per cent sodium hydroxide solution and make up the volume to one litre. Stir the solution with 20g activated charcoal for 30 minutes and filter. Repeat the charcoal treatment for three times or more if the blank value (i.e. the inoculated blank value is high the charcoal treatment may be done once more).

Protein hydrolysate solution suggested is an alternate for casein hydrolysate solution normally used. For preparation of the conventional casein hydrolysate solution see Free sample method vitamin assay published by Association of Vitamin chemist.

11. Standard Folic acid solution:

Dissolve 10mg of pure pteroyl glutamic acid in 100ml of 0.8 per cent sodium biocarbonate solution and store at 2-4°c.

12. Folic acid standard solution:

(To be prepared on the day of use) Dilute the stock standard folic acid solution to give folic acid concentration of 100pg/ml.

13. Ascorbic acid Phosphate buffer:

(To be prepared on the day of use) Prepare a 5% solution of ascorbic acid in water and stir the solution with activated charcoal (about 400mg/100ml) for 15 minutes and filter (an approximately) 40 per cent solution would be obtained by this procedure) Add to this filterate an equal volume of 0.2m sodium phosphate buffer PH6.1, and adjust the PH to 6.1 with 10 per cent sodium hydroxide. The solution is further diluted to two fold, to obtained an approximately 1 per cent ascorbic acid in 0.05m phosphate buffer.

Maintenance of the culture:

The culture is maintained for tightly trasfer in agar stands prepared as follows:

Anhydrous glucose	-10g
Sodium accetate, 3H20	-17g
DEFCO peptone	- 5g
Salt solution A	-2.5ml
Salt solution B	-2.5ml

In about 400ml distilled water. Adjust the solution to pH 6.8 using 40 per cent sodium hydroxide and make up the volume to 500ml. To the above solution add 7.5g of agar and dissolve agar by heating the mixture. While the solution is still hot disperse approximately 10 ml of it to different tubes (6 x 3/4). Plug the tube with cotton and sterlize them by autoclaving for 15 minutes at 15lb pressure. Store the tubes at 2-4°c when not in use.

Preparation of inoculum

The inoculum is to be prepared on the day prior to the day of assay by transferring cells from the stock culture tube to a sterile inoculum tube which is prepared in the same way as above by omitting addition of agar.

Preparation of washed inoculum

In order to wash the organism, free of contamination with folic acid, transfer the incubulaed inoculum into a sterile 50ml centrifuge tube plugged with cotton. The cotton should be placed and held in position by a rubber band, that it is not sunnked into the tube during centrifugation. Alternatively sterile screw capped centrifuged tubes can be used.

Centrifuge the inoculum and discard the supernatant suspend the cells in 10ml of sterile normal saline and centrifuge again. After repeating this process of washing two or more times, finally suspend the cells in sufficient amount of sterile normal saline to yield a slightly opalescent suspension.

Preparation of basal medium

Glucose	-4gms
Sodium acetate 3H20	-5.6gms
Protein hydrolysate solution	-10ml
L.Tryptophan solution	-5ml
L.cystine	-5ml
DL.alanine solution	-1ml
Peptone solution	-1ml
Salt solution A	-5ml
Salt solution B	-5ml
Adenine guanine, uracil solution	-1ml
Xanthine solution	-1ml
Vitamin solution	-2ml

Dissolve in about 70ml of water adjust the PH to 6.8 using 40 per cent sodium hydroxide and make up the volume to 100ml and filter.

Preparation of serum sample for assay

To 0.5ml serum and 0.5ml of 0.05ml sodium phosphate buffer pH 6.1 (prepared by diluting 4 fold the 0.2m sodium phosphate buffer) and add one per cent ml of one per cent and ascorbic acid phosphate buffer pH 6.1 autoclave, the mixture for 10 minutes at 15 lbs pressure cool and make up the volume to 30-50ml to obtain final concentration approximately 60-80pg of folic acid/ml and filter.

Assay procedure:

Set up light duplicate sets of test tube and to each tube add 1ml of 0.25% ascorbic acid phosphate buffer. Prepared by diluting 4 fold of the 1% ascorbic acid phosphate buffer. Two sets of tubes serve as zero blank (one as the inoculated blank, and one as the un inoculated blank). To the other tubes add successively in duplicate 0.5 1, 1.5, 2.0, 3.0 and 4.0ml of folic acid working standard equivalent to 50, 100, 150, 200, 300, 400pg of folic acid respectively. Make up the volume of each tube including zero balank to a total volume of 5ml with distilled water.

Test solution:

Five tubes would be required (singly or in duplicate) for each of the sample to be analysed. Add to these

tubes respectively 0.5, 1.2, 3.4° of the diluted serum extract extract prepared as discribed earlier. Add 1ml of 0.25% ascorbic acid phosphate buffer, to all the tubes and make the total volume 5ml with distilled water.

To all the (standard tests) add 5ml of double strength basal medium plug the tubes with cotton and auto clave for 12 minutes at a pressure of 12lb/square inch cool the tubes (except the set of tubes which serve as uninnoculated blank) with one or two drop of the washed innoculum prepared as earlier incubate the tube at 37°c for 36 to 42 hours and at the end of this period measure turbidity in a calorimeter using 660 filter and setting the instrument for zero optical density with inoculated blank. Plot the optical density against concentration of folic acid. Read from the standard curve the concentration of folic in the test solution.

Calculation:

For a valied assay the concentration per ml of the test solution at different level should agree with in 10-15%. Average the value obtain with different levels of the test solution and folic acid content/ml of serum.

Preparation of food sample for the assay

The method of food folate assay was based on that of Lakshiah and Ramastri (1969) with slight modification. Each food sample (6-10g) was homogenished in 40ml of 2m phosphate buffer.

Preparation of 2M po4 buffer:-

$Na_2 Hpo_4 2H_2O$ = 35.589g/1 litres

$NaH_2po_4 H20$ = 31.202g/liters

Mix 720ml g Na_2H po4 2io 280ml of

Na H_2po4 = $2H_2O$

Add to this 1000ml of H20 adjust PH 7.2 Add 10g of asoorbic acid) At PH 7.2 contains 0.3% ascorbate.

The volume was made up to 50ml with 1 buffer. The homgenate was autoclave at a low preasure 15lb of 15 minuts after cooling to room temperature, the homognenate was adjust to 4.5. The final volume of the extract was made upto 100ml with distilled H_2O and filterated.

Determination of q total folate in foods

For the determination of total folate activity 1.0ml of the above filterate was diluted are 3.5ml 0.2m acetate buffer par PH 4.5.

Preparation of acetable buffer

Sodium acetate an hydrus	=	16.4g/lit
Glacial aceetic acid	=	11.5ml/lit
For PH 4.4 acetic acid	=	Na acetate.
71.0ml	=	39ml
PH 31.0ml	=	20ml

Adjust Ph to 4.5

Add 0.5ml of 100m Mecrcapito ethanol or 2% ascorbic acid buffer. 0.2ml plasma was added to course conjucase to hydro lifse poly gêutamate to give total folate concentration of the test sample. The tube was layord with a few drops of toluene incubated overnight at 37°C. After incubation the samples were diluted suitably before folate determination

APPENDIX B

PROTEIN, IRON AND ASCORBIC ACID CONTENT IN THE DIET OF THE EXPECTANT WOMEN

No.	Protein in g	Iron in mg	Ascorbic acid in mg
1.	26.7	25.4	22.6
2.	29.2	20.0	16.9
3.	28.7	27.4	21.0
4.	32.3	15.0	26.7
5.	26.6	18.0	20.7
6.	34.4	17.5	20.6
7.	39.3	18.5	38.1
8.	33.3	19.5	12.0
9.	26.0	15.4	38.0
10.	32.3	37.0	29.1
11.	27.3	14.6	22.0
12.	28.4	28.4	29.7
13.	35.4	22.0	23.6
14.	38.3	21.7	16.5
15.	23.4	16.5	14.4
16.	29.0	14.8	18.5
17.	24.4	14.0	28.3
18.	21.7	15.3	24.0
19.	26.6	14.4	23.0
20.	27.7	12.6	29.0
	Mean = 30.3 S.D. = 30.3±5.24	Mean = 19.4 S.D. = 19.4 ± 5.9	Mean = 22.0. S.D. = 22.0± 7.8

APPENDIX C

SERUM IRON LEVELS OF THE EXPECTANT WOMEN

No.	Serum iron before supplementation in µg/100ml	Serum iron after supplementation µg/100ml
1.	33	67
2.	42	75
3.	27	58
4.	50	100.0
5.	33	75
6.	33	79
7.	46	92
8.	42	83
9.	27	58
10.	46	91
11.	34	67
12.	33	58
13.	42	88
14.	50	75
15.	27	67
16.	34	71
17.	31	75
18	42	89
19.	46	92
20.	23	71
	Mean = 37.6	Mean = 76.0
	S.D. = 37.6±6.4	S.D. = 76 ± 11.0

APPENDIX D

HAEMOGLOBIN LEVELS OF THE EXPECTANT WOMEN

No.	Before supplementation Haemoglobin g/100 ml	After supplementation Haemoglobin g/100ml
1.	8.2	10.3
2.	8.5	11.6
3.	7.7	9.4
4.	9.1	12.0
5.	8.9	10.3
6.	8.0	10.9
7.	9.1	12.4
8.	8.9	11.5
9.	8.9	11.8
10.	7.9	9.8
11.	8.5	11.3
12.	8.2	11.8
13.	8.2	11.5
14.	8.4	10.0
15.	8.9	10.8
16.	7.7	9.8
17.	8.8	11.3
18.	8.5	10.1
19.	8.4	9.8
20.	8.0	11.8
	Mean = 8.38	Mean = 10.97
	S.D. = 8.38 ±	S.D. = 10.97±0.74

% = 25.6

APPENDIX E

SERUM FOLIC ACID LEVELS OF THE EXPECTANT WOMEN

No	Serum folic acid level before supplementation in ng/ml	Serum folic acid level after supplementation in ng/ml
1.	3.6	11.5
2.	4.0	11.6
3.	4.4	12.4
4.	3.2	10.4
5.	4.4	12.0
6.	2.4	10.8
7.	2.4	8.4
8.	2.8	9.4
9.	3.6	10.4
10.	3.2	10.0
11.	4.4	12.0
12.	4.4	10.8
13.	3.2	10.1
14.	4.0	11.2
15.	2.8	8.8
16.	2.4	8.4
17.	3.0	8.6
18.	3.4	9.6
19.	3.3	8.8
20.	2.8	8.4
	Mean = 3.3	Mean = 10.1
	S.D. = 3.3 ± 0.68	S.D. = 10.1 ± 1.32